A FIELD, CULTURE, AND CYTOLOGICAL STUDY OF *PORPHYRA GARDNERI*, *PORPHYRA NEREOCYSTIS* AND *PORPHYRA THURETTII* (RHODOPHYTA, BANGIOPHYCIDAE)

by

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A biologist should strive to be as diverse in outlook as Humboldt and Darwin, to observe with the intensity of Agassiz, but above all, at risk of being thought an iconoclast, to be ever wary of dogma.
ABSTRACT

A field, culture and cytological investigation has been made of the life histories of *Porphyra gardneri* (Smith et Hollenberg) Hawkes, *P. nereocystis* Anderson and *P. thuretii* Setchell et Dawson, three superficially similar epiphytic red algae (Rhodophyceae, Bangiales).

*Porphyra gardneri* was found from February through November, but is basically a spring-early summer species. *Porphyra nereocystis* is an autumn-winter species and *P. thuretii* is a winter-early spring species. The reported number of host species and geographical distribution is increased for all three species.

A light microscope study of spermatogenesis and carposporogenesis was undertaken to characterize the spermatangium and carpogonium and to follow their division sequence. The spermatangium in *Porphyra gardneri* and probably *P. thuretii* is formed by the production of a new wall layer around a transformed vegetative cell. In contrast, no new wall layer is formed by *P. nereocystis* spermatangia, making them difficult to distinguish from vegetative cells.

The carpogonium of *P. gardneri* and *P. thuretii* is easily recognized because it differentiates bipolar prototrichogyynes. In *P. nereocystis* the carpogonium does not form prototrichogyynes and can only be distinguished from vegetative cells by its elongate, rectangular shape.
On the basis of these observations and reports in the literature I suggest that the species of *Porphyra* can be separated into at least two groups based on their spermatangial and carpogonial morphology; the more advanced group has carpogonia with prototrichogynes and clearly defined spermatangia due to the deposition of a new wall layer, whereas the more primitive group lacks these features.

The first division in both spermatogenesis and carposporogenesis is periclinal. This is discussed with respect to the division sequences reported by other workers.

The transfer of *Porphyrella gardneri* Smith & Hollenberg to *Porphyra gardneri* is explained on the basis of the observed mode of carpogonium formation and carposporangial division.

Of the three species studied, only *Porphyra gardneri* produces monospores. Under all photoperiod and temperature regimes tested monospores germinated into the foliose phase. At an ultrastructural level monosporogenesis in *Porphyra gardneri* is characterized by the production of abundant small and large fibrous vesicles. The economic implications of monospore production are discussed.

Field observations of *Porphyra gardneri* suggest that photoperiod is an important factor involved in inducing the formation of spermatangia and carpogonia.

A conchocelis phase is reported for the first time for *Porphyra gardneri* and *Porphyra thuretii*. Under all photoperiod and temperature regimes tested carpospores germinated into the conchocelis phase.

In *P. gardneri* and *P. thuretii* short days favoured
conchosporangial branch formation; however, no such photoperiodic effect was observed for P. nereocystis. Although my results were inconclusive, reports in the literature indicate that temperature is a critical factor in conchospore release.

Amoeboid movement of conchospores is reported for Porphyra thuretii and discussed with respect to the occurrence of this phenomenon in the Rhodophyta generally.

The epiphyte-host attachment zone of Porphyra gardneri and P. nereocystis was examined with the light microscope. The basal rhizoids of P. gardneri penetrate deep into the medulla of the Laminaria blade, whereas the rhizoids of P. nereocystis make only a superficial penetration of the Nereocystis stipe.

The spermatia of Porphyra gardneri and P. nereocystis were examined ultrastructurally and found to have many similarities with Florideophycidae spermatia.

The occurrence of fertilization in the life history of Porphyra gardneri is documented with the light and electron microscopes. A diploid chromosome number 2n=8 is reported for the carpospores and vegetative cells of the conchocelis phase of P. gardneri. This is the first unequivocal report of sexual reproduction in the genus Porphyra.

Suspected spermatia attached to the carpogonia and linked to them via fertilization canals have been observed in P. nereocystis and P. thuretii suggesting that sexual reproduction also occurs in their life histories. Reports of sexual reproduction in other members of the Bangiophycidae are evaluated in light of my observations.
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of specimens of *Porphyra gardneri*.

My parents interest in my studies has been an inspiration. The encouragement and patient understanding of Denise Bonin has made the task of writing this thesis tolerable.
Despite the great amount of investigation that the genus *Porphyra* has received, there is not a single species for which the complete life history, both morphological and cytological, is known. Higita's (1967b) work on *Porphyra yezoensis* Ueda is the most thorough study to date. Unfortunately he did not present a detailed documentation of the fertilization process, which is the major feature of the *Porphyra* life history requiring verification.

One of my primary objectives in this study was to rectify this situation by attempting to answer the much disputed question of the occurrence of sexual reproduction in the genus. The other main objective was to characterize 3 superficially similar epiphytic, monostromatic *Porphyra* species by obtaining as much information about their life histories and basic biology as possible.

Three approaches to these problems were taken. The first was a field study to determine the seasonal occurrence of the foliose phase and the time of spore formation. The second was a culture study of spore formation and the effect of selected environmental factors on spore formation and germination. The third approach was a cytological study to obtain chromosome counts for all spore types and life history stages to see if there was any change in the ploidy level, thus indirectly indicating the occurrence of fertilization and meiosis. A light and electron microscope study of sporogenesis by the
foliose thallus was undertaken because I felt that it might give direct evidence of the occurrence of fertilization.

Initially I began working with *Porphyra nereocystis* Anderson. It is one of the largest *Porphyra* species, and is reportedly very palatable (Hus, 1902). It therefore seemed to be a plant with potential commercial value. Its epiphytic habit also intrigued me, and I was curious to see if any special life history modifications had been made to adapt it to its 'host' plant, *Nereocystis luetkeana* (Mertens) Postels et Ruprecht.

During the first year of my field studies I became aware of *Porphyra gardneri* (Smith et Hollenberg) Hawkes¹, which superficially resembles *Porphyra nereocystis* and had been considered by Gardner (in: Collins et al. 1919) and Kylin (1941) to be a dwarf or young form of this species. In view of this, and because no life history information had been published on this alga since it was described by Smith and Hollenberg (1943), I decided to include it in my study. This was a fortunate decision because it proved to be the most convenient plant to work with. As a result, the major part of this thesis deals with *Porphyra gardneri*.

During the second year of my field observations I collected *Porphyra thuretii* Setchell et Dawson on the stipe of *Nereocystis*. This was the first time that this species had

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¹When I began my investigation, this plant was known as *Porphyrella gardneri* Smith et Hollenberg. As a result of my observations of the mode of carpogonium formation and division (to be discussed in Part II) I transferred this species to the genus *Porphyra* (Hawkes, 1977b).
been found north of Oregon (Conway et al. 1975). Because so little was known about the ecology and life history of this alga I decided to include it in my study.

I have presented the thesis in four parts: Part I - Field and herbarium studies; Part II - Spermatogenesis and carposporogenesis; Part III - Culture studies; and Part IV - Cytological studies. Part II is one aspect of the cytological studies which I have presented separately, near the beginning of the thesis, because it is essential in justifying the transfer of Porphyrella gardneri to the genus Porphyra.
The morphological life history of most *Porphyra* species consists of an alternation between a foliose thallus and a filamentous phase. I will be referring to these life history stages as the foliose thallus or phase, and the conchocelis phase, respectively.

There is a plethora of terminology which has been applied to the reproductive structures of *Porphyra*. In her classic work on the Bangiophycidae, Drew (1956) presented a spore classification scheme which included *Porphyra*. Richardson (1972) proposed a scheme specifically for *Bangia* and *Porphyra*.

As a result of my observation (Hawkes, 1977a, 1978) that sexual reproduction occurs in the life history of *Porphyra gardneri*, a reassessment and standardization of terminology was necessary. The following is a summary of the terms I have used for the reproductive structures and spore types which I encountered in the 3 *Porphyra* species I studied. For a detailed summary of the original sources of these terms as well as other terminology that has been applied to the reproductive structures of the foliose phase see Conway et al. (1975, p. 189).

**Foliose Phase**

*Spermatangium* - produces the spermatia by a series of periclinal and anticlinal divisions. Synonymous
with $B$-spore mother cell (Conway et al. 1975), packet (Hus, 1902), and antheridium (Hus, 1902; Kurogi, 1972).

Spermatium - the male gamete. Haploid. Synonymous with $B$-spore (Conway et al. 1975), and antherzoids (Hus, 1902).

Carpogonium - formed by the transformation of a vegetative cell, may or may not produce prototrichogynes. Synonymous with mother cells (Drew, 1956) and $\alpha$-spore mother cells (Conway et al. 1975; Hawkes, 1977b).

Prototrichogyne - receptive protuberance of the carpogonium. Length varies with the species and not all species produce them. Monostromatic species produce 2 per carpogonium, whereas distromatic species produce only one.

Carposporangium - following fertilization the carpogonium becomes the carposporangium. Produces the carpospores by a series of periclinal and anticlinal divisions. The possibility exists that this can occur without fertilization. Synonymous with packet, sporocarps (Hus, 1902), cystocarps (Kurogi, 1972), and packets of $\alpha$-spores (Conway et al. 1975; Hawkes, 1977b).
**Carpospore** - released from the carposporangium and germinates into the conchocelis phase. Diploid, although haploid carpospores may form in some cases. Synonymous with $\alpha$-spores (Conway et al. 1975; Hawkes, 1977b).

**Monospore** - asexual spore produced by the foliose phase of several *Porphyra* species by the transformation of a vegetative cell without any divisions. Following release a monospore germinates back into the foliose phase. Synonymous with neutral spore (Kurogi, 1961). In some of the Japanese literature (eg. Kurogi, 1953b) the conchospores were referred to as monospores. The term monospore has also been applied to spores produced by the conchocelis phase which germinate to give more conchocelis phase (Conway and Cole, 1977).

**Conchocelis Phase**

**Conchosporangial branch** - typically an uniseriate branch which is wider than the vegetative conchocelis filaments and consists of a linear series of conchosporangia. Characterized by a thick cell wall compared to the vegetative filaments. Each conchosporangium contains a single stellate chloroplast. The available evidence suggests that
the conchosporangium is the site of meiosis (Giraud and Magne, 1968; Kito, 1974). Conchosporangial branch is synonymous with monosporangial branch (Kurogi, 1953b).

Conchospores - formed by 2-4 divisions of the conchosporangium in some species (Migita and Abe, 1966; Migita, 1967b; 1974). It is still not clear whether only one conchospore per conchosporangium is produced by some species. Referred to as monospores in some of the Japanese literature (eg. Kurogi, 1953b).

A brief justification of my selection of terms for the reproductive structures formed by the foliose phase is in order. Functionally the spermatia, carpogonia and carpospores of *Porphyra gardneri* are homologous with their counterparts in the Florideophycidae. In view of this, and in an attempt to be consistent with previous work (Papenfuss, 1955), and to standardize the terminology in the two subclasses, the terms spermatium, spermatangium, carpogonium and carpospores have been used.

Differences in the position and mode of formation of the spermatia and carpospores have been pointed to as criteria characterizing the Bangiophycidae and Florideophycidae (Fritsch, 1945, p. 437; Drew, 1951). In the former, several spermatia are produced per spermatangium, whereas in the latter only one is produced. After fertilization in *Porphyra gardneri* the entire zygote divides to produce several carpospores per
carposporangium, but in the Florideophycidae the zygote directly or indirectly gives rise to the gonimoblast whose filaments then produce a single carpospore per carposporangium. It should be noted that the details of spermatium production really only apply to the Bangiaceae, and apparently not all Bangiophycidae. For example, in *Smithora* only one spermatium per spermatangium is produced. It is felt that these differences are not of sufficient magnitude to warrant creating new terms for the reproductive structures in *Porphyra*.

The slight protuberances of the carpogonium found in most species of *Porphyra* (Table VI) have been referred to as pseudo-trichogynes (Dangeard, 1927), prototrichogynes (Tseng and Chang, 1955), 'trichogyne-like' (Conway and Cole, 1973) and trichogynes (Kunieda, 1939). In *Porphyra gardneri* these specialized protuberances of the carpogonium act as the receptive site for the spermatia and seem to be responsible for the specificity of spermatium attachment. Functionally they are homologous with the trichogynes of the Florideophycidae; however, because they are not as well developed the term prototrichogyne would seem most appropriate.
PART I - FIELD AND HERBARIUM STUDIES

Introduction

Before attempting any culture work or cytological investigation I felt that it was of the utmost importance to familiarize myself with *Porphyra gardneri*, *P. nereocystis*, and *P. thuretii* in the field. The purpose of the field study was to determine the seasonal occurrence, type and time of spore formation, 'host' species, and geographical distribution for each species. I hoped that this information would enable me to fix thalli at the right time for cytological study of chromosome numbers and sexual reproduction. Furthermore, because these 3 species are epiphytes, I thought that some knowledge of the seasonal development of the 'host(s)' would be essential to a complete understanding of their life histories. The following is a brief characterization of the 3 species and a summary of previous investigations of them.

*Porphyra gardneri* is a foliose, monostromatic red alga which grows epiphytically on several members of the Laminariales (Figure 1a). In the original description of it, Smith and Hollenberg (1943, as *Porphyrella gardneri*) reported that in early summer thalli from Point Joe on the Monterey Peninsula, California were either vegetative or producing monospores. By late summer spermatia and spores they tentatively called carpospores were being produced. The spermatia were said to occur in packets as is typical of *Porphyra*, but the carpospores were reported to occur singly.
Smith and Hollenberg (1943, p. 215) stated, "The carpogonia are formed by a cell division in which there is a curving wall, quite similar to the curving cell walls producing the monosporic spores of Erythrotrichia and the cells thus formed are liberated singly." These single spores formed by an unequal cell division were the basis for establishing the genus Porphyrella (Bangiophycidae), with Porphyrella gardneri as the type species (holotype, G. M. Smith 39-12, DS 306401 in UC). The fate of these spores was not determined by Smith and Hollenberg.

Subsequent workers have reported the occurrence of Porphyra gardneri (as Porphyrella gardneri) elsewhere in California (Smith, 1944; Abbott and Hollenberg, 1976), in Oregon (Doty, 1947; Markham and Celestino, 1976), Washington and British Columbia (Scagel, 1957, 1973; Widdowson, 1974) and Alaska (Wynne, 1972; Lindstrom, 1977). However, no further information concerning the life history of this alga has been published.

Porphyra nereocystis is a monostromatic member of the Bangiophycidae which grows epiphytically on the stipe of Nereocystis luetkeana (Mertens) Postels et Ruprecht. It is one of the largest species of Porphyra (Figure 1b), reaching up to 3.7 m in length and 0.7 m in width (UC 96517, from Esquimalt, Vancouver Island). It was first reported by Anderson (1891, name only; description published in Blankinship and Keeler, 1892) based on material from Santa Cruz or the Farallon Islands, California. No holotype exists. Hus (1900) noted that Pyropia californica J. Agardh is a synonym of
**Porphyra nereocystis.**

*Porphyra nereocystis* is the only species I studied which has been investigated by other workers to any extent. It has been reported in California (Howe, 1893; Kylin, 1941; Smith, 1944; Abbott and Hollenberg, 1976), Oregon (Doty, 1947), Washington (Kylin, 1925; Krishnamurthy, 1972; Mumford, 1973a), British Columbia (Collins, 1913; Scagel, 1957, 1973; Widdcson, 1974; Conway *et al.* 1975) and Alaska (Setchell and Gardner, 1903; Johansen, 1971; Lindstrom, 1977).

Hus (1902) was the first to comment on the palatability of *P. nereocystis* and its use by the Chinese in California. The commercial potential of the plant has recently received extensive investigation by Woessner (1974) and Woessner *et al.* (1977).

The conchocelis phase has been cultured by Krishnamurthy (1969a), Mumford (1973a), Conway *et al.* (1975) and Conway and Cole (1977). Conway and Cole (1975) briefly reported on the ultrastructure of the vegetative cell of both the foliose thallus and the conchocelis phase.

Despite a considerable amount of study, the life history of this alga is still not well known. For example, there are conflicting reports of its seasonal occurrence. Anderson (in Blankinship and Keeler, 1892) only indicated that it "...is seldom found until past midsummer, when the long stems of *Nereocystis* are well grown." Smith (1944) and Abbott and

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*Kylin mistakenly referred specimens of *Porphyra gardneri* to *Porphyra nereocystis*.
Hollenberg (1976) reported that the foliose phase is an annual and is usually found from November to June. Both Woessner et al. (1977) and Arasaki (1974) agree that it is a winter species. In contrast, Conway et al. (1975) reported the season of occurrence as summer through winter, and Conway and Cole (1977) indicated it is summer.

There are also conflicting reports of the degree of host specificity exhibited by *P. nereocystis*. Anderson (in Blankinship and Keeler, 1892) indicated that it was most frequently found on *Nereocystis*, although not exclusively limited to it. Hus (1902) reported it growing on rocks. Abbott and Hollenberg (1976) indicated that it is occasionally found on other Laminariales, but Woessner (1974) felt it was exclusive to *Nereocystis*.

*Porphyra thuretii* is another monostromatic, epiphytic *Porphyra* about which very little is known, particularly its range of host plants or its seasonal occurrence. Paul C. Silva has indicated (on a note attached to the lectotype, 10 Dec., 1971) that the original publication of *P. thuretii* was intended to be in Dawson (1944) which was published in July 1944. However, the species was actually published first by Smith (1944) who included it in *Marine Algae Of The Monterey Peninsula California*, which appeared early in 1944. Smith did not cite a type collection but stated that the type locality was Pacific Grove. Dawson (1944) designated Setchell #5161 as the type, indicating that it was from Pacific Grove; however, according to the label on specimens of that number in the UC herbarium, and to Setchell's field book, his #5161 was
collected at Carmel Bay. A plant from this 29 May 1900 collection (UC 791973) was designated as the lectotype by P. C. Silva in September 1949 (Figure 1c). It was found growing epiphytically on *Gracilaria sjoestedtii* Kylin. Dawson (1944) noted that in addition to the type locality, the species was also known from Santa Cruz and Monterey Bay. Hus (1902) reported this species under the name *Porphyra leucosticta* Thuret.

*Porphyra thuretii* was subsequently reported from Oregon (Doty, 1947). It has recently been reported in British Columbia for the first time¹ (Conway et al. 1975), based on a collection I made at my study site in Barkley Sound, Vancouver Island. Dawson (1952) reported *P. thuretii* from several locations in Mexico, and indicated that the plants Taylor (1945) collected in Costa Rica and referred to as *Porphyra naiadum* Andersen, were actually *P. thuretii*.

Both Smith (1944) and Dawson (1944) reported that *P. thuretii* grows on *Gracilaria sjoestedtii* or on rocks. Dawson (1952) stated that the plants from Mexico were saxicolous or rarely epiphytic. The plant reported by Conway et al. (1975) was growing on the stipe of *Nereocystis luetkeana*.

The season of occurrence has been reported as the spring (March to May) (Hus, 1902, as *P. leucosticta*; Smith, 1944; Scagel (1973) recorded *P. thuretii* from Barkley Sound, but Conway et al. (1975) indicated that these specimens are probably more correctly identified as *Porphyra abbottae* Krishnamurthy and *P. fucicola* Krishnamurthy.
Materials And Methods

Most of the field work was carried out near the Bamfield Marine Station on the west coast of Vancouver Island, British Columbia (Figure 2a). A study site off Diana Island in Barkley Sound (48° 50.1'N, 125° 11.1'W) was selected (Figure 2b) because it is semi-exposed to the open Pacific Ocean and has a sheltered channel between it and Diana Island, where a boat can be landed under most conditions throughout the year. A 'Zodiac' inflatable boat was used for all field work.

At the study site P. gardneri reached optimal development on Laminaria setchellii Silva. Most observations were made on specimens collected from this 'host' from May 1974 through May 1977.

Other sites in Barkley Sound where Porphyra gardneri was collected were: Ross Islets, Leach Islet, Execution Rock, and Cape Beale. See Appendix I for a complete list of my collecting stations, their location, and dates of collection.

Porphyra nereocystis was variable in its abundance at the study site from year to year so it was necessary to collect it at other sites in the Barkley Sound area as well. These included the kelp beds off: Aguilar Point, Cable Beach, Second Beach, and Leach Islet. At the study site P. nereocystis was observed from June 1974 through April 1977.

Porphyra thuretii was not observed at the study site until 1975 and was never abundant enough to make a detailed monthly
study of it. Recently (April 1977) it was found in abundance at Leach Islet, a much more exposed location in Barkley Sound. *Porphyra gardneri* could be collected on low tides (0.5 m or less), whereas *P. nereocystis* and *P. thuretii* could be obtained at all but the highest tides simply by pulling up a *Nereocystis* plant. Despite this, SCUBA was frequently used and proved invaluable for making *in situ* observations and collecting fresh material for chromosome counts and electron microscopy. *Porphyra gardneri* also grows on several subtidal hosts which could be reached only by using SCUBA. SCUBA was also needed to anchor experiments using artificial *Nereocystis* plants and an *in situ* culture chamber for conchocelis filaments. When collections were made at the study site, surface seawater temperature was measured.

When the opportunity arose, specimens were collected from other localities. In the case of *Porphyra gardneri* these were: **British Columbia**: Langara Island, Queen Charlotte Islands; Gordon Islands, Queen Charlotte Islands; Tree Islets; Brooks Peninsula; Grassy Island, Kyuquot Sound; Hot Springs Cove; and Botanical Beach, Port Renfrew. **Oregon**: Harris Beach. **California**: Ano Nuevo Island; Point Joe and Pescadero Point, Monterey Peninsula; and Mission Point, Carmel. Localities outside the Barkley Sound area where *Porphyra nereocystis* was collected included: **British Columbia**: Langara Island, Queen Charlotte Islands; Bolkus Islands, Skincuttle Inlet, Queen Charlotte Islands; Tree Islets; Hope Island; Grassy Island, Kyuquot Sound; Botanical Beach; Whiffen Spit, Sooke; and South Pender Island. **California**: Pebble Beach, Monterey Peninsula.
Other sites in British Columbia where Porphyra thuretii was collected included: Rennell Sound, Queen Charlotte Islands and Volcanic Cove, Kyuquot Channel, Vancouver Island. The purpose of observing these other sites was to see if there were any population differences in the time and type of spore formation. The field study of Porphyra gardneri at its type locality (Point Joe, Monterey Peninsula) was essential in order to confirm that the plants at the study site were the same taxon.

At the study site and the Bolkus Islands, Queen Charlotte Islands, where abundant Porphyra nereocystis was encountered, several Nereocystis plants were collected in order to get a quantitative assessment of the vertical distribution of P. nereocystis along the stipe. Starting at the pneumatocyst, samples were taken at 1 m intervals down the stipe. All the P. nereocystis plants in each vertical meter were collected and dried at 70°C for 1 week and a dry weight was taken.

A series of artificial host experiments was run to see if it was the host plant itself, or just the unique position in the environment which it occupies that the epiphyte requires. Three types of artificial Nereocystis stipes were made, using manila rope (3-strand, 15 mm diameter), vinyl tubing (Fisher brand, 11 mm outside diameter) and surgical rubber (Fisher brand, 11 mm outside diameter) for the 'stipe'. At one end the 'stipe' was lashed to a 4 l sealed plastic bottle for floatation. The 'plants' were then anchored to rock climbing pitons (Cassin - Italy, 250 mm long with ring 47 mm diameter) which were driven into crevices in the rocky bottom at a depth of 7-10 m. Polypropylene rope (13 mm diameter) was used to tie
the 'plants' to the piton ring. These artificial _Nereocystis_ stipes were put out in the field in September 1974 and August 1975.

Artificial _Laminaria_-like plants were made from heavy nylon mesh cut into strips. They were also anchored using pitons, and were placed in the _Laminaria_ zone at approximately the 0.4 m tide level.

In addition to the field study, specimens in numerous herbaria (Table I) were examined to supplement field observations and to confirm that I had correctly identified the species being studied.

**Results**

**A. _Porphyra gardneri_**

1. Seasonal Occurrence Of Epiphyte And 'Host'

A brief consideration of the annual growth and decay of _Laminaria setchellii_ is essential to an understanding of the establishment and seasonal occurrence of _Porphyra gardneri_. At the study site the blade of _L. setchellii_ was worn back almost to the stipe by November. During late November or early December, the meristematic transition zone commenced forming a new blade, which was initially a characteristic heart shape (Figure 3a). Growth was rapid and within a month the blade began to split distally (Figure 3b). This splitting continued as the blade grew and resulted in the formation of the typical
lacerated blade by February or March (Figure 3c).

During the first months of growth the new blade was relatively free of epiphytes. Growth of diatoms and ectocarpalean epiphytes became obvious by early summer; they appeared first on the distal portions of the blade which contained the oldest tissue. During the autumn the blade became increasingly epiphytized and worn away. This blade erosion resulted in the disappearance of a suitable substrate for *Porphyra gardneri*.

The first thalli of *Porphyra gardneri* appeared at the end of February at the study site. Their distribution was very striking, being restricted to the margins of the *Laminaria* blade (Figure 3c). The number of *P. gardneri* thalli continued to increase rapidly throughout the spring, and the margins of *Laminaria setchellii* soon became densely lined with the plant (Figure 1a). These thalli of *Porphyra gardneri* had a characteristic loose gelatinous appearance along their distal margin (Figure 3d) due to a massive production and release of monosporangia (to be discussed in Part III). The first thalli to produce spermatangia were observed during late April (Figure 4a). They were easily recognized by the fine, colourless patch work margin that the spermatangia form. About a month after the appearance of the spermatangia the lateral submarginal regions became slightly more deeply pigmented than the central vegetative region of the thallus, due to the production of carposporangia (to be discussed in Parts II and III).

Thalli growing in the spring and early summer can reach a
length of 130 mm and a width of 60 mm, but typically they are 20-70 mm long and 10-30 mm wide. The largest thalli occurred on *Eisenia arborea* Areschoug and *Laminaria setchellii* which were growing subtidally. Later in the summer the thalli were generally much smaller than those found earlier in the season. The number of thalli declined in the autumn as the *L. setchellii* blade eroded. At this time several small thalli appeared on the surface of the *Laminaria* blade instead of being restricted to the margins.

As long as old *Laminaria* thallus is present *Porphyra gardneri* will continue to grow (Figure 4b). Most *P. gardneri* was gone by September-October, but I collected some as late as November.

The thallus morphology and reproduction of *Porphyra gardneri* at the other sites where it was collected in British Columbia were similar to those observed at the study site (Figures 4c, 4d, and 4e). Specimens from Botanical Beach (Figure 4c) tended to have more ruffled margins than study site plants but were similar in all other respects. Because these sites were visited only once it is impossible to make comparisons of the seasonal occurrence or reproduction.

The field study of the Monterey plants, combined with culture and cytological work (to be discussed in Parts II, III, and IV), conclusively demonstrated that they are the same taxon which is present at the study site. However, there were differences in the abundance of thalli, and in the time of spermatangium and carpogonium formation between the plants at these two sites. *Porphyra gardneri* was more abundant at Point
Joe (type locality) in November than it was at the study site. Also the thalli at Point Joe (Figure 4f) as well as at Pescadero Point and Mission Point were generally much smaller (30 mm long average, up to 70 mm) than thalli from farther north. An examination of herbarium specimens from California, Oregon, Washington, British Columbia and Alaska, in several herbaria (CSUH, DS, GJH, GMS, RS, UBC, UC, UCSC) confirmed these observations.

Most thalli collected at Point Joe in July 1976 were monosporic, this being confirmed by culture work done at the Hopkins Marine Station. Some thalli had spermatangial lateral and monosporic distal margins, whereas others were spermatangial, carposporangial and monosporic. The frequency of carposporangia is low compared to more northern populations. The predominance of monosporic thalli in July is in sharp contrast to the situation at the study site where most thalli were carposporangial and spermatangial. An examination of herbarium specimens collected in the Monterey area revealed that they were almost always monosporic. In fact, the type specimen is exclusively monosporic, a curious fact considering that the genus Porphyrella was based on the details of carpogonium formation and carpospore release.

2. 'Host' Species

Porphyra gardneri is not restricted to one host, having been observed on fifteen laminarialean and one rhodophycean algae (Table II). Saxicolous specimens were not found. Throughout its range it reaches optimal development on the
margins of Laminaria setchellii or other Laminaria species. The 'host' species grow in the tidal range between approximately +1.4 m to -3.0 m. Porphyra gardneri is small (10-20 mm long) on 'host's' like Egregia menziesii (Turner) Areschoug, which are in the upper part of this range. In the early spring months P. gardneri can be found in abundance on the 'leaflets' of Egregia, but by May no thalli were found on this 'host'. Porphyra gardneri thalli on Eisenia arborea were generally larger and became reproductive earlier than those on Laminaria setchellii.

On all the 'host' species, P. gardneri was typically associated with the thallus margins. One exception to this trend was the host Postelsia palmaeformis Ruprecht, where the thalli of P. gardneri were growing on abraded portions of the stipe.

3. Artificial Host Experiment

None of the attempts to grow Porphyra gardneri on the artificial host plant was successful.

4. Geographical Distribution

From my own collections, herbarium specimens and reports in the literature it is clear that Porphyra gardneri has a wide distribution. Scagel (1957) and Dawson (1961) reported it (as Porphyrella gardneri) to extend from northern British Columbia to the Monterey Peninsula. Sparling (1971, as Porphyrella gardneri) extended the southern range to Hazard
Canyon, San Luis Obispo County, California (35° 18'N, 120° 53'W). Specimens on the leaflets of _Egregia laevigata_ Setchell (AHF 77063) from Punta Banda, Baja California, Mexico (31° 44'N, 116° 44'W) have been examined and are referred to _Porphyra gardneri_. This apparently isolated population represents a great extension of the southern limit of _P. gardneri_. Punta Banda falls within one of the areas of cold water upwelling described by Dawson (1951) along the Baja California coast. Several other typically more northern, cooler water algae are found there as well (Abbott and North, 1972).

Wynne (1972) reported _Porphyra gardneri_ (as _Porphyrella gardneri_ ) from Amchitka Island in the Aleutian Islands, Alaska. Collections deposited in the University of British Columbia Phycological Herbarium extend the range well into northern Alaska and west along the Aleutian Islands to Murder Point, Attu Island (52° 48'N, 173° 11'E, UBC 7949). Specimens of _Porphyra nereocystis_ reported by Zinova¹ (1940) on _Laminaria longipes_ Bory from Bering Island in the Commander Islands (55° 12'N, 165° 58'E) are referrable to _Porphyra gardneri_ and represent its western distributional limit. (A specimen collected by E. Kardakova on 30 January 1930, and deposited in the Botanical Institute of the Academy of Science of U.S.S.R. in Leningrad has been examined. UBC 56455 is a photograph of this specimen). No _Porphyra_ species epiphytic on _Laminaria_ margins have been reported for the Sea

¹Author's name transliterated on the original as Sinova.
of Okhotsk (Zinova, 1954), Sakhalin Island (Vozzhinskaya, 1964), or the Kuril Islands (Nagai, 1941). Tokida (1960) and Ohmi (1963) do not mention any Japanese species of *Porphyra* on *Laminaria*, and Dr. M. Kurogi (personal communication) has confirmed this. *Porphyra gardneri* is also absent from the Strait of Georgia, British Columbia. A list of representative specimens of *P. gardneri* from throughout this geographical range is given in Appendix II.

E. *Porphyra nereocystis*

1. Seasonal Occurrence Of Epiphyte And 'Host'

*Nereocystis luetkeana* is an annual, although a few plants can survive for as long as 18 months. It first appeared at the study site at the beginning of March. The plants were essentially epiphyte-free during the period of rapid stipe growth. In June and July, growth of the bryozoan *Membranipora membranacea* was noted on the laminae and pneumatocyst. *Ulva stenophylla* Setchell et Gardner, *U. fenestrata* Postels et Ruprecht and species of *Enteromorpha* appeared on the pneumatocyst during August. By July most of the *Nereocystis* plants had reached the surface and stipe growth was much reduced. The stipes showed signs of abrasion, but were not epiphytized.

Once *Nereocystis* reached the end of its stipe growth phase, establishment of epiphytes was rapid. By late September to early October a bacterial and diatom 'scum' followed by ectocarpalean epiphytes became established on the stipe.
Throughout the autumn and winter the plants became progressively more heavily epiphytized. Breakdown and erosion of the stipe weakened the plants and thereby contributed to the almost complete elimination of the *Nereocystis* beds during the winter and spring. A few plants, which survived the winter storms intact, were easily recognized by their dense coating of epiphytes.

At the study site *Porphyra nereocystis* first appeared on the *Nereocystis* stipes in late November to early December. It grew on the upper portion of the stipe which floats on the surface at low tide. The young thalli have a characteristic long, narrow ribbon shape which slowly tapers to a point (Figure 5a). The margins are straight, and without wrinkles or folds. Large, mature thalli up to 2.7 m long (Figure 1b) were found in January and February.

During January, the lateral margins of the thalli became colourless due to the production of spermatangia (Figure 5b). These marginal spermatangial strips varied in width from 10 mm to 90 mm. They began to erode in the distal region of the thallus and continued to erode in a basal direction, leaving a distal tip of vegetative cells and carposporangia (Figure 5c). Long, narrow, submarginal streaks of spermatangia also formed, and were joined in places to the marginal strips (Figure 5d). Spermatangia were never observed in small microscopic patches as reported by Mumford (1973a) and Conway et al. (1975). By the end of January or early February, carpogonia and carposporangia began forming in the distal and lateral submarginal areas of the thallus (to be discussed in Part II).
In older thalli the carposporangia often gave the distal tips a mottled appearance (Figure 5e).

Porphyra nereocystis lasted from November to July or August in the field. I have collected it in abundance in the spring and early summer when it is always associated with a Nereocystis plant from the previous year.

A number of other Porphyra species were also observed on the stipe of Nereocystis along with Porphyra nereocystis. These included: Porphyra gardneri (UBC 55942), P. miniata (C. Agardh) C. Agardh (UBC 57219), P. perforata J. Agardh (UBC 57222), P. smithii Hollenberg et Abbott (UBC 57220), and P. thuretii (UBC 57201).

Porphyra nereocystis appeared in the Strait of Georgia approximately two months earlier than it did at the study site. New thalli were collected as early as September at Sidney, Vancouver Island (UBC 1434) and South Pender Island (UBC 57194), and in October at Friday Harbor, San Juan Island, Washington (UBC 57195). Porphyra nereocystis also appeared earlier in California than it did at the study site (J. Woessner, personal communication, August 1977). I collected young thalli in November at the Monterey Peninsula (UBC 57196).

At the other sites along the outer coast of British Columbia the appearance of thalli and their reproductive status at the time of collection suggests that they have a seasonal occurrence similar to the study site plants.
2. Vertical Distribution

Porphyra nereocystis varies in its size and abundance depending on its position on the Nereocystis stipe. Most plants are concentrated on the upper 3 m of stipe, with the largest plants typically being in the first 1-2 m (Table III). At the lower end of the distribution the thalli are small (10-100 mm) and vegetative. Another curious feature is that on most stipes the thalli are limited to one side of the stipe.

3. 'Host' Species

Porphyra nereocystis was found exclusively on the stipe of Nereocystis with the exception of a few thalli found growing on the heavily epiphytized blades of Macrocystis integrifolia Bory (UBC 57197). Although there are reports in the literature that P. nereocystis can grow saxicolously, I never observed this.

4. Artificial Host Experiment

The best material tested for imitating a Nereocystis stipe was surgical rubber. The 'plant' I put out in the field in 1975 lasted 14 months. It supported a magnificent growth of algae, some of which included: Ulva stenophylla, Hollenbergia subulata (Harvey) Wollaston, Alaria marginata Postels et Ruprecht and Desmarestia ligulata var. ligulata (Lightfoot) Lamouroux. In November, a few small (30-40 mm long) thalli appeared on the artificial stipe (UBC 57221). They had the typical shape of young P. nereocystis, but none of them grew beyond this stage, making positive identification
5. Geographical Distribution

From my own collections, reports in the literature and herbarium specimens, the known distribution of Porphyra nereocystis is: Staraya Bay, Unalaska Island, Aleutian Islands, Alaska (53° 37.4'N, 165° 30.6'W, UBC 26776), northeast to Box Point, Montague Island, Alaska (59° 58'N, 147° 22'W, UBC 25836) and south to San Luis Obispo County, California (35° 10'N, 120° 45'W) (Dawson, 1961). Drift specimens are occasionally found in southern California (Abbott and Hollenberg, 1976).

Zinova (1940) reported that Porphyra nereocystis grows on Laminaria longipes and Nereocystis luetkeana at Bering Island, Commander Islands, U.S.S.R. (55° 12'N, 165° 58'E). I have been able to examine these specimens, which are deposited in the Botanical Institute of the Academy of Science of U.S.S.R. in Leningrad. As I indicated earlier, the specimen from Laminaria longipes is actually Porphyra gardneri; however, the specimen from Nereocystis is referable to P. nereocystis (UBC 57206, is a colour slide of this plant). Some workers (P. A. Lebednik, personal communication) feel that this report of Nereocystis luetkeana in the Commander Islands was unknowingly based on drift specimens. I feel that this would be worth re-investigating because Zinova (1940) specifically stated that Nereocystis grows in the sub-littoral zone and forms "thick copses which make it difficult or impossible for a boat to pass through." I have attempted to obtain further information on
this point, but without success.

A list of representative specimens of *Porphyra nereocystis* from throughout its geographical range is given in Appendix II.

C. *Porphyra thuretii*

1. Seasonal Occurrence

This little known alga was first encountered at the study site in February 1975 on the stipe of *Nereocystis luetkeana* along with *Porphyra nereocystis*. The one fertile plant from this collection (UBC 52019) was identified by Dr. T. F. Mumford, Jr. I have subsequently collected this species on several occasions.

*Porphyra thuretii* was collected in January, but it was not abundant until March or early April. It was not encountered after May. The most characteristic feature is the strongly ruffled margin (Figure 6a), which readily distinguishes vegetative thalli from *P. nereocystis*. Superficially the ruffled margins give *P. thuretii* a resemblance to *P. miniata*; however, the two can readily be separated on the basis of their monostromatic and distromatic constructions, respectively.

A few thalli producing spermatangia were observed in January, but most did not do so until February or March. The spermatangia are first noticeable as a thin, colourless marginal band at the tip of the thallus (Figure 6b). This band gradually increased in length basally, and on older thalli patches and short streaks of spermatangia formed at the thallus apex and in the lateral submarginal areas (Figures 6c and 6d).
The specific time of the initial stages of carpogonium formation is not known. Fertile thalli were collected in January, but most did not form carposporangia until February or March.

*Porphyra thuretii* in the Barkley Sound area is typically 150-220 mm long and 50-80 mm wide, although thalli up to 390 mm by 105 mm did occur. Herbarium specimens (UC, GMS) of *P. thuretii* from the Monterey Peninsula region were found to be generally larger (up to 770 mm long and 230 mm wide) and have less pronounced marginal ruffling than the specimens from Barkley Sound (Figures 2c and 6d). It should be noted that the Monterey plants were collected later in the year (May-June) and had been floating in the drift, so this may be an explanation for their larger size. Specimens collected in April were more typical of the size of thallus I have observed in Barkley Sound (Figure 6e).

2. *Host* Species

*Porphyra thuretii* occurs on 7 different host species, and in one case was found on rock, confirming the reports of Dawson (1944) and Smith (1944). It has been observed on 1 seagrass, 3 phaeophycean and 3 rhodophycean algae (Table IV). In the Barkley Sound region *P. thuretii* was observed growing epiphytically only on members of the Laminariales. In contrast, specimens from the Monterey region were epiphytic on members of the Rhodophyceae.
3. Artificial 'Host' Experiment

One plant of *Porphyra thuretii* (UBC 57199) which appeared on the artificial *Nereocystis* plant grew to 146 mm in length and reached reproductive maturity.

4. Geographical Distribution

In British Columbia, *Porphyra thuretii* has been collected at Rennell Sound, Queen Charlotte Islands (53° 22.65'N, 132° 30.7'W, UBC 55302), Volcanic Cove, Kyuquot Channel, Vancouver Island (49° 58.7'N, 127° 13.9'W, UBC 54446) and two sites in Barkley Sound, Vancouver Island (48° 50.1'N, 125° 11.1'W, UBC 52019; 48° 49.8'N, 125° 14.4'W, UBC 57200).

Doty (1947) reported it once at Chetco Cove in Oregon (42° 00'N, 124° 18'W).

In California, *Porphyra thuretii* is known only from Santa Cruz (36° 58'N, 122° 00'W) (Dawson, 1944), the Monterey Peninsula (36° 30'N, 121° 57'W, UC 95596) and Carmel Bay (36° 33'N, 121°56'W, UC 791973).

Dawson (1952) reported *P. thuretii* from several locations in Pacific Baja California, Mexico. These specimens, deposited in the herbaria of UC and AHF, were examined and in my opinion represent a different taxon because of their small size, shape, and spermatangium and carposporangium distribution.

A representative specimen list of *Porphyra thuretii* is given in Appendix II.
Discussion

A. *Porphyra gardneri*

Although it has been found at the study site throughout 10 months of the year, *Porphyra gardneri* is a spring-summer species. Its monospore cycle results in the re-establishment of the population to some extent in the autumn, enabling it to persist longer than most *Porphyra* species. The predominance of the monospore cycle in plants from Monterey may enable the foliose phase to exist year-round in this area. Further seasonal studies are required to confirm this suggestion.

It has been noted that the time and frequency of spermium and spore formation varies with latitude. Culture studies of *Porphyra* (Iwasaki, 1961) and *Bangia* (Richardsen and Dixon, 1968; Dixon and Richardson, 1969; Sommerfeld and Nichols, 1973) have shown that photoperiod is important in controlling monospore and carpospore formation. Therefore it seems reasonable to suggest that variation in the times of spermangium and carpogonium formation is the result of the annual photoperiod variation with latitude. In Table V the duration of daylight on the 21st day of each month is compared for 35°N latitude and 48°50.1'N (study site). Data for 35°N have been obtained from the Smithsonian Meteorological Tables (List, 1966), whereas study-site data have been taken from daily sunrise and sunset tables for Victoria, British Columbia (National Research Council, Astrophysics Branch, 1974). Latitude 35°N is used because it is the closest latitude to 36°30'N (Monterey Peninsula, type locality) for which data are
given. There is approximately a 10 minute difference in the
duration of daylight between these two latitudes on the 21st
June, but this is accurate enough for the purposes of the
present discussion.

At the study site spermatangia first appeared when the
daylight was approximately 14 hr. Carpogonia, then
carposporangia, formed a short time after this when a slightly
longer daylight was reached, estimated to be between
14 hr 30 min and 15 hr. As will be seen from Table V, this
duration of daylight is only just reached at 35°N on 21st June.
This strongly suggests that photoperiod is responsible for the
low frequency of carpogonium formation in the Monterey plants.
Whether this is a truly photoperiodic or just a photosynthetic
effect will be discussed in Part III. Higher seawater
temperature may also be a factor. Sommerfeld and Nichols
(1973) found that in Bangia a higher temperature favoured
production of monospores rather than carpospores.

The variation in thallus size of Porphyra gardneri with
vertical position on the shore is an interesting phenomenon
which may be the result of growth inhibition by prolonged
exposure. Kurogi (1961) noted that growth of Porphyra
vezcensis was more rapid at lower intertidal levels.

As a result of my study, P. gardneri is now known from a
considerable number of 'host' species, all but one of which are
in the Laminariales. Whether there is a requirement for a
specific compound produced by the Laminariales is not known.

The striking marginal distribution of P. gardneri on the
blades of Laminaria setchellii is probably the result of the
tearing of the *Laminaria* blade producing a wound site in which spore germination and rhizoid attachment can occur. This also appears to be the case for the other 'host' plants, where *P. gardneri* is found growing on areas of the thallus where there has been wounding caused by abrasion or some other means. In her recent work on the red algal parasite *Harveyella mirabilis* (Reinsch) Schmitz et Reinke, Goff (1975) found that spore establishment depended on the presence of a wound area on the host plant caused by grazing isopods and amphipods.

The absence of epiphytes on young *Laminaria* blades may be due to rapid growth and sloughing off of surface material or production of exudates; some of which (e.g. phenolics) are known to be toxic (Craigie and McLachlan, 1964; McLachlan and Craigie, 1966; Sieburth and Jensen, 1969).

The phenomenon of epiphytism and host specificity is an intriguing one. Harlin (1973a) demonstrated that it was the unique position that the host plant occupies in the environment which the obligate epiphyte *Smithora naiadum* (Anderson) Hollenberg requires. The fact that *Porphyra gardneri* was not found on rock and would not grow on the artificial *Laminaria* suggests that it may have a physiological requirement for the host.
E. *Porphyra nereocystis*

My field studies indicate that *Porphyra nereocystis* is a winter species. This is in agreement with the reports of Smith (1944), Abbott and Hollenberg (1976) and Woessner et al. (1977). The reports of Conway et al. (1975) that it occurs from summer through winter and of Conway and Cole (1977) that it is a summer species are attributable to two phenomena. The first is that because *Porphyra nereocystis* is a subtidal plant it is not 'burnt off' during the spring months as happens to other winter *Porphyra* species (Mumford, 1975). As a result it can persist well into the summer provided that its host plant is not carried away. Without collecting at a single site year round it is easy to get a different concept of the seasonal occurrence.

The second phenomenon which has caused confusion is the much earlier appearance of *P. nereocystis* in the Strait of Georgia and vicinity than on the outer coast. In the Strait it is an autumn plant. When I first encountered it this early I thought I was dealing with a different species; however, subsequent study has not supported this conclusion.

Just what conditions in the Strait of Georgia region are responsible for altering the seasonal occurrence of *P. nereocystis* are not clear, but they are probably involved with triggering conchospore release (to be discussed in Part III). Most workers (e.g. Bird et al., 1972) have indicated that the seawater temperature is a critical factor required for conchospore release. Parsons (1965) has noted that, because of the stabilizing effect of freshwater input from the Fraser
River, the spring phytoplankton bloom occurs 4-6 weeks earlier in the Strait of Georgia than on the outer coast. Perhaps the factors involved here are also affecting *P. nereocystis*. The *Nereocystis* plants in the Strait also appear earlier in the year and begin to senesce earlier than outer coast plants. Another difference between the Strait and the outer coast is that the low tides in the spring and summer occur in the middle of the day in the Strait as opposed to early in the morning on the outer coast (Krishnamurthy, 1969b; Mumford, 1975). This results in higher desiccation and insolation for plants floating on the surface at low tide in the Strait. *Porphyra nereocystis* in the Strait also becomes reproductive earlier than it does on the outer coast.

*Porphyra nereocystis* is remarkably 'host' specific, being restricted essentially to *Nereocystis luetkeana*. I have never observed it growing on rocks and agree with Mumford (1973a) that such reports are probably misidentifications of *Porphyra miniata*. Several specimens in the UBC herbarium reported to be saxicclous in mid-intertidal tide pools were found to be *P. miniata*. The young thalli figured by Conway et al. (1975, Fig. 19a, UBC 33206) as *P. nereocystis* are referrable to *P. miniata*. Misidentification of *P. gardneri* and *P. miniata* as being *P. nereocystis* has been responsible for reports of *P. nereocystis* on hosts other than *Nereocystis*. For example, Kylin (1941) mistook *P. gardneri* on *Pterygophora californica* Ruprecht for a young or dwarf form of *P. nereocystis*. My studies have also shown that a number of other *Porphyra* species are capable of growing on the stipe of
Nereocystis and could potentially be mistaken for P. nereocystis.

Porphyra nereocystis was found only on Nereocystis which had terminated its rapid stipe growth. Such plants were not encountered until the autumn and winter and were characterized by a heavy layer of diatom and ectocarpalean epiphytes. As with P. gardneri, it is felt that a wound on the stipe, caused by abrasion or grazing, is important for conchospore establishment.

The reason for P. nereocystis usually being restricted to one side of the stipe becomes obvious when the kelp bed is observed at low tide. At this time, the upper 3-5 m of the stipe is floating at the surface. The half of the stipe that is exposed to the air experiences a great amount of desiccation, which would tend to inhibit the growth of P. nereocystis.

The epiphytic habit of Porphyra nereocystis has obvious advantages. The plant is on a substrate which is relatively free from grazing, and which keeps the plant constantly submerged but near the surface and adequate illumination.
C. *Porphyra thuretii*

My field observations indicate that *Porphyra thuretii* is a winter-early spring species, as has been reported by Smith (1944) and Dawson (1944).

At present the distribution of *P. thuretii* appears to be rather patchy; however, I do not think this is actually the case. This species has probably been overlooked because it grows in rather exposed areas where collectors seldom go early in the year. It is also possible to mistake it for *P. nereocystis*, *P. miniata* or *P. perforata*.

The fact that *Porphyra thuretii* grew on the artificial *Nereocystis* suggests that it does not have a physiological dependance on the host plant, but just requires the physical position in the environment which the host offers. This conclusion is further substantiated by the fact that it was found on hosts as diverse as *Phyllospadix* and *Nereocystis*.

It would be desirable to make a seasonal study of *P. thuretii* at the Monterey Peninsula in order to see if the differences between these plants and study site plants, in the degree of marginal ruffling and thallus size, are significant.
Table I. Herbaria from which specimens were examined

<table>
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<th>Abbreviation</th>
<th>Herbarium Description</th>
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<tr>
<td>AHF</td>
<td>Allan Hancock Foundation, University of Southern California</td>
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<td>CSUH</td>
<td>California State University at Humboldt</td>
</tr>
<tr>
<td>DS</td>
<td>Dudley Herbarium, Stanford (specimens now in UC)</td>
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<td>GJH</td>
<td>Private Herbarium of George J. Hollenberg</td>
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<tr>
<td>GMS</td>
<td>Gilbert Morgan Smith Herbarium (Hopkins Marine Station)</td>
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<td>PS</td>
<td>Private Herbarium of Robert B. Setzer</td>
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<td>UCSC</td>
<td>University of California at Santa Cruz</td>
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Table II. 'Host' plants of *Porphyra gardneri*

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<tr>
<th>Host</th>
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<tr>
<td><strong>Phaeophyceae</strong></td>
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<td>UBC 54894</td>
<td>midrib</td>
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<td>AHF 77063</td>
<td>tips of leaflets</td>
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<td><em>Egregia menziesii</em> (Turner) Areschoug</td>
<td>UBC 54889</td>
<td>tips of leaflets</td>
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<td><em>Fisania arborea</em> Areschoug</td>
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<td><em>Hedophyllum sessile</em> (C. Agardh) Setchell</td>
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<td><em>Laminaria groenlandica</em> Rosenvenge</td>
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<td><em>L. setchellii</em> Silva Farlow</td>
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<td><em>L. sinclairii</em> (Harvey)</td>
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<tr>
<td><em>L. yezoensis</em> Miyabe Farlow</td>
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<td><em>Lessoniopsis littoralis</em> (Farlow et Setchell) Reinke</td>
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</tr>
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<td><em>Postelsia palmaeformis</em> Ruprecht</td>
<td>UBC 54886</td>
<td>torn blade margin</td>
</tr>
<tr>
<td><em>Ptterygophora californica</em> Ruprecht</td>
<td>UBC 55751</td>
<td>stipe</td>
</tr>
<tr>
<td><em>Rhodophyceae</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Schizytemenia pacifica</em> Kylin</td>
<td>Abbott 2516</td>
<td>thallus margin in GMS</td>
</tr>
</tbody>
</table>

Note: The Acc. no. column lists the accession numbers for each host plant.
Table III. Biomass (grams dry weight) of *Porphyra nereocystis* per vertical meter of *Nereocystis* stipe.

<table>
<thead>
<tr>
<th>Meter</th>
<th>Study site</th>
<th>Queen Charlotte Islands</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (top)</td>
<td>98</td>
<td>43</td>
</tr>
<tr>
<td>2</td>
<td>46</td>
<td>67</td>
</tr>
<tr>
<td>3</td>
<td>18</td>
<td>11</td>
</tr>
<tr>
<td>4</td>
<td>negligible</td>
<td>negligible</td>
</tr>
</tbody>
</table>

(total of 3 *Nereocystis* plants) (total of 4 *Nereocystis* plants)
<table>
<thead>
<tr>
<th>Host</th>
<th>Acc. no.</th>
<th>Location on host</th>
</tr>
</thead>
<tbody>
<tr>
<td>Artificial host</td>
<td>UBC 57199</td>
<td>upper stipe</td>
</tr>
<tr>
<td>Saxicolous</td>
<td>UBC 55302</td>
<td>upper subtidal</td>
</tr>
<tr>
<td>Sea grasses</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Phyllospadix</em> sp.</td>
<td>UBC 54447</td>
<td>on the leaf</td>
</tr>
<tr>
<td>Phaeophyceae</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Egregia menziesii</em> (Turner)</td>
<td>UBC 57198</td>
<td>on tips of leaflets</td>
</tr>
<tr>
<td>Areschoug</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Eisenia arborea</em> Areschoug</td>
<td>UBC 57202</td>
<td>blade margins</td>
</tr>
<tr>
<td><em>Nereocystis luetkeana</em></td>
<td>UBC 57200</td>
<td>stipe</td>
</tr>
<tr>
<td>(Mertens) Postels et</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ruprecht</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rhodophyceae</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Gastroclonium coulteri</em></td>
<td>GMS 1410</td>
<td>general</td>
</tr>
<tr>
<td>(Harvey) Kylin</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Gracilaria sjoestedtii</em></td>
<td>UC 791970</td>
<td>general</td>
</tr>
<tr>
<td>Kylin</td>
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<td></td>
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<tr>
<td><em>Microcladia coulteri</em></td>
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<tr>
<td>Harvey</td>
<td></td>
<td></td>
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<tr>
<td>(tentative identification</td>
<td></td>
<td></td>
</tr>
<tr>
<td>from fragment of host)</td>
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</table>
Table V. Annual variation in duration of daylight (hr. and min.) on the 21st day of the month at 35°N and 48° 50.1'N (study site).

<table>
<thead>
<tr>
<th>Month</th>
<th>35°N (hr, min)</th>
<th>Study site (hr, min)</th>
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<td>10, 11</td>
<td>8, 58</td>
</tr>
<tr>
<td>February</td>
<td>11, 08</td>
<td>10, 32</td>
</tr>
<tr>
<td>March</td>
<td>12, 09</td>
<td>12, 12</td>
</tr>
<tr>
<td>April</td>
<td>13, 16</td>
<td>14, 00</td>
</tr>
<tr>
<td>May</td>
<td>14, 09</td>
<td>15, 28</td>
</tr>
<tr>
<td>June</td>
<td>14, 31</td>
<td>16, 07</td>
</tr>
<tr>
<td>July</td>
<td>14, 10</td>
<td>15, 32</td>
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<tr>
<td>August</td>
<td>13, 18</td>
<td>14, 04</td>
</tr>
<tr>
<td>September</td>
<td>12, 12</td>
<td>12, 17</td>
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<tr>
<td>October</td>
<td>11, 07</td>
<td>10, 32</td>
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<tr>
<td>November</td>
<td>10, 11</td>
<td>8, 59</td>
</tr>
<tr>
<td>December</td>
<td>9, 48</td>
<td>8, 17</td>
</tr>
</tbody>
</table>
Figure 1a. Monosporic thalli of *Porphyra gardneri* growing on the blade margins of *Laminaria setchellii*. The type specimen (DS 306401 in UC).

Figure 1b. A 2.7 m, fertile specimen of *Porphyra nereocystis* collected at the study site (UBC 57180).

Figure 1c. The lectotype of *Porphyra thuretii* (UC 791973).
Figure 2a. The field work was carried out in Barkley Sound on the west coast of Vancouver Island (inset). The study site was located off Diana Island (circled), near the Bamfield Marine Station (from Canadian Hydrographic Service Chart #L-3001).

Figure 2b. Aerial photograph of Diana Island and the study site. The direction of the open Pacific Ocean is indicated by the arrow. (from B. C. Government air photograph #BC 7238-182).
Figure 3a. Characteristic heart-shaped new blade growth of Laminaria setchellii (UBC 54895).

Figure 3b. Early stage in the splitting of the blade of L. setchellii (UBC 57204).

Figure 3c. Mature, lacerated blade of L. setchellii with thalli of Porphyra gardneri along its margins (UBC 54896).

Figure 3d. Monosporic thalli of P. gardneri (UBC 54819).
Porphyra gardneri

Figure 4a. Thallus of *Porphyra gardneri* which has spermatangia, carpogonia and young carposporangia along its lateral margins. Monospores are still being released along the distal margin (UBC 54821).

Figure 4b. Old, lacerated, and epiphytized thallus of *Laminaria setchellii* with a few thalli of *Porphyra gardneri* on its margins (arrows). The new *Laminaria* blade is just beginning to grow (UBC 57205).

Figures 4c-e. Morphological variation in thalli of *P. gardneri* which are spermatangial and carposporangial. Note the characteristic pattern formed by the spermatangia along the lateral margins. Figure 4c: UBC 54138, from Botanical Beach; Figures 4d and 4e: UBC 54891, from Cape Beale.

Figure 4f. Thallus of *P. gardneri* from the type locality. This plant is releasing monospores along its distal margin and spermatia and carpospores along its lateral margins (UBC 54818).

Scale bar = 10 mm on Figures 4a, 4c, 4d, 4e, 4f.
Porphyra nereocystis

Figure 5a. Young vegetative thalli are characteristically long and strap shaped, tapering gradually to a point (UBC 57187).

Figure 5b. The formation of spermatangia begins at the lateral margins and proceeds basally. These regions of the thallus are easily recognized by their pale yellow to white colour (UBC 57188).

Figure 5c. As a result of spermatium liberation, the lateral spermatangial margins begin to erode. This starts at the apex and proceeds basally. The remaining apical portion of the thallus consists of vegetative cells and carposporangia (UBC 57184).

Figure 5d. Spermatangia forming marginal and submarginal streaks which are oriented in an apex to base direction (UBC 57180).

Figure 5e. The tips of older thalli often have a slightly mottled appearance caused by abundant carposporangia (UBC 57192).
**Porphyra thurii**

**Figure 6a.** Morphological variation in thalli which were growing on the stipe of *Nereocystis luetkeana* in Barkley Sound. Note the characteristic ruffled margins (UBC 57200).

**Figure 6b.** The production of spermatangia begins in the apical marginal region of the thallus (UBC 52109).

**Figures 6c and 6d.** On older thalli short streaks of spermatangia form submarginally. Note the characteristic pattern that results (UBC 57201 and UC 95596 respectively).

**Figure 6e.** Specimen from the Monterey Peninsula which resembles specimens from Barkley Sound in size and spermatangial pattern.
PART II - SPERMATOGENESIS AND CARPOSPOREGENESIS

Introduction

Hus (1902) was the first to use the number of spermatia per spermatangium and carpospores per carposporangium as a taxonomic criterion for delimiting Porphyra species. He thought that an undifferentiated vegetative cell functioned as the carpogonium, and that two anticlinal divisions (referred to by Hus as 'cruciate' division) of a vegetative cell gave rise to a group of four spermatangia.

Hus devised a spore formula by considering the spermatangium and carposporangium to be cubes with 'a' and 'b' representing the horizontal coordinates and 'c' the vertical. A point which has been overlooked by all recent workers, with the exception of Krishnamurthy (1972), was that Hus considered each carposporangium to be equivalent to four spermatangia. Hus' notation, applied to a cube 4x4x2, would be: 32 carpospores a/4, b/4, c/2 or 8 spermatia 1/2 a/2, 1/2 b/2, c/2 (Hus, 1902, plate 22, fig. 25). As Krishnamurthy (1972) has pointed out, subsequent workers have dropped the 1/2 in the spermatangium formula and applied the formula equally to both carposporangia and spermatangia. I have followed this system because I could not find any indication that a carpogonium is

\[^{1}\]Hus (1902) and many subsequent workers used the term 'packet' in place of spermatangium and carposporangium. See terminology section for further discussion.
equivalent to four spermatangia.

Judging by reports in the literature, summarized by Drew (1956), recognition of the carpogonium in *Porphyra* has been an area of considerable confusion. According to Drew, Berthold (1882) considered the carpogonia of *P. leucosticta* Thuret in *Le Jolis* to be slightly larger and paler than vegetative cells. The carpogonia Berthold (1882) figured also had slight bipolar protuberances which did not push out the outer wall. Berthold also illustrated cells with long bipolar protuberances which caused a protrusion of the outer wall. He apparently considered these cells to be old unfertilized carpogonia.

Hus (1902) did not adequately characterize the spermatangium or carpogonium of the *Porphyra* species he studied. He thought they were formed by undifferentiated vegetative cells and reported that he could distinguish vegetative division from spermatangium division by the wall thickness. Drew (1956) noted that there was too much variation in wall thickness to be able to distinguish vegetative from reproductive divisions.

Kunieda (1939) reported carpogonia with prototrichogynes in *Porphyra tenera*. Dangeard (1927) illustrated undifferentiated carpogonia in *P. linearis* Greville (as *P. umbilicalis f. linearis* (Greville) Harvey) and carpogonia with prototrichogynes in *P. umbilicalis* (Linnaeus) J. Agardh. Conway and Cole (1973) described 'carpogonia-like' cells with 'trichogyne-like' projections in *P. papenfussii* Krishnamurthy. Based on this distromatic species, Conway et al. (1975, fig. 1) showed the carpogonium with one prototrichogyne. No indication
was given of the appearance of the carpogonium in monostromatic species. Kurogi (1961), in his excellent investigation of 4 monostromatic *Porphyra* species, reported that the carpogonium had bipolar prototrichogynes.

In addition to the differing reports about the appearance of the carpogonium, Drew (1956) pointed out that there are conflicting reports of the orientation of the first division of the carposporangium. One group of workers believed the first two divisions were anticlinal and at right angles to each other (often referred to as cruciate divisions) (Hus, 1902; Krishnamurthy, 1959, 1969b, 1972; Conway, 1964b), whereas others have reported the first division of the carposporangium as being periclinal (in the plane of the thallus) (Janczewski, 1873; Berthold, 1882; Ishikawa, 1921; Grubb, 1924; Dangeard, 1927; Kurogi, 1961). Tanaka (1952) indicated that the initial division could be either anticlinal or periclinal depending on the species. Conway et al. (1975) did not indicate the division sequence of the carposporangium.

Despite a considerable amount of observation of spermatangial development at the light microscope level (Janczewski, 1873; Thuret and Bornet, 1878; Berthold, 1882; Hus, 1902; Ishikawa, 1921; Grubb, 1924; Tanaka, 1952; Krishnamurthy 1959, 1972; Mumford, 1973a; Conway et al. 1975), the appearance and division sequence of the spermatangium of *Porphyra* has not been well documented. These studies did little more than indicate that the spermatia are formed by repeated division of the spermatangium. The data are contradictory in that Janczewski (1873), Thuret and Bornet
(1878), Hus (1902), Ishikawa (1921) and Grubb (1924) maintained that the first division of the spermatangium was periclinal, whereas Tanaka (1952) and Krishnamurthy (1959) thought it was anticlinal. As Drew (1956) pointed out, these various discrepancies are due perhaps to the difficulty in distinguishing the spermatangium from a vegetative cell.

It was apparent to me that Drew's (1956, p. 595) statement, "Little attention has been paid to the method of distinguishing normal cell-division from spore formation..." was still valid. I therefore decided to try to characterize the spermatangium and carpogonium and follow their division sequence in detail at the light microscope level for the three species I was studying.

For the genus *Porphyrella* Smith and Hollenberg (1943) reported that the carpogonium was formed by a curving cell division similar to that of monospore division in *Erythrotrichia*, and that the carpospores were released singly. My preliminary examination of specimens from the study site indicated that the carpogonia and carpospores were not forming in this manner. These specimens fit the description of *Porphyra gardneri* (*Porphyrella gardneri* at that time) in all other respects. A detailed study of carpogonium and carpospore formation in plants at the study site and type locality was therefore essential to confirm that the genus *Porphyrella* was incorrectly described.
Materials And Methods

Spermatangial and carposporangial thalli were collected at the study site and marginal and submarginal pieces (approx. 5mm²) were cut out and fixed in a solution of glutaraldehyde (50%), phosphate buffer pH 7.2, and millipore filtered seawater (1:4:4) (McBride and Cole, 1969) for 1.5 hours. Whenever possible material was fixed immediately in the field.

Fixation was followed by dehydration in a graded ethanol series and infiltration with glycol methacrylate (Polysciences JB-4). Serial sections (Henry, 1977) 2-3 um thick were cut on glass knives using a Sorval JB-4 microtome. Sections were stained with toluidine blue-0 in benzoate buffer or distilled water (Feder and O'Brien, 1968) mounted in Euparal (GBI Labs Ltd., Heaton St, Denton, Manchester) and examined on an Olympus light microscope (Model FHA) with planapochromatic objectives. Photographs were taken with a Nikon Microflex (Model AFM) automatic photographic attachment using Kodak Panatomic-X film.
Results

A. Porphyra gardneri

1. Spermatogenesis

The division sequence leading to the formation of mature spermatangia was followed in surface view and transverse section. Vegetative cells have a single stellate chloroplast, central pyrenoid, and peripherally located nucleus (Figure 7a). During late April the spermatangia began forming along the thallus margin. The first stage that vegetative cells went through in the transition to a spermatangium was the production of a new wall layer (Figure 7b). The first division of the spermatangium was periclinal (a/1, b/1, c/2) (Figure 7c). The second and third divisions were anticlinal and at right angles to each other (Figures 7d and 8a). These were followed by anticlinal and periclinal divisions (Figures 7e-f) until a mature spermatangium containing 64 spermatia (a/4, b/4, c/4) was formed (Figure 7g). An immature spermatangium is shown in surface view in Figure 7h, and a mature spermatangium in Figure 7i. This division sequence is summarized in Figure 8a.

2. Carposporogenesis

The spermatangia, carpogonia, and carposporangia (Figure 9b) are readily distinguished from vegetative cells in surface view (Figure 9a).

By early May carpogonia began forming in the submarginal
region of the thallus. No curving, *Erythrotrichia*-like walls were involved in their formation. In transverse section the carpogonia were easily distinguished from vegetative cells by their characteristic spindle- or lemon-shape due to the presence of bipolar prototrichogynes (Figure 9c). The carpogonium contained a single stellate chloroplast, pyrenoid, and abundant floridean starch grains. In some cases two carpogonia were closely appressed (Figure 9d). This appeared to result from carpogonium differentiation immediately following a vegetative cell division before a thick wall had formed between the daughter cells. In determining the number of carpospores per carposporangium it was necessary to ascertain that the carpospores being observed were actually derived from a single carpogonium and not two closely appressed ones.

Following fertilization of the carpogonium (or perhaps without it in some cases; to be discussed in Part IV), the first division of the carposporangium was periclinal (a/1, b/1, c/2) (Figure 9e). Many of the carposporangia did not divide beyond this stage, but some underwent second and third anticlinal divisions to form 4 carpospores (Figures 9f, 9g, and 9h). In some carposporangia these two divisions appeared to occur in the same plane (a/1, b/2, c/2, Figure 8b), but in most cases they were oriented at right angles to each other (Figure 8b). The latter carposporangia were easily seen in surface view (Figures 9g and 9h). Occasionally further division to produce 8 carpospores (a/2, b/2, c/2) occurred (Figures 9i and 9j). The division sequence of
carposporogenesis is summarized in Figure 8b.

3. Examination Of Type Locality Plants

In view of the mode of carpogonium formation and division sequence of the carposporangium observed in study site plants it was apparent to me that they belonged to the genus *Porphyra*, not *Porphyrella*. Examination of carpogonia and carposporangia of herbarium specimens from Alaska to northern California revealed that they were identical to study site plants. Therefore it was necessary to determine whether the plants at the type locality on the Monterey Peninsula, California, were the same taxon.

From the original description, herbarium specimens, and thorough notes made by Dr. Hollenberg on specimens in his herbarium, it was possible to confirm that both taxa had the following characteristics in common: similar host plants, a marginal distribution on *Laminaria*, a monospore cycle, 64 spermatia/spermatangium, similar basal region morphology and attachment, vegetative blade thickness 25-35 μm, and vegetative thalli minutely wrinkled. The only difference between type locality plants and those from the rest of the coast was the reported mode of carpogonium formation and carposporangium division.

In order to solve this problem, specimens were collected in July 1976 at Point Joe (type locality), Pescadero Point and Mission Point, California. The number of carpogonia and carposporangia was low compared to more northern populations; however, their mode of formation and division was identical
with that observed in specimens from the study site and other areas along the coast. This observation combined with field, culture and chromosome studies (see Parts I, III, IV) on the type locality population conclusively demonstrated that it was the same taxon as was found at the study site. It seems probable that the small number of carpogonia and carposporangia produced by thalli at the type locality is the reason that their formation has been overlooked in the past.

B. *Porphyra nereocystis*

1. Spermatogenesis

Spermatogenesis was difficult to follow in *Porphyra nereocystis* because the spermatangia were not very distinct from vegetative cells. Unlike *P. gardneri*, no new wall was laid down around the spermatangium prior to its first division. Furthermore, the spermatangia that had undergone a series of divisions were not well defined, making it difficult to determine the number of spermatia in mature spermatangia.

In Figure 9k a vegetative portion of the thallus is shown in surface view. The young spermatangia were difficult to distinguish from vegetative cells until they had undergone several divisions (Figure 9l).

Spermatangia with 4 \((a/1(2), b/2(1), c/2)\) or 8 \((a/2, b/2, c/2)\) spermatia were starting to lose pigmentation. Subsequent divisions occurred (Figure 10a) until a mature spermatangium \((a/4, b/4, c/8)\) containing 128 spermatia was reached (Figures 10b and 10c). This is a maximum number; variations in
the final number of spermatia/spermatangium were observed.

2. Carposporogenesis

At the study site carpogonium formation began in January in the submarginal region of the thallus. In transverse section the carpogonium was difficult to recognize because it did not form prototrichogynes. The carpogonia were characterized as being rectangular and elongate in transverse section (Figure 10e) compared to the square-shaped vegetative cells (Figure 10d). They had a single stellate chloroplast, central pyrenoid and peripheral nucleus. Carposgonial regions of the thallus could also be recognized by the presence of spermatia forming fertilization canals to the carpogonia (to be discussed in Part IV) and by periclinal divisions of the carposporangia.

Frequently 2 closely appressed carpogonia were observed (Figure 10f), a phenomenon which may lead to misinterpretation of the number of carpospores per carposporangium.

The first division of the carposporangium was periclinal (a/1, b/1, c/2) (Figure 10g). It is felt that this division normally follows fertilization (see Part IV). Both the second and third divisions were anticlinal and at right angles to each other (Figure 10h). Carposporangia at this stage were easily detectable in surface view (Figure 10i). Subsequent division occurs rapidly to produce 8 carpospores/carposporangium (a/2, b/2, c/2) (Figures 10h and 10j). Most carposporangia released carpospores at this stage (see Part III). Further division along the 'c'-axis of the carposporangium (Figure 10l) occurred
frequently on older thalli and resulted in a deeper pigmentation. Suspected vegetative cells and incompletely divided carposporangia which had lost their pigmentation were mixed in with the mature carposporangia and gave the thallus a mottled appearance (Figure 5e).

I was unable to find carposporangia with 32 carpospores \((a/4, b/4, c/2)\) as has been typically reported for Porphyra nereocystis (Hus, 1902; Smith, 1944; Krishnamurthy, 1972; Mumford, 1973a; Abbott and Hollenberg, 1976; Conway et al. 1975). Groups of carpospores that superficially appeared to have this spore formula (Figures 10k and 11a) were too large to have been derived from a single carposporangium. Carposporangia \(a/4, b/2\) appeared to form although I could not unequivocally determine this. I suspect that the carposporangia reported to be \(a/4, b/4\) are actually made up of 2 closely appressed \(a/4, b/2\) carposporangia.

C. *Porphyra thuretii*

1. Spermatogenesis

Due to a limited amount of material of *Porphyra thuretii* it was not possible to follow spermatogenesis in the same detail as was done for *P. gardneri*. The early division sequence is shown in Figures 11b and 11c. The spermatangium is well defined in transverse section, but I have not been able to determine if this is due to the formation of a new wall layer prior to division.

Figure 11d shows an intermediate stage in spermatangial
division. Mature spermatangia contained 64 spermatia (a/4, b/4, c/4) (Figures 11e and 11f).

2. Carposporogenesis

In transverse section the carpogonia of *Porphyra thuretii* were seen to possess slight prototrichogynes (Figure 11g). This needs to be re-examined because the material I studied was not fixed until 2 days after being collected. My studies of *P. gardneri* indicated that immediate fixation in the field was essential for good carpogonium and spermatangium preservation. The degree of prototrichogyne development in *P. thuretii* may therefore be greater than I have reported.

The carpogonium contained a single stellate chloroplast, central pyrenoid and peripheral nucleus. The first division of the carposporangium (following a suspected fertilization; see Part IV) was periclinal (a/1, b/1, c/2) (Figure 11h). The subsequent division sequence (Figures 11i and 11j) was the same as that observed in *P. gardneri* and *P. nereocystis* (early stages). The mature carposporangium contained 8 carpospores (a/2, b/2, c/2) (Figures 11k and 11l), although carpospore release could occur any time after the first division of the carposporangium. The distal region of older thalli typically had a mottled, deeply pigmented appearance due to the abundant mature carposporangia.
Discussion

This study of spermatogenesis and carposporogenesis has yielded three major results:

1. The genus Porphyrella is not valid.
2. An attempt has been made to characterize the carpogonium and spermatangium of Porphyra.
3. The classical 'cruciate' division sequence of the carposporangia and spermatangia as proposed by Hus (1902) does not occur in the Porphyra species examined.

Examination of the mode of carpogonium formation and division in Porphyra gardneri demonstrated that the carpogonia are not formed by unequal divisions and the carpospores are not released singly. The carposporangial division sequence is typical of the genus Porphyra. Furthermore, the small and variable number of carposporangial divisions are not unique to P. gardneri. Kurogi (1961) has reported and figured the same division pattern for Porphyra kuniedai Kurogi. In view of this, the genus Porphyrella as described by Smith and Hollenberg (1943) is not valid. Therefore the following nomenclatural change was made (Hawkes, 1977b):

Porphyra gardneri (Smith et Hollenberg) comb. nov.


Description: Thalli monostromatic 25-35 um thick, one chloroplast per cell. Thalli up to 130 mm long
and 60 mm wide, but typically 20-70 mm long and 10-30 mm wide. Reproducing asexually by monospores. Mature spermatangia containing 64 spermatia (a/4, b/4, c/4) maximum. Carpogonium formed by the transformation of a vegetative cell and characterized by bipolar prototrichogynes. Carposporangium undergoing one to three or rarely seven divisions to form two (a/1, b/1, c/2), four (a/1(2), b/2(1), c/2) or eight (a/2, b/2, c/2) carpospores. The life history is heteromorphic, released carpospores germinating into a conchocelis phase. Conchocelis phase with pit plugs as is typical of all Porphyra species that have been examined (Lee and Fultz, 1970; Bourne et al. 1970; Cole, 1972b).

Spore formation by unequal division is characteristic of the Erythropeltidaceae, whereas spore formation by repeated division to produce several spermatia/spermatangium or carpospores/carposporangium is typical of the Bangiaceae. On the basis of Smith and Hollenberg's (1943) report of the occurrence of both these modes of spore formation in Porphyra gardneri, Drew (1956) suggested that this species had affinities with both families. This, however, is not the case; P. gardneri is typical of the Bangiaceae in all aspects of spore formation.

Only one other species has been referred to the genus Porphyrella, this being Porphyrella californica Hollenberg, described by Hollenberg (1945) from Santa Cruz Island and
several places along the mainland coast, southern California. He separated it from *Porphyra gardneri* on the basis of its saxicolous habit and shape of thallus. No mention was made of uneven cross walls in carpogonium formation in this species.

The holotype of *P. californica* (GJH 1353) consists of one thallus permanently mounted on a glass slide. An examination of it revealed abundant spermatangia along the thallus margin. Also in the marginal region were larger pigmented cells, which are probably carpogonia and carpospores. No evidence of uneven crosswalls was seen. In view of this it is suspected that *Porphyrella californica* should be transferred to *Porphyra*; however, until further investigation of it is made, nothing conclusive can be said about its taxonomic status.

There are at least three different carpogonial morphologies in the genus *Porphyra*. Hawkes (1977b) pointed out that in those species for which prototrichogynes have been reported, there are two carpogonial types. Monostromatric species have carpogonia with 2 prototrichogynes located on the sides of the carpogonium next to the thallus surface, whereas in distromatric species the carpogonium has only one prototrichogyne, located next to the thallus surface. Judging by reports in the literature (Table VI), most *Porphyra* species have carpogonia with prototrichogynes. The prototrichogynes are produced by the developing carpogonia and function as specialized receptive sites for spermatium attachment and fertilization (see Part IV). They give the carpogonia a characteristic elongate lemon-shape and cause the outer cell wall of the thallus to protrude to varying degrees depending on
how well developed they are. The degree of protrusion appears to vary depending on the species. Porphyra papenfussii and P. tasa (Yendo) Ueda are unique amongst the species listed in Table VI because of their extremely long prototrichogynes. Examination of these prototrichogynes at the E. M. level is needed. The long protuberances reported by Berthold (1882) and Joffé (1896) to be formed by old unfertilized carpogonia were not seen in Porphyra gardneri.

A third type of carpogonial morphology is displayed by Porphyra nereocystis in which no prototrichogynes are formed. The only feature to differentiate the carpogonia from vegetative cells is their slightly more elongated, rectangular shape. Only Dangeard (1927) and Tseng and Chang (1955) have specifically noted that a few Porphyra species lack prototrichogynes (Table VI). Preliminary observation of other species from the Northeast Pacific Ocean suggests that some of them lack prototrichogynes. Further investigation of this is needed.

Dangeard (1927) was the first to report that some Porphyra species possessed prototrichogynes, whereas others lacked them (Table VI). Tseng and Chang (1955) noted that there was variation in the occurrence of the prototrichogyne in the three Porphyra species they studied. They suggested that all Porphyra species have the ability to form prototrichogynes but that this may be suppressed in those which possess a thick, tough cell wall. Another explanation which I am proposing is that those species lacking prototrichogynes have never had the ability to produce these more specialized receptive areas and
may therefore be more primitive. This needs to be studied in other *Porphyra* species in order to completely characterize the carpogonium of this genus.

Groups of 2 carpogonia were occasionally noted in *P. gardneri* and *P. nereocystis*, but groups of 4 carpogonia resulting from a 'cruciate' division of a vegetative cell as reported by Conway *et al.* (1975) were not seen. This phenomenon appears to be limited to *P. papenfussii* and *P. schizophylla* Hollenberg.

The spermatangium in *Porphyra gardneri* can be distinguished from vegetative cells by the new wall layer that is laid down around it prior to division. This is the first time this feature has been reported for *Porphyra* spermatangia, although Scott and Dixon (1973a) previously reported it for the tetrasporangia of *Ptilota hypnoides* (Harvey) Kylin. The new wall layer gives the spermatangium a well defined appearance, making it simple to identify all the spermatia produced by one spermatangium. In contrast, the spermatangium of *Porphyra nereocystis* lacked the new wall layer making it difficult to identify the products of a single spermatangium. It is interesting to note that this less specialized spermatangium is associated with the simple carpogonium of *P. nereocystis*, adding further evidence in support of the theory that it is a more primitive member of the genus. In *P. gardneri* the more advanced carpogonium is associated with the more specialized spermatangium. Thus, this preliminary study suggests that there may be a correlation between the type of carpogonium and spermatangium. Study of other members of
the genus is needed to confirm this.

The report by Janczewski (1873) of spermatia and carpospores occurring in the same sporangium remains an enigma. This may have been a misinterpretation by Janczewski. Such a phenomenon was not observed in the 3 species I studied.

Once the carpogonium and spermatangium have been recognized, the subsequent division sequence can be followed. In all 3 species and for both the spermatangia and carposporangia the initial divisions were identical. The first division is periclinal and no 'cruciate' divisions as first reported by Hus (1902) and later by Krishnamurthy (1959) occurred. It is possible that the division sequence is different for other Porphyra species; however, this should be re-investigated, particularly in those cases where cruciate divisions have been reported.

Hus (1902), Krishnamurthy (1972), and Conway et al. (1975) reported mature carposporangia of Porphyra nereocystis contained up to 32 carpospores (a/4, b/4, c/2). I was unable to identify carposporangia of this size. On older thalli I observed carposporangia a/4, b/2, c/2-4, but they were never larger than this. This is the first report of carposporangia larger than c/2 in Porphyra nereocystis.

In the Japanese Porphyra species carposporangia have surface dimensions a/2, b/2 or a/4, b/2; there are no reports of a/4, b/4 (Kurogi, 1972). Kurogi (1972) noted that the close contact of 2 carposporangia often occurs and superficially resembles a single carposporangium of a/4, b/4. Kurogi detected this phenomenon by comparing the dimensions of these
large 'carposporangia' with those of smaller carposporangia and vegetative cells. They should all have similar dimensions; however, the suspected a/4, b/4 'carposporangia' were too large to be derived from a single carpogonium.

I agree with Kurogi's (1972) view and think that the phenomenon of 2 closely appressed carposporangia has been responsible for the reports of carposporangia a/4, b/4. Other species from the Northeast Pacific Ocean have been reported to have carposporangia of these dimensions, for example, *Porphyra kanakaensis* Mumford (Mumford, 1973b) and *P. perforata* (Hus, 1902; Conway et al. 1975). Carposporogenesis should be re-examined in these species to see if carposporangia as large as a/4, b/4 really occur.

In all 3 species I studied there was some variation in the number of spermatia or carpospores per sporangium. Kurogi (1961) noted that the age of the thallus and ecological conditions could cause such variation. Furthermore, he observed that species with thick thalli generally had larger division numbers of spermatangia and carposporangia in transverse section than those with thin thalli. In view of this variation, Krishnamurthy (1972) suggested that less emphasis should be put on the division formulae of spermatangia and carposporangia as taxonomic criteria, a trend which is generally accepted (Mumford, 1973a; Conway et al. 1975). In addition I would like to emphasize that the division formula is meaningless unless the products of the division of a single spermatangium or carposporangium can be recognized with certainty.
Table VI. Summary of the species of *Porphyra* which possess prototrichogynes, and those which do not.

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Without prototrichogynes</strong></td>
<td></td>
</tr>
<tr>
<td>1. Monostromatic</td>
<td></td>
</tr>
<tr>
<td><em>Porphyra dentata</em> Kjellman</td>
<td>Tseng and Chang (1955)</td>
</tr>
<tr>
<td><em>P. linearis</em> Greville</td>
<td>Dangeard (1927) - as <em>P. umbilicalis f. linearis</em> (Greville) Harvey</td>
</tr>
<tr>
<td><em>P. nereocystis</em> Anderson</td>
<td>Hawkes (this thesis)</td>
</tr>
<tr>
<td><strong>With prototrichogynes</strong></td>
<td></td>
</tr>
<tr>
<td>1. Monostromatic</td>
<td></td>
</tr>
<tr>
<td><em>Porphyra angusta</em> Ueda</td>
<td>Kuroqi (1961)</td>
</tr>
<tr>
<td><em>P. capensis</em> Kützing emend. Agardh</td>
<td>Graves (1969)</td>
</tr>
<tr>
<td><em>P. crispata</em> Kjellman</td>
<td>Tanaka (1952)</td>
</tr>
<tr>
<td><em>P. gardneri</em> (Smith et Hellenberg) Hawkes</td>
<td>Hawkes (1977b, and this thesis)</td>
</tr>
<tr>
<td><em>P. katadai</em> Miura</td>
<td>Miura (1968)</td>
</tr>
<tr>
<td><em>P. kuniedai</em> Kurogi</td>
<td>Kuroqi (1961)</td>
</tr>
<tr>
<td><em>P. leucosticta</em> Thuret in Le Jolis</td>
<td>Janczewski (1873)</td>
</tr>
<tr>
<td><em>P. perforata</em> J. Agardh</td>
<td>Berthold (1882)</td>
</tr>
<tr>
<td><em>P. suborbiculata</em> Kjellman</td>
<td>Rosenvinge (1909)</td>
</tr>
<tr>
<td><em>P. tanegashimensis</em> Shinmura</td>
<td>Kornmann (1961a)</td>
</tr>
<tr>
<td><em>P. tenera</em> Kjellman</td>
<td>Dr. T. F. Mumford (personal communication)</td>
</tr>
<tr>
<td><em>P. tenuipedalis</em> Miura</td>
<td>Tanaka (1952)</td>
</tr>
<tr>
<td><em>P. umbilicalis</em> (Linnaeus) J. Agardh</td>
<td>Shinmura (1974)</td>
</tr>
<tr>
<td><em>P. vezoensis</em> Ueda</td>
<td>Ishikawa (1921)</td>
</tr>
<tr>
<td><em>P. sp.</em> (amethystea Kützing?)</td>
<td>Kunieda (1939)</td>
</tr>
<tr>
<td><strong>2. Distromatic</strong></td>
<td></td>
</tr>
<tr>
<td><em>Porphyra bulbopes</em> (Yendo) Okamura</td>
<td>Miura (1961)</td>
</tr>
<tr>
<td><em>P. papefussii</em> Krishnamurthy</td>
<td>Dangeard (1927)</td>
</tr>
<tr>
<td></td>
<td>Krishnamurthy (1972)</td>
</tr>
<tr>
<td></td>
<td>Conway and Cole (1973)</td>
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<tr>
<td></td>
<td>Conway et al. (1975)</td>
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</tbody>
</table>
P. schizophylla Hollenberg in Smith and Hollenberg

P. tasa (Yendo) Ueda

Krishnamurthy (1972) as P. norrisii Krishnamurthy
Conway et al. (1975)
Nagai (1941)
Tanaka (1952)

Tanaka (1952) figures prototrichogynes for this species but does not mention them in the description.

Tseng and Chang (1955) reported they could not find prototrichogynes in this species.
Porphyra gardneri - spermatogenesis, light microscopy

Figure 7a. Vegetative cell with large central pyrenoid and single stellate chloroplast. The nucleus is peripherally located.

Figure 7b. New wall layer (arrow) laid down around spermatangium prior to the first division.

Figure 7c. The first division of the spermatangium is periclinal.

Figures 7d-f. Subsequent divisions of the spermatangium.

Figure 7g. Mature spermatangium 4 spermatia deep in transverse section.

Figure 7h. Surface view of an immature spermatangium.

Figure 7i. Surface view of a mature spermatangium.
Porphyra gardneri

Figure 8a. Spermatogenesis. The division sequence leading to a mature spermatangium of 64 spermatia. Note the production of a new wall layer (indicated by a thicker line) prior to division, and the absence of any 'cruciate' divisions.

Figure 8b. Carposporogenesis. Division sequence which produced 2, 4 or 8 carpospores per carposporangium. The upper carposporangium containing 4 carpospores is most obvious in surface view and is shown in Figures 9g and 9h. The division pathway indicated by solid arrows is most common.
**Porphyra gardneri** - carposporogenesis

**Figure 9a.** Surface view of vegetative cells.

**Figure 9b.** Surface view of spermatangia (lower left), carpogonia and carposporangia.

**Figure 9c.** Transverse section of the thallus showing two carpogonia. Note the bipolar prototrichogynes and attached spermatium (arrow).

**Figure 9d.** Transverse section showing two closely appressed carpogonia. Note attached spermatium (arrow).

**Figure 9e.** Transverse section showing that the first division of the carposporangium is periclinal.

**Figure 9f.** Transverse section showing the orientation of the third and fourth divisions of the carposporangium. Shown in surface view in Figures 9g and 9h. See also Figure 8a.

**Figure 9g.** Surface view of carposporangium that has undergone three divisions and contains 4 carpospores. See also Figure 8a.

**Figure 9h.** As in Figure 9g, but a different plane of focus.

**Figure 9i.** Transverse section through a carposporangium containing 8 carpospores (only 4 visible).

**Figure 9j.** Surface view of a carposporangium containing 8 carpospores (only the top 4 are visible).

**Porphyra nereocystis** - spermatogenesis

**Figure 9k.** Surface view of the foliose phase showing vegetative cells with a single stellate chloroplast.

**Figure 9l.** Surface view of spermatangia which have undergone a few divisions.
**Porphyra nereocystis** - spermatogenesis

Figure 10a. Transverse section through an immature spermatangium.

Figure 10b. Surface view of mature spermatangial region of the thallus. Individual spermatangia are not clearly delimited.

Figure 10c. Transverse section through a mature spermatangium which is 8 spermatia deep.

**Porphyra nereocystis** - carposporogenesis

Figure 10d. Transverse section through a vegetative cell region of the thallus. Note the single stellate chloroplast, central pyrenoid and lateral nucleus.

Figure 10e. Transverse section through a carpogonium. Note its slightly more elongate shape compared to the vegetative cells.

Figure 10f. Transverse section showing two closely appressed carpogonia. Note the spermatium (arrow) and fertilization canal.

Figure 10g. Transverse section of two carposporangia showing that the first division is periclinal.

Figure 10h. Transverse section of two carposporangia. The one on the left is at the stage shown in surface view in Figure 10i, whereas the one on the right is at the stage shown in Figure 10j. This division sequence is the same as that shown for *Porphyra gardneri* in Figure 8b.

Figure 10i. Surface view of a carposporangium containing 4 carpospores.

Figure 10j. Surface view of a carposporangium containing 8 carpospores (only the top 4 are visible). See also Figure 10h.

Figure 10k. Surface view of mature carposporangia. It is difficult to determine the boundaries of a single carposporangium.

Figure 10l. Transverse section showing two closely appressed carposporangia which have undergone more than one division in the plane of the thallus.
Figure 11a. *Porphyra nereocystis*, transverse section through a region of mature carposporangia. It is difficult to determine the original boundaries of a single carposporangium.

*Porphyra thuretii* - spermatogenesis

Figures 11b and 11c. Surface view showing early divisions of the spermatangia.

Figure 11d. Transverse section through an immature spermatangium.

Figure 11e. Mature spermatangium with surface dimensions a/4, b/4 and containing a total of 64 spermatia.

Figure 11f. Transverse section through a mature spermatangium.

*Porphyra thuretii* - carposporogenesis

Figure 11g. Transverse section through a carpogonium which appears to have very slight bipolar prototrichogynes.

Figure 11h. Transverse section showing that the first division of the carposporangium is periclinal.

Figure 11i. Transverse section of a carposporangium that has undergone second and third anticlinal divisions. A surface view is shown in Figure 11j.

Figure 11j. Surface view of a carposporangium which has undergone three divisions.

Figure 11k. Surface view of a mature carposporangium containing 8 carpospores (only the top 4 are visible). See Figure 11l.

Figure 11l. Transverse section of a mature carposporangium.
PART III - CULTURE STUDIES

Introduction

The first culture studies of *Porphyra* were made by Janczewski (1873) and, although crude by current standards, demonstrated that the carpospores of *Porphyra purpurea* (Roth) C. Agardh (as *P. laciniata* (Lightfoot) J. Agardh) and *P. leucosticta* germinated into a filamentous phase. Thuret and Bornet (1878) and Berthold (1882) obtained similar results; however, Berthold thought that the filamentous growths were abnormal and concluded that the carpospores remained unchanged until they germinated into the foliose phase. Yendo (1919) germinated the carpospores of several Japanese *Porphyra* species and found they also produced a filamentous phase. Grubb (1924), Kylin (1922), Dangeard (cited from Drew, 1956, p. 574) and Rees (1940) obtained the same results with European *Porphyra* species.

Kunieda (1939) thought that the filamentous phase was an anomaly and suggested that the entire carpogonium is released from the thallus and that the carpospores pass the summer within it and are finally released in the autumn when they germinate to give the foliose phase. Okamura *et al.* (1920) reported that immature carpospores germinated to produce rhizoid-like filaments which functioned to absorb nutrients for the foliose thallus growth. They stated that mature carpospores germinated directly into foliose thalli. Ueda (1929) suggested that the carpospores germinated immediately
into small plants which lasted through the summer to produce mcnosspores in the autumn and thereby produce the foliose thalli again. Kusakabe (1929) supported this view.

Drew (1949, 1954a, 1954b) was the first to point out that the filamentous phase resulting from carpospore germination was already known to phycologists as Conchocelis rosea Batters. This shell-boring alga was first described by Batters (1892) from material collected off the coast of Scotland.

Drew's findings stimulated a number of investigations of various Porphyra species (Graves, 1955; Hollenberg, 1958; Krishnamurthy, 1959; Conway, 1964a, 1964b, 1964c). These culture and cytological studies did not determine the environmental parameters responsible for optimal growth and reproduction. Such information is essential to efficient Porphyra cultivation and it is therefore no surprise that the Japanese are the leaders in this area, having amassed a great amount of data on the biology of the conchocelis phase.

Kurogi (1953a, 1953b) was one of the first investigators to elucidate the complete morphological life history of Porphyra. He germinated the carpospores of 4 species, and obtained the conchocelis phase. The conchocelis phase formed conchosporangial branches which produced conchospores that germinated into the foliose phase. Kurogi followed this pioneering work with a series of excellent studies on the effects of photoperiod, light intensity, water temperature,

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*In some of the Japanese literature the conchosporangial branch is referred to as a monosporangial branch and conchospores are called mcnosspores.*

These studies have enabled the Japanese to effectively manipulate the life histories of their Porphyra species to improve cultivation and have enabled them to develop such novel techniques as quick-freezing sporelings on nets to -20°C and storing them for later cultivation (Migita, 1964, 1966, 1967a; Migita et al., 1971; Miura, 1975).

Another unique approach that has been taken to the study of nutrient requirements by the foliose thallus is to characterize the bacterial flora of the thalli, then introduce isolated strains into axenic cultures of thalli, and observe how the bacteria affect growth (Tsukidate, 1970, 1971, 1974).

A few investigators outside Japan have also been interested in the specific conditions of spore formation and release by the conchocelis phase. Tseng et al. (1963) studied the effect of temperature and photoperiod on conchosporangial branch formation and conchospore release in six species of
Porphyra, and Dring (1967) and Rentschler (1967) have investigated the effect of photoperiod on conchosporangial branch formation by Porphyra tenera.

Iwasaki (1961), working with Porphyra tenera, was the first to follow the complete life history in vitro using well defined conditions and growth medium. Kornmann (1960, 1961a, 1961b) has done some excellent culture work on the European Porphyra species. Chen et al. (1970) and Bird et al. (1972) have succeeded in completing the life histories of Porphyra miniata and P. linearis respectively, through 5-6 generations.

Because of the difficulties of maintaining the foliose thallus in culture, there have been few studies of the environmental parameters responsible for spermatium, carpospore and monospore formation. Iwasaki (1961) pointed out the importance of photoperiod in inducing carpospore formation. Kurogi (1961) suggested that temperature plays an important role in controlling monospore production. Bird et al. (1972) and Bird (1973) found that P. linearis sporulated under a variety of temperatures and photoperiods and suggested that sporulation was determined by the age of the thalli rather than by an environmental stimulus.

My culture studies of Porphyra gardneri, P. nereocystis, and P. thuretii were intended to complement my field studies in

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1In some of the Japanese literature these spores which are produced by the foliose thallus and which germinate to produce more foliose thalli, are referred to as neutral spores. The term monospore in some of the earlier Japanese literature refers to conchosporas.
order to obtain a detailed picture of the morphological life histories of these three algae. I had the following objectives: to identify the reproductive bodies produced by the foliose thallus and see if different photoperiods could affect their germination as has been reported for Bangia (Richardson, 1970); to determine if P. gardneri and P. thuretii have a conchocelis phase and if so to characterize the general features of the conchocelis phase morphology and growth; and to determine the specific factors responsible for conchosporangial branch formation and conchospore release.

Materials And Methods

Most culture experiments were carried out with material from the study site, although some spore germination studies used material from other sites as a check for any population differences in the time or type of spore formation. The other sites included: Porphyra gardneri: Second Beach and Execution Rock in Barkley Sound; Botanical Beach, Port Renfrew; Point Joe, Monterey Peninsula. Porphyra nereocystis: Aquilar Point, Second Beach, Cable Beach and Cape Beale in Barkley Sound; Botanical Beach, Port Renfrew; Friday Harbor, San Juan Island. Porphyra thuretii: Leach Islet, Barkley Sound.

Preliminary monospore and carpospore germination studies were carried out under a photoperiod of 12:12 (light:dark), a temperature of 10°C and a light intensity of 25-35 ft-c. Sylvania or Westinghouse P48T12 Cool White 40 W fluorescent tubes were used for illumination. The suitability of these
conditions for growing bangiophyceae algae was determined in previous culture studies (Hawkes, unpublished B.Sc. Honours thesis).

Small thallus pieces were placed in 100x15 mm disposable Nalgene Petri plates with 50 ml of Provasoli's enriched seawater medium (Provasoli, 1971) and GeO₂ (10 µg/ml) to control diatom growth (Lewin, 1966). Seawater used for all experiments was taken from the seawater system at the Bamfield Marine Station and had a salinity which varied from 30-32%. Cultures were examined after 1-2 days and, if spore release was sufficient, the thallus pieces were removed and germination and development of the spores were observed.

Sub-cultures for further experiments were obtained by transferring spores to fresh Petri plates and culture medium.

In addition to the standard culture conditions, a number of other conditions were used in experiments on spore germination and conchocelis phase growth and reproduction. Pyrex culture dishes 100x80 mm, 450 ml were used in some of these experiments.

Monospores and carpospores of *Porphyra gardneri* and carpospores of *P. nereocystis* were germinated under photoperiods of 8:16, 12:12, 16:8 and temperatures of 7°, 10° and 15°C. Both low (25-35 ft-c) and high (150-200 ft-c) light intensities were used.

In experiments on the development of monospores of *Porphyra gardneri*, cultures were aerated or agitated and pieces of host plant were added to some to see if growth could be enhanced. Conchospore cultures of both *P. gardneri* and
P. thuretii were maintained at 10°C, 12:12 and aerated. A light intensity of 200-300 ft-c was used for these cultures.

The growth of the conchocelis phase of P. gardneri and P. nereocystis in egg shell, clam shell (Saxidomus giganteus) and oyster shell (Crassostrea gigas) was observed.

For studies of the effect of temperature and photoperiod on conchocelis branch formation, the following conditions were used: 7°, 10°, 15°C and 8:16, 12:12, 16:8, 25-35 ft-c. Only 10°C was used for P. thuretii.

Temperature and photoperiod were also the main factors which were manipulated in an attempt to obtain conchospore release. Cultures were maintained on a temperature gradient table at temperatures from 7°-20°C. In another series of experiments cultures were transferred to different temperature and photoperiod regimes. These included: Porphyra gardneri: from 10°C, 12:12 to 7°C, 12:12 and 7°C, 8:16; Porphyra nereocystis: from 15°C, 16:8 to 13°C, 11:13 and 10°C, 8:16; from 10°C 12:12 to 10°C, 10:14 and 7°C, 9:15; from 10°C, 16:8 to 7°C and 10°C, 8:16; Porphyra thuretii: from 10°C, 12:12 to 7°C, 9:15.

Porphyra nereocystis conchocelis phase was subjected to several other conditions in an effort to induce conchospore release. These included: pH over the range 7-10, salinity from 20-40%, desiccation, agitation, and host plant exudate. Cultured conchocelis phase was also placed in a special plexiglass culture tube and put out in the field at the study site in the autumn at a depth of 7 m. The design of the culture tube was based on that of the 'Biomonitor' which is
Results

A. *Porphyra gardneri*

1. Monospores

Soon after *Porphyra gardneri* first appeared in the field in February the cells in the marginal zone developed a fine granular appearance which partially obscured the stellate chloroplast. The granular appearance was best developed in those cells which were on the margin. Following the appearance of the granular cell contents, the entire distal margins began to release thousands of monospores which were 25-30 um in diameter (Figure 12a).

In culture the monospores germinated after 1-2 days by sending out a long rhizoidal protuberance (Figure 12b). The function of this rhizoid is probably to ensure that the spore is anchored in the 'host' tissue before the foliose thallus commences growth. Following rhizoid elongation, a series of parallel divisions formed an uniseriate thallus (Figures 12c and 12d). At the 4-5 cell stage divisions occurred at right angles to the initial ones to form the foliose, monostromatic thallus (Figures 12e and 12f). Sporelings grew well in culture initially, but when they reached the size shown in Figure 12f growth ceased and they eventually died.
This prolific monospore production explains the great increase in *P. gardneri* abundance in the field throughout the spring months. During May, thalli releasing monospores along their distal margin and spermatia and carpospores along their lateral margins were common. By June and July, spore production became almost exclusively carposporic, although the presence of a few bipolar sporelings indicated that some monospores were still being produced. In the autumn, monospore production again became predominant, but not to the same extent as it was in the spring.

In culture aeration was found to be essential for good monospore germination. Under all photoperiods and temperatures tested, monospores underwent bipolar germination to produce the foliose phase; however, at 16:8 the number of monospores germinating was considerably lower than at 12:12 or 8:16.

Monospores were being produced by plants in the other populations examined. Culture work done at Hopkins Marine Station confirmed that most of the thalli collected at Point Joe (type locality) in July 1976, were exclusively monosporic.

The failure of sporelings to reach maturity suggested they may have a physiological requirement for the 'host' plant. In view of the occurrence of *Porphyra gardneri* on 15 laminarialean algae and because one of the major photosynthates in this group is mannitol, I tested the effect of mannitol on sporeling growth. Parker (1966) reported a mannitol concentration of 3.6% (w/v) for *Macrocystis pyrifera* (Linnaeus) C. Agardh. Using this concentration as a guideline a 1.8% and 0.9% solution of d(-) mannitol was tested; however, no enhancement
of growth occurred.
In another experiment 1 cm² pieces of Laminaria thallus were added to the monospore cultures. A control without Laminaria was also run. Monospores in the cultures containing host tissue germinated before those in the control, but the resulting sporelings did not grow to maturity.

2. Spermatangium And Carpo gonium Formation

Variation in the time of spermatangium and carpogonium formation with latitude (discussed in Part I) suggested that photoperiod was important in inducing their formation. To determine if this is a photoperiodic or photosynthetic response, monosporic thalli attached to the 'host' plant were kept in 20 l containers at 10°C, 12:12 and 16:8, 300-400 ft-c. Spermatia formed under both photoperiods. Unfortunately the thalli died before carpospores were formed.

3. Carpospore Germination - The Conchocelis Phase

At the study site the first carpospores were produced by May. They were viable whether they had been formed by one or more divisions. Whether subsequent divisions occurred after the spores had been released is not known. Released carpospores were 14-20 um in diameter (Figure 12g) and germinated in 2-3 days to give conchocelis filaments that were 4-7 um wide. The cells of the conchocelis phase contained parietal chloroplasts (Figure 12h). Under all temperatures and photoperiods tested, carpospores germinated into the
conchocelis phase. Carpospores were produced by all of the other populations which were examined. The number of carpospores produced by the population at the type locality was low compared to more northern populations, but they were viable and germinated into the typical conchocelis phase.

The conchocelis phase grew prolifically and soon formed numerous tuft-like colonies. Propagation of filaments by fragmentation occurred readily. Single spore-like swellings were formed in some cases (Figure 12i), but no evidence of them functioning as monosporangia was seen.

After 10–12 weeks conchosporangial branches formed. They were initially short and stubby (Figure 13a), but later became long and branched (Figure 12j). By 24 weeks conchospore release occurred (Figure 13a). Released conchospores were 22–30 μm in diameter and after 2–3 days they underwent bipolar germination to produce the foliose phase (Figure 13b). Aeration was found to be essential for good conchospore germination.

When grown in shell, the conchocelis phase had a characteristic pinnately branched morphology (Figure 13c). Growth in the eggshell was poor compared to the prolific growth in the clam and oyster shells. In older portions of the conchocelis phase the branches became irregularly lobed.

The ability of the conchocelis phase to survive in total darkness was tested. After 8 months of darkness the conchocelis filaments were pale pink, but were alive and grew well when returned to the light.
4. Conchosporangial Branch Formation

The effects of photoperiod and temperature on conchosporangial branch formation were examined. Conchosporangial branches were irregularly branched and varied in size, length and branch density, making quantitative assessment of their abundance difficult. The technique used by Dring (1967) on Porphyra tenera conchocelis phase was considered impractical because, as Richardson (1970) has pointed out, it does not determine the actual number of conchosporangia. The technical difficulties encountered in trying to obtain this data proved insurmountable. In each culture the presence or absence of conchosporangial branches was noted and a qualitative assessment of abundance made.

At 10°C, conchosporangial branches formed at 8:16, and 12:12. One or two branches formed at 16:8, but most cultures under long days remained vegetative. Conchosporangial branches formed at all 3 temperatures, but because of culture chamber malfunctions it was not possible to determine precisely the optimal temperature for conchosporangial branch formation. The trend was for conchosporangial branches to form sooner at 10°C and 15°C than at 7°C.

5. Conchospore Release

Although it was easy to induce conchosporangial branch formation, getting them to release conchospores consistently proved impossible. Conchospore release occurred in cultures maintained at 7°C, 10°C and 8:16, 12:12, with the most prolific
release occurring at the latter photoperiod. A small conchocelis release was induced by transferring a culture from 10°C, 12:12 to 7°C, 12:12.

E. *Porphyra nereocystis*

1. Carpospore Germination - The Conchocelis Phase

No evidence of monospore production by the foliose thallus was found.

Carpospores were produced by all populations of *Porphyra nereocystis* which were examined. Carpospores were released from carposporangia which had undergone varying degrees of division (both c/2 and c/4, see Part II). Released carpospores were 8.0-15 µm in diameter (Figure 13d). After 2-3 days they germinated to produce conchocelis filaments 4-6 µm across (Figure 13e), the cells of which contained parietal chloroplasts. Under all temperatures and photoperiods tested, carpospores germinated into the conchocelis phase. No production of monospores by the conchocelis phase was observed, although it readily regenerated from fragments of vegetative filaments.

A number of irregular swellings formed at both the base and apex of filaments (Figures 13f and 13g). Beaded branches 11-15 µm across (Figure 13h) and square-celled branches 13-17 µm across (Figure 13i) also formed and were initially mistaken for conchosporangial branches. These two branch types formed under all photoperiod regimes tested. Whether they have any specific function such as the production of monospores is
not known. They appear to form a transition from vegetative filaments to conchosporangial branches (Figure 13j).

Conchosporangial branches appeared after 10-15 weeks and were typically candelabra-shaped (Figures 13k and 14a). The branches were uniseriate, 16-25 μm across. Each cell of the conchosporangial branch had a stellate chloroplast, thick cell wall and pit plugs between adjacent cells (Figure 14b). If a cluster of conchosporangial branches was removed from the vegetative filaments it would continue to grow, producing either more conchosporangial branches or vegetative filaments. The older portions of some conchosporangial branches were two cells thick (Figure 14c).

When grown in shell the vegetative conchocelis filaments developed pinnate branching (Figure 14d). Conchosporangial branches initially formed in the shell and then grew out of it to form small colonies on the shell surface. The vertical orientation of these branches suggested that they were phototropic. To test this, a culture dish was covered so that light was admitted at one end only. Under this treatment all conchosporangial branches grew toward the light, indicating they are positively phototropic.

At the end of 8 months in complete darkness, vegetative conchocelis filaments were as deeply pigmented as those grown in the light.

The conchocelis phase was observed in the field growing in barnacles which were on the stipe of an old Nereocystis plant. It was taken back to the laboratory and the surface layers of barnacle dissolved by placing them in a 1% solution of Na₂EDTA.
for 12 hr (Prud'homme van Reine and van den Hoek, 1966). Small patches of conchocelis filaments were removed and transferred to culture dishes. This isolate grew well and formed beaded, square-celled and conchosporangial branches typical of *Porphyra nereocystis*. The general morphology of this conchocelis phase and its failure to release conchospores suggests that it is referrable to *Porphyra nereocystis*.

2. Conchosporangial Branch Formation

The effects of temperature and photoperiod on conchosporangial branch formation were examined. At 10°C, conchosporangial branches formed under all 3 photoperiods tested. They first appeared in cultures at 15°C and 16:8, or 12:12. At 8:16 most conchosporangial branches formed at 15°C. Conchosporangial branches which formed at 15°C were short and stubby.

3. Conchospore Release

None of the attempts to induce conchospore release succeeded. Because *Porphyra nereocystis* appeared in the late autumn it was suspected that the decreasing daylength and seawater temperature were critical factors; however, none of the transfer experiments, from higher temperatures and longer photoperiods to cooler temperatures and shorter photoperiods, resulted in conchospore release.

The possibility that some chemical cue from the aging *Nereocystis* caused conchospore release was investigated by
placing pieces of mature stipe, or stipe exudate, in with the conchocelis phase. Neither of these treatments was successful.

The conchocelis phase of some *Porphyra* species has been found in the intertidal zone (Drew and Richards, 1953; Mumford, 1973a) suggesting that desiccation could be a factor affecting conchospore release. No conchospores were released by conchosporangial branches subjected to 1, 2 and 3 hr of drying at 20°C.

Because the conchocelis phase grows in a calcareous substrate it was suspected that there may be a specific pH required for conchospore release. Six cultures with a pH ranging from pH 7-10 were tested. At pH 10 the culture died. The other cultures survived but the pH quickly changed to pH 8-9.

Changes in salinity had no effect in triggering conchospore release.

The conchosporangial branches put out in the field in the culture tube also failed to produce conchospores.

C. *Porphyra thuretii*

1. Carpospore Germination - The Conchocelis Phase

No evidence of monospore production by the foliose thallus of *Porphyra thuretii* was found.

Released carpospores were 15-22 μm in diameter (Figure 14e) and germinated after 2-3 days to give a conchocelis phase (Figure 14f). The filaments were 4-5 μm across and in general morphology and colour they were distinct
from those of *Porphyra gardneri* and *P. nereocystis*. In culture the vegetative filaments formed long, fine, hair-like 'tufts' which were purple-gray.

Early in my studies I collected thalli which I could not identify. Herbarium specimens and conchocelis phase cultures were made from this material for future study. The conchocelis phase was so distinct that I was later able to refer it to *Porphyra thuretii*.

The vegetative filaments developed irregular and beaded branches (Figure 14g). After 8-14 weeks conchosporangial branches formed (Figure 14h). Initially they were short, but in older cultures they formed long candelabra-shaped colonies (Figure 14i) which were positively phototropic.

In one experiment at the end of 12 weeks, abundant conchospores formed within the conchosporangial branches (Figure 14j) followed by conchospore release (Figure 14k). Conchospores were not observed at the time of their formation or release, so the details of these processes are not known. Freshly released conchospores exhibited pronounced amoeboid changes of shape and movement (Figures 15a-d). Following this the conchospores rounded up (14-17.5 um in diameter) and within a few days germination occurred. In the initial stage of germination a long rhizoidal protuberance was formed (Figure 15e). The rhizoid did not develop to the same extent as it did in *Porphyra gardneri* sporelings. Following the initial rhizoid elongation, a few short, broad rhizoidal protuberances formed (Figure 15f) and partially coalesced to produce a basal pad (Figures 15g, 15i, 15j).
Growth of the foliose thallus began with a series of parallel divisions which resulted in an uniseriate filament (Figure 15g). Sporelings remained uniseriate only to about the 4-7 cell stage, at which time a series of perpendicular divisions resulted in a biseriate thallus (Figure 15h) and eventually gave rise to a parenchymatous, monostromatic thallus (Figures 15i and 15j). In these young sporelings there was a definite apical growth zone. In culture, thalli grew to about 1 mm in length before growth ceased.

2. Conchosporangial Branch Formation

The effect of photoperiod on conchosporangial branch formation was studied. Conchosporangial branches formed first at 8:16 then at 12:12. Cultures at 8:16 consisted almost entirely of conchosporangial branches. In 1 out of 3 experiments a couple of conchosporangial branches formed at 16:8, but most cultures remained vegetative at this photoperiod.

3. Conchospora Release

Conchospora release occurred under two conditions tested: at 10°C, 12:12, and by transferring from 10°C, 12:12 to 7°C, 9:15. Subsequent exposure of other cultures to these conditions failed to induce conchospora release indicating that other factors were also involved.
Discussion

1. Monospores

*Porphyra gardneri* is the only *Porphyra* species known from the Northeast Pacific Ocean which has such a predominant monospore cycle. Although Conway *et al.* (1975) reported monospore production by small, high intertidal plants in British Columbia they did not specify which species this was observed in. *Porphyra nereocystis* and *Porphyra thuretii* do not form monospores, a situation I think applies to most species in this area. This is in contrast to 10 Japanese species of *Porphyra* which are known to produce monospores (Kurogi, 1972; Shimamura, 1974). In its prolific monospore production *P. gardneri* is similar to *Porphyra kuniedai* and *P. yazdensis* which produce monospores on thalli up to 70 mm in length (Kurogi, 1972), and to *P. tanegashimensis* Shimamura which liberates monospores through all seasons (Shimamura, 1974).

Why monospore production should be so prevalent in the Japanese species is an intriguing question. A study of the environmental conditions under which the monospore-forming species grow and the factors triggering monospore production may provide insight into this phenomenon.

My studies demonstrated that monospore production by *Porphyra gardneri* was predominant in the spring and autumn. This implies that both shorter photoperiods and lower temperatures may play a role in inducing monospore formation. Kurogi (1961) stated that water temperature seems to be closely related to monospore liberation by the foliose thalli of
Porphyra kuniedai and P. tenera. In culture experiments with Bangia, Sommerfeld and Nichols (1973) demonstrated that water temperature affected monospore production. Kurogi (1961) also indicated that there is a correlation between nutrients and monospore production by *P. vezoensis* and *P. tenera*. In some species, thallus size determines whether monospores are produced. In *Porphyra angusta* Ueda for example, thalli more than 2 mm high do not form monospores even when conditions in the field are suitable (Kurogi, 1961).

The production of monospores, spermatia and carpospores on the same thallus in *Porphyra gardneri* in the early summer is not a unique phenomenon. Kurogi (1961) reported this for *P. kuniedai* and *P. vezoensis*.

Because of its monospore cycle, *Porphyra gardneri* could potentially be one of the most valuable species on our coast for commercial cultivation, assuming that a method of growing it on a synthetic substrate can be developed. Japanese species of major economic importance have a monospore cycle (Kurogi, 1972).

2. Spermatangium And Carpogonium Formation

My initial field observations of spermatangium and carpogonium formation in *Porphyra gardneri* (see Part I) suggested that photoperiod was responsible for their formation. The formation of spermatangia at a photoperiod of 12:12 in culture (less than what appeared to be the critical inducing daylength), indicates that spermatangium formation is probably a photosynthetic effect rather than a truly photoperiodic
response as defined by Richardson (1970). Critical experimental studies are needed to answer this question and to determine why some cells produce spermatangia whereas others form carpogonia. Photoperiod certainly seems to be an important factor. Fujiyama and Suto (cited from Ogata, 1975) and Iwasaki (1965) reported that when the daylength was greater than 12 hr light, spermatangia and carpogonia formed.

Kurogi (1961) tried to correlate spermatangium and carpogonium formation in *P. kuniedai* with water temperature; however, he concluded that this was not the only factor inducing sexual reproduction.

When experimental technique is sufficiently refined to permit manipulation of the reproductive status of the foliose thalli in culture, a new era in *Porphyra* cultivation will begin. It will then be possible to attempt hybridization and selection to improve the currently cultivated *Porphyra* species. Such studies may already be going on in Japan (Dr. T. F. Mumford, personal communication).

3. Effect Of Photoperiod On Monospore And Carpospore Germination

Germination of monospores of *Porphyra gardneri* and carpospores of *P. gardneri*, *P. nereocystis* and *P. thuretii* under different photoperiods showed that once a particular spore type is induced, its fate cannot be changed by photoperiod. This is in agreement with the work of Bird et al. (1972) and Mumford (1973a) on *Porphyra*, and Cole (1972a) on *Bangia*. In contrast, Richardson (1970) reported that in *Bangia*
released carpospores underwent bipolar germination when cultured under a photoperiod of less than 12 hr light, and unipolar germination to give the conchocelis phase when cultured under a photoperiod with more than 12 hr light. Conway (1964b, 1966) has suggested that under certain conditions carpospores of Porphyra can germinate into bipolar sporelings instead of the conchocelis phase. In such cases I think that one should be very careful that all of the spores being tested are of the same type. Kurogi (1961) and Hawkes (1977b) have both noted that monospores and carpospores can be present on the same thallus.

4. The Conchocelis Phase

This is the first report of a conchocelis phase in the life histories of Porphyra gardneri and P. thuretii, establishing that they have heteromorphic life histories. Of the Porphyra species which have been cultured, only Porphyra sanjuanensis Krishnamurthy (Conway, 1973; Conway et al. 1975) and Porphyra subtumens J. Agardh ex Laing (Conway and Wylie, 1972) lack a conchocelis phase.

Irregular swellings of vegetative conchocelis filaments and branch types which superficially resemble conchosporangial branches have been reported by several workers (Iwasaki, 1961;  

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1The report by Conway et al. (1975) that Porphyra thuretii has a conchocelis phase was based on my work.

2It should be noted that if the mode of spermatium formation reported by Conway and Wylie (1972) is correct, then this species does not belong in the genus Porphyra.
Iwasaki and Matsudaira, 1963; Conway, 1964b; Conway and Cole, 1977). Conway and Cole (1977) reported that these produced monosporangia. Iwasaki and Matsudaira (1963) found that when Porphyra tenera conchocelis phase was maintained under continuous illumination it formed squarish branches which released monosporangia. I cannot definitely conclude that the swellings I observed are monosporangia because they were never seen to produce monosporangia. Vegetative propagation of Porphyra nereocystis conchocelis filaments in my cultures occurred by fragmentation rather than by monospore production. This was also observed by Mumford (1973a) in his cultures. Chytrid infection of the conchocelis phase can also produce spore-like swellings (Arasaki, 1960, referring to Yendo's (1919) work; Migita, 1973,) which could be mistaken for monosporangia.

Under certain conditions, the beaded branches produced by Porphyra nereocystis and Porphyra thuretii may produce monosporangia, or they may have other functions such as playing some role in the shell-boring process. Krishnamurthy (1969a) misinterpreted these beaded branches as being conchosporangial branches.

Fukuhara (1968), in his studies of several Porphyra species of Hokkaido, noted that the conchocelis phase of the species belonging to the subgenus Porphyra could be divided into two types; one in which the vegetative filaments and conchosporangial branches are a different colour and one in which they are the same colour. In the 3 species I studied the conchosporangial branches were more deeply pigmented than the
vegetative filaments but were not markedly different in colour.

The pinnately branched morphology of the conchocelis filaments grown in shell was striking and poses the question of how the shell matrix causes this morphological change. Ogata (1959; 1961) presented some fascinating photographs of the vertical growth pattern of the conchocelis phase in shell and noted that the morphology was affected by the type of shell. I found that the degree and regularity of branching varied with the shell type. Several other Porphyra species develop pinnate branching in shell (Kurogi, 1953a, 1953b; Drew, 1954a; Kornmann, 1960; Kurogi and Sato, 1962a, 1962b, 1967; Migita and Kim, 1970; Mumford, 1973a, 1975). Migita and Kim (1970) found that under diffuse light conchocelis filaments developed the pinnately branched morphology, whereas under unidirectional light both the vegetative filaments and conchosporangial branches showed a definite positive phototropism. Mumford (1975) also reported a positive phototropism for the conchosporangial branches of Porphyra brumalis Mumford. A physiological study is needed to elucidate the mechanism of this phototropic response.

Drew (1954a) referred to the branches she observed in the shell matrix as 'fertile-cell rows' and those on the shell surface as 'plantlets'. Migita (1961, 1962) has pointed out that they are actually one continuous structure and should be referred to as the conchosporangial branches. My observations are in agreement with Migita's conclusion. Migita (1962) also reported that the conchosporangial branches could revert to vegetative filament growth which could re-penetrate the shell.
I also observed this phenomenon in *P. nereocystis*.

Ogata (1961) used pH sensitive stains to determine that the internal pH of conchocelis filaments was pH 5-6. He speculated that this mild acid production was responsible for dissolving the shell; however, the actual physiological mechanism of this process needs to be elucidated.

The ability of the conchocelis phase to survive prolonged darkness is another area in which physiological study is needed and should provide interesting insight into adaptations of the conchocelis. Sheath *et al.* (1977) have recently reported the effects of darkness and low light on chloroplast ultrastructure, pigmentation and photosynthesis in the conchocelis phase of *Porphyra leucosticta*. Ogata (1961, 1971) observed that conchocelis continued growing even after the shell it was in was embedded in liquid paraffin. He concluded from this that the conchocelis was able to use the shell matrix as a carbon source.

The conchocelis phase has been observed in barnacles or shells in the field a number of times (Batters, 1892; Rosenvinge, 1931; Drew and Richards, 1953; van den Hoek, 1958; Miura and Ito, 1959; Migita, 1959b; Bird, 1973; Mumford, 1973a, 1975). At the study site the predominant calcareous substrate was provided by crustose coralline algae. In view of Drew and Richards (1953) report of *Lithothamnion laevigatum* Foslie (reported as *Lithothamnion laevigata*) being a suitable conchocelis phase substrate, a thorough survey should be made before crustose corallines are ruled out as a potential substrate for the conchocelis phase.
5. Conchosporangial Branches

Conchosporangial branch formation by *Porphyra gardneri* and *Porphyra thuretii* was most abundant under a photoperiod of 12 hr light or less, whereas in *P. nereocystis* no optimal photoperiod was observed. The studies of most workers on other *Porphyra* species indicate that photoperiod is of major importance to conchosporangial branch induction. Dring (1967) and Bentschler (1967), both working with *Porphyra tenera*, showed that conchosporangial branch initiation was a short-day, phytochrome-mediated response. My results with *P. gardneri* and *P. thuretii* are similar to those of Dring (1967) and Iwasaki and Sasaki (1972), in that conchosporangial branches formed under all photoperiods tested, but were rare under long days and abundant under short days. It is not known if phytochrome is involved. Richardson (1970), however, reported that conchosporangial branch production by *Bangia* was an 'all or nothing' response.

The data of Japanese investigators support the hypothesis that photoperiod is a major factor in conchosporangial branch induction. The main conclusion of several workers (Kurogi, 1959; Iwasaki, 1961; Kurogi and Sato, 1962a, 1962b; Kurogi et al. 1962; Iwasaki, 1965; Iwasaki and Sasaki, 1972; and Chihara, 1975) is that conchosporangial branch formation in the Japanese winter *Porphyra* species is most abundant in cultures subjected to 12 hr or more dark per 24 hr. In the field such conditions are encountered in the autumn and winter when the foliose phase of most Japanese *Porphyra* species appears. In *P. umbilicalis*, an autumn species, Kurogi and Sato (1967) found that
Ccnchosporangial branches were most abundant at 16:8.

Iwasaki and Sasaki (1972), in an experiment with Porphyra suborbiculata Kjellman forma latifolia, showed that it was photoperiod rather than the total amount of light received (photosynthetic effect) which was responsible for ccnchosporangial branch formation.

The apparent lack of an optimal photoperiod for ccnchosporangial branch induction in Porphyra nereocystis remains an enigma. It must be pointed out that the branches formed were assumed to be conchosporangial branches, but because none of them released conchospores this is perhaps a premature assumption. The only report in the literature of photoperiod having no effect on conchosporangial branch formation is that of Bird et al. (1972) for Porphyra linearis.

Other factors can have a modifying effect on the photoperiodic induction of conchosporangial branches. Rentschler (1967) found that while temperature changes did not induce conchosporangial branches, they did influence the duration from photoperiodic induction until the appearance of conchosporangial branches. In Porphyra nereocystis I observed that conchosporangial branches first appeared in those cultures at a higher temperature. In many Porphyra species it has been shown that conchosporangial branches formed over a range of temperatures, but that there was usually an optimal temperature (Kurogi and Hirano, 1956a, 1956b; Iwasaki and Sasaki, 1972). Furthermore, Kurogi and Hirano (1956b) and Kurogi and Akiyama (1966) noted that this temperature was not always the optimal temperature for conchospore liberation. These are general
trends and should not be assumed to be true for all Porphyra species. For example, Kurogi and Sato (1967) reported that P. umbilicalis conchocelis formed conchosporangial branches equally as well at 10°C as at 20°C.

Light intensity has also been shown to have a modifying effect on conchosporangial branch production (Kurogi and Hirano, 1955a, 1955b; Conway, 1964b). In Rhodochorton, Knaggs (1966a, 1966b, 1967) demonstrated that light intensity, or a combination of light intensity and nutrient level, was responsible for tetrasporangium initiation; however, West (personal communication) has pointed out that this response was only investigated under inductive daylengths (less than 12:12).

6. Conchospore Release

Only Porphyra tenuipedalis Miura (Miura and Ito, 1959, as Porphyra sp.; Miura, 1961) and P. miniata (Krishnamurthy, 1969a, as P. cuneiformis (Setchell et Hus) Krishnamurthy) have been reported to produce the foliose phase directly from the conchosporangial branch without conchospore release. It is unfortunate that the formation of conchospores in the conchosporangia was not observed in either Porphyra gardneri or P. thuretii. It has been generally assumed that each cell of the conchosporangial branch functioned as a conchosporangium by releasing its entire contents as a single conchospore (Conway and Cole, 1977); however, Migita and Abe (1966), working with Porphyra tenera and P. yezoensis, observed that each conchosporangium produced 2-4 conchospores. Migita (1967b, 1974) has subsequently
studied this in further detail (see Part IV for more discussion). Kornmann (1961b) illustrated more than one conchospore per conchosporangium for Porphyra purpurea. This observation is of considerable importance because it provides good circumstantial evidence that the division of the conchosporangium to form conchospores is the site of meiosis and not the first division to form the conchosporangial branch as suggested by Conway et al. (1975). An interesting point is that shell-living conchocelis typically produced two conchospores per conchosporangium whereas free-living conchocelis often produced four (Migita and Abe, 1966).

In my experiments conchospore release proved to be impossible to trigger consistently. Conchospore release by Porphyra gardneri was optimal at 7-10°C, which correlated reasonably well with temperature conditions in the field when P. gardneri first appeared (Table VII). In Table VII study site surface seawater temperatures are based on only a single measurement each month, but the general trend is the same as that for Amphitrite Point (Hollister, 1968), a nearby site where daily measurements are made.

Although I was unable to demonstrate conclusively that temperature was responsible for conchospore release by Porphyra gardneri and P. thuretii, work which has been done on other Porphyra species points to temperature as the primary factor triggering release (Tseng and Chang, 1956, Tseng et al. 1963; Kurogi and Hirano, 1956a, 1956b; Migita and Abe, 1966; Kurogi and Akiyama, 1966; Kurogi and Sato, 1967; Kurogi et al. 1967; Bird et al. 1972; Shinmura, 1974; Migita, 1974;
Migita (1974) reported that conchocelis phase cultured without changing the medium for 3 months failed to form conchospores even when it was transferred to an inducing temperature. My failure to obtain consistent conchospore release may be attributable to such a nutrient-related phenomenon.

In contrast to most reports, Iwasaki (1961) did not observe any direct correlation between temperature and the life history phases of Porphyra tenera. He did note that low light intensity or continuous illumination inhibited conchospore release. Other workers have also reported that light intensity has a modifying effect on conchospore release (Kurogi and Hirano, 1955a, 1955b; Saito, 1956; Kurogi and Akiyama, 1965; Iwasaki and Sasaki, 1972; Kurogi et al. 1962). Several workers have reported that conchospore liberation is highest in the morning (Yamasaki, 1954b; Suto et al. 1954; Kurogi and Hirano, 1955b; Tseng and Chang, 1956).

Photoperiod is a secondary factor mediating conchospore release. The work of Kurogi (1959), Iwasaki (1961), Kurogi and Sato (1962a, 1962b), Kurogi et al. (1962), and Kurogi et al. (1967) has shown that there is an optimal photoperiod for conchospore release. In contrast, Bird et al. (1972) found that photoperiod variation did not affect conchospore liberation by P. linearis.

Other factors that have been reported to have an effect on conchospore release are agitation (Tseng and Chang, 1956) and desiccation (Graves, 1969). Saito (1955) correlated vertical
turbulence in the water column with an increase of conchospores in the surface water. Kurogi (1953b) and Kurogi and Hirano (1956a) both reported a 1-2 week periodicity in conchospore liberation but were unable to correlate it with spring tides as reported by Takeuchi et al. (1954).

Although my attempts to induce conchospore release by Porphyra nereocystis failed, workers in California (J. Woessner, personal communication August, 1977) have recently succeeded in triggering release by subjecting the conchocelis to a temperature of 12°C.

Judging from reports in the literature I think that the combination of specific temperature, photoperiod and light intensity are all important for conchospore release, with temperature being of primary importance.

Further culture studies under much more critically controlled conditions than I was able to maintain must be done before we will have a thorough understanding of the process of conchosporangial branch induction and conchospore release in the 3 species I studied. From my experience, Porphyra thuretii would be the best one to subject to further investigation because it responded faster and more definitely to different photoperiod regimes than did the other 2 species.

7. Conchospores

Of particular interest was the photographic documentation of amoeboid change of shape and movement by conchospores of Porphyra thuretii. These two phenomenon are not unknown in the Rhodophyta, having previously been reported for: monospores of
Bangia (Reinke, cited from Fritsch, 1945, p. 431; Berthold, 1882; Kylin, 1922; Sommerfeld and Nichols, 1970), Porphyra (Berthold, 1882; Kunieda, 1939; Kurogi, 1961; Graves, 1969), Erythrocladia (Nichols and Lissant, 1967), Helminthora (Svedelius, 1917), Liagora (von Stosch, 1965) and Acrochaetium (Rosenvinge, 1909, as Chantransia; Dangeard, 1929; Stegenga and Borsje, 1976; Stegenga and Vroman, 1976); for released 'carpogonia' of Porphyra papenfussii (Conway and Cole, 1973); for Porphyra spermatia (Kunieda, 1939); for carpospores of Porphyra (Janczewski, 1873; Okamura et al. 1920; Kylin, 1922; Graves, 1969), Bangia (Sommerfeld and Nichols, 1970), Helminthora (Thuret and Bornet, 1867), Platoma (Kuckuck, cited from Fritsch, 1945, p. 602), Scinaia (Chemin, 1927), and Bonnemaisonia (Chemin, 1929); and for tetraspores of Liagora (von Stosch, 1965), and Acrochaetium (Stegenga and Vroman, 1976).

Some rhodophycean spores exhibit gliding movements with no amoeboid changes of spore shape. Such locomotion has been reported by Dangeard (cited from Fritsch, 1945, p. 602) for Acrochaetium carpospores; by Rosenvinge (1927) for monospores of Erythrotrichia, tetraspores of Antithamnion, Ceramium, and Dumontia, and carpospores of Ceramium, and Polysiphonia, and spermatia of Phyllophora; and by Chemin (cited from Fritsch, 1945, p. 602) for monospores of Erythrotrichia and Goniotrichum. Recently Lin et al. (1975) made a detailed study of Porphyridium which exhibited gliding movements with only slight changes of shape. They reported that this phenomenon has also been observed in Chrootheca mobilis Pascher and
Amoeboid changes of shape and movement by conchospores have previously been reported for *Porphyra vezoensis* (Migita, 1972) *P. angusta*, *P. kuniedai* (Kurogi, 1961), *P. miniata* (Chen et al. 1970), *P. smithii* Hollenberg et Abbott (McDonald, 1972), and *Bangia* (Sommerfeld and Nichols, 1970).

The purpose of listing all of these examples is to point out that most are from the Bangiophycidae and Nemaliales. Is this just a coincidence or is there some evolutionary link between these two groups of Rhodophyta?

Neither the function nor mechanism of the amoeboid movements is known, although Berthold (1882) pointed out that light had an influence. A possible function could be to increase the probability of the spore finding a suitable substrate for attachment and germination. The photographs of *P. vezoensis* conchospores published by Migita (1972) suggest this may be the case. The ultrastructural study of Lin et al. (1975) on *Porphyridium* showed that there was a production and polar expulsion of mucilage from the moving cells, although it was not definitely established that this was the cause of the movement.
Table VII. Annual trend in surface water temperature (°C) at the study site (48° 50.1′N, 125° 11.1′W) and Amphitrite Point (48° 55.2′N, 125° 32.2′W).

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<tr>
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Porphyra gardneri

Figure 12a. Monospores being released along the margin of a thallus collected at the study site.

Figures 12b-f. Various stages in the development of the foliose thallus from a monospore. Note the long rhizoidal protuberances.

Figure 12g. Released carpospores.

Figure 12h. Carpospore germination to give the conchocelis phase.

Figure 12i. Single spore-like swelling on the conchocelis filament.

Figure 12j. Conchosporangial branches.
Porphyra gardneri

Figure 13a. Conchocelis filaments, conchosporangial branch (lower right) and released conchospore (arrow).

Figure 13b. Bipolar sporeling resulting from conchospore germination.

Figure 13c. Characteristic pinnately branched morphology of the conchocelis phase growing in oyster shell.

Porphyra nereocystis

Figure 13d. Released carpospores.

Figure 13e. Carpospore germination to give the conchocelis phase.

Figure 13f. Basal spore-like swelling of the conchocelis phase.

Figure 13g. Apical spore-like swelling of the conchocelis phase.

Figure 13h. Irregular and beaded-branch types.

Figure 13i. Square-celled branch.

Figure 13j. Small conchosporangial branch connected to the vegetative filament by a square-celled branch.

Figure 13k. Conchosporangial branches.
Porphyra nereocystis

Figure 14a. Closeup of conchosporangial branches showing the single stellate chloroplast in each conchosporangium.

Figure 14b. Pit plugs (arrows) between conchosporangia in the conchosporangial branch (treated with chromosome fixative and stain).

Figure 14c. Divisions within the conchosporangial branch.

Figure 14d. Characteristic pinnate branching of the conchocelis phase growing in oyster shell. Note the irregular swellings.

Porphyra thuretii

Figure 14e. Released carpospores.

Figure 14f. Carpospore germination to give the conchocelis phase.

Figure 14g. Vegetative conchocelis filaments, beaded branches and young conchosporangial branch (arrow).

Figure 14h. Cluster of conchosporangial branches showing single stellate chloroplast in each conchosporangium.

Figure 14i. Candelabra-shaped cluster of conchosporangial branches showing their positive phototropism.

Figure 14j. Conchosporangial branch with conchospores.

Figure 14k. Released conchospores.
Porphyra thurstonii

Figure 15a-d. Conchospores exhibiting amoeboid change of shape and movement. Figures 15a and 15b taken 90 seconds apart. Figures 15c and 15d taken 45 seconds apart.

Figure 15e. Young bipolar sporeling resulting from conchospore germination. Note rhizoidal protuberance.

Figures 15f-15g. Older, uniseriate sporelings starting to form basal pad.

Figure 15h. Biseriate stage of sporeling growth.

Figures 15i-15j. Older sporelings. Note the basal rhizoidal pad in Figure 15j.
PART IV - CYTOLOGICAL STUDIES

General Introduction

The first three parts of this thesis presented a detailed discussion of the morphological life histories of Porphyra gardneri, P. nereocystis and P. thuretii. The primary purpose of Part IV is to document the cytological life histories of these 3 species in as much detail as possible, with emphasis on Porphyra gardneri and the occurrence of sexual reproduction in its life history.

I also wanted to examine the attachment of Porphyra gardneri and P. nereocystis to their host plants, and observe the ultrastructure of monosporogenesis in P. gardneri.

Materials And Methods

Cytological studies were done on material collected at the study site. The specimens of Porphyra gardneri used in the fertilization study were collected in May, and those of P. nereocystis and P. thuretii in January.

For chromosome counts material was fixed in 95% ethanol:glacial acetic acid (3:1), and stained with aceto-iron-haematoxylin-chloral hydrate (Wittmann, 1965). Spermatangial material could be fixed at any time to obtain a chromosome count because of the large number of divisions occurring and because the chromosomes in the mature spermatia
are condensed. The best counts were obtained just as the chromosomes were beginning to condense. The highly condensed chromosomes in the mature spermatia did not give reliable counts because they tended to clump. Spermatial chromosome counts were made on type locality plants of *Porphyra gardneri*.

In the case of vegetative and carposporangial material it was necessary to fix thalli every hour for 24 hours to determine when mitosis was occurring. This was found to begin at sunset and continue for 1-2 hours. Pringle and Austin (1970) obtained similar results with another *Porphyra*-species. Several workers have emphasized that mitosis occurs most frequently at night (Kito, Yabu and Tokida, 1967; Migita, 1967b; Yabu, 1969a, 1970; Kito, Ogata and McLachlan, 1971).

Methods of isolation and conditions of growth of the conchocelis phase in culture have been described in Part III. Cultured conchocelis was fixed at hourly intervals for 24 hours. Dividing cells were infrequent in the conchocelis phase compared to the foliose thallus. Most divisions were found in material fixed in the dark period, primarily in the first few hours of darkness. Ramus (1969) in work with *Pseudogloioeidiolea* and Sommerfeld and Nichols (1970) in work on *Bangia*-conchocelis phase also found that the frequency of cell division was highest during the dark period.

In an attempt to increase the number of cells in metaphase, conchocelis phase cultures were treated with 0.05%, 0.1% and 1% colchicine for 24 hours. This compound is known to arrest metaphase in higher plants (Klein and Klein, 1970); however, it had no effect on the conchocelis phase.
Material for the attachment study and Feulgen staining was fixed and embedded in methacrylate as described in Part II. For Feulgen staining, serial sections (Henry, 1977) 3-6 um thick were cut. An aldehyde blockade was carried out using a saturated solution of 5,5-dimethylcyclohexane-1,3-dione (Dimedone) for 18 hours at room temperature. The slides were then rinsed in running water for 30 minutes (Feder and O'Brien, 1968). Hydrolysis was carried out in 1N HCl at 60°C for 20 minutes. Slides were then transferred to Schiff's reagent (made according to the recipe of Sharma and Sharma, 1965) for 3 hours. This was followed by 3 successive washes of 0.5% potassium metabisulfite (2 minutes in each), then a rinse in running water for 10 minutes. Sections were lightly counterstained for 1 minute in a 1% aqueous solution of Fast Green (Feder and O'Brien, 1968).

For electron microscopy material was postfixed in 2% OsO₄ in phosphate buffer pH 7.2 (1:1) for 1 hour, followed by dehydration in ethanol and infiltration with Spurr's resin. Sections were cut on glass knives using a Reichert Om U3 ultramicrotome. They were stained with uranyl acetate in 50% ethanol (Dawes, 1971) and lead citrate (Reynolds, 1963) and examined with a Carl Zeiss EM-10 electron microscope.

Material for scanning electron microscopy was fixed and dehydrated using the above procedure. Thallus pieces were then run through a graded series of ethanol:amyl acetate (3:1, 1:1, 1:3), 0.5 hr in each and 0.5 hr in 100% amyl acetate. Specimens were critical point dried, mounted on stubs, gold coated and examined with a Cambridge Stereoscan Type 2A
scanning electron microscope.

A. Epiphyte-'Host' Attachment

Introduction

The known epiphytic species of Porphyra have been summarized by Ohmi (1963) and Tckida (1960). The nature of the epiphyte-'host' relationship in these Porphyra species has received little investigation. Grubb (1923) described the attachment of Porphyra purpurea (as P. umbilicalis var. laciniata) to Fucus and concluded that the basal rhizoids penetrated host cells. Kito (1966) reported that the basal rhizoids of P. katadai Miura (as Porphyra sp.) penetrated deeply into the tissue of its host Grateloupia filicina var. porracea (Mertens) Howe. Fukuhara (1968) found that the basal rhizoids of P. onoi Ueda penetrated the host tissue whereas those of P. pseudocrassa Yamada et Mikami did not.

There have been no physiological studies of the epiphyte-'host' interaction in the epiphytic Porphyra species.

The restriction of Porphyra nereocystis to essentially one host species and the failure of P. gardneri sporelings to develop into mature thalli in culture (see Part III) suggests that they may have a physiological dependency on the 'host'. As the most probable region of nutrient exchange in such a relationship would be the attachment zone, a light microscope study of this region was made.
Results

1. Porphyra gardneri

Transverse sections were cut through the basal portion of Porphyra gardneri and the Laminaria blade margin. The rhizoidal filaments which form the base of the thallus of P. gardneri spread out slightly on the Laminaria blade margin to form a basal pad (Figure 16a). The rhizoidal filaments in the central portion of this pad penetrate deeply into the medulla (Figures 16a and 16b), and in some cases they appear to penetrate the medullary cells, although an E. M. examination is needed to verify this.

2. Porphyra nereocystis

Sections were cut through the basal region of Porphyra nereocystis and the stipe of Nereocystis in a plane perpendicular to the stipe axis. As in P. gardneri, the basal rhizoidal filaments spread out to form a pad on the stipe surface (Figure 16c). This pad is much larger than that formed by P. gardneri. In the center of the pad the rhizoidal filaments make a slight indentation into the cortex of the Nereocystis stipe (Figures 16c and 16d), but there is no extensive rhizoidal penetration of the stipe tissue.
Discussion

The intimate attachment of *Porphyra gardneri* to the *Laminaria* blade suggests that there may be a transfer of metabolites from the *Laminaria* to the *Porphyra* thalli. Studies by Lüning *et al.* (1972) have shown that most *Laminaria* photosynthates are transported in the medulla tissue.

In contrast to *Porphyra gardneri*, the attachment of *P. nereocystis* is superficial, and the lack of significant rhizoidal penetration of stipe tissue suggests there may not be exchange of materials at the attachment zone. It should be noted though, that Harlin (1973b) demonstrated that a penetration of host tissue is not a prerequisite for exchange of material. The case may also be that *Nereocystis* is exuding compounds into the water where they are picked up by *P. nereocystis*. The mechanism determining the host specificity of *P. nereocystis* may be acting at an earlier stage in development such as the time of initial conchospore settlement and germination.

Ultrastructural and physiological studies are needed to elucidate the nature of the association between the epiphyte and 'host' in these obligate epiphytic *Porphyra* species.
B. Monosporogenesis

Introduction

*Smithora naiadum* and *Porphyropsis coccinea* (Areschoug) Rosenvinge are the only members of the Bangiophycidae in which monosporogenesis has been examined at an ultrastructural level (McBride and Cole, 1971; McDonald, 1972, respectively). There has not been any ultrastructural investigation of monospores in *Porphyra*, a surprising fact in view of the great importance of the monospor cycle in commercially cultivated *Porphyra* species. It was therefore decided to make some preliminary ultrastructural observations of monosporogenesis in *Porphyra gardneri*.

Results

Monosporic thalli were easily recognized in the field by their gelatinous margins releasing monospores (see Part I). Under the light microscope the monosporangia were slightly larger than vegetative cells and more spherical in shape. They had fine granular contents and the stellate chloroplast was somewhat obscured (see Part III). Tanaka (1952) also noted that the monospores of *Porphyra okamurai* Ueda had more granular contents than the vegetative cells.

The ultrastructure of the distal portion of monosporangial thalli was examined in transverse section. The first
indication that the vegetative cells were undergoing a transition to monosporangia was an increase in dictyosome activity (Figures 17 and 19a) which resulted in the production of small fibrous vesicles. Mitochondria were found in association with these dictyosomes (Figure 19a). Each monosporangium contained a single stellate chloroplast with central pyrenoid and central to lateral nucleus. Floridean starch granules were abundant. The number of fibrous vesicles increased prolifically in older monosporangia (those toward the releasing margin) (Figure 18). These abundant fibrous vesicles appear to be the numerous granules observed in the monosporangia at the light microscope level. The small fibrous vesicles appeared to coalesce to form larger fibrous vesicles (Figures 18 and 19c) as well as release their contents between the plasmamembrane and inner cell wall, resulting in a layer of fibrous material (Figure 19b).

In the more mature monosporangia the number of small and large fibrous vesicles increased (Figure 19c), and the large fibrous vesicles became extracytoplasmic. This phenomenon was associated with monosporangia that were near the releasing margin.

Unfortunately only the initial stages of monospore release were observed, and were characterized by an apparent dissolution of the cell wall layers beneath the outer cuticle.
Monosporogenesis in Porphyra gardneri is characterized by the production of small and large fibrous vesicles which appear to be produced primarily by dictyosome activity. This ultrastructural study has demonstrated that monospores are more than undifferentiated vegetative cells which have dissociated from the thallus, as has been suggested by Dixon (1970) for monospores of Bangia and Porphyra.

Ultrastructurally the fibrous vesicles appear the same as those reported in Smithora monosporangia (McBride and Cole, 1971) and in other red algal spermatia, carpospores and tetraspores (see Part IV, spermatogenesis, for a complete summary). McBride and Cole (1971) reported the production of 2 different types of vesicles during monosporogenesis in Smithora. In Porphyra gardneri I observed only the one type, as did McDonald (1972) in Porphyropsis monosporogenesis.

Released monospores lack a cell wall (observed, but picture omitted) and are surrounded by a layer of mucilaginous material which no doubt has its origin in the fibrous vesicles. This mucilage layer may be a mucopolysaccharide (Peyrière, 1970) and appears to aid in monospore adhesion to the substrate. Histochemical study is needed to determine the chemical nature of the material produced by the fibrous vesicles. Another aspect which needs to be examined is whether cell wall breakdown at the time of monospore release is the result of enzymatic digestion. Such enzymes may be released by the fibrous vesicles.
C. Sexual Reproduction In *Porphyra gardneri* ¹

Introduction

The occurrence of sexual reproduction in the genus *Porphyra* has long been debated by phycologists. It was first reported by Berthold (1882) for *Porphyra leucosticta*, and several subsequent investigators of other species concurred (Ishikawa, 1921; Grubb, 1924; Hamel, 1924; Dangeard, 1927; Kunieda, 1939; Magne, 1952; Tseng and Chang, 1955; Kuroqi, 1961, 1972; Yabu and Tokida, 1963; Migita, 1967b; Giraud and Magne, 1968; and Yabu, 1969b). However, Hus (1902) reported that he could find no evidence of sexual reproduction, and other workers thought that the evidence for it was inconclusive (Drew, 1954a; Krishnamurthy, 1959; Conway, 1964a, 1964b; Graves, 1969; Dixon, 1970, 1973; Conway and Cole, 1973, 1977; Conway *et al.* 1975). Conway (1964a) suggested that because of the lack of conclusive evidence of sexual reproduction the two types of spore formed in 'packets' in *Porphyra* should not be referred to as spermatia and carpospores. She proposed the terms $B$-spore to replace spermatium and $\alpha$-spore to replace carpospore. In addition to this, the terms 'carpospore' mother cell (Dixon, 1973) and $\alpha$-spore mother cell (Conway *et al.* 1975; Hawkes, 1977b) have been used instead of

¹This portion of the thesis has been accepted for publication in Phycologia and is 'in press' in V. 17(3).
The term \( B \)-spore has met with criticism (McDonald, 1972) because the non-pigmented cells that it refers to do not germinate and are therefore not spores.

The majority of cytological studies have provided evidence of sexual reproduction. Magne (1952) reported a diploid chromosome number for the carpospores of \( P. \) linearis and subsequent workers have obtained diploid counts for the carpospores in 22 other species (Table VIII). Diploid chromosome counts have also been reported for the vegetative cells of the conchocelis phase in 9 Porphyra species (Table VIII).

There is some difference of opinion regarding the site of meiosis. According to Ishikawa (1921), Dangeard (1927) and Tseng and Chang (1955) the first division of the fertilized carpogonium is meiotic and the carpospores are haploid, however, Mijita (1967b), Giraud and Magne (1968) and Kito (1974) found that the division to form the conchosporangial branch is the site of meiosis.

The data which have been pointed to as evidence against the occurrence of sexual reproduction consists of Krishnamurthy's (1959) report that all life history stages of \( P. \) purpurea (as \( P. \) umbilicalis var. laciniata) are haploid, and a report by Conway and Cole (1973) that the conchocelis

1 The studies which are being reported in this thesis indicate that there is no longer any reason to continue to use the terminology proposed by Conway (1964a) for Porphyra gardneri. The terms spermatium, carpogonium, prototrichogyne and carpospores, which have previously been applied to Porphyra, will be used. See the terminology section for further comments.

2 In contrast Kito et al. (1971) obtained a diploid count for the carpospores of this species.
phase of *P. papenfussii* is haploid. The reports of Janczewski (1873) that both carpospores and spermatia can originate from the same carpogonium led Drew (1954a) and Conway et al. (1975) to express doubt that they play a role in sexual reproduction.

1. The Spermatium

As a first step in attempting to clarify this problem, the nature of the spermatium in *Porphyra* would seem to be of considerable importance. Krishnamurthy (1959) commented on the similarity of mature spermatia of *Porphyra* with those in the Florideophycidae, and pointed out the need for further cytological investigation of the spermatium.

Spermatangial development has been studied by several investigators using the light microscope (Janczewski, 1873; Thuret and Bornet, 1878; Berthold, 1882; Hus, 1902; Ishikawa, 1921; Tanaka, 1952; Krishnamurthy, 1959, 1972). These studies did little more than indicate that the spermatia are formed by repeated division of the spermatangium, and the data are contradictory in that Janczewski (1873), Thuret and Bornet (1878) and Ishikawa (1921) maintained that the first division of the spermatangium was periclinal (in the plane of the thallus) whereas Tanaka (1952) and Krishnamurthy (1959) thought that it was anticlinal. Hus (1902) reports that there is a 'cruciate' division to give four spermatangia, and that the first division of each spermatangium was periclinal. As Drew (1956) pointed out, these various interpretations are perhaps due to the difficulty in distinguishing the spermatangium from a vegetative cell.
Little is known about the internal structure of the spermatium in Porphyra. Hus (1902) was of the opinion that, except for the larger number of divisions that produce the spermatia, there is little to differentiate them from the carpospores. Krishnamurthy (1959) suggested that the spermatia and carpospores were homologous based on the fact that they are formed by repeated divisions. Most workers mention the fact that the spermatia tend to become less pigmented as they develop. Magne (1952), Krishnamurthy (1959), Hawkes (1977b) and Mumford and Cole (1977) report that the nucleus of the mature spermatium is in a condensed state. Peyrière (1974) mentions that G. and J. Feldmann have seen abundant fibrous vesicles in the spermatia of Porphyra leucosticta.

Rather surprisingly only a few ultrastructural studies of Bangiophycidae spermatia have been made (McBride, 1972 - Smithora; McDonald, 1972 - Bangia). In contrast to this, numerous ultrastructural studies have now been made of spermatium development in several genera of the Florideophycidae (Brown, 1969 - Batrachospermum; Kugrens and West, 1972 - Levriniella and Erythrocystis; Kugrens, 1974 - Janczewskia; Peyrière, 1971, 1974 - Griffithsia, Polysiphonia, Laurencia, Polyneura and Furcellaria; Scott and Dixon, 1973b - Etilota; Simon-Bichard-Bréaud, 1971, 1972a, 1972b - Bonnemaisonia; Young, 1977 - Bonnemaisonia). These studies indicated that the characteristic features of spermatium formation and maturation are the production of fibrous vesicles, and a general reduction in chloroplast size and complexity (in those which have chloroplasts). In most cases
the nucleus of the mature spermatium is in a condensed state. Starch is usually absent in the mature spermatium (Young, 1977).

If the spermatium in Porphyra is in fact functioning as a male gamete, as has been classically stated (Berthold, 1882), then one would expect it would perhaps be ultrastructurally similar to the spermatium in the Florideophycidae. A study of spermatium development in Porphyra gardneri was undertaken as the first step in attempting to determine if sexual reproduction occurs in the life history of this alga.

2. Reports Of Fertilization

The most vital piece of information that has not been conclusively demonstrated, until my work with Porphyra gardneri, is the actual occurrence of a sexual fusion. Derbes and Solier (1856) and Koschtsug (according to Drew, 1956) were of the opinion that the spermatia and carpospores were gametes which underwent fusion after they had been released. Krishnamurthy (1959) obtained no evidence of such fusions, and neither have subsequent workers.

Drew (1956) summarized the literature dealing with sexual reproduction in the Bangiophycidae. She noted that most reports on Porphyra indicated that a cell in the foliose thallus is transformed into a carpogonium which is then fertilized by a spermatium, and that the carpospores are produced following this fusion. Drew noted that there were two different mechanisms of fertilization described, based on the presence or absence of a prototrichogyne produced by the
carpogonium. She indicated that in those species with prototrichogynes the spermatium is engulfed by it (Joffe, 1896 and Kunieda, 1939 are cited as examples), whereas in those without prototrichogynes a fine canal-like connection exists between the spermatium and carpogonium (Berthold, 1882 and Dangeard, 1927 are cited). It should be noted that the carpogonia Berthold (1882) figured did have slight bipolar protuberances (although he did not show the wall protruding), and that Rosenvinge (1909), working on the same species, did observe carpogonia with prototrichogynes. Similarly, Dangeard (1927) also figured spermatia with fertilization canals into the prototrichogynes in one of the two species he studied.

Hawkes (1977b) pointed out that in most monostromatic species there is a prototrichogyne at each pole of the carpogonium, whereas in distromatic species, for which prototrichogynes have been reported, there is only one, located on the side of the carpogonium next to the thallus surface.

Several workers (Joffe, 1896; Rosenvinge, 1909; Dangeard, 1927; Magne, 1952; Tseng and Chang, 1955; Kurogi, 1961) have figured spermatia attached to carpogonia via fertilization canals. Conway and Cole (1973) reported suspected spermatia in association with the prototrichogyne of *P. papenfussii* but they did not observe fertilization canals. Similarly, Krishnamurthy (1959) observed spermatia on the thallus surface of *P. purpurea* (as *P. umbilicalis* var. *laciniata*) but did not see fertilization canals. He did report seeing canals but without spermatia over top of them. He said that they ended blindly in the mucilaginous sheath of the thallus or in the intercellular
layer. Graves (1969) reported occasional carpogonia with prototrichogynes in *P. capensis* Kützing emend. Agardh but felt that the phenomenon was not common and never found spermatia associated with the prototrichogynes.

Drew (1956) summarized the cytological evidence for fertilization up to that time. It consisted of a report by Joffé (1896) of two nuclei in a cell, and Dangeard's (1927) illustration of two adjacent cells, one of which has a spermatium attached to its cuter wall and a fertilization canal. In this cell what Dangeard interpreted as a male nucleus is in contact with the carpogonial nucleus. In the neighbouring cell there is a single large nucleus which is assumed to be a fusion nucleus. Magne (1952) reported a nucleus in prophase in the fertilization canal connecting the spermatium with the carpogonium, and was the first to report diploid carpospores. The only recent work has been done by Yabu and Tokida (1963) on *P. yezoensis* and Yabu (1969b) on *P. tenera*. They figure a male and female nucleus in close contact with each other in the carpogonium just prior to fusion.

3. Reports Of Fungal Infection Of *Porphyra*

Dangeard's (1927) report of spermatia forming fertilization canals led Kunieda (1939) to suggest that what Dangeard had observed was not a sexual fusion but the parasitization of the *Porphyra* thallus by an oomycetous fungus. His only basis for making this statement was that he had observed wall penetrations in the vegetative cell region which
he thought were caused by a fungus. As has been pointed out by Fritsch (1945, p. 434), Kunieda did not provide any convincing evidence for this fungus theory. A point that has been overlooked by Dixon (1970, 1973) is that Kunieda did believe that a sexual fusion occurred. Kunieda thought that the prototrichogyne engulfed the entire spermatium and disagreed with Dangeard's concept that the contents of the spermatium reached the carpogonium by a fertilization canal. Recently Krishnamurthy (1977) also suggested that a fungal infection could be mistaken for the fertilization of a carpogonium; however no convincing evidence was presented. Hus (1902) added to the scepticism surrounding the reports of fertilization. He commented that bacteria infesting the walls of all regions of thalli he examined formed narrow lines perpendicular to the surface of the fronds, which he said reminded him of the fertilization canals figured by Berthold (1882). No evidence was given.

The only fungi that have been reported to infect the Porphyra thallus are species of Pythium (Oomycetes) (Arasaki, 1947, 1962; Tsuruga and Nitta, 1960; Fuller et al. 1966; Sasaki and Sato, 1969; Kazama and Fuller, 1970; Sasaki and Sakurai, 1972; Sakurai et al. 1974; Takahashi et al. 1977) and a chytrid (Arasaki, 1960; Arasaki et al. 1960; Migita, 1969, 1973; Sparrow, 1969). In the case of the Pythium infection (known as the 'red rot' disease in Japan), the fungal hyphae penetrate the host cells and cause a rapid plasmolysis of them, resulting in the formation of red pigment crystals (Ogata, 1975). The infection is visible to the unaided eye and does
not bear any resemblance to the reports of fertilization. In the case of the chytrid, only settled zoospores at their initial stage of infection could be mistaken for a spermatium forming a fertilization canal. It is interesting to note that Arasaki (1960) speculated that Yendo's (1919) report of male and female swarming gametes from Porphyra conchocelis may be attributable to a chytrid infection, but that he did not make a similar statement regarding Berthold's (1882) and Dangeard's (1927) reports of fertilization. Furthermore, Ogata (1975), in a discussion of the pathology of Porphyra, made no mention that the reports of these workers are attributable to a fungal infection.

In view of the poor documentation that the reported spermatia and their fertilization canals were actually a fungal infection, it became apparent to me that the method of fertilization as proposed by Berthold (1882) merited closer examination. It was therefore decided to try to find fertilization in Porphyra gardneri and to examine it using the light, transmission and scanning electron microscopes in an attempt to resolve the problem.
Results

1. Spermatogenesis - Electron Microscopy

Some general ultrastructural features of spermatogenesis were observed in transverse section. The vegetative cell (Figure 20a) contained a stellate chloroplast with a centrally located pyrenoid. The nucleus was laterally located. The new wall that was laid down around a vegetative cell undergoing the transition to a spermatangium can be clearly seen in Figure 20b. Ultrastructurally the immature spermatia did not differ much from vegetative cells (Figures 20c and 20d). The chloroplast was still well developed and floridean starch grains were present. However, around the time of the final division to give a mature spermatangium large fibrous vesicles appeared (Figure 21a). At higher magnification (Figure 21b) small fibrous vesicles were apparent as well. These appeared to be releasing their contents to the outside of the spermatium, as well as contributing to the formation of the large fibrous vesicle. Also at this stage of maturation the chloroplast was considerably reduced in size and structural complexity. It had a degenerate appearance, there being only a few thylakoids which were often distorted, and a few to several plastoglobuli. Mitochondria were also present, and the nucleus possessed a nuclear membrane. Prior to spermatium liberation the large fibrous vesicle became extracytoplasmic (Figure 21c), and the nuclear membrane began to disappear. The mature spermatium (Figure 22) contained a nucleus which was in a highly condensed state, and lacked a nuclear membrane. Small
fibrous vesicles were abundant and a reduced chloroplast was present. Most spermatia did not possess floridean starch grains. At the time of release, there was no cell wall around the spermatium; it was surrounded by a plasmamembrane and the contents of the large vesicles. The released spermatia varied from 3-5 um in diameter, most being 4 um.

2. Fertilization - Light Microscopy

While examining the early stages of carpogonium formation in order to assess the validity of the genus Porphyrella (Hawkes, 1977a, 1977b), numerous examples of suspected 'spermatia' attaching over the prototrichogynes of the carpogonia were observed (Figure 23a). They had a very strong similarity to Berthold’s (1882) illustrations of fertilization in Porphyra leucosticta. These 'spermatia' were only found attached to the prototrichogynes, and they were in the 3-5 um in diameter size range of the released spermatia. In addition to this, there were fertilization canals connecting the 'spermatia' with the inside of the carpogonium (Figures 23b and 23c). Their specific attachment and penetration strongly suggested that they were spermatia; however, it was impossible to prove this conclusively without using the electron microscope.
3. Fertilization - Electron Microscopy

Two scanning electron micrographs of the thallus surface of *Porphyra gardneri* are shown in Figures 24a and 24b. The suspected spermatia are clearly visible over the prototrichogynes. Some have a sunken and shrivelled appearance, probably as a result of having already released their contents into the carpogonium. Cyanophyta are also present on the thallus surface.

A low magnification micrograph of a transverse section through one of these spermatia and the carpogonium is shown in Figure 25a. Other examples at a higher magnification to show the contents of the spermatia are shown in Figures 25b-25d. In these, the presence of a reduced, degenerate looking chloroplast typical of that found in the mature spermatium demonstrates that they are spermatia and not fungi. Mitochondria and non-membrane bound nuclear material are present as well. In Figures 25b and 25c a thin layer of wall material can be seen around the spermatium.

The next point to clarify is whether anything is transferred from the spermatium to the carpogonium. Figure 26 shows two empty spermatia and their fertilization canals. The spermatial nuclear material has been transferred to the carpogonium. The diameter of the fertilization canal was 0.6-0.9 μm in the sections examined. Figure 27 shows another transverse section of a carpogonium in which the prototrichogynae and central pyrenoid are distinct. The plane of section does not go through the fertilization canal; however, the presence of a slight deposit of wall material in
the prototrichogyne below the spermatium indicates that a fertilization canal is present.

Some spermatia that have transferred their nuclear material retain what appears to be the remains of a chloroplast (Figures 28a and 28b). These chloroplasts have a few distorted thylakoids and some plastoglobuli. Following the transfer of the nuclear material, the fertilization canal is rapidly plugged by new wall material deposited around it (Figure 28b). This appears to originate from dictyosome activity in the apex of the carpogonium.

Figure 28c is a transverse section through an empty spermatium and apex of the carpogonium. Unfortunately the plane of section does not go through the fertilization canal. In the apex of the carpogonium is a small reduced chloroplast typical of the chloroplast found in the mature spermatium. Another example of this is shown in Figure 28d. It would appear that these chloroplasts have been transferred from the spermatium to the carpogonium along with the spermatial nuclear material.

Carpogonia which contained what appeared to be highly condensed spermatial nuclear material were observed (Figure 29), but because of the apparent lack of a nuclear membrane around the spermatial nucleus I was unable to follow the details of fusion with the carpogonial nucleus at an ultrastructural level. The first division of the presumed fusion nucleus, and cytokinesis to give 2 carpospores, is shown in Figures 30a and 30b. The fertilization canal is still visible.
4. Chromosome Counts

Evidence that a fusion of spermatial nuclear material with the carpogonial nucleus does occur has been obtained from chromosome counts. Hawkes (1977b) reported that the vegetative cells of the foliose thallus and the spermatia have 4 chromosomes \( n=4 \) (Figures 31a and 31b). In both the carpogonial regions and vegetative cells of the conchocelis phase a diploid chromosome number \( 2n=8 \) has been obtained (Figures 31c, d and 31e, f).

5. Feulgen Staining

In an attempt to clarify the details of the transfer of the spermatial nuclear material to the carpogonium, transverse sections of carpogonial regions of the thallus were stained using the Feulgen technique. Initially the stain was used on the spermatia. Figure 32a shows a spermatangium which has undergone the maximum number of divisions, but the nucleus in most spermatia is still in a diffuse interphase state. The condensing of the nucleus is complete in Figure 32b. The released spermatia likewise have condensed nuclear material which stains a dense purple (Figure 32c). These observations agree with the ultrastructural observations of spermatogenesis.

In Figure 33a a spermatium containing condensed nuclear material can be seen attached to the prototrichogyne. No fertilization canal is present. In Figure 33b the spermatial nuclear material is in a diffuse state and a fertilization canal is present. Figures 33c and 33d show condensed
spermatial nuclear material and fertilization canals are present. The correct time sequence for Figures 33b-d is not known. It may be that the spermatial nuclear material decondenses in order to code for an enzyme required to make the fertilization canal, and then re-condenses for the transfer to the carpogonium. Another explanation would be that the fertilization canal enzymes are present (or at least coded for) in the released spermatium, and that the spermatial nuclear material remains condensed until the canal is complete, this being followed by de-condensation and transfer to the carpogonium.

Once transfer has occurred the spermatial nuclear material shows up in the carpogonium as a small densely staining purple mass (Figure 33e). It is not uncommon to see from one to three of these in addition to the carpogonial nucleus (Figures 33f and 33g). Occasionally up to 4 or 5 have been observed (Figures 33h and 33i).

On the basis of the diploid chromosome number, and the fact that dense spermatial nuclear masses are not found in subsequent stages, it is assumed that one of them fuses with the carpogonial nucleus, and that the rest degenerate. It has not been possible to observe this fusion; however, carpogonia with large suspected fusion nuclei have frequently been seen (Figure 33j). As has been shown at an ultrastructural level, the first division of the fusion nucleus is periclinal (Figure 33k). The chloroplast and pyrenoid then divide followed by cytokinesis (Figure 33l) to give 2 carpospores. Subsequent divisions to give 4 and 8 carpospores per
carposporangium are shown in Figures 33m–c. A summary of the division sequence involved in carpospore formation by *Porphyra gardneri* has been reported previously (Hawkes, 1977b) (see Part II).

**Discussion**

1. Spermatogenesis

The main features of spermatogenesis in *Porphyra gardneri* are essentially the same as those reported by McDonald (1972) for *Bangia fuscorubra* (Dillwyn) Lyngbye, and similar in some respects to the report of McBride (1972) for the spermatia of *Smithora naiadum*. Unfortunately neither of these workers indicated if the nuclear membrane remained intact in the mature spermatium.

There are some striking similarities between spermatogenesis in the Florideophycidae and in *Porphyra gardneri*. The most characteristic feature that they have in common is the production of small and large fibrous vesicles, and the release of the large fibrous vesicle prior to spermatium liberation. The reduction in chloroplast size and complexity in the mature spermatium is another feature they have in common. Kugrens (1974) notes that this is a general phenomenon in male gametes of algae in several groups. Another similarity is the general absence of starch grains in the mature spermatia. Kugrens (1974) has pointed out that one possible explanation for the reduction in chloroplast structure
and storage product is to prevent the male gamete from developing on its own, thereby bypassing sexual reproduction.

Most workers report that the nucleus of the mature spermatium in the Florideophycidae contains condensed chromatin (Grubb, 1925; Fritsch, 1945, p. 596; Kugrens and West, 1972; Peyrière, 1974; Young, 1977), as is the case in *P. gardneri*. Scott and Dixon (1973b) were uncertain of the nuclear condition in *Ptilota* spermatia, but judging from their micrographs, condensed chromatin is present. The nucleus in *Janczewskia* spermatia (Kugrens, 1974) does not contain condensed chromatin.

The disappearance of the nuclear membrane in the mature spermatium of *Porphyra gardneri* is rather unexpected. This has previously been reported at the light microscope level in several Florideophycidae (Grubb, 1925; Fritsch, 1945, p. 596) and at the ultrastructural level by Scott and Dixon (1973b). Kugrens (1974) questioned this because he found it difficult to explain why the nuclear membrane would disappear in the spermatium when there is little nuclear membrane breakdown during mitosis in the Rhodophyta. The case may be that the nuclear membrane is particularly sensitive at this stage of development and that it therefore was not preserved by the fixation procedure used. Alternatively, in those cases where an intact nuclear membrane has been reported, perhaps older released spermatia should have been examined to see if the membrane does eventually disappear.

For the Florideophycidae spermatia, many workers report only dictyosome involvement in the formation of the fibrous vesicles (Peyrière, 1971; Scott and Dixon, 1973b;
Simon-Bichard-Breaud, 1972a); however, Kugrens and West (1972), Kugrens (1974) and Young (1977) have indicated that the ER is involved in the early stages in the species they studied. The fibrous vesicles in the spermatia of *P. gardneri* may be mostly derived from ER as few dictyosomes were observed during spermatogenesis.

Reports on the chemical nature and function of the fibrous vesicles have been summarized by Scott and Dixon (1973b). They appear to contain a mucopolysaccharide, although more critical analysis is required to confirm this. One suggested function of the fibrous vesicles is that they may cause an increase in the osmotic pressure resulting in the rupturing of the spermatangial wall and thereby aiding in spermatium release. Likewise, the mucopolysaccharide may aid in spermatium attachment to the trichogyne. Recently, mucilage strands have been shown to be involved in spermatium release and dispersal in *Tiffaniella snyderae* (Farlow) Abbott (Fetter, 1977).

The widespread occurrence of similar fibrous vesicles in other red algal spores such as the monospores of *Smithora naiadum* (McBride and Cole, 1971), *Porphyropsis coccinea* (McDonald, 1972) and *Porphyra gardneri* (see Part IV), carpospores of *Porphyra nereocystis* (Hawkes, unpublished observation), *Levringiella* and *Erythrocytis* (Kugrens and West, 1973, 1974) and *Polysiphonia novae-angliae* Taylor (Wetherbee and Wynne, 1973) and tetraspores of *Ptilota hypnoides* (Scott and Dixon, 1973a) indicates that they must have an important function.
2. Fertilization

This is the first conclusive documentation of the occurrence of a sexual fusion in a species of *Porphyra*, and except for Magne's (1960) excellent work on sexual reproduction in *Rhodochaete parvula* Thuret it is the only unequivocal report of fertilization in the Bangiophycidae. It confirms that the classical interpretation by Berthold (1882) of sexual reproduction in *Porphyra leucosticta* is correct. Similarly, the observations by Boneminge (1909), Ishikawa (1921), Dangeard (1927), Magne (1952), Tseng and Chang (1955), Kurogi (1961), Yabu and Tokida (1963), Migita (1967b), and Yabu (1969b) of the fertilization process in other *Porphyra* species appear to be basically correct. The reports of Ishikawa (1921), Dangeard (1927) and Tseng and Chang (1955) of the first division of the carpogonium being meiotic are probably incorrect.

The mechanisms of fertilization proposed for *Porphyra* by Derbès and Solier (1856), Koschtschug (cited from Drew, 1956), Suto (1963) and Kunieda (1939) are probably incorrect. Dixon's (1973) suggestion that fertilization and the division of the carpogonium to produce carpospores are separated in time by several or even many vegetative cell divisions is likewise not the case.

The reports by Krishnamurthy (1959), Conway and Cole (1973, and in press) and Mumford and Cole (1977) of a haploid chromosome number in the vegetative cells of the conchocelis phase of *Porphyra* are not necessarily incorrect. In view of the tremendous adaptability exhibited by the *Porphyra* life
history it is possible that carpospore formation could occur parthenogenetically. Similarly, the report by Sommerfeld and Nichols (1970) of a haploid chromosome number in the conchocelis of *Bangia* may be due to a parthenogenetic development of carpogonia. Rosenvinge (1909) observed the formation of carposporangia in a population of plants which did not produce spermatia and in which he could not find fertilization canals. He suggested the carpospores developed aposgamously. Kurogi (1972) has pointed out there are some species for which he has never observed fertilization canals.

Drew (1954a), Krishnamurthy (1959) and Dixon (1973) have expressed doubt that the number of carpospores which are produced are all the product of sexual fusions. The case may be that some carpospores can form parthenogenetically whereas others are the result of fertilization. Further investigation of this point is required. Certainly in *Porphyra gardneri* the fusions are extremely abundant, there often being several fertilization canals into one carpogonium. Multi-penetrations have also been reported by Rosenvinge (1909) and Dangeard (1927) for *Porphyra umbilicalis*.

The other possible explanation for the occurrence of a haploid chromosome number in the vegetative cells of the conchocelis phase is that meiosis occurs during the first division of the fertilized carpogonium as has been reported by Tseng and Chang (1955); however, there is no evidence that this takes place.

Although the site of meiosis in *Porphyra gardneri* has not been investigated yet it probably occurs in the conchospore
branch as has been reported for other *Porphyra* species by Migita (1967b), Giraud and Magne (1968) and Kito (1974).

In view of the large number of *Porphyra* species for which fertilization has been reported at the light microscope level, and in which diploid chromosome numbers for the carpospores or vegetative cells of the conchocelis phase have been reported, it would seem reasonable to suggest that sexual reproduction in the genus is a common occurrence. The application of techniques such as have been used in this study of *Porphyra gardneri* should be helpful in demonstrating fertilization in other *Porphyra* species. It is expected that future investigations of other *Porphyra* species will show that the typical cytological life history found in the genus is the same as that outlined by Migita (1967b) in his excellent work on *Porphyra vezcensis*.

The report by Janczewski (1873) of spermatia and carpospores occurring in the same sporangium remains an enigma. This may have been a misinterpretation by Janczewski. Such a phenomenon was not observed in *Porphyra gardneri*.

According to Fritsch (1945, p. 592) the carpogonium and trichogyne in the Florideophycidae (except certain Nemaliales) lack chloroplasts. Fritsch (1945, p. 597) also notes that the spermatium in the Florideophycidae transfers its contents to the trichogynes as a result of wall dissolution in the region of contact. This differs from the situation in *Porphyra gardneri* in which the carpogonium possesses a well developed chloroplast and in which the contents of the spermatium reach the prototrichogyne via a fertilization canal.
A detailed examination and comparison of fertilization at the ultrastructural level in suspected primitive members of the Florideophycidae (for example; *Acrochaetium* and other Nemaliales) and other members of the Bangiophycidae may provide interesting insight into the evolution of the female reproductive system in these two groups of Rhodophyta.

Although this investigation has answered the major question of the occurrence of sexual reproduction in the life history of *Porphyra gardneri*, it points out several areas in need of further study. What changes does the spermatium undergo from the time of release to the time of attachment to the prototrichogyne? One change observed in this study of *P. gardneri* is that the attached spermatium possesses a thin wall which was not present at the time of liberation. Fritsch (1945, p. 597) reports that in *Batrachospermum* and *Nemalion* the spermatial nucleus divides once it contacts the trichogyne. Such a phenomenon was not observed in *P. gardneri* but the possibility exists that it does divide once it enters the carpogonium. The decondensing of the spermatial nuclear material after attachment to the prototrichogyne needs to be explained because it would seem to argue against the mature spermatial nucleus being in a prophase state as has been assumed by most workers (Grubb, 1925; Fritsch, 1945, p. 596; Drew, 1951; Magne, 1952; Krishnamurthy, 1959; Mumford and Cole, 1977).

Histochemistry may help to clarify changes that the vegetative cells undergo in the transformation to a carpogonium. The cuticle of vegetative cells of
**Porphyra umbilicalis** has been studied biochemically (Hanic and Craigie, 1969; Gunawardena and Williamson, 1974) and found to contain a high percentage of protein and glycoprotein. A similar investigation of wall chemistry in the prototrichogyne region may provide insight into mechanisms of the specificity of spermatium attachment. An approach along the lines of the recent work carried out by Evans et al. (1977) on egg-sperm recognition in Fucoids may be appropriate here.

With modified Feulgen procedures that can be used with electron microscopy (Jurand et al. 1958; Bryan and Brinkley, 1963, 1964) it should be possible to follow the spermatial nuclear material in the carpogonium at an ultrastructural level. With such a technique it may be possible to observe the formation of the fusion nucleus.

The known morphological and cytological life history of **Porphyra gardneri** is summarized in Figure 34.
D. Evidence Of Sexual Reproduction In *Porphyra nereocystis* And *Porphyra thuretii*

**Introduction**

Following the detailed investigation of sexual reproduction in *Porphyra gardneri* I made a preliminary survey of *P. nereocystis* and *P. thuretii* for evidence of sexual reproduction in their life histories.

**Results**

1. *Porphyra nereocystis*

   The spermatium of *Porphyra nereocystis* is ultrastructurally similar to the spermatia of *P. gardneri* and the Florideophycidae. Figure 35 shows a spermatium in a spermatangium near the thallus margin. Note the reduced chloroplast with plastoglobuli, central nucleus, and abundant small and large fibrous vesicles.

   At the light microscope level spermatia were observed attached to the wall over the carpogonia and fertilization canals were present (Figures 36a and 36b). The cell wall of *P. nereocystis* is thicker than in *P. gardneri* and as a result the fertilization canals are longer. Note also that the attachment of spermatia is not as specific as in *P. gardneri*, perhaps due to the absence of prototrichogynes. An electron
micrograph of a transverse section through a spermatium and associated fertilization canal is shown in Figure 36c.

Evidence that fertilization occurs has been obtained from chromosome counts. The spermatia and vegetative cells of the foliose thallus have 3 chromosomes, \( n=3 \) (Figures 37a and 37b), whereas the carposporangia and conchocelis phase have 6 chromosomes, \( 2n=6 \) (Figures 37d,e and 37f,g).

2. *Porphyra thuretii*

Spermatia attached to the slight prototrichogyne of the carpogonium and associated fertilization canals were observed at the light microscope level (Figures 36d and 36e).

The spermatia have 2 chromosomes, \( n=2 \) (Figure 37c). Unfortunately I have not had sufficient material or time to obtain counts for the carpospores or the conchocelis phase.
Discussion

This preliminary investigation of Porphyra nereocystis and P. thuretii suggests that sexual reproduction is a common occurrence in their life histories. My evidence combined with that of other workers strongly suggests that sexual reproduction in the genus Porphyra is a widespread phenomenon.
Table VIII. The species of *Porphyra* for which diploid chromosome numbers have been reported for the carpospores or vegetative cells of the conchocelis phase.

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1. Diplcid Carpospores</strong></td>
<td></td>
</tr>
<tr>
<td><em>Porphyra abbottae</em> Krishnamurthy</td>
<td>Mumford and Cole (1977)</td>
</tr>
<tr>
<td><em>P. dentata</em> Kjellman</td>
<td>Yabu (1971)</td>
</tr>
<tr>
<td><em>P. gardneri</em> (Smith et Hollenberg) Hawkes</td>
<td>Hawkes (1977a, 1977b, and this thesis)</td>
</tr>
<tr>
<td><em>P. kanakaensis</em> Mumford</td>
<td>Mumford and Cole (1977)</td>
</tr>
<tr>
<td><em>P. kata</em> Miura</td>
<td>Kito (1966) - as <em>P. sp.</em></td>
</tr>
<tr>
<td><em>P. kinositai</em> (Yamada et Tanaka)</td>
<td>Yabu (1972)</td>
</tr>
<tr>
<td><em>P. kuniedai</em> Kurogi</td>
<td>Yabu (1972)</td>
</tr>
<tr>
<td><em>P. linearis</em> Greville</td>
<td>Yabu (1971)</td>
</tr>
<tr>
<td><em>P. miniata</em> (C. Agardh) C. Agardh</td>
<td>Magne (1952)</td>
</tr>
<tr>
<td><em>P. moriensis</em> Ohmi</td>
<td>Kito et al. (1967) - as <em>P. umbilicalis</em> f. <em>linearis</em> (Greville)</td>
</tr>
<tr>
<td><em>P. nereocystis</em> Anderson</td>
<td>Harvey</td>
</tr>
<tr>
<td><em>P. okapurai</em> Ueda</td>
<td>Kito et al. (1967) - as <em>P. amplissima</em> (Kjellman)</td>
</tr>
<tr>
<td><em>P. onoi</em> Ueda</td>
<td>Setchell et Hus</td>
</tr>
<tr>
<td><em>P. perforata</em> J. Agardh</td>
<td>Yabu (1970) - as <em>P. amplissima</em></td>
</tr>
<tr>
<td><em>P. pseudolinearis</em> Ueda</td>
<td>Kito et al. (1971)</td>
</tr>
<tr>
<td><em>P. purpurea</em> (Roth) C. Agardh</td>
<td>Kito et al. (1967)</td>
</tr>
<tr>
<td><em>P. schizophylla</em> Hollenberg in Smith and Hollenberg</td>
<td>Hawkes (this thesis)</td>
</tr>
<tr>
<td><em>P. seriata</em> Kjellman</td>
<td>Yabu (1969a)</td>
</tr>
<tr>
<td><em>P. tenera</em> Kjellman</td>
<td>Yabu (1972)</td>
</tr>
<tr>
<td><em>P. umbilicalis</em> (Linnaeus) J. Agardh</td>
<td>Mumford and Cole (1977)</td>
</tr>
<tr>
<td><em>P. yezoensis</em> Ueda</td>
<td>Yabu (1969b)</td>
</tr>
</tbody>
</table>

*References:*
- Yabu (1969a)
- Kito (1968)
- Mumford (1975)
- Mumford and Cole (1977)
- Giraud and Magne (1968) as *P. umbilicalis* var. *laciniata* (Lightfoot) Thuret
- Kito et al. (1971)
- Kito et al. (1967)
- Hawkes (this thesis)
P. spp. # 1 and 2

2. **Diploid Conchocelis**

- *Porphyra gardneri*
- *P. kanakaensis*
- *P. miniata*
- *P. perforata*
- *P. pseudolinearis*
- *P. purpurea*
- *P. schizophylla*
- *P. tenera*
- *P. yezoensis*

Yabu (1971)

Hawkes (1977a, and this thesis)
Mumford and Cole (1977)
Kito et al. (1971)
Mumford and Cole (1977)
Kito (1974)
Giraud and Magne (1968) - as *P. umbilicalis var. laciniata*
Mumford and Cole (1977)
Kito (1974)
Kito (1967)
Migita (1967b)
Kito (1974)
Figure 16a. Transverse section of the blade margin of *Laminaria setchellii* and the base of *Porphyra gardneri*. The rhizoidal filaments can be seen penetrating into the medulla of the *Laminaria* blade.

Figure 16b. Closeup of the rhizoidal penetration into the medulla.

Figure 16c. Transverse section through a *Nereocystis* stipe and the base of a *Porphyra nereocystis* attached to it.

Figure 16d. Closeup of the central region of the attachment disc.

Scale bar = 100 μm on all Figures.

List Of Abbreviations Used On Figures For Part IV.

Carpogonial nucleus (cn); chloroplast (C, c); cell wall (CW, cw); dictyosome (D, d); floridean starch (FS, fs); large fibrous vesicle (LFV, lfv); medulla (ME); mitochondrion (MI, mi); *Nereocystis* (NE); nucleus (N, n); *Porphyra* (PO); pyrenoid (P, p); small fibrous vesicle (SFV, sfv); spermatial nuclear material (m); spermatium (S).
Figure 17. Tranverse section of *Porphyra gardneri* thallus, through a young monosporangium. Note dictyosomes, small fibrous vesicles and layer of fibrous material being formed on the outside of the plasmamembrane.
Figure 18. Transverse section of *Porphyra gardneri* thallus through a monosporangium which has developed beyond the stage shown in Figure 17. Small fibrous vesicles are abundant and large fibrous vesicles are beginning to form.
Figure 19a. Dictyosome activity producing small fibrous vesicles.

Figure 19b. Closeup of a monosporangial wall showing the depositon of fibrous material along its inner surface (arrow).

Figure 19c. Monosporangium in the final stages of differentiation prior to monospore liberation. Both small and large fibrous vesicles are abundant.
Porphyra gardneri - spermatogenesis, electron microscopy
(transverse sections)

Figure 20a. Vegetative cell of the fcliose thallus.

Figure 20b. The first division of the spermatangium is nearly complete. Note the new wall layer (arrow) that has been laid down in the transition from a vegetative cell.

Figures 20c-d. Later division stages of the spermatangium.

Scale bar = 5 um on all Figures.
**Porphyra gardneri** - spermatogenesis, electron microscopy (transverse sections)

Figure 21a. Final divisions in the formation of a mature spermatangium. Note the production of large fibrous vesicles.

Figure 21b. Spermatium from a mature spermatangium. It contains a nucleus, mitochondria, reduced chloroplast, several small and one large fibrous vesicle. Note small fibrous vesicle emptying its contents into the large fibrous vesicle (arrow).

Figure 21c. Two spermatia in a spermatangium near the releasing margin. The large fibrous vesicle has just become extracytoplasmic.

Scale bar = 1 um in Figures 21b-c.
Figure 22. Mature spermatium just prior to release. Note the nucleus with highly condensed chromatin and no nuclear membrane, reduced chloroplast with numerous plastoglobuli, mitochondrion and abundant small fibrous vesicles.
Porphyra gardneri - fertilization, light microscopy (transverse sections)

Figure 23a. Spermatium attached to the prototrichogyne of the carpogonium.

Figure 23b. Two spermatia with fertilization canals going into the carpogonium.

Figure 23c. Spermatia attached to both prototrichogynes (note fertilization canals).

Scale bar = 10 um on all Figures.
Figures 24a-b. Two scanning electron micrographs of the thallus surface of *Porphyra gardneri* showing several spermatia attached to the prototrichogynes.
Porphyra gardneri - fertilization, electron microscopy (transverse sections)

Figure 25a. Low magnification view of a spermatium attached to the prototrichogyne.

Figures 25b-d. Three examples of spermatia attached to the prototrichogyne prior to the formation of the fertilization canal. They contain a characteristic reduced chloroplast, mitochondria and non-membrane bound nuclear material.

Scale bar = 2 um on Figures 25b-d.
Porphyra gardneri - fertilization

Figure 26. Transverse section through a carpogonium and two spermatia which have transferred their nuclear material down the fertilization canal into the carpogonium.
Porphyra gardneri - fertilization

Figure 27. Transverse section through a carpogonium showing both prototrichogynes, central pyrenoid and lateral nucleus. The presence of a small deposit of wall material (arrow) in the prototrichogyne below the spermatium indicates that a fertilization canal has been formed, although it is not visible in the plane of section. Suspected Cyanophyta are also visible on the thallus surface (double arrow).
Porphyra gardneri - fertilization (transverse sections)

Figures 28a-b. Two spermatia which have transferred their nuclear material down the fertilization canal into the carpogonium, but which still have the remains of a chloroplast left in them.

Figures 28c-d. Prototrichogyne region of the carpogonium with a reduced chloroplast which is thought to have come from the spermatium during the transfer of nuclear material.

Scale bar = 1 μm on all Figures.
Porphyra gardneri - fertilization

Figure 29. Transverse section through a carpogonium which has the remains of a spermatium attached to the prototrichogyne, and what may be the highly condensed spermatial nuclear material (arrow).
Porphyra gardneri - carposporogenesis
(transverse sections)

Figure 30a. Fertilized carpogonium showing the two daughter nuclei resulting from the first division of the fusion nucleus. The fertilization canal is still present although the spermatium has disintegrated.

Figure 30b. The first division of the carposporangium is periclinal and produces 2 carpospores (note the fertilization canal still present in the wall).

Scale bar = 5 um on all Figures.
Porphyra gardneri - chromosome counts

Figure 31a. Spermatium, n=4.
Figure 31b. Vegetative cell of the foliose thallus, n=4.
Figures 31c-d. Carpospore, 2n=8.
Figures 31e-f. Vegetative cell of the conchocelis phase, 2n=8.

Scale bar = 5 µm on all Figures.
Porphyra gardneri - Feulgen staining
(transverse sections)

Figure 32a. Mature spermatangium; most spermatia still have nuclei in a diffuse interphase state (arrow).

Figure 32b. Mature spermatangium right on the releasing margin. The nuclear material is highly condensed (arrow).

Figure 32c. Released spermatia with highly condensed nuclear material (dark dots).

Scale bar = 10 um on all Figures.
Porphyra gardneri - Feulgen staining
(transverse sections)

Figure 33a. Carpogonium and attached spermatium which contains condensed nuclear material. No fertilization canal is present.

Figure 33b. Carpogonium and carpogonial nucleus laterally located. The attached spermatium contains nuclear material in a diffuse state, and a fertilization canal is present.

Figures 33c-d. Two examples of spermatia containing condensed nuclear material in close association with the fertilization canal.

Figure 33e. Carpogonium with attached empty spermatium and a densely staining mass of spermatial nuclear material in the carpogonium close to the fertilization canal.

Figures 33f-i. Several examples of carpogonia with empty spermatia on their outer walls and from 1-5 densely staining masses of spermatial nuclear material inside.

Figure 33j. Carpogonium with large, suspected fusion nucleus (note empty spermatium and fertilization canal).

Figure 33k. The first division of the fusion nucleus prior to cytokinesis (see also Figure 30a).

Figure 33l. Two carpospores produced by the first division of the carposporangium (see also Figure 30b).

Figures 33m-o. Subsequent stages of division to produce 4 (Figure 33m) or 8 (Figure 33o) carpospores.

Scale bar = 10 μm on all Figures.
Figure 34. Diagram summarizing the known morphological and cytological life history of *Porphyra gardneri*. It is suspected that meiosis occurs in the conchosporangium at the time of conchospore formation.
PORPHYRA GARDNERI LIFE HISTORY

MONOSPORE

Spermatangium

Spermatium (n)

Carpogonium

Fertilization (2n)

Foliose thallus (n)

Conchosporangium

Conchosporangial branch

Conchoecilis phase (2n)

Conchosporangium

Conchosporangium

Conchosporangium
Figure 35. *Porphyra nereocystis* spermatium in a spermatangium near the releasing margin. The spermatium contains abundant small and large fibrous vesicles, a reduced chloroplast, mitochondria, and central nucleus.
**Porphyra nereocystis**

*Figures 36a-b.* Transverse section of the foliose thallus through carpogonia and suspected spermatia forming fertilization canals.

*Figure 36c.* Electron micrograph of a transverse section through a suspected spermatium and fertilization canal.

**Porphyra thuretii**

*Figure 36d.* Transverse section of the foliose thallus through a carpogonium and suspected spermatium.

*Figure 36e.* Transverse section of the foliose thallus through a carposporangium that has undergone the first division. A suspected spermatium and fertilization canal are present.

Scale bar = 10 μm on Figures 36a-b, 36d-e.
Figure 37a. *Porphyra nereocystis* spermatia, n=3.

Figure 37b. *Porphyra nereocystis* vegetative cells of the foliose thallus, n=3.

Figure 37c. *Porphyra thuretii* spermatium, n=2.

Figure 37d,e *Porphyra nereocystis* carpospore, 2n=6. (only 5 chromosomes visible).

Figure 37f,g *Porphyra nereocystis* ccncchosporangial branch, 2n=6.

Scale bar = 5 um on all Figures.
GENERAL SUMMARY

Field, culture and cytological studies of 3 epiphytic Porphyra species have yielded four major results:

1. The genus Porphyrella is invalid and Porphyrella gardneri has therefore been transferred to the genus Porphyra.

2. Porphyra gardneri, P. nereocystis and P. thuretii, 3 superficially similar species, have been clearly characterized on the basis of their seasonal occurrence, host species, vegetative and reproductive morphology, growth in culture, and chromosome numbers.

3. The spermatangium and carpoconium of these 3 species have been characterized, and their division sequence followed in detail. From these observations I have concluded that there are two basic types of carpoconial and spermatangial morphology in the genus Porphyra, and that the classical 'cruciate division' sequence as reported by Hus (1902) may not be as common as previously thought.

4. The occurrence of sexual reproduction in the genus Porphyra has been unequivocally demonstrated for the first time.
As is always the case with research, my studies have raised more questions than they have answered. Major topics requiring further investigation include:

1. The nature of the epiphyte-'host' relationship. Physiological studies are needed to elucidate what determines host specificity.

2. Does meiosis occur in the conchosporangium? Cytological study of conchosporogenesis both at the light and electron microscope level is needed to establish that this is where meiosis occurs.

3. What are the environmental conditions and physiological mechanisms responsible for spermatangium and carpogonium induction?

4. Genetic studies along the lines of those which have been done on Gracilaria (van der Meer, 1977; van der Meer and Todd, 1977) may provide insight into what determines sexuality and how the various patterns of spermatangia and carpocsporangia on the thallus are established. Of particular interest here are species like Porphyra kataîaî (Kurogi, 1972), P. brumalis-Mumford, and several other species listed by Mumford (1975), which have the spermatangia on one half of the thallus and the carpocsporangia on the other.
5. Can *Porphyra* hybrids be produced? Such results were reported by Sutc (1963), but his experiments were not well documented.

6. How widespread is sexual reproduction in other members of the Bangiophycidae? Based on McDonald's (1972) ultrastructural observations of the spermatia of *Bangia fuscospurpurea*, the reports of Rosenvinge (1909), Dangeard (1927), and Cole (personal communication, 3 May, 1978) of fertilization canals connecting spermatia with carposonia, and Yabu's (1967) report of fertilization and a diploid chromosome number in the carpospores, sexual reproduction would certainly seem to be occurring in *Bangia*. In the Erythropeltidaceae, McBride (1972) has reported on the ultrastructure of the spermatia in *Smithora*. They have some similarities with the spermatia of *Porphyra gardneri* and *Bangia fuscospurpurea* and may therefore be functional male gametes. McBride also reported the attachment of a spermatium of *Erythrotrichia boryana* (Montagne) Berthold over a carpospore-like cell in the filamentous thallus of this alga. No fertilization canal was observed. In view of this evidence a re-investigation of the life histories of these algae as well as other members of the Bangiophycidae would be worthwhile.
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APPENDIX I

Porphyra Collecting Stations And Dates Of Collections

British Columbia:

Aguilar Point, 48° 50.4′N, 125° 08.3′W, 31 MAY 1974, 2 APR 1977.

Bolkus Islands, Skincuttle Inlet, Queen Charlotte Islands, 52° 19.4′N, 131° 14.8′W, 27 MAR 1976.

Botanical Beach, 48° 32′N, 124° 27′W, 11 JUL 1975.

Brooks Peninsula, 50° 06.7′N, 125° 54.5′W, 25 JUN 1975.

Cable Beach, 48° 49.6′N, 125° 09.1′W, 8 AUG 1974.

Cape Beale, 48° 48′N, 125° 13′W, 28 JUL 1976.

Execution Rock, 48° 48.8′N, 125° 10.6′W, 10 MAY 1974.

Gordon Islands, Queen Charlotte Islands, 52° 06.1′N, 131° 08.7′W, 1 APR 1976.

Grassy Island, Kyuquot Sound, 49° 55.4′N, 127° 15.0′W, 14 MAY 1975.

Hope Island, 50° 54′N, 127° 57′W, 15 APR 1975.

Hot Springs Cove, 49° 21.5′N, 126° 15.8′W, 13 MAY 1975.

Langara Island, Queen Charlotte Islands, 54° 13.54′N, 132° 57.7′W, 29 MAR 1976.

Leach Islet, Barkley Sound, 48° 49.8′N, 125° 14.4′W, 2 APR 1977, 17 MAY 1977.

Rennel Sound, Queen Charlotte Islands, 53° 22.65′N, 132° 30.7′W, 30 MAR 1976.

Ross Islets, Barkley Sound, 48° 52.2′N, 125° 09.5′W, 5 JUN 1974.

Second Beach, Barkley Sound, 48° 48.9′N, 125° 10′W, 9 MAY 1974.

South Pender Island, 48° 44.0′N, 123° 11.2′W, 4 SEP 1977.

Study site, Barkley Sound, 48° 50.1′N, 125° 11.1′W, JUN 1974 through MAY 1977.

Tree Islets, Queen Charlotte Sound, 50° 58.9′N, 127° 42.6′W, 24 JUN 1975.
Volcanic Cove, Kyuquot Channel, 49° 58.7'N, 127° 13.9'W, 15 MAY 1975.

Whiffen Spit, Sooke, 48° 21.2'N, 123° 44.2'W, 15 OCT 1973.

Oregon:

Harris Beach, 42° 04'N, 124° 18'W, 23 SEP 1975.

California:

Ano Nuevo Island, 37° 06'N, 122° 20'W, 2 NOV 1975.

Mission Point, Carmel, 36° 32.5'N, 121° 56'W, 13 JUL 1976.

Pebble Beach, Monterey Peninsula, 36° 34'N, 121° 57'W, 4 NOV 1975.

Fescadero Point, Monterey Peninsula, 36° 33.8'N, 121° 57.4'W, 12 JUL 1976.

Point Joe, Monterey Peninsula, 36° 36.6'N, 121° 57.4'W, 3 NOV 1975, 10 JUL 1976.
APPENDIX II

Representative specimens of Porphyra gardneri, P. nereocystis, and P. thuretii

Porphyra gardneri

Monocentric

Alaska:

Murder Point, Attu Island, 52° 48'N, 173° 11'E, UBC 7947, 26 JUN 1960; Bird Cape, Amchitka Island, 51° 39.5'N, 178° 38.5'E, UBC 54202, 27 JUN 1960; Top Camp, Amchitka Island, 51° 34.7'N, 178° 50.5'E, UBC 54203, 17 AUG 1970; Cape Agagdak, Adak Island, 52° 00'N, 176° 35'E, UBC 7783, 13 JUN 1960; Harbor Point, Lituya Bay, Sitka, 57° 08'N, 135° 15'W, UBC 23401, 29 JUN 1965; Cape Muzon, Dall Island, 54° 40'N, 132° 41'W, UBC 21469, 13 JUN 1965.

British Columbia:

Langara Island, Queen Charlotte Islands, 54° 13.54'N, 132° 57.7'W, UBC 54883, 29 MAR 1976; Plover Island, 50° 55'N, 127° 58'W, UBC 1501, 26 JUN 1953; Garden Island, Kyuquot, 50° 01'N, 127° 21'W, UBC 4342, 27 MAY 1959; Diana Island, Barkley Sound, 48° 50.1'N, 125° 11.1'W, UBC 54133, 23 FEB 1975; UBC 54127 and UBC 54134, 28 MAR 1975; UBC 54819, 16 APR 1976; Execution Rock, Barkley Sound, 48° 48.8'N, 125° 10.6'W, UBC 54129, 10 MAY 1974; Botanical Beach, 48° 32'N, 124° 27'W, UBC 54884, 14 MAY 1976.

Washington:

Watch Point, Mukkaw Bay, 48° 20'N, 124° 40'W, UBC 24882, 2 JUN 1966.

Oregon:

Indian Beach, Clatsop County, 45° 56'N, 123° 59'W, UBC 47328, 14 MAY 1972; Chapman Point, Clatsop County, 45° 55'N, 123° 58'W, UBC 47584, 12 JUN 1972; Short Sand Beach, 45° 45'N, 123° 58'W, UBC 24883, 6 JUN 1966.

California:

Pillar Point, San Mateo County, 37° 30'N, 122° 30'W, RS 474, 10 JUL 1968; Point Joe, Monterey Peninsula, 36° 36.6'N, 121° 57.4'W, GJH 2672, 18 JUN 1939; UBC 54814, 10 JUL 1976; Pebble Beach, Monterey Peninsula, 36° 34.4'N, 121° 57'W, GMS 12461, 7 MAY 1973; Mission Point, Carmel, 36° 32.5'N, 121° 56'W, GMS 4763, 16 MAR 1966; Estero Point, San Luis Obispo County, 35° 28'N, 120° 58'W, AHF 80437, 6 AUG 1974.
Mexico:

Punta Banda, Baja California, 31° 44'N, 116° 44'W, AHF 77063, 25 JUL 1971.

Spermatangial and carposporangial

Alaska:

Wingham Island, 60° 01'N, 144° 23'W, UBC 25459, 4 JUL 1966; Klokachef Island, 57° 25'N, 135° 53'W, UBC 23397, 30 JUN 1965; Loran Station, Biorka Island, 56° 51'N, 135° 32'W, UBC 10291, 11 JUL 1960.

British Columbia:

Tasu Narrows, Tasu Sound, Queen Charlotte Islands, 52° 44.5'N, 132° 06'W, UBC 53651, 17 AUG 1970; Tree Islets, 50° 58.9'N, 127° 42.6'W, UBC 54888, 24 JUN 1975; Cape Scott, 50° 47'N, 128° 25'W, UBC 35742, 11 AUG 1968; Solander Island, 50° 06.5'N, 127° 56.3'W, UBC 53836, 25 JUN 1975; Lynne Rock off Cautious Point, 50° 04.3'N, 127° 33.4'W, UBC 36036, 16 AUG 1968; Diana Island, Barkley Sound, 48° 50.1'N, 125° 11.1'W, UBC 54891, 13 MAY 1976; UBC 54820, 16 JUN 1975; UBC 54136, 17 JUN 1975; UBC 54130, 19 JUL 1974; UBC 54137, 8 AUG 1975; UBC 54122, 14 SEP 1974; UBC 54132, 17 OCT 1974; Cape Beale, 48° 47'N, 125° 13'W, UBC 54891, 28 JUL 1976; Botanical Beach, 48° 32'N, 124° 27'W, UBC 54133, 11 JUL 1975.

Washington:

Waadah Island, 48° 23'N, 124° 36'W, UBC 8767, 15 AUG 1958.

Oregon:

Indian Beach, Clatsop County, 45° 56'N, 123° 59'W, UBC 49873, 1 JUL 1973; Jockey Cap Rock, Clatsop County, 45° 51'N, 123° 58'W, UBC 48333, 8 JUL 1972.

California:

Trinidad Harbor, Humboldt County, 41° 03'N, 124° 09'W, CSUH 07353, 12 APR 1972; Bodega Head, Sonoma County, 38° 18'N, 123° 03'W, UBC 20010, 21 JUN 1963; Tomales Bay, Marin County, 38° 14'N, 122° 55'W, AHF 55944, JUN 1915; Año Nuevo Island, 37° 06'N, 122° 20'W, Hansen 1631 in UCSC (filed under Laminaria sinclairii), 8 AUG 1972; Davenport, 37° 00'N, 122° 11'W, GMS 2083, 6 JUL 1965; Point Joe, Monterey Peninsula, 36° 36.6'N, 121° 57.4'W, UBC 54818 and UBC 54887, 10 JUL 1976; Pescadero Point, 36° 33.8'N, 121° 57'W, UBC 54892, 12 JUL 1976; Mission Point, Carmel, 36° 32.5'N, 121° 56'W, UBC 54815, 13 JUL 1976.
Porphyra nereocystis

Alaska:


British Columbia:

Langara Island, Queen Charlotte Islands, 54° 13.54′ N, 132° 58.6′ W, UBC 56677, 29 MAR 1976; Masset, Graham Island, Queen Charlotte Islands, 54° 00′ N, 132° 09′ W, UBC 50229, 10 MAR 1973; Off N. W. coast of Calvert Island, 51° 42′ N, 128° 05′ W, UBC 42946, 13 APR 1970; Tree Islets, 50° 58.9′ N, 127° 42.6′ W, UBC 54105, 24 JUN 1975; Hope Island, 50° 54′ N, 127° 57′ W, UBC 57182, 15 APR 1975; Amphitrite Point, Vancouver Island, 48° 54′ N, 125° 33′ W, UBC 39820, 8 MAY 1969; Aguilar Point, Barkley Sound, 48° 50.4′ N, 125° 08.3′ W, UBC 57183 and 57184, 2 APR 1977; Cable Beach, Barkley Sound, 48° 49.6′ N, 125° 09.1′ W, UBC 57181, 8 AUG 1974; Second Beach, Barkley Sound, 48° 48.9′ N, 125° 10′ W, UBC 57186, 9 MAY 1974; Diana Island, Barkley Sound, 48° 50.1′ N, 125° 11.1′ W, UBC 57185, 12 DEC 1976, UBC 57186 and 57189, 23 JAN 1977, UBC 57187 and 57190, 23 FEB 1975; Leach Islet, Barkley Sound, 48° 49.8′ N, 125° 14.4′ W, UBC 57191, 2 APR 1977; Botanical Beach, 48° 32′ N, 124° 27′ W, UBC 50652, 25 APR 1974, UBC 57193, 11 JUL 1975; Esquimalt, Vancouver Island, 48° 28′ N, 123° 26′ W, UC 96517, no date.

British Columbia - Strait of Georgia

Maude Island, 49° 16′ N, 124° 05′ W, UBC 11583, 15 AUG 1959; Tugboat Island, 49° 09′ N, 123° 41′ W, UBC 56603, 29 DEC 1976; Gowlland Point, South Pender Island, 48° 44.0′ N, 123° 11.2′ W, UBC 57194, 4 SEP 1977; Sidney, Vancouver Island, 48° 39′ N, 123° 24′ W, UBC 1434, 26 SEP 1917.

Washington:

Friday Harbor, San Juan Island, 48° 32′ N, 123° 00′ W, UBC 57195, 27 OCT 1973; Whidbey Island, 48° 13′ N, 122° 46′ W, UBC 1244, no date.

California:

Pebble Beach, Monterey Peninsula, 36° 34′ N, 121° 57′ W, UBC 57196, 4 NOV 1975.
Porphyra thuretii

**British Columbia:**

Shields Bay, Rennell Sound, Queen Charlotte Islands, 53° 22.65'N, 132° 30.7'W, UBC 55302, 30 MAR 1976; Volcanic Cove, Kyuquot Channel, Vancouver Island, 49° 58.7'N, 127° 13.9'W, UBC 54446, 15 MAY 1975; Diana Island, Barkley Sound, 48° 50.1'N, 125° 11.1'W, UBC 57203, 23 JAN 1977; UBC 52019, 23 FEB 1975; UBC 57202, 29 APR 1975; Leach Islet, Barkley Sound, 48° 49.8'N, 125° 14.4'W, UBC 57200 and 57201, 2 APR 1977.

**California:**

Pacific Grove, Monterey County, 36° 37.6'N, 121° 55'W, UC 95610, 23 APR 1897; UC 95598 and 95596, JUN 1901; UC 95612, no date; Pebble Beach, Monterey Peninsula, 36° 34'N, 121° 57'W, GMS 1406, 24 JUN 1943; Carmel Bay 36° 33'N, 121° 56'W, UC 791973 and 791970, 29 MAY 1900; GMS 6467, 23 JUN 1939.