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ANALIPUS JAPONICUS (HARV.) WYNNE (PHAEOPHYTA):

STUDIES OF ITS BIOLOGY AND TAXONOMY

by

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Abstract

A culture and light microscope study was made of the development, morphology and anatomy of Analipus japonicus (Harv.) Wynne (Phaeophyta). Its life history was observed in culture and followed in the field over a period of two years. Cytological studies employing microspectrophotometry to measure relative DNA content confirmed the life history to be an alternation of isomorphic generations - verifying the work of Abe (1935a,b, 1936) which has been questioned in the literature. The unilocular plants proved to be diploid. Meiosis occurs in the unilocular sporangia, resulting in the formation of haploid unispores. Haploid plurilocular plants were the only plants of A. japonicus found in British Columbia.

A survey of herbarium specimens and collections outside British Columbia showed that only plurilocular populations occur in most of the eastern portion of A. japonicus' geographic range (Alaska, B.C., Washington, Oregon, California). Unilocular plants occur in Japan and in one site in California. Prior to this study the rarity of this stage of A. japonicus' life history had not been realised.

The ecology of A. japonicus in British Columbia was studied at a site in the Strait of Georgia and at two sites in Barkley Sound on the west coast of Vancouver Island.

A comparison was made of A. japonicus and the

subantarctic alga Caepidium antarcticum J.Ag. (to which A.japonicus has been likened in the literature). Although many similarities exist between them, it is concluded that these plants are generically distinct. The affinities of Caepidium will remain unclear until life history data are available.

It is concluded from an evaluation of the order Ralfsiales (into which A.japonicus has been recently placed) that this order should not be recognised. The placement of A.japonicus into the Ralfsiaceae is evaluated and considered inappropriate. The use of the family Heterochordariaceae for A.japonicus and the placement of this family in the Ectocarpales are advocated.

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GENERAL INTRODUCTION,

The brown alga Analipus japonicus (Harv.) Wynne is found in the intertidal regions of the northern Pacific. Despite its considerable geographic range there is little published information on this plant. Prior to this research, a number of important questions about the biology of A.japonicus were outstanding:-

- the life history, which had been reported to be an alternation of isomorphic generations (Abe, 1935a,b, 1936), needed verification (Bold and Wynne, 1978).
- this plant had not been grown in culture to reproductive maturity and there were no studies of its development beyond the early stages of disc formation.
- there were no studies of the ecology or phenology of A.japonicus in the eastern Pacific.
- the similarities between A.japonicus and the subantarctic alga Caepidium antarcticum had been noted in the literature by a number of workers (e.g. Skottsberg, 1921; Kylin, 1940), but no comparative studies had been performed. The most detailed work on Caepidium antarcticum was that of Skottsberg (1907, 1921).
- the taxonomic position of A.japonicus was unclear. Although it had been placed in the Ralfsiales by Nakamura (1972), this order has not been widely accepted and the placement of A.japonicus varied between authors (e.g. Abbott and Hcllenberg, 1976; Lindstrom, 1977; Bold and Wynne, 1978).

Thus, the objective of this study was to attempt to

answer some fundamental questions about the biology of A. japonicus.

Various approaches were taken in this research. A field study in British Columbia at sites in the Strait of Georgia and on the west coast of Vancouver Island provided information about the seasonality, population dynamics, and autecology of this plant. Collections south of British Columbia to Point Conception, California, the southern limit of this plant, resulted in information on the phenology of A. japonicus in other parts of its range and further observations of its habitat and growth. An herbarium survey was made of A. japonicus plants from throughout its range to obtain phenological data. Culture studies were carried out to study (1) the life history of this plant, and (2) the effect of selected environmental factors on spore germination and development. A cytological study was performed to obtain information on the ploidy levels of A. japonicus plants, and to attempt to identify the site of meiosis and the occurrence of fertilisation. Development of A. japonicus and formation of reproductive structures were followed by a light microscope study. A comparative study was made of the morphology and anatomy of A. japonicus and Caepidium antarcticum. The taxonomic position of A. japonicus was evaluated particularly with respect to the Ralfsiales and Ralfsiaceae where it has been placed most recently (Bold and Wynne, 1978).

This research is presented in six interrelated

chapters in the following sequence:- (1) the development and morphology of the plant, (2) the phenology and distribution of A.japonicus in the eastern North Pacific, (3) its life history and cytology, (4) the autecology of A.japonicus at Bath Island, (5) a comparative study of A.japonicus and Caepidium, and (6) an evaluation of the Ralfsiales, Ralfsiaceae and the taxonomic position of A.japonicus.

Terminology

In Analirus japonicus, swarmers from the plurilocular reproductive organs presumably can function either as spores or gametes. It is not appropriate to assign the terms sporangia or gametangia to these reproductive organs until one knows the fate of their products. For this reason, the term plurangium (pl. plurangia) has been employed to refer to a plurilocular reproductive structure that may bear either spores or gametes (Abbott and Hollenberg, 1976). Where the fate of the reproductive swarmers is known, the terms plurispores or gametes are used (for asexual or sexual swarmers respectively).

The plurilocular structures of Caepidium antarcticum also are referred to as plurangia as their function and plcidy level remain unknown.

CHAPTER 1.

Development, morphology and anatomy of Analipus japonicus
(Harv.) Wynne

Introduction

Members of the brown algal genus Analipus Kjellman possess a distinctive, perennial, profusely-lobed crust from which arise erect, percurrent axes. The erect axes bear either plurilocular or unilocular reproductive organs on separate plants. There are two species in this genus; A. filiformis (Rupr.) Papenfuss has simple axes and A. japonicus possesses unbranched laterals which clothe the upright axes. Amongst the few other genera in the Phaeophyta which show an equivalent elaboration of crustose and erect systems in the same generation of the life history are the monotypic Caepidium antarcticum J.Ag. (see Chapter 5) and Stereocladon rugulosus (Bory) Hariot (Skottsberg, 1907, 1921).

Early taxonomic treatments of A. japonicus (as Chordaria abietina Rupr. ex Farlow) dealt exclusively with characteristics of the erect axes and placed this plant in the Chordariales. Later, the presumed life history of this plant resulted in it being placed into the genus Heterochordaria and the monotypic Heterochordariaceae. Some recent work has seen A. japonicus placed in the Ralfsiaceae and the newly-established Ralfsiales, on the basis of its germination pattern, chloroplast features and life history. The taxonomic position of this plant is discussed in detail in Chapter 6.

The most complete discussion of the development and

morphology of A. japonicus has been that of Inagaki (1958) in his systematic study of the Chordariales in Japan. Abe (1935a) described germination and early development of swarmers from uni- and plurilocular structures. In a paper in which Heterochordaria was included in Analipus and the new combination of A. japonicus recognised, Wynne (1971) compared the two species of Analipus, particularly their reproductive organs and crust structure. Wynne (1972) illustrated germlings of A. japonicus up to a six-week stage in culture, describing germination of plurispores and formation of the initially discoidal crust.

This morphological and anatomical study has been undertaken to examine the development and reproduction of A. japonicus. It is the first part of a consideration of the biology of this plant and its systematic affinities within the Phaeophyta.

Materials and Methods

Specimens used in this study were collected from June 1977 to September 1979 along the Pacific coast from British Columbia to central California. Monthly observations and collections were made at Bath Island (Strait of Georgia, British Columbia - $49^{\circ}09'N$, $123^{\circ}40'W$) and in Barkley Sound near the Bamfield Marine station (west coast of Vancouver Island, British Columbia - $48^{\circ}50'N$, $125^{\circ}09'W$). The plants bearing unilocular sporangia were collected at City Point (Mission Point) Monterey County, California ($36^{\circ}33'N$, $123^{\circ}56'W$). Collections of plants were made either at low tide or by use of SCUBA. (Refer to Appendix I for collection sites and dates.)

Plant material was either fixed in the field or fixed within 48 hours of returning to the laboratory, having been transported from the field wrapped in damp newspaper in an ice-cooled chest. Fixation was in either 2-5% formaldehyde/seawater or 4-5% glutaraldehyde/seawater. Material was embedded in glycol methacrylate using the Polysciences JB4 kit, and sectioned at $2-5\mu m$ on a Sorval JB4 microtome. Sections mounted on glass slides were stained with:-

- (1) toluidine blue O (TBO) in benzoate buffer (Feder and O'Erien, 1968) following the procedure of McCully, Goff and Adshead (1980).
- (2) periodic acid-Schiff's (PAS) (Jensen, 1962) sometimes

counterstaining this with aniline blue (PAS/AN). Dimedone blocking of background aldehydes preceded the PAS procedure (Feder and O'Brien, 1968).

- (3) alcian blue (AB) at pH 0.5 (Parker and Diboll, 1966).

Cultures were started from fertile plurilocular and unilocular material using the hanging drop technique (Wynne, 1969) and were grown in Provasoli's enriched seawater medium. (Refer to Chapter 3 for details of culture methods.)

Results and Discussion

Germination of spores and growth in culture

Both plurispores (Figure 1a) and unispores (Figure 1b) of A. japonicus followed a similar germination sequence. After swimming in culture medium for 1/2-4 hours, the spores rounded up on the coverslip or dish, having become progressively more sluggish in their movements and eventually losing their flagella.

The spores soon showed signs of germination. A germination tube was produced first, giving the spores a pear-like shape (Figure 1c). As growth proceeded a short filament was produced, and the spore was usually evacuated resulting in an empty spore wall and a short tube before the first new wall was laid down. The cytoplasm did not, however, always leave the germ tube (Figure 1d). After

forming a filament of 1-3 new cells beyond the germination tube, longitudinal and oblique divisions occurred giving the germlings a club-like appearance. As growth and divisions continued, the disc took on a rosette-like appearance (Figure 1e). By about the 12-cell stage, the original spore and germination tube were obscured from view by new growth (in culture at about day 15 at 10°C).

At this stage in development, the cells began to divide in the plane parallel to their attachment, forming a multilayered crust. Colourless, uniseriate hairs were produced by the crust as it developed (Figure 1e and f). If the young germlings were dislodged from their substrate the basal cells of the crust became very elongated showing their rhizoidal nature (Figure 1f). Wynne (1972 - figure 16) illustrates a young crust showing this condition.

As the crusts developed in culture, they often produced filaments (Figure 1f and 2a). Initially these filaments were uniseriate but they could be distinguished from hairs as they possessed pigment, and also, as they developed some became bi- or multiseriate. On contact with the substrate these filaments formed a pad or disc of cells - a secondary disc - which in turn produced hairs and could also develop filaments. Figures 2a and b show such a situation with a number of secondary or subsidiary discs resulting from one initial crust. Although I was not able to observe the early development of crusts in the field (due to failure of settlement on plates - Chapter 4), this

growth pattern may be a means of vegetative growth, enabling the crust to colonise the substrate more rapidly. It would help to explain the spread and growth of the crust in the field at times when recruitment from spores is not occurring.

As the crusts grew in culture they eventually became detached from their substrate - either coverglass, glass slide or base of the culture dish. The crusts from culture had a looser and less compact appearance than those from the field (compare Figure 2c with 3b). I think that this can be attributed to the lack of firm attachment in culture conditions and perhaps also the lack of water movement. The crusts in culture consisted of an almost cylindrical branched thallus and produced erect axes from the tips of their branches (Figure 2c). The upright axes in culture developed lateral branches in some cases, but generally remained shorter and possessed fewer laterals than their field counterparts (compare Figure 2d with 3a). Reproductive maturity of cultured erect axes occurred when the plants were considerably smaller than field collected material.

Morphology and development of the crust

The crust of A. japonicus (Figure 3b) has been clearly illustrated by Setchell and Gardner (1903 - pl. 18, figure 16, 17), Okamura (1913 - pl. CXIV, figure 6) (who described it as "tuberous and lobed"), and most recently by Wynne

(1971 - figure 4). The crusts have a multilayered appearance in the field as lobes of the flattened, branching axes often grow over each other (Figure 4c).

Inagaki (1958) described the discoid base of A. japonicus as having a medullary layer of "parenchymatous cells which are irregularly polygonal", a cortex consisting of "2-3 layers of cubic cells", and rhizoids "issuing from the marginal portion of the primary discoid base". Setchell and Gardner (1903) also refer to the crust as being parenchymatous in construction. Nakamura (1972) states that the germlings develop into minute parenchymatous discs by successive cell divisions. Wynne (1972), however, describes the manner of growth of the prostrate system as being "a broad multi-axial 'front' of assurgent filaments".

The crust has a clearly filamentous construction when seen in longitudinal section with prominent assurgent (or obliquely curving) filaments (Figure 4a). The crust has a cortex of 3-4 cell layers on the uppermost side and a less distinct cortex of 2-3 cell layers on the lower side (Figure 4a and c). Frequently the lower marginal cells extend forming rhizoidal attachment with the substrate or crust layer beneath (Figure 4c). Growth of the crust occurs at the margins with divisions occurring around the tip of each lobe (Figure 4a). Cells internal to the meristematic zone elongate and expand. Hairs occur in the crust although in mature field collected material they are infrequently encountered. These are colourless and appear to arise at

the base of the cortical layer (Figure 4b).

The unstained cells of the crusts possess golden brown bead- or droplet-like contents. When stained with TBO these bodies stain bright green. This material is spread throughout the cells of the crust cortex and medulla, staining particularly densely in the cells of the upper cortex as seen in Figures 4a and 4b. The green, turquoise colour reaction with TBO indicates the presence of phenolic materials. The function and origin of this material is unclear. The staining reaction is very similar to that of the material found in the terminal cortical cells of the erect axes in A. japonicus.

These cellular bodies have been recorded in brown algae by many workers from the time of Naegeli (1847), and a variety of names have been applied to them including the terms physodes and fucosan vesicles. In a review of these structures, Ragan (1976) argues that the term fucosan vesicles should no longer be used as this implies that the contents are carbohydrates, which has not been established. Although there have been a number of studies on these structures, and their nature has been debated for over a century, many questions remain concerning their origin, function, and composition (Ragan, 1976). In a study of the physodes of Dictyota, Evans and Holligan (1972b) conclude that these subcellular bodies originate in the plastids, suggesting that they might be the products of photosynthesis. Their histochemical studies showed the

physodes to be phenolic or tannin-like in nature. McCully (1966) states that the presence of these bodies in the thallus of Fucus "seems correlated with apparent photosynthetic activity, i.e., they are mainly in the epidermis and outer layers of the cortex. But this correlation does not apply for holdfast fibres where they are also plentiful and where photosynthesis is probably minimal.". Of the number of functions which have been suggested for physode material in brown algae (Ragan, 1976), the most plausible for the material present in the crust of A. japonicus would appear to be a metabolic reserve substance, or as an anti-grazing compound.

Development of erect axes and lateral branches

The erect axes arise out of the crust from the tips of the lobes (Figure 2c). Initially the upright axis has a narrower diameter than the lobe from which it arises thus appearing to come from a depression in the crust tip. The collar of crust tissue at the base of the upright axis becomes less obvious as the axis increases in size.

Upright axes are at first simple but soon become covered in short lateral branches. Rarely the main axes are branched and even less frequently lateral branches are forked. In the case of the main axis, it appears that branching occurs after damage or injury to the plant, presumably affecting the apical meristem and resulting in a lateral branch becoming dominant.

In the transition zone between the crust and the upright axis, medullary cells become elongated and, instead of terminating in cortical cells of the crust, rise into closely packed parallel filaments (Figure 5c). There is a transition between the rounder almost isodiametric cells of the crust medulla and the rectangular and elongated cells of the upright axis (Figure 5d). At the borders of the medulla the cells of the filaments divide outwards giving rise to short cortical filaments terminated by club-shaped cells (Figure 5c).

At the apex of the erect axis of A. japonicus there is a fan of cortical or assimilating filaments terminating the closely packed medullary filaments. This can be seen most clearly in longitudinal sections of young material (Figure 5a and b). The region of division and elongation occurs primarily at the base of cortical filaments at the apex (arrows in Figure 5b). However, the process of elongation affects the cells below this point. As the plant grows, medullary cells are stretched apart, leaving a hollow central region traversed by only a few very elongated medullary cells (Figure 5a and e).

The cortex and medulla in A. japonicus are well defined but the subcortex is less clearly distinguishable. I have interpreted the 1-3 layers of rounded cells borne on medullary filaments and which give rise to cortical filaments to constitute the sub-cortex (Figure 6a).

In her study of the Mesogloioaceae, Parke (1933) uses

two models of thallus construction - the central thread type (e.g. Mesogloia, Myriocladia, Acrothrix), and the multiple strand thallus (e.g. Eudesme, Castagnea, Myriogloea, Cladosiphon). Analipus is most like Chordaria in this treatment and fits most closely into the multiple strand model. Parke (1933) considers that the five genera in the Mesogloioaceae (which includes Chordaria) all possess the three tissue types, although in defining the sub-cortex states that it is "an intermediate and variable zone lying between the medulla and cortex". According to Fritsch (1945), however, Chordaria does not possess a sub-cortical layer.

Parke (1933) also describes secondary filament systems in this family which can arise from the cortex, sub-cortex and medulla. Fritsch (1945) uses the term hyphae for the secondary filaments and in the case of Chordaria describes them as issuing from medullary cells and growing both upwards and downwards in the thallus. In the most detailed anatomical study of A. japonicus to date (Inagaki, 1958) there is no mention of secondary filaments.

From my examination of serial sections and of squashes of material previously soaked in sodium carbonate, it appears that secondary filaments do occur in A. japonicus, arising from medullary cells (Figure 7a-d). I have not been able to identify any secondary filament systems in the cortex or sub-cortex. It is very difficult to trace secondary filament systems from their points of origin in

A. japonicus. The medulla of this plant is tightly bound and when material is squashed the cortical filaments are relatively easily dislodged while the medulla and sub-cortex remain firmly bound together.

Longitudinal sections of erect axes indicate that formation of laterals is initiated in the sub-cortex of the erect axis. Figure 6a shows a longitudinal section of a sterile erect axis, which can be compared with Figure 6b, where the first stages of lateral formation can be seen. Divisions occur in the subcortical zone of the erect axis cutting off cells which either produce cortical filaments to the surface, or if internally located become elongated and form the medulla of the branch (Figure 6c). As the lateral grows, just as in the growth of the main axis, the medullary tissue gets pulled apart, and the branch becomes hollow. Once initiated, growth of the lateral follows essentially the same pattern as that of the main axis with the zone of divisions being at the apex of the branch in the 'fan' of actively dividing cells. The divisions occur at the base of the assimilatory filaments in the sub-cortical region (arrows in Figure 6c).

As the main axis and lateral branches develop, terminal cells of the cortical filaments become enlarged and often club-shaped (Figures 6a-c). The filaments are 3-5 cells in length and interspersed with them are hairs which arise in the sub-cortex (Figure 10c). The terminal cells of cortical filaments are filled with golden-brown contents

which stain an intense green with TBO, indicating the presence of phenolic compounds.

Reproductive Organs

(a) Unilocular sporangia

Unilocular sporangia are borne primarily on the lateral branches, although they can also be found on the main axis of the uprights (Figure 8a). The sporangium develops from a cell cut off by a sub-cortical cell and is surrounded by sterile cortical filaments 3-6 cells in length (Figures 8b and c). The sporangium is immersed amongst these filaments until maturity. In freshly collected fertile material unilocular sporangia were easily detached from the subcortex. When viewed with a dissecting microscope, dislodged ripe sporangia and emptied sporangia were seen on the surface of the thallus. No empty sporangia were seen in sectioned material suggesting that this dislodgement may take place prior to release and aid in dispersal of the spores.

Staining with TBO, AB, and PAS shows distinct zones in the sporangia. In material stained with TBO (Figures 8b and d) the central part of the sporangium, which later rounds up to form the spores, stains as a speckled pink/purple zone. Surrounding this lightly stained central region is a darkly stained purplish band. Between this darkly staining band and the sporangial wall is a thin lightly stained pink

layer. This layer is not very noticeable in younger sporangia but, as they mature, it becomes more prominent particularly around the apex of the sporangium where it forms a 'dome' or 'cap'. The sporangial wall stains light-purple. Once the spores have rounded up, TBO staining results in light pink/purple spores surrounded by a more darkly staining purple border.

When unilocular material is stained with AB the pattern is very similar (Figure 8e). Each spore is only lightly stained turquoise blue and is surrounded by a more darkly staining border. Around the spores in the centre of the sporangium is a layer of blue-staining material. The sporangial wall stains quite densely with AB. Between the wall and the layer surrounding the spores is a thin layer (not seen in Figure 8e) which as with TBO is lightly stained. This is again the layer which forms the cap at the tip of the sporangium as it matures.

The PAS stained material possessed similar layers (Figures 8a, c and f). The central cytoplasm, and later the spores, stained a dense pink/magenta and were outlined with a more darkly staining border. Immediately surrounding the spore mass the layer which was darkly stained with TBO and AB was virtually unstained. This region was occasionally speckled with patches of pale pink. Between this essentially unstained layer and the sporangial wall (thin but brightly staining) was a bright pink layer and it is this which forms the cap at the tip of the sporangium.

Toluidine blue is a thiazin dye and stains metachromatically high molecular weight compounds with free carboxyl, sulphate or phosphate groups (Barka and Anderson, 1963; McCully, 1966). Particularly, the pink/purple stain with TBO is regarded to be characteristic of sulphated polysaccharides often referred to as fucoidin in brown algae. Percival (1979) prefers, for simplicity, to call the sulphated polysaccharides of the Phaeophyta 'fucans'. She notes that various names have been applied to these compounds and that in fact one is dealing with "a family of polydisperse heteromolecules containing in addition to fucose, varying proportions of galactose, mannose, xylose and glucuronic acid" (Percival, 1978). These compounds have a strong affinity for Mg ions, and as these ions are highly hydrated, Percival (1979) considers that they play an important role in the protection of these plants from desiccation. Alcian dyes differentiate and locate sulphated and non-sulphated polysaccharides. Alcian blue at pH 0.5 complexes exclusively with sulphate groups as the carboxyl groups are not dissociated below pH 1.0 (Parker and Diboll, 1966).

The pink/magenta reaction of PAS is quite specific for polysaccharides having free hydroxyl groups on two vicinal carbon atoms (Hotchkiss, 1948; McCully, 1966). Alginic acid is a carboxylated polysaccharide found in the Phaeophyta which possesses free vicinal hydroxyl groups and is thus PAS positive. Percival (1979) notes that alginic acid has a

high affinity for Ca ions and that this feature gives this material a high gel strength. Wasserman (1948, 1949) showed that alginic acid can undergo a salting out process in which the pore size of the gel is greatly decreased, and that it can also act as an excellent ion-exchange resin. Alginates (salts of alginic acid) have been found intercellularly and in cell walls (Evans and Holligan, 1972a) and are considered to have structural as well as ion-exchange functions (Mackie and Preston, 1974).

Using the TBO metachromasy (Percival, 1964; McCully, 1966, 1968a; Evans and Holligan, 1972a; Evans, Simpson, and Callow, 1973; Toth, 1974, 1976) one can conclude that within the unilocular sporangia of A. japonicus layers can be identified with the following composition:- (1) the sporangial wall, possessing both alginic acid and fucans (PAS, AB, and TBO positive), (2) a thin layer immediately beneath the wall which encircles the sporangium and also expands at the apex of the sporangium to form a dome or cap consisting of primarily alginic acid (PAS positive, TBO and AB lightly stained), (3) a layer primarily of fucans with possibly traces of alginic acid (TBO and AB darkly stained, mainly PAS negative but with a few patches of stain), and (4) spores surrounded with a thin layer containing both alginic acid and fucans (PAS, TBO and AB positive).

A number of workers have speculated on the ecological and functional significance of polysaccharide material, particularly the hydrophilic alginic acid and fucans within

brown algae. In particular, protection against desiccation, and the osmotic buffering provided by these materials is stressed (McCully, 1966, 1968b; Percival, 1964, 1978, 1979). These compounds have also been implicated in the release of unispores. Knight (1923) suggested that in Pilayella release of spores from the unilocular sporangia was affected by a rapid intake of water resulting in an increase in the volume of the mucilaginous material surrounding the mass of spores. In a study of Pilayella, Markey and Wilce (1976) suggested two factors are involved in the release process - both the swelling of mucilaginous material and a weakening of the inner part of the sporangial wall. In a study of unilocular sporangia of species of Elachista, Hecatonema and Pilayella, Loiseaux (1973) suggested that the mucilage surrounding the spores may act as an osmoticum, thus increasing osmotic pressure. In a study of propagule release in several brown algae, Toth (1976) observed spore release and the changes occurring in cell wall structure. Toth suggests that the production and hydration of carbohydrates may produce an internal pressure in the sporangia. Enzymatic breakdown of the wall particularly in the region of the apex allows the spores to be released from this point of weakness as swelling of polysaccharides occurs. Toth suggests that timing of release may occur with reimmersion in seawater after the low tide exposure and desiccation, the subsequent rapid hydration of the mucilaginous polysaccharides

resulting in the sporangium bursting.

As can be seen in Figures 9a and 9b unilocular sporangia of A. japonicus dehisce at the sporangial tip. The release of spores from sporangia observed in the laboratory was rapid. The sporangial wall ruptures at the tip (Figure 9b shows a flap of wall bent back after release) and a mass of spores oozes out - sometimes leaving a few spores or apparently uncleaved cytoplasm behind (Figure 9b). The mass of spores swells as it leaves the sporangium forming a mushroom-like cloud. Within a minute or less of rupturing the sporangial wall, spores on the outside of the spore mass begin to break away. Eyespots in the spores can be clearly seen in later stages of development within the sporangium and are very distinct after release of the spore mass.

(b) Plurangia

In A. japonicus plurangia are borne primarily on the laterals although they can also be found on the main axis of the upright plant. They result from the transformation of the sterile cortical filaments by transverse, longitudinal and sometimes oblique divisions (Figures 10 and 11). They form usually uni- to biseriate (occasionally multiseriate) filaments which are terminated by 1-3 sterile cells (Figure 10a). At maturity the depth of the plurilocular tissue can be up to 15 cells deep although more often the layer formed is 8-10 cells deep. In cultured material the plants became fertile when much smaller than

their field counterparts and the cortical filaments were not transformed into such a deep fertile layer, consisting instead of a layer of usually 4-8 cells in depth (Figure 10b).

When the plurangia release their swarmers the laterals where they were appear white (Figure 3a). This is because the entire photosynthetic tissue is shed from the fertile areas with only colourless sub-cortex and medulla remaining. No regeneration of the cortex occurs and thus the result of reproduction via plurangia is loss of the laterals and often of the entire erect axis.

Swarmers release from the plurilocular organs (Figures 11a-d) can occur very rapidly particularly in material which has been dried by emersion and then is reimmersed in water. The process of release apparently consists of at least two stages. The sterile terminal cells become detached from the plurangia although they are still laterally connected to one another. Thus in sections of releasing material one can find ribbons of sterile cells (Figure 11b and c). These curl backwards as if released from tension when they are detached from the plurangia. Once the swarmers have been shed empty plurangia cannot be found. The walls appear to disintegrate and only fragments remain (Figures 11b-d).

Jaasund (1964) suggested that in Ralfsia verrucosa the sterile layer above the sporangia facilitated release as these laterally connected cells remained adjoined and broke

away from the rest of the tissue bending back with some sporangia. Edelstein, Chen and McLachlan (1968) reported that in Ralfsia fungiformis a similar release process occurs. In this species there are also overlying sterile cells which "form a firm layer which holds the sporangia together" and that when the sorus matures this layer splits. They reported that the sporangia completely disintegrate on spore release and noted that this is not the characteristic mode of plurilocular release reported for the Phaeophyta. Release is most often reported to occur through an apical pore.

With TBO (Figures 11a and b, 10b) and AB (not shown) the plurangia stain in the following way - swarmers are stained pink/purple and turquoise blue respectively, and are surrounded within the plurangial wall by a more darkly staining layer. The plurangial wall also stains intensely as does the matrix surrounding the plurangia with these two stains indicating the presence of sulphated polysaccharides. On staining with PAS (Figures 10a, c and d) the swarmers stain a bright pink/magenta, but the border between the swarmer and the wall is unstained - implying the presence of only sulphated polysaccharides such as fucans, and not carboxylated polysaccharides such as alginic acid. The plurangial walls stain with PAS and the matrix outside the walls surrounding the plurangia is not stained. Thus the material surrounding the swarmers within the plurangia and surrounding the plurangia is sulphated

polysaccharide or fucan. It seems likely that this material plays an important role in the release process. The rapidity of the release and the shattering of the plurangial walls suggests that a swelling or pressure build-up due to the hydration of these compounds could be important. This may be particularly so after desiccation and then reimmersion in water, or when an increase in the hydration of these materials occurs after a break appears in the protective surface sterile layer. A swelling of the matrix would force the plurangial walls to break down and also force back the roof of sterile cells allowing a more rapid release of the swarmers into the environment.

In each plurilocular plant, virtually all the cortical tissue in most laterals is converted to plurilocular organs and thus huge numbers of swarmers are produced. The loss of cortical tissue on swarmer release makes the plant vulnerable to entry of disease agents (fungi were found in tissue of released laterals and were causing disruption of the cells, thus deleteriously affecting the plant), and an increase in epiphytes (normally rare) was also found on the parts of the plant where release had taken place. The post-reproductive phase of plurilocular plants varies in length. In some plants the whole plant is forming sporangia virtually synchronously. In other individuals, extension and new lateral formation is occurring at the apex whilst laterals lower on the axis are fertile and shedding swarmers.

Conclusions

A summary of the life history of Analipus japonicus is presented in Figure 20. The development sequence of Analipus japonicus which I observed in culture and in field material may be summarised as follows. A swarmer released from either a unilocular sporangium or plurangium settles and germinates. Within ten days a distinctive discoidal pad of cells has formed from an initially one to three-celled germling. The disc of cells develops to form a pseudoparenchymatous crust. The assurgent filaments of the crust rise to form an upper cortical layer or grow downwards forming a basal cortex from which rhizoids will often arise. Erect axes are produced from the tips of the crust axes, as these cylindrical and branching axes extend along the substrate. The anatomy of the erect axis of this plant is similar to that of Chordaria. It differs from Chordaria, however, in possessing plurangia as well as unilocular sporangia on separate individuals. Swarmer release from the plurangia is rapid and results in complete loss of the cortical tissue from the fertile branches, as all of this tissue except for a terminal layer of 1-3 cells in depth is converted to plurangia. The unilocular sporangia arise from the subcortex and are found immersed among sterile cortical filaments. In both types of reproductive organs there is considerable production of polysaccharide material. The studies of other workers have

implicated such material to be involved in the release process in brown algae. Both the terminal cortical cells of the erect axes and the cells of the crust cortex and medulla are rich in phenolic materials. The function of these 'physodes' is not known although in other brown algae workers have postulated their role in storage and as anti-grazing compounds (Ragan, 1976).

Figure 1a. Settled plurispores of A.japonicus. (525x)

Figure 1b. Settled unispores of A.japonicus. (525x)

Figure 1c. Germinating plurispores. (600x)

Figure 1d. Developing discs (from plurispores) with evacuated (e) and non-evacuated (n) germination tubes. (690x)

Figure 1e. Discs developed from unispores showing empty spore wall (s - arrow) and hair (h). (540x)

Figure 1f. Developing disc detached from base of dish with cluster of hairs (h), filament (f) and rhizoids (rh). (675x)

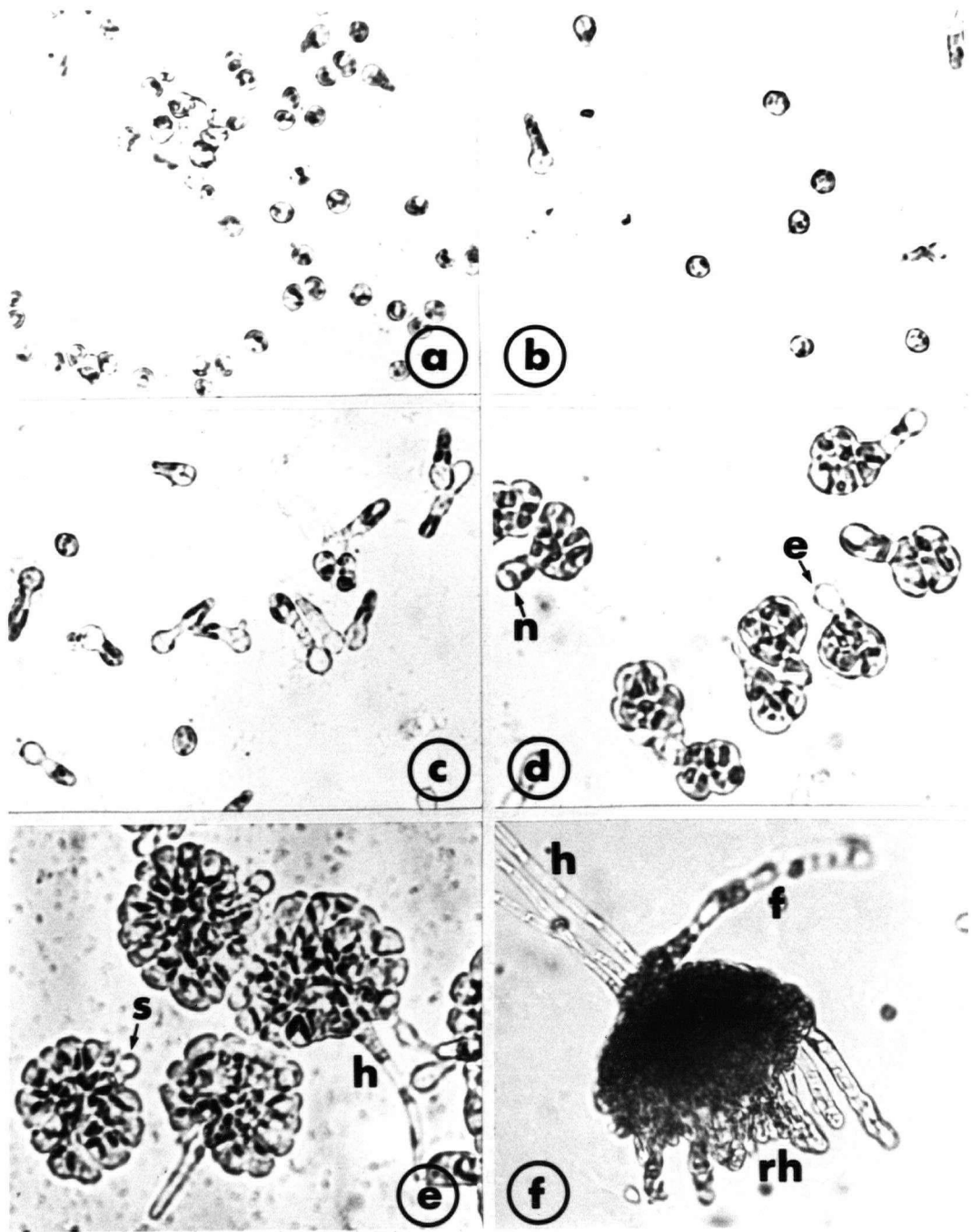


Figure 2a. Primary disc (pd) with filaments (f) and secondary discs (sd). (370x)

Figure 2b. Developing crusts on surface of coverslip. Crusts with secondary discs indicated by arrows. (2.75x)

Figure 2c. Developing erect axes from crust. Arrows point to transition region between crust and erect axis. (7.5x)

Figure 2d. Erect axes with lateral branches grown in culture. (1.5x)

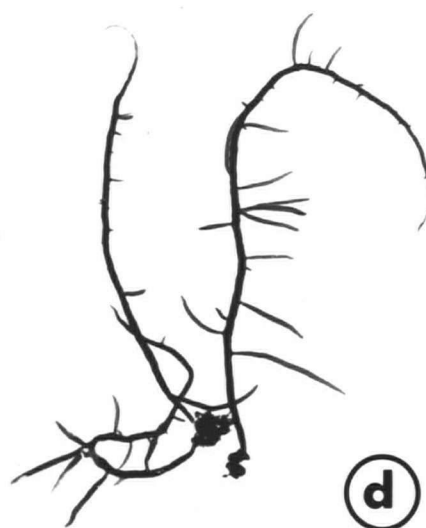
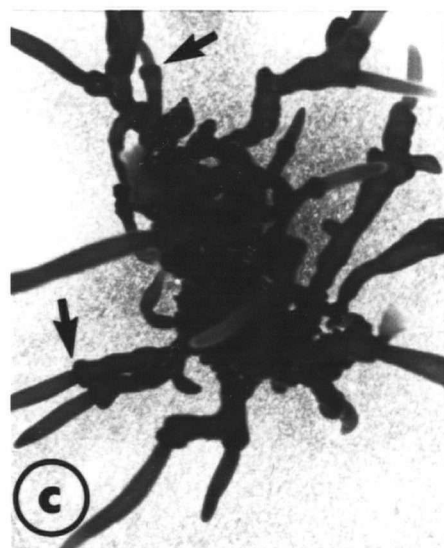
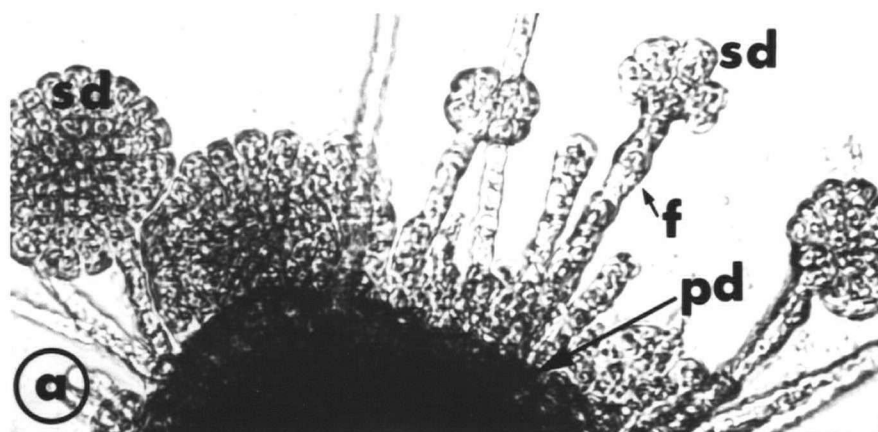


Figure 3a. Herbarium specimen of A. japonicus showing cleared lateral branches where plurangia have released swarmers.

Figure 3b. Crusts of A. japonicus. (2.75x)

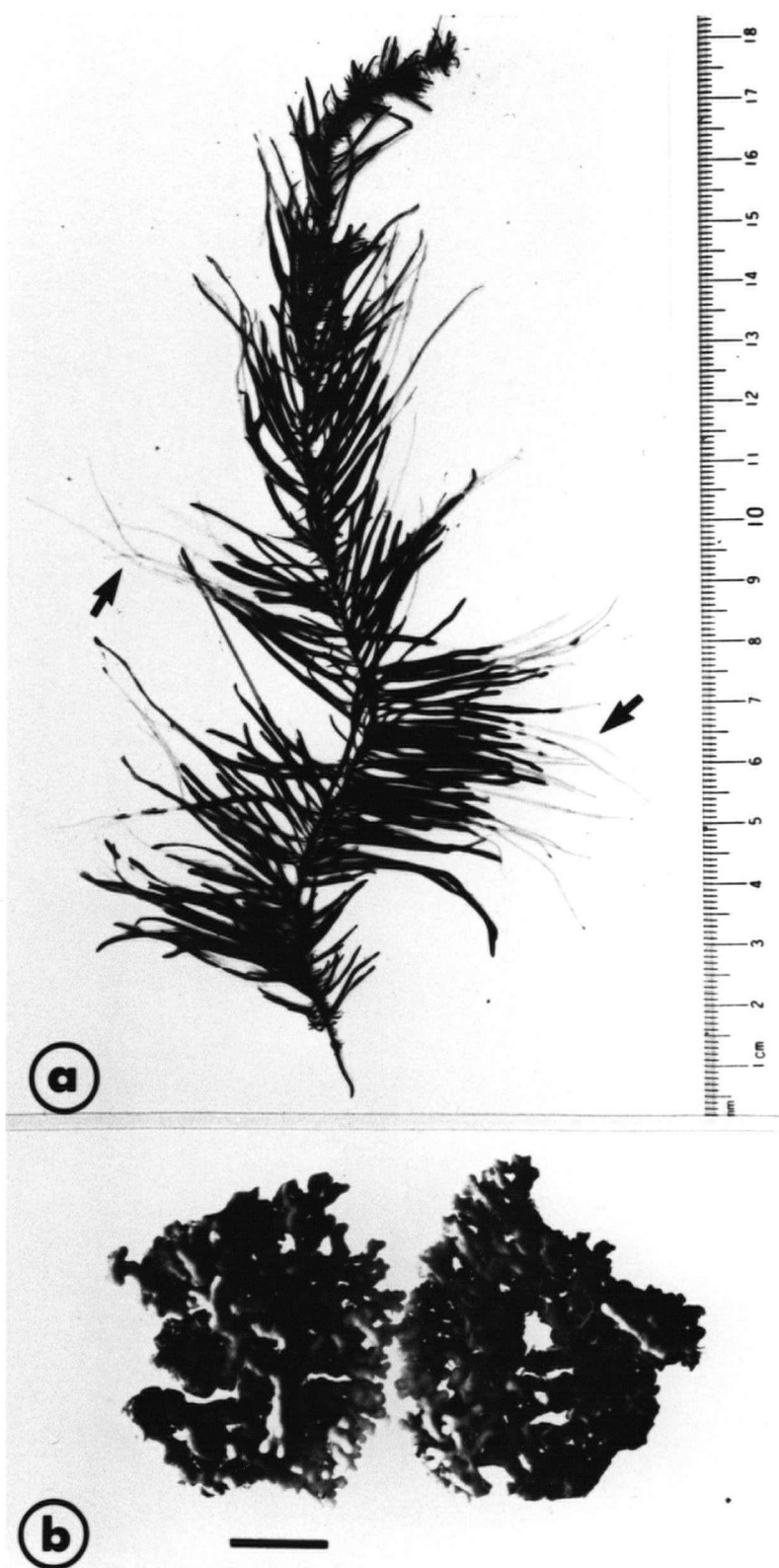


Figure 4a. Longitudinal section of A.japonicus crust. UC = upper cortex; M = medulla; LC = lower cortex. TBO stain. (260x)

Figure 4b. Cross-sectional view of crust with hairs (h) developing beneath cortex. TBO stain. (740x)

Figure 4c. Cross-section of overlying A.japonicus crust showing rhizoids (rh - arrows) developing from lower cortex. PAS stain. (250x)

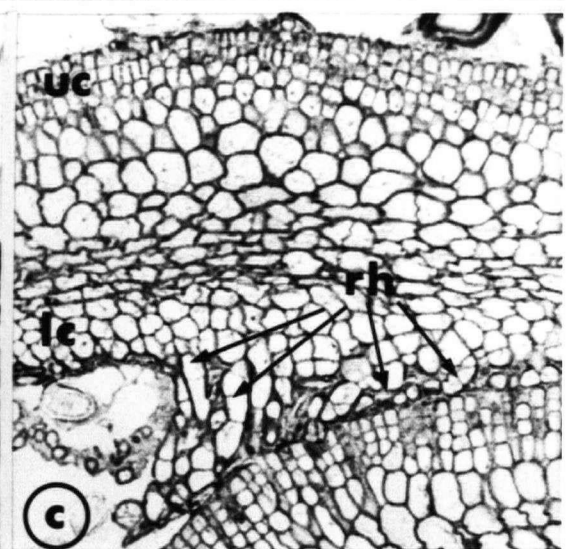
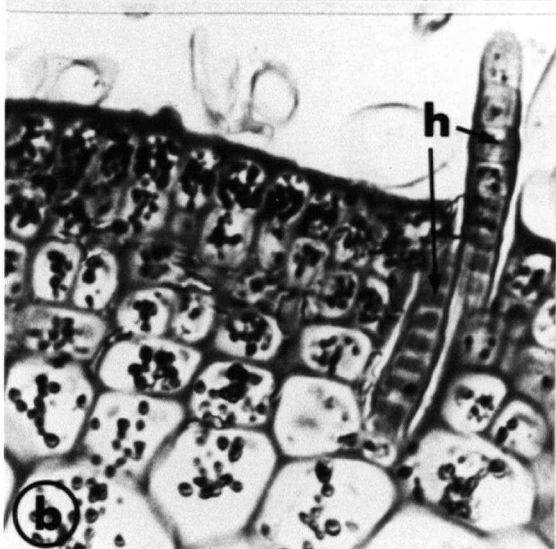
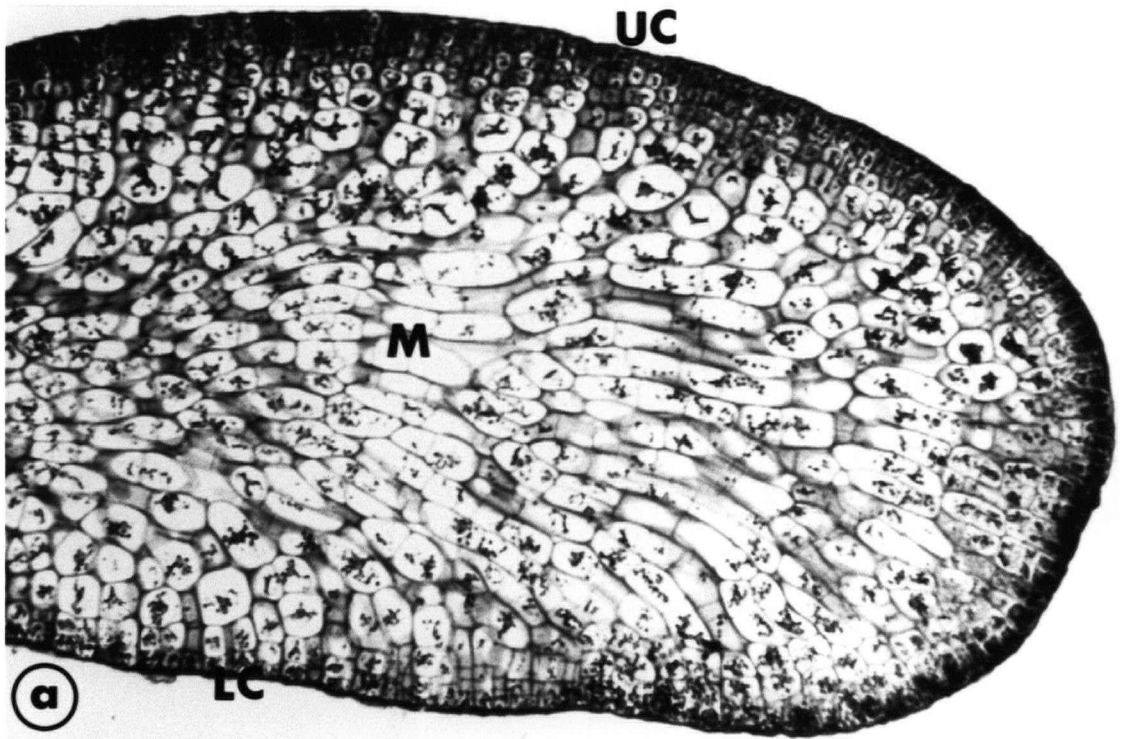


Figure 5a. Longitudinal section through apex of an erect axis of A. japonicus. Note hollowed central region (hc). TBO stain. (230x)

Figure 5b. Apex of erect axis of A. japonicus. Arrows point to zone of divisions at base of cortical filaments. TBO stain. (785x)

Figure 5c. Longitudinal section through region of transition between erect axis and crust. EM = erect axis medulla; CM = crust medulla; EC = erect axis cortex; CC = crust cortex. TBO stain (175x)

Figure 5d. View of transition between crust medulla (CM) and medulla of erect axis (EM). TBO stain. (475x)

Figure 5e. Longitudinal view of central region of erect axis showing medullary cells which are being split apart (arrow) as axis becomes hollow (hc). TBO stain. (475x)

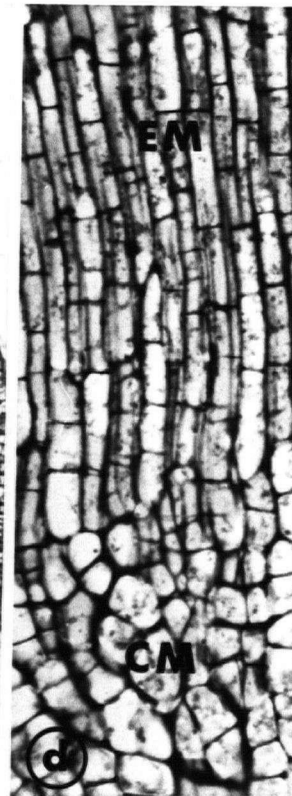
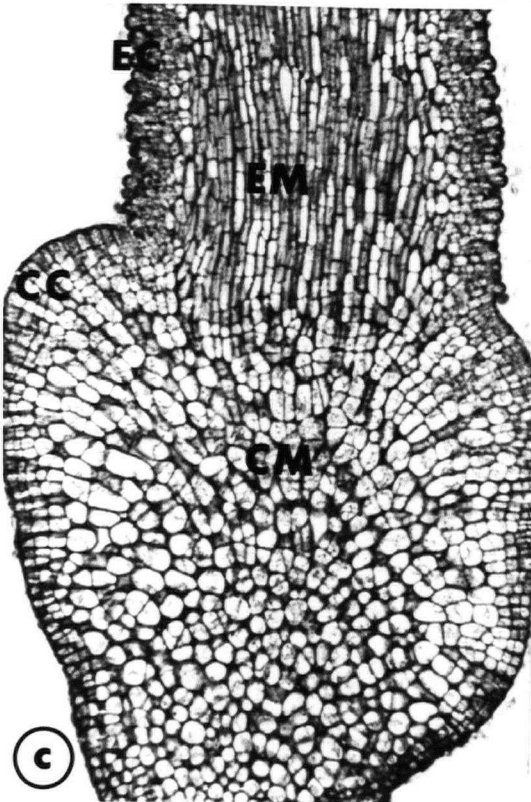
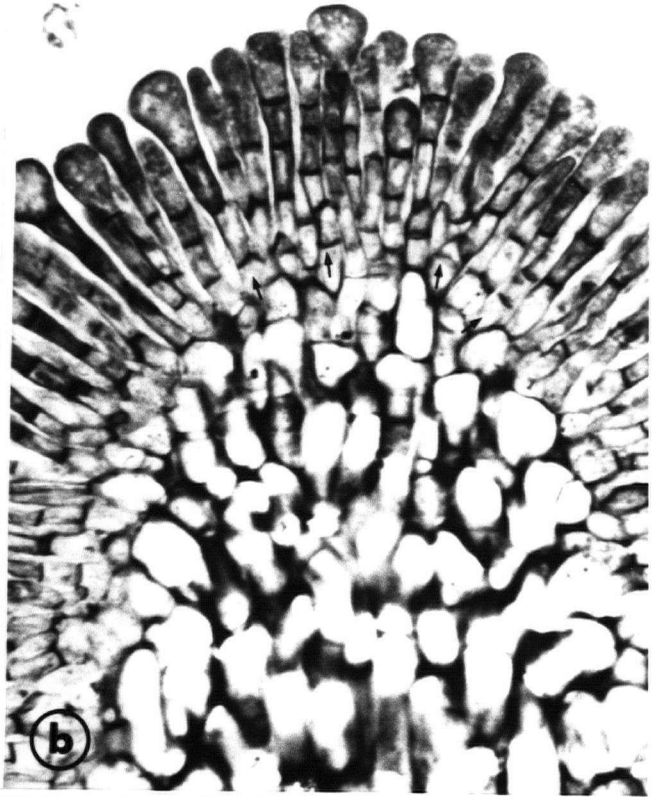
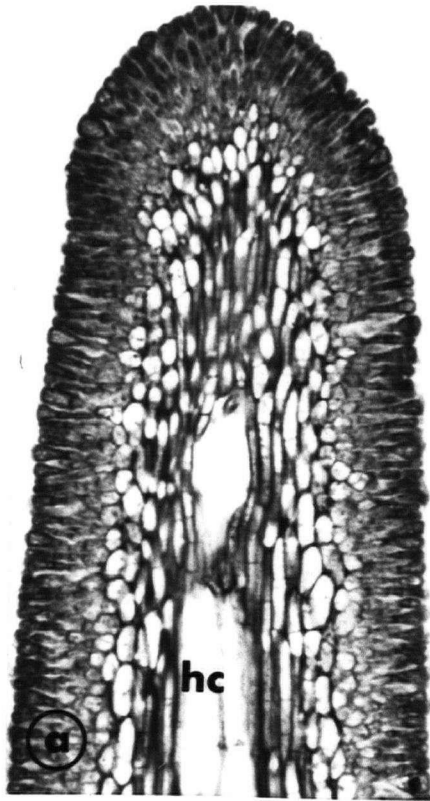


Figure 6a. Longitudinal section of a sterile erect axis of A. japonicus. C = cortex; SC = sub-cortex; M = medulla. TBO stain. (550x)

Figure 6b. Longitudinal section of erect axis showing developing lateral branch. Arrows point to zone of divisions in subcortex. TBO stain. (550x)

Figure 6c. Developing lateral branch. Arrows point to zone of divisions at base of cortical filaments in sub-cortex. TBO stain. (550x)

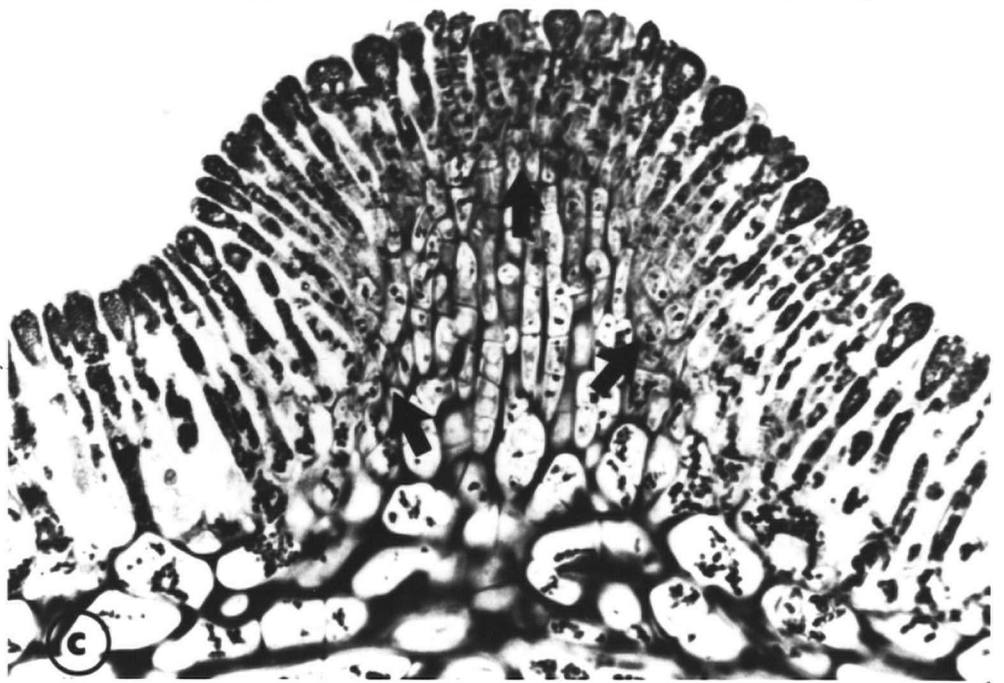
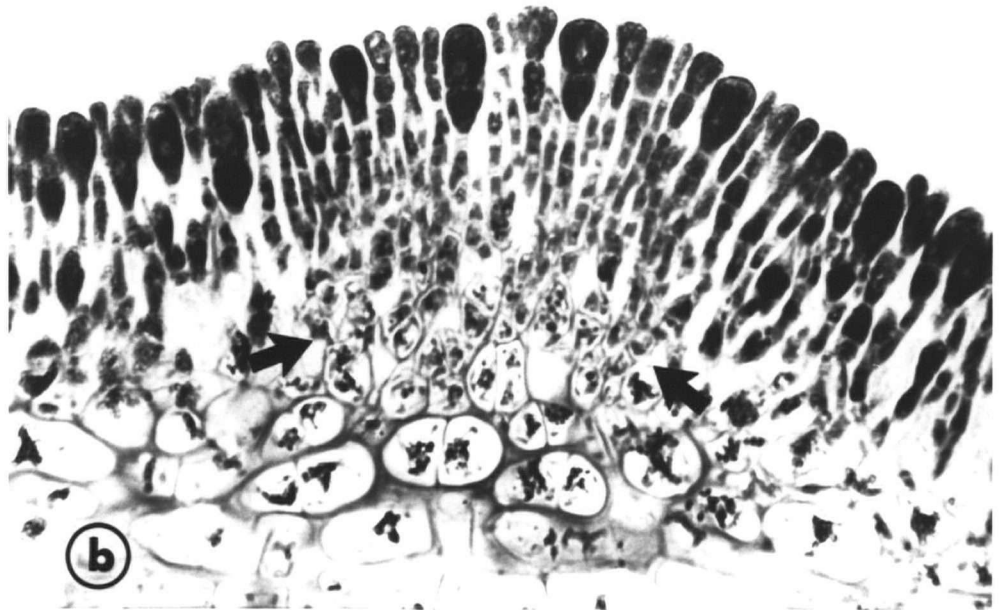
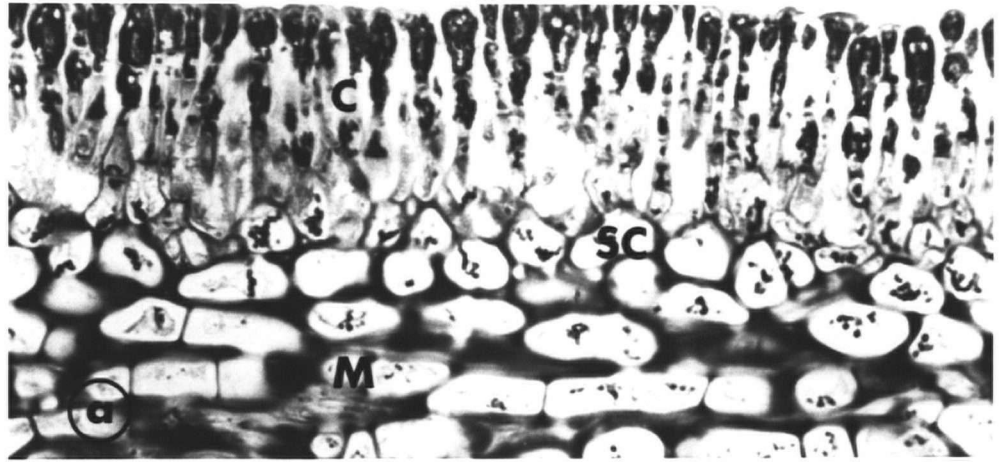


Figure 7. Secondary filament systems in erect axis of A.japonicus. Arrows point to secondary filaments; hc = hollow central region of axis.. (550x)

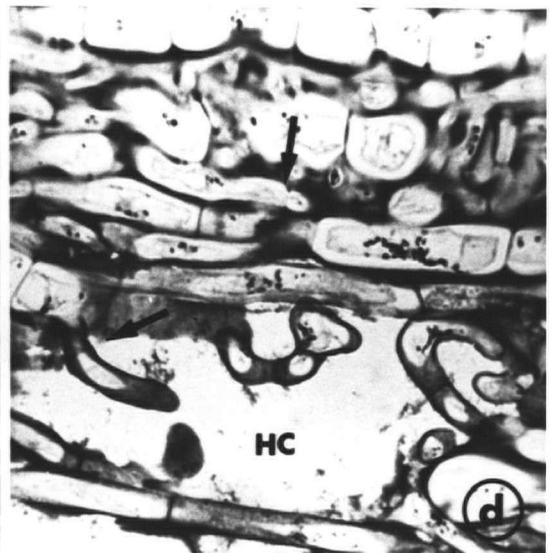
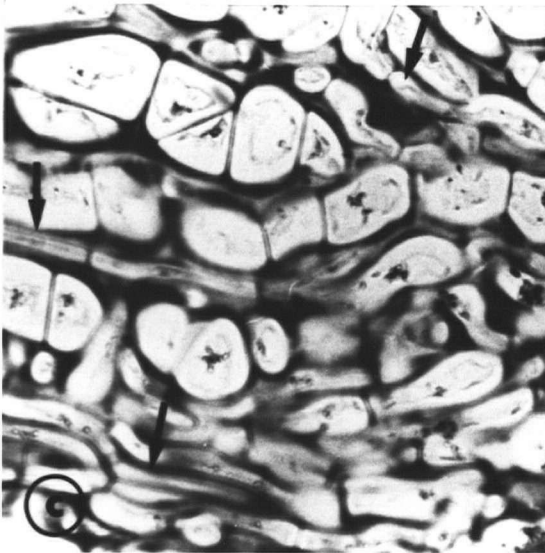


Figure 8a. Longitudinal section of unilocular lateral branch of A. japonicus. PAS stain. C = cortex; SC = sub-cortex; M = medulla; U = unilocular sporangia. (215x)

Figure 8b. Unilocular sporangia. TBO stain. SF = sterile filaments; 1,2,3,4 = numbers assigned to layers within the sporangia. (625x)

Figure 8c. Unilocular sporangia. PAS stain. (labels as for b.) (625x)

Figure 8d. Unilocular sporangia in cross-sectional view showing layers of staining. TBO stain. (625x)

Figure 8e. Unilocular sporangia in cross-section. AB stain. (625x)

Figure 8f. Unilocular sporangia in cross-section. PAS stain (625x)

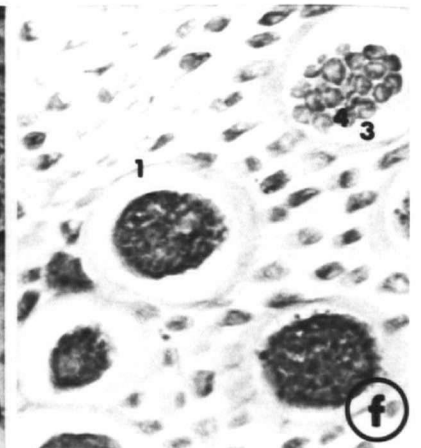
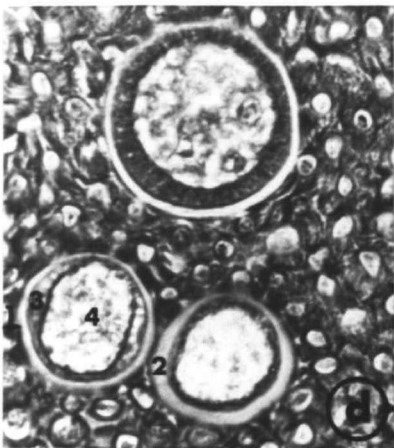
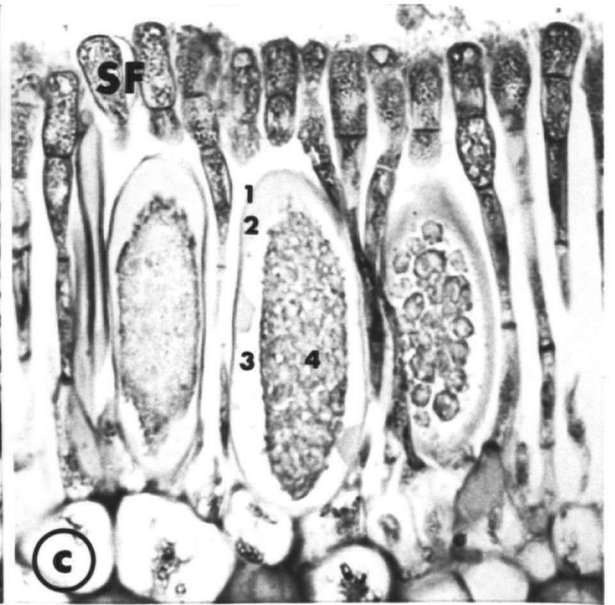
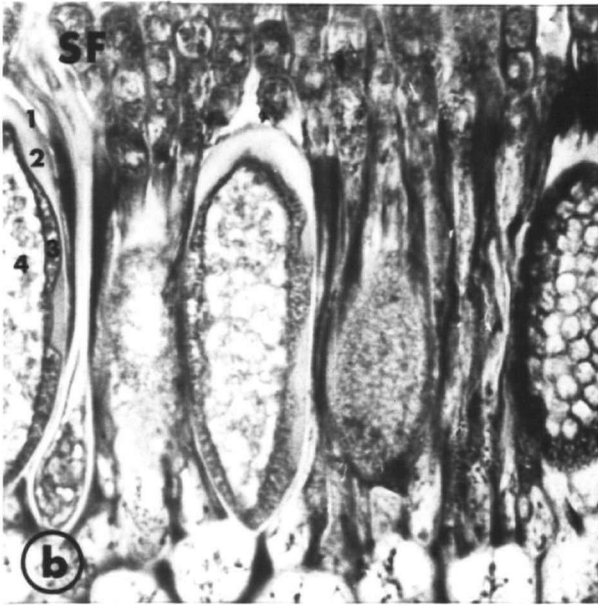
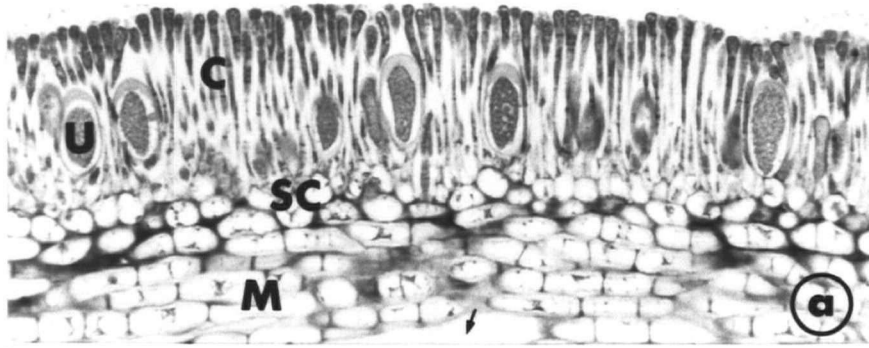


Figure 9. Release of spores from unilocular sporangia.
(b) arrow pointing to flap of sporangial wall torn
back during release. (750x)

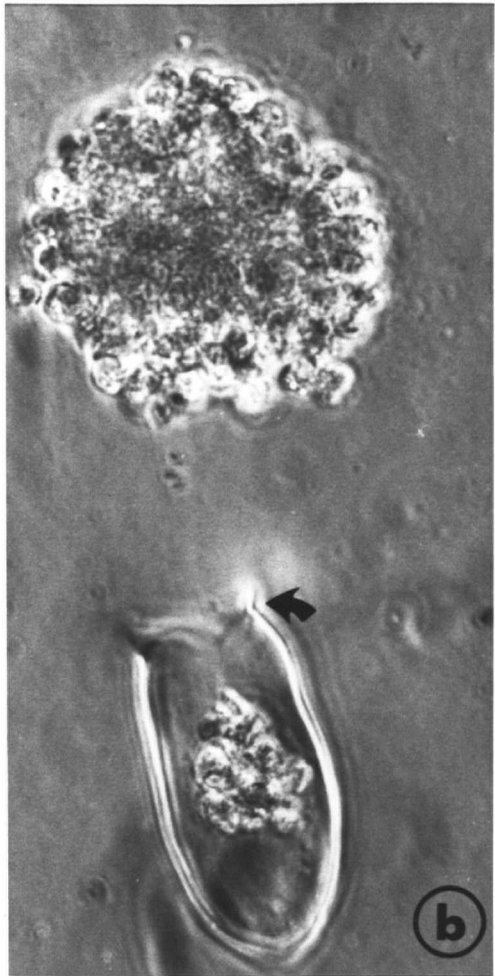
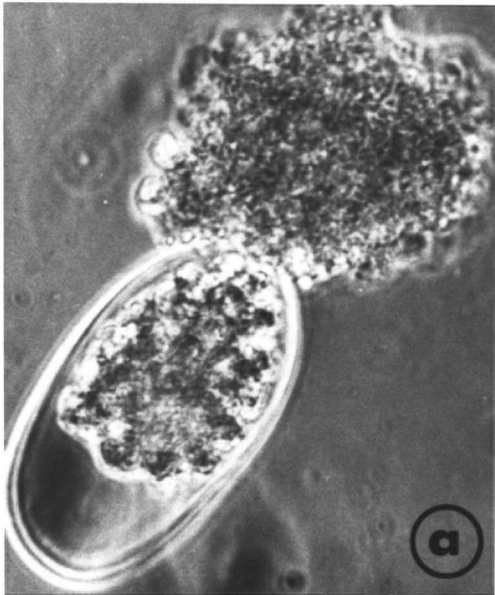


Figure 10a. Longitudinal section of plurilocular axis in A. japonicus. TS = terminal sterile cells; P = plurangia; SC = sub-cortex; M = medulla; hc = hollow central region of axis. PAS stain. (190x)

Figure 10b. Longitudinal section of plurilocular tissue grown in culture. S = spore germinating; large arrow points to thick surface layer of mucilage-like material. TBO stain. (650x)

Figure 10c. Section through lateral branch showing developing plurangia (dp) and hairs (h). PAS stain. (600x)

Figure 10d. Plurangia. PAS stain. (900x)

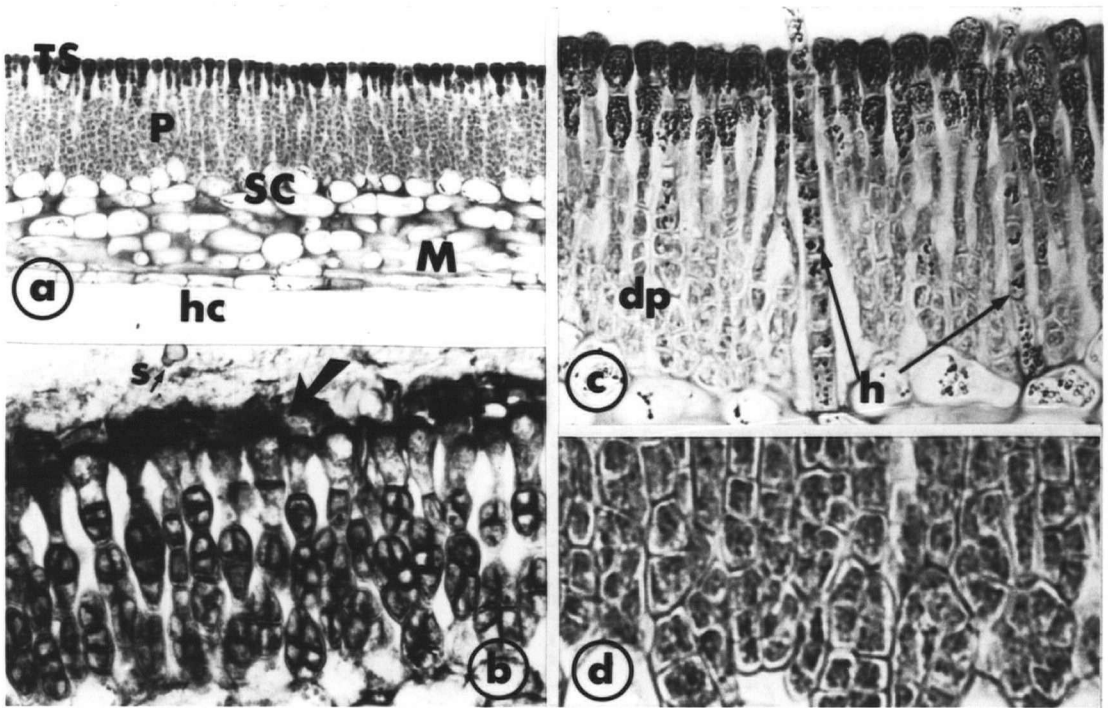
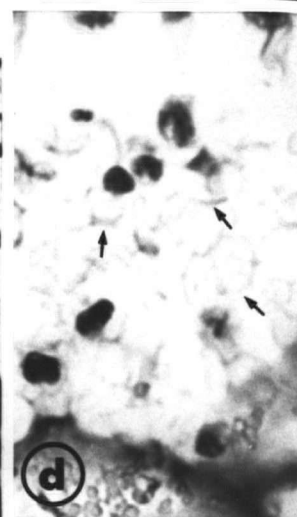
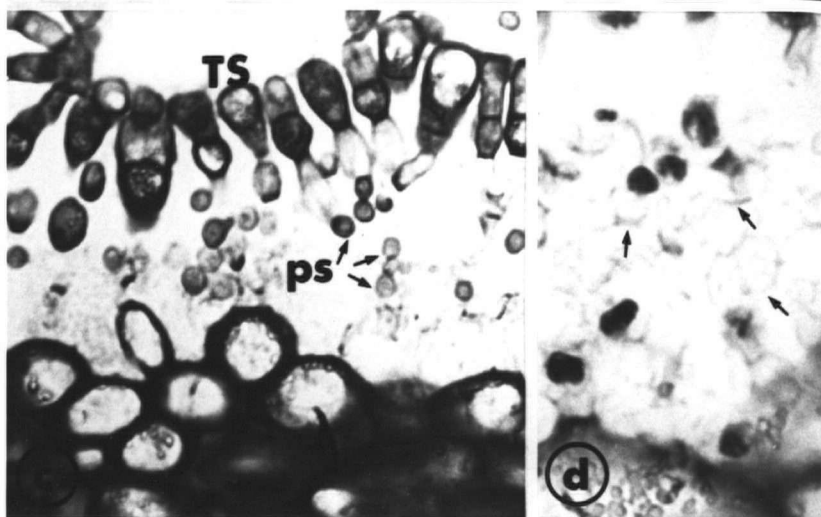
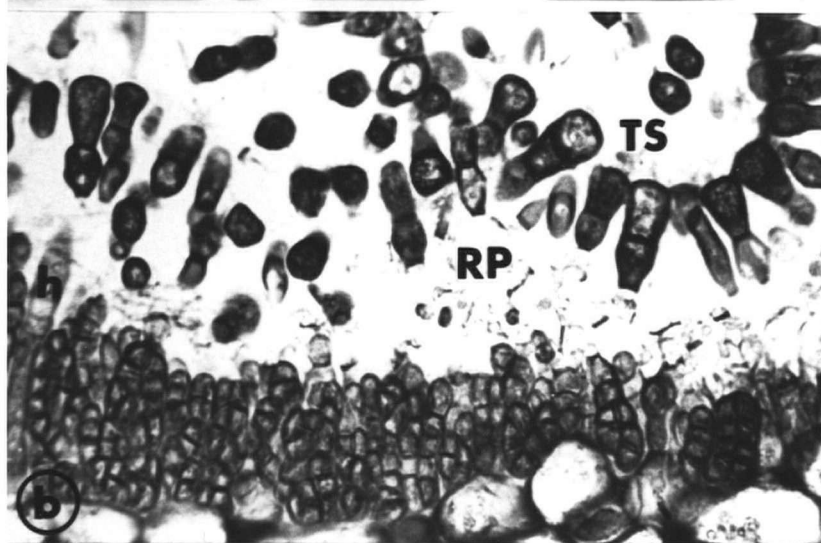
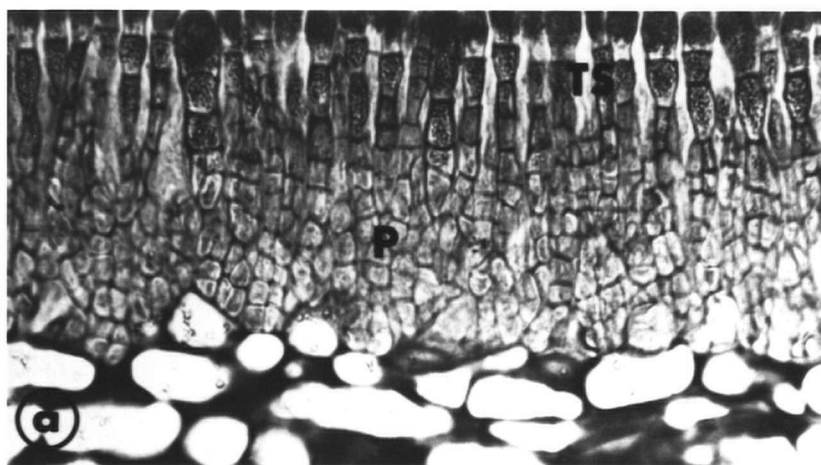


Figure 11. Plurangia and stages in their release.

- (a) mature plurangia
 - (b) release of swimmers has begun - terminal sterile cells (TS) curving back; hair (h) showing origin in the sub-cortex; RP = releasing plurangia
 - (c) plurangia have released their contents - some swimmers from the plurangia (ps) can be seen amongst the plurangial wall fragments.
 - (d) plurangial wall fragments (arrowed) remaining after release of swimmers.
- TBO stain. ((a)-(c) 550x, (d) 750x)



CHAPTER 2.

The phenology and distribution of Analipus japonicus
(Harv.) Wynne in the eastern Pacific

Introduction

Analipus japonicus is widely distributed in the northern Pacific having been recorded from Japan (the type locality), the Kuriles, Saghalien, the Japan Sea coast of Siberia, the sea of Okhotsk, Kamschatka, the Bering Sea, the Aleutian Islands, and the Pacific coast of North America from Alaska to Point Conception, California (Farlow, 1875; Okamura, 1910, 1933; Setchell and Gardner, 1903, 1925; Tokida, 1954; Inagaki, 1958). Despite this considerable geographic range there is little published on the phenology or ecology of this plant.

The observations which have been published on the phenology of A. japonicus - the growth of the upright axes from the crust and the seasonality of reproduction - are contradictory and suggest that A. japonicus exhibits different patterns of seasonal growth in different parts of its range:- Japan - "Sporangia of both kinds, March-May" (Okamura, 1910); "In May or June the fronds usually fall off from the base which remains adhering to the rocks and gives rise to new fronds again in September or October." (Inagaki, 1958); "...develops in Asamushi from winter to spring..." (Abe, 1935a); Saghalien - "This is one of the most common and ubiquitous seaweeds in Saghalien. It flourishes from early spring to late summer." (Tokida, 1954); California - "...erect axes disappearing in late fall, new axes arising from perennial base the following

spring." (Abbott and Hollenberg, 1976). When I began this study there was no published account or observations on the phenology of A.japonicus in British Columbia.

Descriptions of the habit of A.japonicus which have been published also vary considerably, describing it to be an alga which grows on rocks or stones in the upper intertidal (Okamura, 1910; Abbott and Hollenberg, 1976), in the middle or lower intertidal region (Setchell and Gardner, 1925; Kawabata, 1936; Nagai, 1940; Tokida, 1954), at or just below low water (Setchell and Gardner, 1903), or, "growing on rocks, stones, or sea mussels, very often in tide pools, between tide marks" (Inagaki, 1958). Taniguti (1962, 1978) describes A.japonicus as forming an important association in the intertidal region with Chordaria flagelliformis in northern Hokkaido in the coastal areas strongly influenced by drift ice. Scagel (1961) recorded the vertical distribution of A.japonicus in the intertidal region from three sites in the vicinity of Queen Charlotte Strait - Hope Island, Deer Island, and Klucksiwi River. These three sites represent a transition from high to low salinity, and also, from exposure to surf action at Hope Island to calmer waters at the other two sites. Scagel found that at Hope Island A.japonicus extends through a considerably larger vertical range and to a much higher elevation than at either of the other two locations, and attributed these vertical elevation differences primarily to the effect of surf action. From studies of

intertidal transects with differing aspect and wave exposure, Saito et al. (1971) concluded that A. japonicus (as Heterochordaria abietina) is a "sun form". They found it was not present at either the most exposed transect or the two most sheltered transects. From their discussion it is not clear why they chose to use the term "sun form" rather than stating that A. japonicus grew most abundantly at the sites of intermediate exposure. Atobe and Saito (1974) studying the effects of wave action on algal zonation at sites in Hokkaido, note that at least 10 algal species including A. japonicus exist at higher elevations along more exposed transects and at lower elevations along more sheltered transects.

In this section of my research I addressed the following questions about A. japonicus in British Columbia:- At what time of the year are the upright axes produced? What is the seasonality of reproduction? What proportions of plurilocular and unilocular plants are found in the field? After the first year of field studies in British Columbia, I extended the scope of this work and sought information about seasonal growth and reproduction of A. japonicus in other parts of its range particularly in the eastern Pacific.

Materials and Methods

1.A. Collections and Observations in British Columbia

Over a 27 month period, 85 collections were made of A. japonicus in British Columbia. This material came from 18 sites (shown in Figure 12 and 13) in the southern portion of the Province. (Refer to Appendix I for a complete list of collection sites with dates of collections.) Regular sampling occurred at three major sites - Bath Island in the Strait of Georgia, and Aguilar House Point and Second Beach, near the Bamfield Marine Station in Barkley Sound on the west coast of Vancouver Island. Whenever possible, collections consisted of more than 50 erect axes with crust material. The collections were preserved and stored in 2-5% formaldehyde/seawater.

Material from the three major sites was used for a detailed phenological study. The plant characteristics used in this study were measured according to the following procedure:-

From each collection, 25 upright plants were chosen at random. (Twenty-five was found to be a suitable sample size after running a test series increasing the sample size and measuring variation in the value of the mean height of the main axis.)

- 1) The number of axes per erect plant was recorded.
- 2) Each plant was scored for the presence/absence of laterals.

3) The height of the main axis was measured for each plant. Measuring $1/3$ of the main axis height down from the apex, the next 1 cm of the main axis was used for characteristics 4-6.

4) The number of laterals occurring in the 1cm of main axis was counted as a measure of lateral density.

5) The lengths of the first five laterals encountered were measured. A mean value for lateral length was calculated when there were three or more laterals measured.

6) The widths of the first five laterals encountered were measured, and a mean value calculated when there were three or more measured.

7) Either laterals or the main axis were sectioned to determine the reproductive status of the plant.

A total of 1125 plants were examined - 700 plants from Bath Island from collections made over 27 months, 225 plants from Second Beach from collections spanning 16 months, and 200 plants from Aguilar House Point collected over 13 months.

1.E. Collections and Observations outside British Columbia

Figure 12 shows the locations at which A. japonicus was collected during this study. In Appendix I, the collection data are given for the material of A. japonicus that came from sites outside British Columbia. An extensive collecting trip was made in late March 1979. Collections were made over an eight-day period from south of the

published southern limit of A.japonicus (Point Conception, California) to Oregon. Thus, beginning in the south at Carpenteria Reef (where no A.japonicus was found), 17 sites were visited at 15 of which A.japonicus was found and collected. Another collecting trip to California was made in early September 1979 when over a four-day period ten sites were visited from Piedras Blancas in the south to Moss Beach (San Mateo County) in the north, and A.japonicus was collected at all sites. Collections were preserved and stored in 2-5% formaldehyde/seawater. Whenever possible, collections consisted of more than 50 erect axes. Material was sectioned, and its reproductive status recorded.

2. Herbarium Survey

Herbarium specimens of A.japonicus were obtained from as many parts of its geographic range as possible. Material for this study came from the following herbaria:- University of British Columbia (UBC); University of California, Berkeley (UC), including specimens now housed at UC from the Dudley Herbarium, Stanford University (DH), and from the Missouri Botanical Garden (MO); Gilbert Morgan Smith Herbarium, Hopkins Marine Station (GMS); University of Washington (WTU); University of Michigan, Ann Arbor (MICH); Farlow Herbarium, Harvard University (FH); and Trinity College Dublin (the type material) (TCD).

Portions of lateral axes were sectioned and the reproductive status of the material was recorded. A total

of 397 specimens with erect axes were examined, with the sample size according to geographic location being as follows:- Alaska (93), British Columbia (191), Washington (33), Oregon (19), California (43), Japan (17), Kamschatka (1). Five specimens consisting of only the crustose portion of A.japonicus were also examined - Japan (1), British Columbia (1), California (3).

Results

1.A. Collections and Observations in British Columbia

From observations made of A.japonicus over the two year study period, it was found that in the southern part of British Columbia this plant is characteristic of moderately exposed to exposed rocky intertidal shores both on the west coast of Vancouver Island and in the Strait of Georgia. Generally it was found not to occur on loose rocks or boulders and was rarely found in tidal pools. The relative tidal height and the vertical extent of the A.japonicus range differed between sites. At some sites, for example Botany Beach (48°32'N, 124°27'W), A.japonicus occurred near Postelsia palmaeformis Rupr. in the upper intertidal region of an exposed shore, and at others, for instance Bath Island and Breakwater Island (49°09'N, 123°40'W), it extended into the lower intertidal/upper subtidal region and was found growing alongside

Cryptopleura violacea (J. Ag.) Kyl. and Lithothrix
aspergillum Gray. In general, A. japonicus occurred in the mid-intertidal regions, frequently on rocky headlands which were more exposed than the surrounding coves and shoreline.

The field site at Bath Island was exposed to surf action from the southeast, the predominant wind direction in winter months and for most of the year. Wind and storms were particularly severe in February of both years in which field work was carried out. Drift logs accumulated in the upper intertidal region at Bath Island. These were dislodged by high tides or storm waves and often caused considerable damage by battering and crushing the intertidal and upper subtidal flora and fauna. These effects of drift log damage were noted by Dayton (1971) in the intertidal shores of Washington and the San Juan Islands. In the summer the freshet of the Fraser River strongly influences the salinity of the Strait of Georgia causing a marked drop over the summer months in the minimum salinity values. (Refer to Appendix III for monthly salinity and temperature data for the Strait of Georgia.) Extreme low tides in the summer occur at Bath Island in the middle of the day (Anon, 1977, 1978, 1979), and the A. japonicus plants can be exposed for up to 5 hours at midday before being reimmersed. The smooth rock shelf of the intertidal region at this site provided little relief from direct sun or wave action as there are few cracks, crevices or shade areas and thus little habitat variation.

At Bath Island the A. japonicus population was distributed through the intertidal region from the upper midtidal to the upper subtidal - a vertical range of 2.75 metres.

The field sites at Aguilar House Point and Second Beach were exposed to surf action year round although the incidence of severe storms was higher in the winter months. (No mid-winter collections were made at Second Beach as winter storms prevented boat landing and access to the collection site.) Both sites were characterised by considerable topographic relief - crevices, cracks, rock faces with varying slopes, surface structure and aspect. They differed markedly in this from the field site at Bath Island. On the west coast, in summer the extreme low tides occur in the early morning. The intertidal regions where A. japonicus occurs are reimmersed by mid-morning. In summer and early autumn on the west coast, morning fog is a common, almost daily, occurrence. The fog serves to moderate the effect of the uncovering of the intertidal region at low tide. The salinity and temperature of the west coast waters are not subject to the extreme seasonal fluctuations that the waters of the Strait of Georgia experience. (Refer to Appendix IV for salinity and temperature data for the west coast of Vancouver Island.)

At both Aguilar House Point and Second Beach, A. japonicus was present on rocky headlands, with Hedophyllum sessile (C. Ag.) Setch. occurring just below the A. japonicus and below that, in the lower reaches of the

intertidal, Lessoniopsis littoralis (Tild.) Reinke, both plants characteristic of exposed habitats. The vertical extent of the A.japonicus zone in both west coast sites was more limited than that at Bath Island, ranging not more than 0.75 m, being found in the mid-upper intertidal and not extending into the lower reaches of this region. The west coast sites were characterised by a more diverse flora and fauna than present in the Strait of Georgia.

No plants of A.japonicus bearing unilocular sporangia were found in the British Columbian collections. Erect axes were present in all months of the year growing from the crusts of A.japonicus although not at all sites. Plants bearing plurangia were also found year round, although not at all sites.

In Figures 14, 15, and 16 the results of the phenological survey for mean axis height and for fertility are presented for the A.japonicus populations from Bath Island, Aguilar House Point and Second Beach, respectively. The summary provided by Table I gives descriptive measures of the samples of Bath Island, Aguilar House Point and Second Beach, respectively, collected from August 1978 to July 1979.

1.E. Collections and Observations outside British Columbia

All plants that were collected outside British Columbia were either sterile or plurilocular except for a small population of unilocular plants found at City Point

California. Dr. Lynda Goff collected an initial set of plants from City Point, California for me, as the only two unilocular plants collected in North America that I had seen were from that site. I collected at City Point two weeks after the first collection by Dr. Goff. The unilocular population was small and very restricted in its extent growing on the faces of some large and apparently unmoving boulders. The plurilocular population was extensive at City Point and appeared to be occupying a greater vertical extent at this site than the unilocular plants.

A feature of City Point and many of the sites visited in California and Oregon was the large amount of sand present nearby the rock shelves or headlands on which A. japonicus was growing. This was particularly noticeable in September in California when, at a number of sites visited, rocks with A. japonicus were partially covered by sand. In places the crustose basal system had been covered completely with only the upper portion of the upright axis and laterals protruding through the deposited sediments.

On the March collecting trip erect axes of A. japonicus were present at virtually all sites where crusts of the plant were found. A. japonicus is a characteristic, and sometimes prominent, component of the intertidal, in general occurring in the middle intertidal regions. On the September collecting trip, erect axes of A. japonicus were found at all sites where the crustose portion was found

except for Moss Beach. Average plant size was greater in September than in March. At both times all the populations sampled with erect axes possessed plurilocular plants.

2. Herbarium Survey

Results of the herbarium survey of A. japonicus are given in Figure 17 (except for the single specimen from Kamschatka - which was plurilocular and collected in August). The results, presented as histograms by regions indicate that there is not an even distribution of herbarium material from throughout the year for all parts of its range. This was a problem for material from Alaska - not surprising given the rigorous physical climate and difficulty of access for collecting - but also for material from Oregon and Washington. This can be attributed to an absence of collections rather than to absence of plant material in the field because this plant does perennate by means of the crustose base. The five specimens consisting of only the base were distributed as follows:- Japan (1-September), British Columbia (1-May), California (1-March, 2-May).

In surveying the specimens from throughout its geographic distribution, the range of plant size encountered was considerable. The largest plants seen were collected on San Juan Island (Washington) - these reached a length of 70 cm with laterals up to 6 cm and were plurilocular. The smallest fertile plants (plurilocular)

were less than 2 cm in height with laterals of 1-2 mm.

Unilocular specimens from North America were restricted to two localities, both very closely situated on opposite sides of a bay - Point Lobos and City Point.

Discussion

The populations of A. japonicus that were studied in British Columbia were found to have certain features in common. The populations showed great variability in plant size at virtually all times of the year. (Witnessing to this are the large standard deviations for the variables mean axis height, lateral length and lateral width - Figures 14, 15, and 16). Erect axes were found to be produced at almost all sites visited throughout the year and thus axis initiation is not synchronous or correlated with particular environmental cues (such as daylength or temperature). The results of culture work reported in Chapter 3 are also consistent with this observation. However, the extent to which the erect axes develop at various sites and the dynamics of the populations studied differed markedly. It seems likely that the variations in patterns of growth and reproduction at the different sites are largely due to differing physical and chemical regimes encountered at these sites.

The following is my interpretation of the events at

Bath Island, referring in particular to Figure 14. The decrease in mean axis height which occurred in both years in February/March can be attributed to increased wave action and possibly storm damage (log movement) in the winter. The largest plants in the population are probably the most vulnerable to tearing or damage because of their greater drag and resistance in the water. In spring and early summer the plants grew rapidly, probably due to increasing light, daylength, and warmer temperatures. The abrupt decrease in axis height in June-July can be related to a combination of factors. The two most important factors appear to be the lowered salinity in the Strait of Georgia (due to the influence of the Fraser River freshet), and the timing of the extreme low water tides (leaving the plants out of water for up to 5-6 hours in the hottest part of the day exposed to both the sun and wind). The marked decline in the general condition of the Bath Island A. japonicus population can be attributed to this combination of stresses. (Refer to Chapter 4 for more detail about this phase of the seasonal growth.) Although the population was clearly in a state of decline in late summer, erect axes were still being produced; their development, however, was apparently being inhibited by the prevailing environmental regime. By October and November the population had recovered, with the mean axis height again increasing. At this time of year, however, available light and temperature are decreasing and so although the plants are growing and

reproducing the maximal growth rates found in spring and early summer are not encountered.

At Aguilar House Point (Figure 15), the population dynamics of A. japonicus, as measured by mean axis height or by the percentage of the plants bearing plurangia, were found to be different from those just discussed for Bath Island. There was no summer decline in the Aguilar House Point populations - in fact, the population showed an increase in mean axis height at the same time that the Bath Island population was at its lowest ebb. At Aguilar House Point, however, there was a decline in the mean axis height over the winter months, and I attribute this to the effects of the rigorous physical environment and storm and wave damage. The tidal cycle, specifically, the timing of the low tides in summer, and the topographic variation at the site, resulted in much less stressful summer conditions than found at Bath Island. Thus, summer conditions at this west coast site were apparently favourable to growth of A. japonicus and allowed the population to reach its highest levels of fertility. The seasonal pattern occurring at Second Beach (Figure 16) appears to be very similar to that at Aguilar House Point - showing the best growth in the summertime. Unfortunately, due to the absence of mid-winter data for this site, a complete picture of the growth of this population cannot be constructed.

The interpretation presented here correlates well with the population variations observed in the course of this

study. It could also help to explain the apparently contradictory reports on A.japonicus phenology in the literature which may be merely reflecting the ability of this plant to grow in varied habitats and its differing response to different physical regimes. This interpretation could be tested by a number of procedures possibly the most direct of which would be reciprocal transplant experiments between populations of A.japonicus from the Strait of Georgia and west coast sites.

A number of workers have recorded the vertical elevation of intertidal plants and animals from all zones of the shore with increasing wave action (e.g. Moore, 1935; Jones and Demetropoulos, 1968; Morton and Miller, 1968). Although at some sites the vertical position of A.japonicus appears to be raised by exposure to wave action (e.g. at Botany Beach), I do not think that the vertical range of this plant in the intertidal region can be entirely explained by relative exposure to wave action. The presence of A.japonicus from high to low intertidal zones shows this plant to have considerable ecological flexibility. In British Columbia, A.japonicus showed the greatest distribution within the intertidal region in the Strait of Georgia. On the west coast, the extent of the intertidal distribution of this plant was rather limited.

In those intertidal organisms where vertical limits of distribution have been studied, both physical and biological factors have been shown to be important. It is

generally understood that the effects of the physical environment predominate in the upper reaches of the intertidal, and as the physical environment becomes relatively less harsh lower in the intertidal region, biological factors become increasingly important in determining the species niche width (Connell, 1972; Chapman, 1973a, 1974; Lubchenco, 1980). For algae, the main factors implicated in determining the lower limits of a species' vertical distribution are competition and herbivory (Dayton, 1975; Hruby, 1976; Lubchenco, 1980). One explanation for the differences described here in the vertical range of A. japonicus in British Columbia, is that A. japonicus is competitively more successful in obtaining substrate (space being the resource which is so critical to the success of sessile intertidal inhabitants) in the less diverse flora and fauna of the Strait of Georgia. In the Strait of Georgia few representatives of the order Laminariales are found. At west coast sites, the kelp zone which occurred below that of A. japonicus is well developed and provides a canopy in the lower intertidal. Possibly the shade cast by these plants, a whiplash affect of the blades moving with wave action, or greater ability of these or other algae or sessile animals to colonise and maintain occupancy of substrate inhibits the extension of A. japonicus into the lower reaches of the intertidal region at these sites. It would be very interesting to test experimentally the role of competition in determining

A. japonicus' lower intertidal limits (e.g. by selective removal of certain species, by substrate clearance or by transplanting A. japonicus from other parts of its distribution and vertical range). Varying competitive success at different sites would go some way to explaining the apparently contradictory comments in the literature about the habit of this plant.

The morphology of A. japonicus specimens surveyed in the herbarium study and in the material which I collected was found to vary considerably. When plants which are 70 cm tall, with laterals up to 6 cm in length and bearing plurangia (e.g. from San Juan Island, Washington, in June) are placed next to plants with erect axes less than 2 cm in height with laterals of barely 2 mm (e.g. from Botany Beach, Vancouver Island, in March) and also bearing plurangia, one is confronted very graphically with the extremes of this variation. The morphology appears to be affected by the habitat experienced and the three B.C. populations which were studied reflect a certain amount of this variation (e.g. in the maximum height attained by the erect axes in the sample, the density of lateral branching and the length and width of laterals - Table I). One characteristic which was found to be very consistent was the axis number. Where branched axes occurred it appeared to be the result of damage to the apex resulting in one or more laterals taking over the apical function and in turn developing laterals.

No definitive statements can be made about the phenology of A.japonicus based solely on the herbarium material examined here. From the data available, however, both from my collections and the herbarium survey, it appears that the phenological patterns are similarly varied in other parts of A.japonicus' range. The occurrence of specimens with erect axes from California collected in ten months of the year (with plurilocular plants in all of these months) shows that the plants do not exhibit exclusively the phenological pattern given in Abbott and Hollenberg (1976) although this may be the predominant one.

The most unexpected finding of this phenological study was the distribution of reproductive structures and the absence of unilocular sporangia (or at least their extreme rarity) over the majority of the range of this plant. The work presented in Chapter 3 shows that the plurilocular plants are haploid, but apparently the swarmers from the plurangia are not functioning as gametes over the majority of the range of the plant.

For each of the three sites studied in British Columbia the proportion of plants bearing plurangia in the population samples closely followed the trends shown by mean axis height. The high incidence of fertile plants in the field and in the herbarium material examined, coupled with the observation (Chapter 1) that each plurilocular plant produces a very large number of reproductive units, indicates that there is a considerable investment of energy

in reproduction. The rapid dieback of the post-reproductive plants further emphasises the primary role of reproduction for the erect axes of A. japonicus.

Figure 12. Map indicating collection sites in British Columbia, Washington, Oregon, and California.

BRITISH COLUMBIA:-

- 1) Bath Island
- 2) Breakwater Island
- 3) Aguilar House Point
- 4) Brady's Beach
- 5) Diana Island
- 6) Haines Island
- 7) Helby Island
- 8) Ross Islets
- 9) Seppings Island
- 10) Second Beach
- 11) Wizard Rock
- 12) Pachena Bay
- 13) Botany Beach
- 14) Sombrio River
- 15) Sooke
- 16) Victoria Breakwater
- 17) Sidney

WASHINGTON:-

- 18) Cattle Point, San Juan Island
- 19) Mar Vista, San Juan Island
- 20) Kalaloch

OREGON:-

- 21) Fogarty Creek
- 22) Boiler Bay
- 23) Marine Gardens
- 24) Devil's Punchbowl
- 25) Yaquina Head
- 26) Cape Arago
- 27) Sunset Beach
- 28) Harris Beach

CALIFORNIA:-

- 29) Trinidad Point
- 30) Westport
- 31) Point Arena
- 32) Anchor Bay
- 33) Moss Beach
- 34) Pescadero Beach
- 35) Pebble Beach
- 36) Pigeon Point
- 37) Davenport Landing
- 38) Point Joe
- 39) Point Pinos
- 40) Stillwater Cove
- 41) City (Mission) Point
- 42) Piedras Blancas
- 43) Montana de Oro

Figure 13. Map of southern side of Barkley Sound indicating locations of study sites near the Bamfield Marine Station.

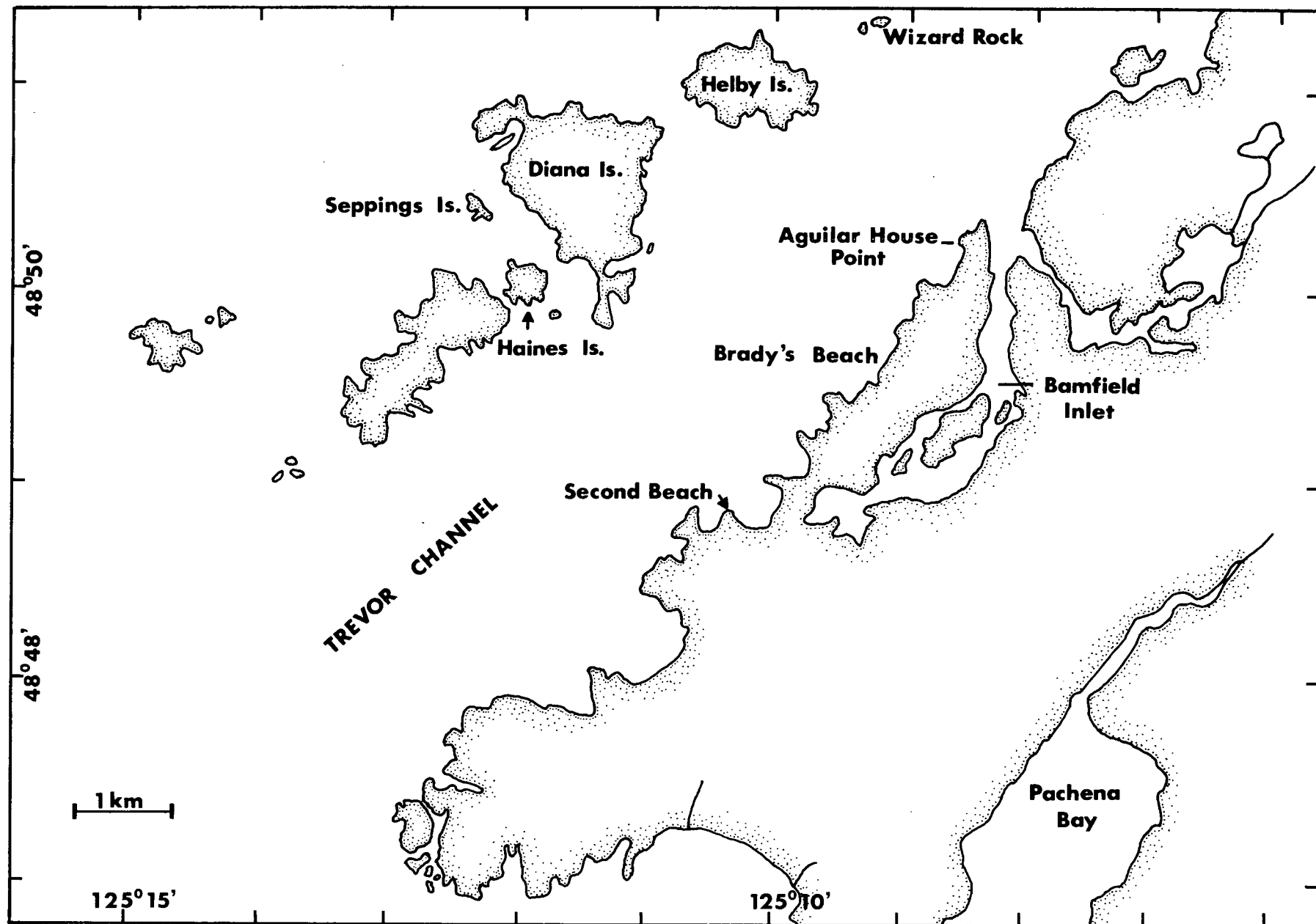


Figure 14. Mean axis height and percentage of plurilocular plants in collections of A. japonicus from Bath Island between July 1977 and August 1979. Error bar indicates one standard deviation of mean.

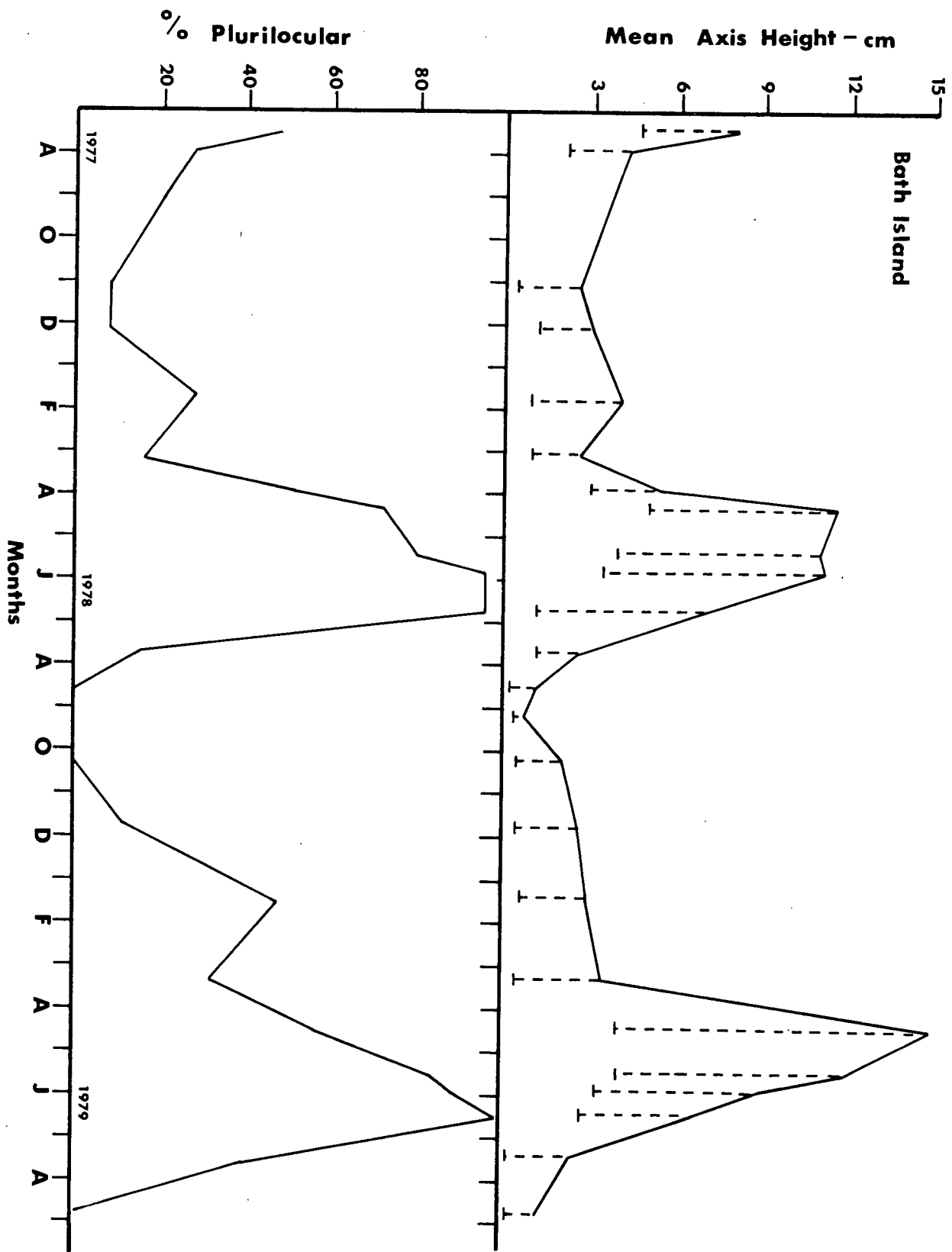


Figure 15. Mean axis height and percentage of plurilocular plants in collections of A. japonicus from Aguilar House Point between August 1978 and August 1979. Error bar indicates one standard deviation of mean.

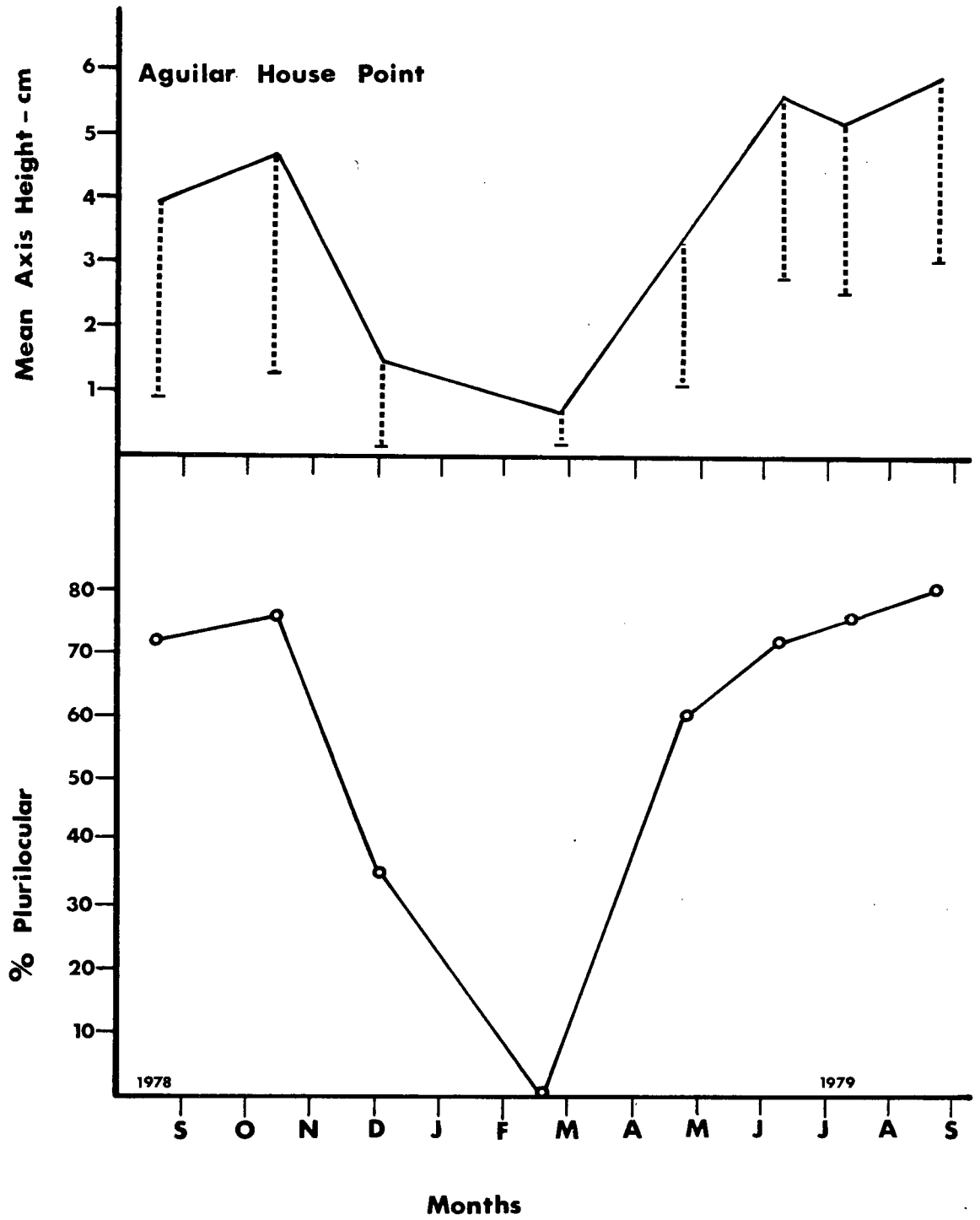


Figure 16. Mean axis height and percentage of plurilocular plants in collections of A. japonicus from Second Beach between May 1978 and August 1979. Error bar indicates one standard deviation of mean.

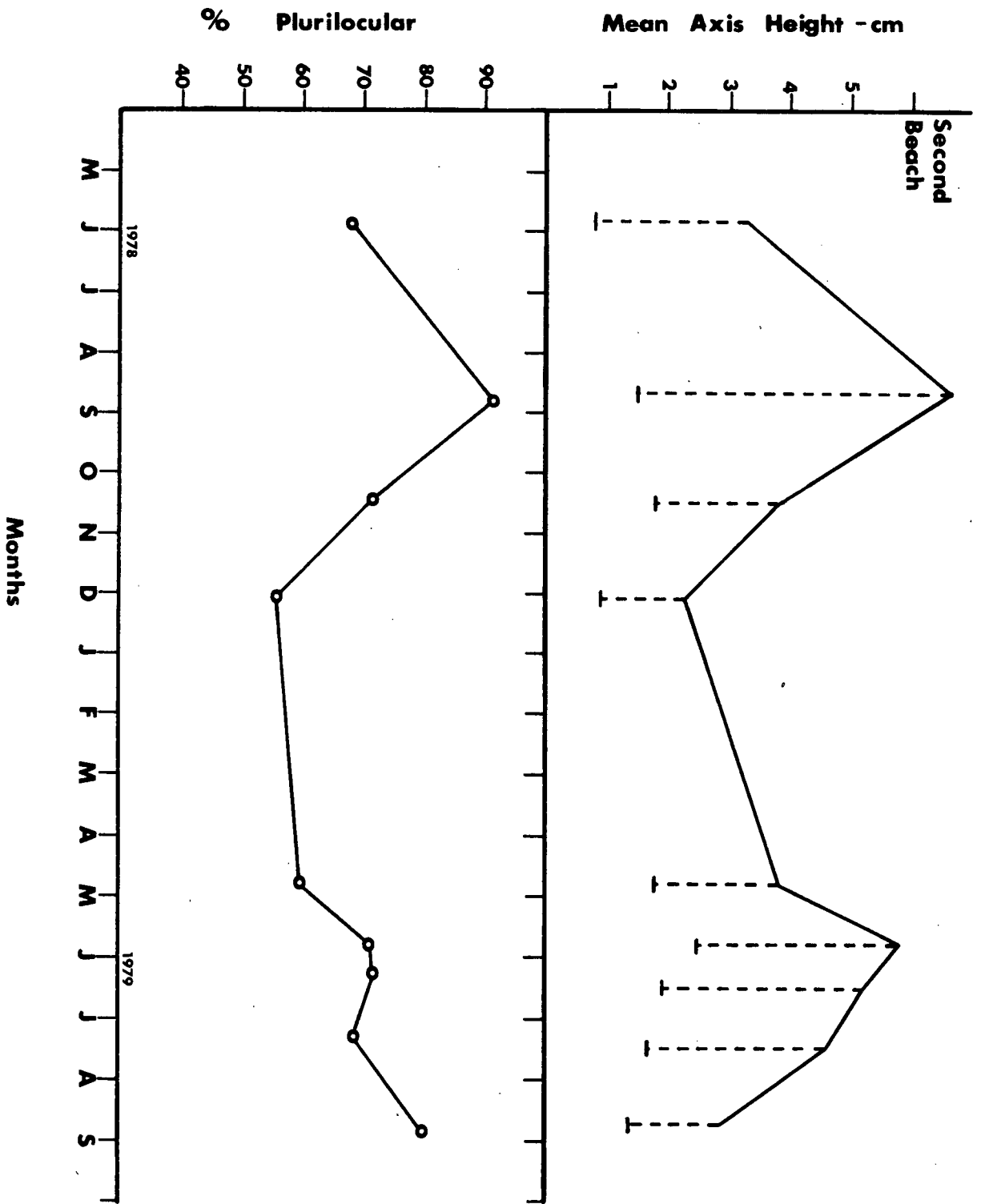


Figure 17. Herbarium survey results showing numbers of sterile, unilocular, and plurilocular specimens examined for each month for six regions of A.japonicus' range.

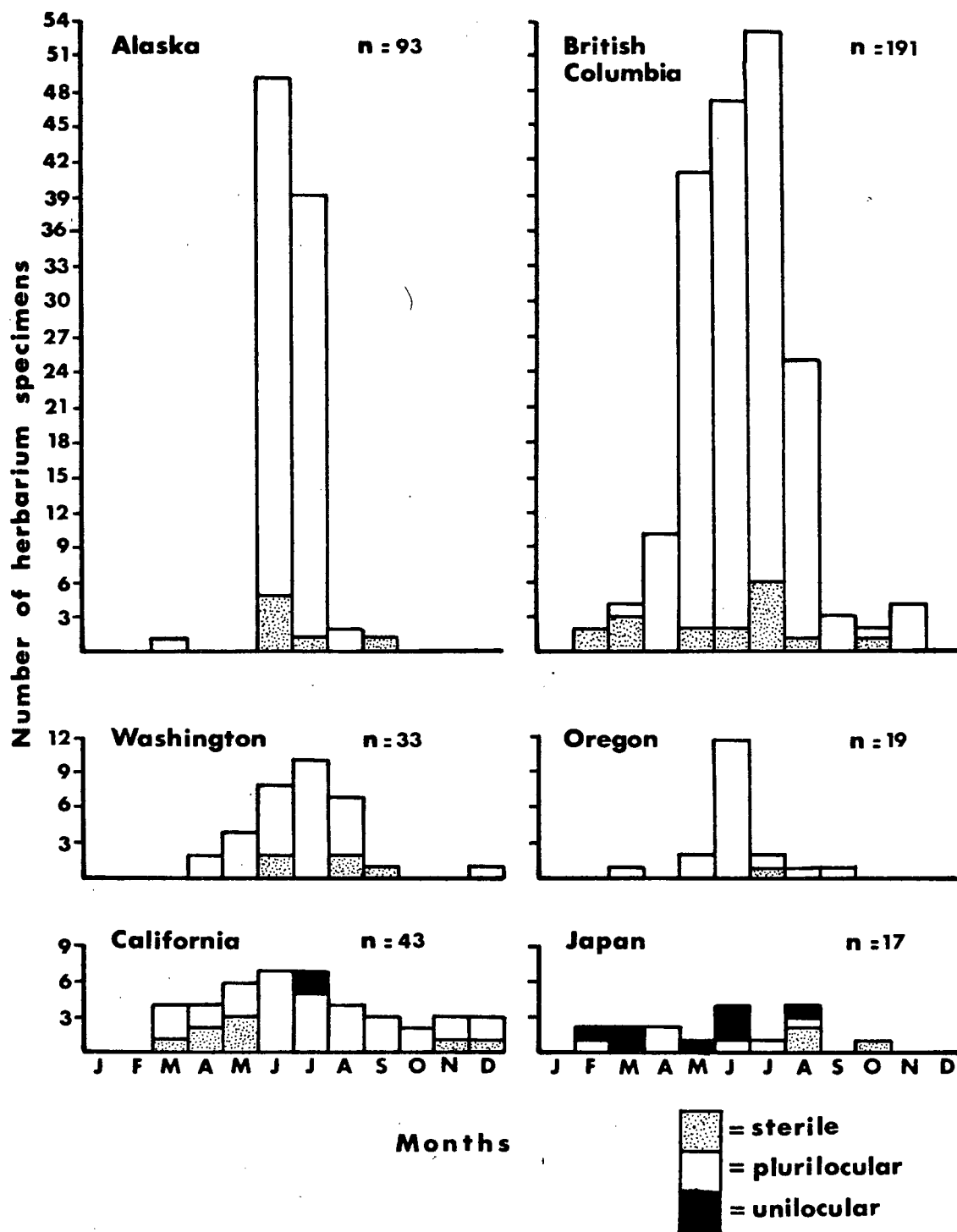


TABLE I:- Descriptive measures of A. japonicus sampled from August 1978 to July 1979 at 3 sites in British Columbia. (Refer to the text for measurement procedures.)

BATH ISLAND

VARIABLE	SAMPLE SIZE	MIN.	MAX.	MEAN	STANDARD DEVIATION
axis:-					
number	250	1.0	2.0	1.03	0.176
height(cm)	250	0.1	33.9	4.70	6.18
lateral:-					
density	250	0	36	5.66	9.14
length(cm)	84	0.1	1.86	0.53	0.39
width(cm)	83	0.05	0.11	0.061	0.014
fertility	250 - 63% sterile, 37% plurilocular				

AGUILAR HOUSE POINT

VARIABLE	SAMPLE SIZE	MIN.	MAX.	MEAN	STANDARD DEVIATION
axis:-					
number	170	1.0	4.0	1.06	0.35
height(cm)	170	0.1	15.0	3.63	2.98
lateral:-					
density	151	0	75	18.55	17.58
length(cm)	104	0.1	1.10	0.41	0.24
width(cm)	104	0.05	0.14	0.071	0.025
fertility	170 - 42% sterile, 58% plurilocular				

SECOND BEACH

VARIABLE	SAMPLE SIZE	MIN.	MAX.	MEAN	STANDARD DEVIATION
axis:-					
number	175	1.0	2.0	1.02	0.13
height(cm)	175	0.4	22.5	4.62	3.34
lateral:-					
density	175	0	83	22.33	19.50
length(cm)	129	0.1	1.46	0.46	0.29
width(cm)	128	0.05	0.16	0.073	0.027
fertility	175 - 29% sterile, 71% plurilocular				

CHAPTER 3,

Life history and cytological studies of Analipus japonicus
(Harv.) Wynne

Introduction

Prior to this research, the life history of Analipus japonicus had not been observed in culture, and although Abe, in reporting the results of cytological studies on A. japonicus in Japan (Abe, 1935a,b; 1936), stated that it exhibits an alternation of isomorphic generations, this had not been fully accepted. Bold and Wynne (1978) suggested that Abe's work needed verification. Further emphasis of the need for a life history study of A. japonicus is given by the recent placement of this plant in the Ralfsiales (Nakamura, 1972; Bold and Wynne, 1978). The life history pattern is one of three criteria used to distinguish this order and yet to date a complete life history involving sexuality has not been shown for any member of the order.

The growth of A. japonicus in culture studies has been reported on by Ikari (1928), Abe (1935a) and Wynne (1972), but these were restricted to the early stages of disc growth. Plants with erect axes have not been grown in culture, and thus the development of reproductively mature plants has not been observed in culture either.

Abe published three papers on the cytology of A. japonicus. Abe (1935a) reported that A. japonicus exhibits an alternation of isomorphic generations, with dioecious plurilocular plants producing swarmers which function as gametes. He reported that the female swarmers are larger than the male, and only the female swarmers can develop

without fusion. Prior to fusion, he also reported that the male gametes swarm about the female gametes. Abe (1935a and b) reported that swarmers from unilocular sporangia could develop with or without fusion and when fusion occurred it could be between swarmers from the same individual or from the two kinds of unilocular plants which he recognised. Ikari (1928) did not observe fusion of swarmers from unilocular sporangia. Abe (1936) reported that the unilocular sporangia are the site of meiosis in A. japonicus and that the unispores and the plurilocular plants are haploid with a chromosome number of $n=20$. He (1936) concluded that A. japonicus exhibits an alternation of isomorphic generations but that when the swarmers from unilocular organs fuse, or swarmers from plurilocular organs develop without fusion, the alternation of generations is suppressed.

Kylin (1937) noted that if A. japonicus does have an alternation of isomorphic generations and the plurilocular plants function as gametophytes, as suggested by Abe, this would mean that it would have to be placed in the Ectocarpales and not in the Chordariales. However, Kylin (1940) continued to place A. japonicus (as Heterochordaria) in the Chordariales, suggesting that it is analogous to Caeridium antarcticum. He (1940) stated that the possibility cannot be dismissed that plurangia of both these plants are diploid and function as vegetative reproductive structures - that is, the same pattern

exhibited by other members of the Chordariales.

In this chapter I discuss the results of culturing and cytological observations on A.japonicus. I wanted to observe the life history of A.japonicus in culture, and to determine: (1) the effect of temperature and photoperiod on the growth of the crustose phase and the production of erect axes; (2) under what conditions the plants become fertile; (3) the response of the plants to various salinity and temperature regimes; and, (4) the effect of light quality on germination and early development. I also wanted to find out if different populations of plurilocular A.japonicus are interfertile and to test Abe's claim that A.japonicus is a strictly dioecious plant, with two distinguishable forms of plurilocular plants corresponding to female and male. In addition, I wanted to determine if fusions occurred between swarmers from unilocular sporangia, a controversial claim made by Abe (1935a,b) and if I could also recognise the two types of unilocular plants distinguished by Abe. In order to test Abe's claim that A.japonicus exhibits an alternation of isomorphic generations, I needed to determine if there was an alternation of caryological generations between unilocular and plurilocular individuals.

Materials and Methods

A. Culture Work

Cultures of A. japonicus were established using fertile field material with either plurilocular or unilocular organs. Plurilocular plants, used to initiate cultures, came from a number of locations:- British Columbia - Aguilar House Point, Bath Island, Botany Beach, Diana Island, Helby Island, Second Beach, Sooke; Washington - Cattle Point, Mar Vista; Oregon - Boiler Bay, Cape Arago, Fogarty Creek, Marine Gardens, Yaquina Head; California - City Point (Mission Point), Point Pinos. The field material with unilocular sporangia came only from City Point, California. (Refer to Appendix I for co-ordinates of collection sites, and to Figure 12 for a map of the collection range.)

The isolation procedure usually employed was the hanging-drop technique of Wynne (1969). On some occasions when field material was immersed in seawater upon arrival at the laboratory, swarmer release would be extremely rapid and dense. In these cases, drops of the swarmer suspension were pipetted onto either glass slides or coverslips, and the swarmers allowed to settle in a moist chamber created by damp paper towels surrounding the slides on the bottom of a closed tray. The slides were rinsed using a squeeze bottle filled with filtered seawater, and then immersed in culture solution. Cultures were initially set up in

disposable polystyrene petri plates - either 60 x 15 mm or 100 x 20 mm. After the material had developed for 6-10 weeks, the coverslips or slides were placed into lidded Pyrex culture containers (#3250) with ca. 200 ml of culture medium.

Both Provasoli's ES medium (West's modification of Provasoli's (1968) medium given in McLachlan (1973) was used) and artificial seawater (a modification of ESAW of Harrison, Waters, and Taylor, 1980 - refer to Appendix V for the composition of the enrichment solution) were used in the culture study. The media were filtered with 0.45 μ m pore-size filter or by glass fiber filter (Reeve Angel). ES medium was used alternately with non-enriched filtered seawater as the maintenance culture media. The natural seawater used for the culture medium was taken either from the seawater system at the Bamfield Marine Station (salinity of 29 - 32 ‰) or collected near Bath Island in the Strait of Georgia (salinity of 18 - 26 ‰). The addition of 2 ml of a stock solution of 250 mg/L germanium dioxide to a litre of culture medium was used to eliminate diatom contaminants from the newly established cultures (Lewin, 1966). The use of germanium dioxide was discontinued when the diatoms had been eliminated. Control cultures without germanium dioxide were set up to check if the germanium dioxide had any inhibitory effect (as has been reported by McLachlan, Chen and Edelstein, 1971a; Tatewaki and Mizuno, 1979). Artificial seawater was used in

the salinity experiments as it enabled solutions to be prepared of known and repeatable salinity. Another culturing method tried was the growth of spore suspensions on agar (ES solidified with 1% agar - Chapman, 1973b) or on agar with liquid ES forming a layer on top.

Cultures were grown at a variety of temperature and photoperiod regimes:- 5° 8:16, 5° 12:12, 10° 12:12, 10° 16:8, 15° 12:12, and, 20° 12:12 were used, although routinely cultures were maintained at the first four of these conditions. The lighting conditions provided by Cool White fluorescent tubes varied between 44-110 $\mu\text{E} \cdot \text{m}^{-2} \cdot \text{sec}^{-1}$. (This variation was due to bulb aging, different positions of dishes within the chambers, and different chambers. To minimise the effects of light variation dishes were moved around within the chambers at each medium change.) Cultures were grown with or without agitation in their early stages. Due to lack of space and equipment, cultures could not be grown or maintained routinely in agitated conditions.

1) Salinity/Temperature experiment

Coverslips with freshly-settled plurispores were placed into culture dishes with 50 ml of culture medium. Artificial seawater was made up to the following salinities - 12.5, 18.5, 25, 30, 37‰ - by varying the amount of the salt solutions and distilled water (Harrison et al., 1980). A modified enrichment medium was used (refer Appendix V) and the same amount of the enrichment medium was used in

each of the five different solutions. Three replicates for each salinity treatment were placed in growth chambers at 5° , 10° , 15° , and 20° C with a photoperiod of 12:12. The growth of the cultures was recorded by photographic sampling at 5, 7, 10, and 14 days after commencement. A camera attached to an Olympus inverted microscope was used to take three photographs of each culture.

2) Effects of light quality on germination and germling growth

Coverslips with freshly settled plurispores were placed in petri dishes with ES medium. The petri dishes were placed in boxes constructed from "Cinemoid" coloured acetate sheeting with the following transmission properties:- red(#14) 370-414 nm (minor component), 588-700 nm; green (#24) 444-590 nm; blue (#32) 370-564 nm, 640-700 nm (minor component). Dishes were also placed in the chamber without coloured filters but shaded by layers of cotton gauze. All dishes received $32-35 \mu E \cdot m^{-2} \cdot sec^{-1}$. Three dishes were set up in each of the four treatments. The cultures were grown at 10° C, 12:12 photoperiod, and the medium was changed every 10-14 days. Growth of these cultures was observed for 6 weeks. In the initial stages growth was followed by photographic sampling (as in 1 above).

3) Fusion of swarmers

The diameter of recently settled swarmers from different populations was measured. In addition to hanging drops with tissue from single plants, small pieces of tissue from

laterals of two plurilocular plants were used for a single hanging drop. The plants used for these two-plant hanging drops were either both from the same site or from different sites. Material from the following sites was tried in combination:- Bath Island/Bath Island, Aguilar House Point/Aguilar House Point, Second Beach/Second Beach, Bath Island/Aguilar House Point, Bath Island/Second Beach, Cape Arago/Cape Arago, Bath Island/Cape Arago, Fogarty Creek/Fogarty Creek, Bath Island/Fogarty Creek, Fogarty Creek/Yaquina Head, Fogarty Creek/Boiler Bay, City Point/City Point. The released swarmers were observed as they settled and any swarmer interactions were noted. Swarmers released from the only unilocular material available (from City Point, California) were observed as they swam and eventually settled.

B. Cytological Methods

Material to be used for cytological work was mostly field collected material and was either field fixed or brought back to the laboratory in a cool chest and fixed within 24 hours of collection. The fixative used was 3 parts 95% ethanol: 1 part glacial acetic acid. Three methods were tried to obtain information about the caryological phases of the life history of A. japonicus:- (1) chromosome staining with (a) acetocarmine, and (b) aceto-iron-haematoxylin-chloral-hydrate, and (2) quantitative DNA staining and measurement with

microspectrophotometry.

(1) (a) Acetocarmine (Jensen, 1962). The acetocarmine squash technique was used on small pieces of plurilocular tissue. Tissue was pretreated by soaking in a 6% solution of sodium carbonate to assist in the spreading of tissue (Naylor, 1959; Roberts, 1966). (Sodium carbonate removes alginic acid - Parker and Diboll, 1966.)

(1) (b) Aceto-iron-haematoxylin-chloral hydrate (Wittmann, 1965). This stain was used on both squashes of plurilocular material and also on cultured germlings. Coverslips with freshly-settled plurispores growing in enriched seawater at 10° C on a 12:12 photoperiod were fixed over a 24 hour period at 1 hour intervals, after 1 day in culture and also after 3 days in culture. No sodium carbonate pretreatment was used with this stain as the chloral hydrate functions to soften the tissue.

(2) Quantitative DNA measurement by microspectrophotometry. Feulgen was used as a quantitative DNA stain. (The quantitative relationship between stain intensity and DNA content is well established e.g. Leuchtenberger, 1958; Wied, 1966.) The relative DNA content of the nuclei was measured using the two-area method (Garcia and Iorio, 1966) with a Zeiss photomicrometer with Kohler illumination and Zeiss filters BG23 and GG14.

Material was prepared for microspectrophotometry according to the following schedule:-

(a) fertile lateral branches were chopped with a razor

blade into pieces ca. 1-2mm, and then placed into a 6% sodium carbonate solution for 1-2 hours.

All the following steps until the dehydration series were performed in centrifuge tubes, with the material being centrifuged and then resuspended with each solution change.

(b) two rinses with distilled water

(c) hydrolysis in 3N HCl at 60° C for 20 minutes (These were the optimal hydrolysis conditions and were determined experimentally.)

(d) repeat (b) to stop hydrolysis

(e) transferred to Schiff's reagent for 6 hours in the dark (-reagent made up according to Sharma and Sharma, 1965)

(f) rinsed in tap water - four changes

(g) rinsed in SO₂ water - three changes of ten minutes each

(h) transferred to distilled water

(i) dehydrated in ethyl alcohol series - 30%, 50%, 70%, 90%, 100%, 100%, 100% - after transferring to glass vials

(j) mounted small pieces of tissue in Euparal, squashing firmly to spread the cells.

The relative DNA content of 40 nuclei was measured for material from each of the types examined:- (1) Bath Island (B.C.) - plurilocular plants, (2) Davenport Landing (California) - plurilocular plants, (3) City Point (California) - plurilocular plants, (4) City Point - unispores in unilocular sporangia, (5) City Point - sterile cells in unilocular plants.

Results

A. Culture Work

The hanging drop technique of Wynne (1969) gave consistently good results. Apart from a thick border of spores about the rim (a hedgerow effect) of the hanging drop, spore settlement on the coverslip or slide surface was generally even. When using drops of spore suspension for settlement there was a tendency for a less even distribution of settled spores. Growth on agar, either with or without a layer of liquid ES medium above, was not good. Growth in the artificial seawater was very similar to that in the ES medium. There was also no apparent difference in the growth of A.japonicus in media with or without germanium dioxide. The cultures which were grown with agitation did not develop differently than those grown without agitation. Developmental differences between agitated and non-agitated cultures may have appeared if the cultures had been grown beyond the early crust stages.

The development and morphology of cultures grown from plurispores and unispores are described in Chapter 1. The cultures initiated with plurispores produced a crustose phase and erect axes in all photoperiod/temperature conditions under which they were maintained, that is, 5° 8:16, 5° 12:12, 10° 12:12, 10° 16:8, 15° 12:12. Cultures initiated with unispores also produced both crust and erect axes in all the conditions in which they were grown, that

is, 5° 8:16, 10° 12:12, 10° 16:8. The cultures initiated with plurispores developed plurilocular sporangia under the conditions listed above. At the time of writing, the cultures initiated with unispores had not developed reproductive structures.

1) Salinity/Temperature experiment

The results of this experiment are summarised by Figure 18 in which five representative germlings have been figured at day 7 after commencement for each of the treatments. The two factors being varied in this experiment can be seen to have interactive effects on the growth of A.japonicus. At 5° C slow growth occurred at all salinities, being particularly slow at the lowest salinity 12.5%. At 15° and 20° C, the effect of the lower salinities was apparently severe. Thus at 15° C and 12.5% and 20° C and 12.5 and 18.5%, the cells have little cytoplasm and apparently are mostly vacuolar space. These cells also have an inflated appearance being considerably larger than cells at the same salinity but lower temperature. In general, the first oblique division of the discoid crust occurred when the germlings reached a 2-3 cell stage. In cultures at 20° C between 12.5 and 30% the germlings continued to divide transversely forming longer filaments before the oblique divisions occurred. In these cultures at 20° C, a number of aberrant germlings were seen where the oblique divisions occurred from several cells in a longer filament resulting in atypical and sometimes branching germlings. These

germlings apparently aborted shortly after this stage and the more normal germlings continued to grow and develop into discoid crusts. (For an example refer to Figure 18 - 20° C at 30‰.) At 20° C and 37‰ the germlings had a normal morphology and were considerably more developed than the germlings at the same salinity but lower temperatures. Growth of cultures beyond day 7 followed a similar pattern to that illustrated in Figure 18. At 12.5‰ only the cultures at 5° and 10° C survived beyond day 14. At 18.5 and 25‰ all cultures survived beyond day 25 except for those at 20° C. At 30 and 37‰ all cultures survived beyond day 25 although some germlings at 30‰ and 20° C were aberrant in their form.

2) Light Quality

Growth in all the treatments was equivalent with no developmental differences distinguishable up to the six week stage.

3) Fusion of swarmers

The size of twenty swarmers from each of four populations were measured, and the data are shown in the following list.

MATERIAL	MEAN DIAMETER (µm)	SD (µm)
City Point unispores	7.325	0.3354
City Point pluriswarmers	6.675	0.3898
Bath Island pluriswarmers	6.7625	0.3086
Fcgarty Creek pluriswarmers	6.6625	0.4236

The mean diameters of the swarmers from the three plurilocular populations were not found to be significantly

different, and were all significantly smaller (0.001 confidence level) than the mean of the unispores measured. Thus I found no evidence to support Abe's claim of anisogametes.

I did not observe any evidence of physiological anisogamy, or swimmers swimming around others (as reported by Abe). I saw no evidence of fusion in the combination hanging drops. The unispores I observed also showed no signs of fusing. Further, I could not identify the two kinds of unilocular or two kinds of plurilocular plants which Abe described in Japan.

B. Cytological Results

(1) (a) Acetocarmine. Results obtained with this method were poor and no chromosome counts were obtained. The primary problem was obtaining division stages in the tissue rather than failure of the stain.

(1) (b) Aceto-iron-haematoxylin-chloral hydrate. This treatment gave better results than the acetocarmine, but the size of the nuclei and cytoplasmic staining prevented counting chromosomes. With plurilocular material it was also difficult to find nuclei in division stages. A 24-hour fixation series of young germlings was carried out in the hope of identifying the time at which nuclear divisions were occurring. The germlings had the advantage of being only 1 cell-layer thick. With this material the cytoplasm

was very dense, and obscured the nuclei.

(2) Quantitative DNA measurement. Results of the microspectrophotometric technique are given in Figure 19. Feulgen microspectrophotometry reveals the relative amount of DNA present in a cell. For a particular ploidy level this will be a range of values depending upon the stage of the cell cycle that the nuclei are in. That is, in haploid tissue DNA values will range between 1x and 2x and in diploid tissue between 2x and 4x. Although absolute DNA content can be calculated using known standards, this is not necessary here as the purpose is to see if there is a difference in ploidy level. The range of values of DNA content for sterile cells from unilocular plants is between 9 and 22, whereas that of the unispores within the unilocular sporangia and also that of all the plurilocular plants falls between 3 and 12. The mean relative DNA content of the plurilocular and unispore nuclei is approximately half as large as that in the sterile cells of the unilocular plants.

Discussion

The culture results indicated that development of erect axes and production of plurilocular reproductive organs can occur in A. japonicus over a range of temperature and photoperiod conditions. This agrees with field observations (reported in Chapter 2) where it was found that erect axes occurred throughout the year (although not necessarily at all sites), and that reproductively mature plants with plurilocular sporangia were also present year round.

The response of germlings to varying salinity/temperature conditions shows A. japonicus to be widely tolerant of these environmental factors. This is also consistent with field observations as A. japonicus in British Columbia is found both in higher salinity waters of the open coast and in lower salinity waters of the Strait of Georgia. Populations of A. japonicus in the Strait of Georgia decline in the summer, unlike other populations studied in B.C. In summer, the Fraser River freshet results in low minimum salinity values in the waters of the Strait of Georgia. This is also a time when water and air temperatures are at their highest. (Refer to Appendix III for salinity and temperature data for the Strait of Georgia.) As borne out by these culture results, the low salinity/high temperature combination is particularly stressful for the plants.

Plants of A.japonicus are known to occur at different tidal heights at different locations being reported from the upper intertidal zone to the upper subtidal zone (refer Chapter 2). The plants at different tidal heights not only experience different amounts of desiccation, but also experience different light quality over the tidal cycle due to the differential absorption of the light by the water column. The results of the light quality experiment suggest that A.japonicus possesses at least the ability to germinate and develop under a range of light quality regimes. It would be interesting to extend this work to see whether adult morphology and reproduction are affected by prolonged exposure to regimes of different light quality.

The use of the Feulgen microspectrophotometric method reported here is the first account of the application of this technique in the Phaeophyta although it has been used on members of the Chlorophyta (Hopkins and McBride, 1976; Hopkins, 1977; Kemp, Doyle, and Anderson, 1979) and Rhodophyta (Hurdelbrink and Schwantes, 1972; Breeman, 1979). This cytological work verifies Abe's (1936) claim that the unilocular sporangia in A.japonicus are the site of meiosis.

Cultures initiated with plurispores were capable of asexually cycling themselves following the life history pattern outlined in Figure 20a. A.japonicus apparently has an exclusively asexual life history for most of its geographic range (Alaska, British Columbia, Washington,

Oregon and much of California). The sexual cycle and alternation of isomorphic generations represented in Figure 20b occurs in Japan and in at least one site in California. (Refer to Chapter 2 for further details about the phenology of A. japonicus.) The cytological work reported here showing the plurilocular plants to be haploid is contrary to Kylin's (1940) suggestion that they are diploid. If Abe (1935a) was correct in maintaining that only female swarmers are capable of apogamous development then the observations reported here are not surprising. Apparently for most of the range of A. japonicus there are no sexual populations. Thus, the plurilocular plants which are found at most sites would be, according to Abe, exclusively female and only able to reproduce asexually. The results given here - that there were not two size classes of swarmers from the plurilocular plants and that these swarmers did not fuse - would be consistent with this. Only where there are unilocular plants would there be the possibility of male plurilocular plants (growing from unispores) and thus the possibility of sexual fusion. If this interpretation is correct, then the only site where the anisogamous plurilocular plants might be found is City Point. Although I did not find anisogamy in the material I examined, I think that this warrants closer attention. If, as at other sites, the asexual portion of the life history of A. japonicus at City Point contributes substantially to the population maintenance and growth, then one would

expect the male plurilocular plants to be relatively rare compared to the female plurilocular plants, particularly as the unilocular population is so limited in its extent..

The life history findings show that A.japonicus should not be placed with the Chordariales which is distinguished as a distinct order "on the basis of the heteromorphic life history in which the haploid microthallus alternates with a diploid macrothallus" (Bold and Wynne, 1978).. (Refer to Chapter 6 for a discussion of the taxonomic position of A.japonicus.)

Figure 18. Summary of effects of varying salinity and temperature on growth of germlings of A. japonicus. Five 7-day-old germlings from each of the twenty experimental conditions are represented here. Scale bar = 20 μm .

$\begin{matrix} T^{\circ} \\ S^{\text{‰}} \end{matrix}$	5°	10°	15°	20°
12.5				
18.5				
25				
30				
37				

Figure 19. Relative DNA values measured by
microspectrophotometry in nuclei of A. japonicus. For
each type of tissue the sample size of nuclei = 40.

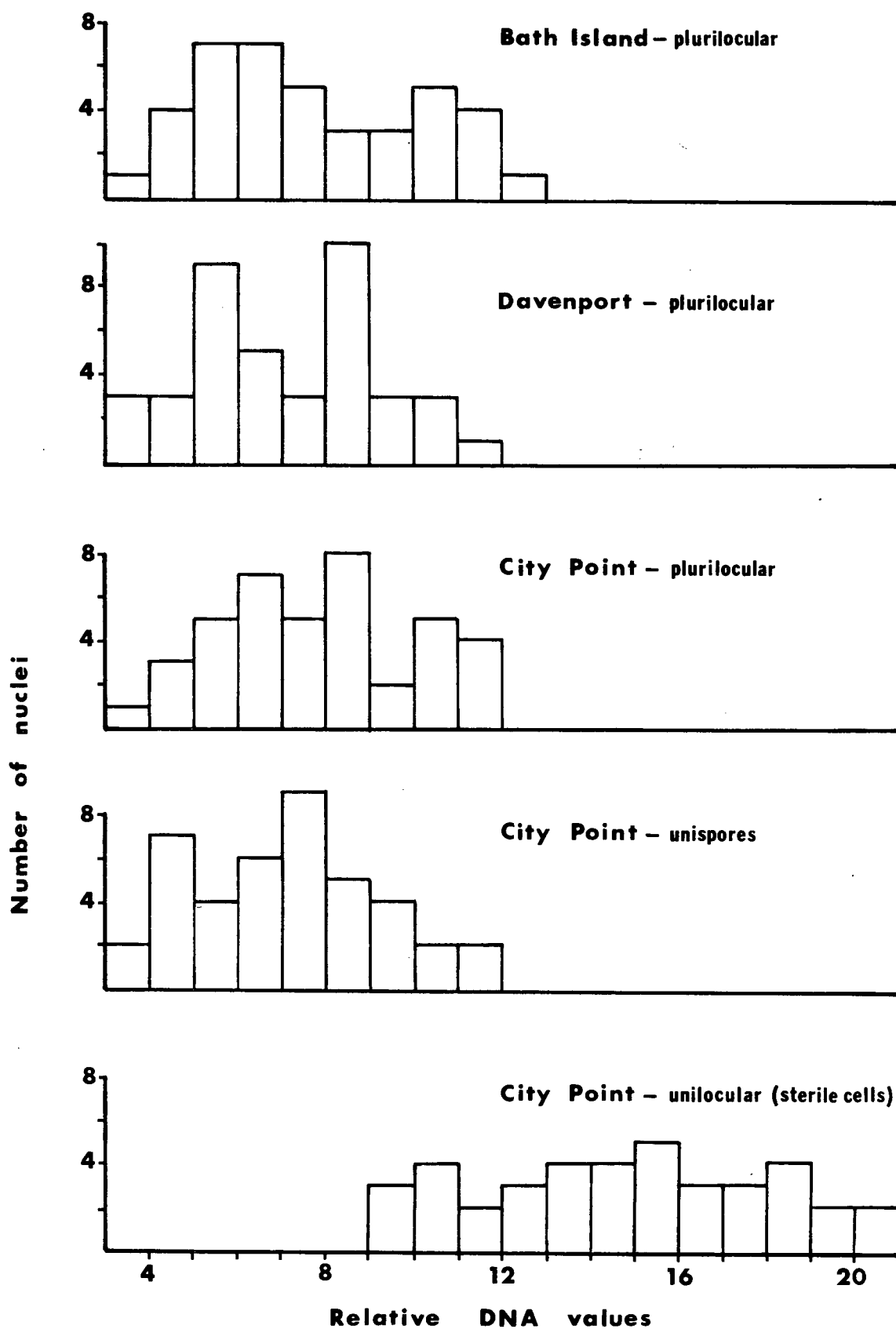


Figure 20. Diagrammatic representation of (a) life history of A.japonicus in culture, and, (b) life history of A.japonicus in nature.

CHAPTER 4,

Autecology of Analipus japonicus at Bath Island, British
Columbia

Introduction

Over a two year period from July 1977 to August 1979, an autecological study was made of a population of Analipus japonicus at Bath Island in the Strait of Georgia, British Columbia. When this work was started there were no studies on the ecology of A.japonicus in the eastern Pacific, and, as noted in Chapter 2, there were contradictory reports in the literature on the phenology of this plant in other parts of its range. In making this study it was hoped to describe dynamics of the A.japonicus population and elements of the community in which it is found at Bath Island. This work should be seen as hypothesis-generating in nature rather than hypothesis-testing, and serve as a baseline for future experimental ecological studies on this plant and the intertidal communities found in the Strait of Georgia.

The seasonal growth of the A.japonicus population and the Bath Island intertidal community was followed by permanent quadrats and transects sampled either by the point intersect method or with underwater photography. As reported in Chapter 2, regular collections were made of A.japonicus from the Bath Island site in order to measure various morphological characters of the plants and in order to follow the phenology of the population. Settlement plates were set out in the study area in the hope of observing early stages of crust development and also to get

an indication of the time of year when most settlement occurs. Substrate within the A.japonicus zone in the intertidal region was cleared to follow recolonisation at different times of A.japonicus' reproductive cycle.

Materials and Methods

Bath Island (49°09'N, 123°40'W) is one of the Flat Top Group, a cluster of small islands located in the Strait of Georgia to the southeast of Gabriola Island (Figure 21). The field site used for this study was on the southeast side of Bath Island on an evenly sloping rock face. The island is located at the widest point of the Strait of Georgia with the field site facing the direction of the prevailing winds, and an uninterrupted stretch of water to the lower mainland of British Columbia.

Quadrats and Transects

Five permanent quadrats (QI-QV, Figure 22) were established at the field site at the beginning of July 1977. An aluminium quadrat frame 0.5 m x 0.5 m was used. Nylon thread was strung tightly across the frame horizontally and vertically, resulting in intersecting lines at 5 cm intervals (a total of 81 intersect points within the frame). A hole was drilled in each of the upper

left and right hand corners of the frame. The five quadrats were located in the field with expansion bolts (secured in the rock substrate after drilling holes with a drill powered with compressed air) which were flagged with bright plastic tape. The frame slotted over the bolts and sampling consisted of recording what occurred at each point intersect. The quadrats were sampled at approximately monthly intervals from July 1977 to August 1979. Refer to Appendix VI for a list of sampling dates for Quadrats I-V.

A permanent transect (PT1-10, Figure 22) running vertically through the intertidal region was established at the beginning of July 1977. It was marked by two expansion bolts placed 12 m apart in the rock substrate and marked with coloured plastic tape. A cotton cord was used as the transect line and this was marked at one metre intervals with plastic tape and at 5 cm intervals with indelible ink. The cord was stretched out between the endpoints and attached to these by 'S' hooks at either end of the cord. A 10 m length of this cord was used for recording what occurred at 5 cm intervals through the length of the line. The transect line was sampled at approximately monthly intervals from July 1977 to July 1979. Refer to Appendix VI for a list of sampling dates of the transect.

Clearing Experiment

As represented in Figure 22 by T1-T12, 12 quadrats were established along a horizontal line. They were marked by expansion bolts, flagged with plastic tape and were 0.3 m x 0.3 m in size. They were sampled photographically, for 19 months (from May 1978 to November 1979) using an underwater camera attached to an aluminium frame. The rectangular base of the frame slotted over bolts marking each quadrat. The camera and strobe flash light source were supported on the upper portion of the frame which was raised on legs at right angles to the base and a set distance from the substrate being photographed. The dimensions of each quadrat that was photographically sampled was 0.2 m x 0.15 m.

Quadrats 1,3,5,7,9, and 11 were left undisturbed during the sampling period. Six quadrats were cleared at low tide by scraping and then by burning with an ethylene torch. Quadrats T2 and T6 were cleared in May 1978, T4 and T8 in June 1978, and T10 and T12 were cleared in August 1978.

Monthly Collections

As described in Chapter 2, collections were made at Bath Island at approximately monthly intervals and were used for a phenological study.

Photographic Quadrats

In addition to the quadrats T1-T12 which were observed photographically as part of the clearing experiment , 8 quadrats (indicated in Figure 22 as Q1-Q8) were followed photographically. These photographs provided a record of crust and upright axis growth.

Settlement Plates

Frames to hold glass microscope slides were constructed from perspex and consisted of a flat rectangular base with strips mounted along both sides to form overlapping edges. Six glass slides were thus held in a horizontal position on the perspex base. The glass slides were ground on a rock polishing turntable with carborundum to roughen their surfaces. The frames with the slides in place were secured at three vertical heights within the A. japonicus range by being slotted over expansion bolts and were held in place by wing nuts.

The plates with the slides were placed in the field site for varying lengths of time and when collected the slides were placed into a 5% formaldehyde/seawater solution. The slides were stained with lactophenol blue and observed microscopically in the laboratory.

The following list gives the dates on which slides were placed in the field and then collected and fixed for examination:-

<u>Date placed in the field</u>	<u>Date collected</u>
7 . IX . 78	7 . X . 78
7 . X . 78	26 . XI . 78
26 . XI . 78	13 . I . 79
13 . I . 79	10 . III . 79
10 . III . 79	12 . V . 79
12 . V . 79	10 . VII . 79

Results

Field Site

Storms and increased wave action affected the Bath Island study site in the winter. Drift logs which accumulate in the upper intertidal region were dislodged by the highest tides or by storm waves and often caused considerable damage by battering and crushing the intertidal and upper subtidal flora and fauna.

Monthly mean, maximum and minimum salinity and temperature values for the surface waters off Entrance Island (49°10'N, 124°10'W) are given in Appendix III. (This is the closest site to Bath Island where daily sampling of surface waters occurs. The limited amount of salinity and temperature data collected at Bath Island closely follows the data from Entrance Island.) The freshet of the Fraser River strongly influences salinity in the Strait of Georgia causing a marked drop in the minimum values measured in the summer months.

The tidal regime at Bath Island is a mixed semidiurnal

type. The extreme low tides in summertime occur around the middle of the day, and the A.japonicus plants can be exposed to the air for up to 5 hours before being re-immersed by the incoming tide (Anon, 1977, 1978, 1979).

The intertidal rock shelf at this site is evenly sloping with little topographical relief - few cracks, crevices or shade areas - and thus, there is little habitat variation. A diagrammatic representation of the field site and the vertical heights above chart datum, of quadrat and transect points are given in Figure 22. The site was surveyed at extreme low water in mid-summer to measure the height of the study points above chart datum.

Access to the site for most of the year was by diving with SCUBA. From May to August some observations and collections were made at extreme low tides.

Quadrats and Transects

A list of species found in the quadrats and transect is given in Appendix VII. Figure 23 and 24 show numbers of A.japonicus plants sampled in quadrats QI-QV over the study period. Figure 25 shows numbers of A.japonicus recorded in the transect over the same period with the upper eight metres of the transect having been divided into four, two-metre lengths and labelled PTI-PTIV (from upper to lower intertidal region) respectively. No A.japonicus plants were recorded in the lowest metre of the transect. In Figure 26 numbers of plants of Sargassum muticum, Rhodomela laris,

and Cryptosiphonia woodii sampled in the transect and quadrats are shown. Figure 27 shows numbers of mussels (Mytilis edulis) and Microcladia borealis plants recorded over the sampling period in both transect and quadrats. These species were important elements of the community (as measured by numbers recorded in quadrats and transect) and the abundance of these species changed over the study period. Figure 31 indicates the distribution over time of eight other species that were frequently recorded in the quadrats and transect with the period of maximum abundance being indicated by shading. Figures 28 and 29 show numbers of annual and perennial plants sampled in each of the quadrats plotted against the time of sampling. In Figure 30 the total number of A.japonicus plants recorded from all quadrats and the mean axis height of A.japonicus from monthly collections from Bath Island (as given in Figure 14) are plotted over the sampling period.

Clearing Experiment

A descriptive summary is presented in Table II of the results of the clearing experiment.

Monthly Collections

Descriptive statistics and graphs of the mean axis height and of fertility of plants collected over the study period are given in Chapter 2 (Table I and Figure 14).

Figure 32 shows the frequency of height classes for the A. japonicus plants collected at Bath Island for the period August 1978 to July 1979.

Photographic Quadrats

As noted in Chapter 2, erect axes of A. japonicus were seen to be produced throughout the year although growth of these axes was greatest in spring and early summer. It was observed that A. japonicus crusts were growing and extending during the winter months and into the spring. Crusts as well as erect axes were deleteriously affected by the late summer conditions at the site. Erect axes in the late summer were a red/tan colour and eroded back to stubbs. These often had algal epiphytes on them, which were not observed at other times of the year. It was not possible to measure growth of crusts throughout the year from the slides as overstorey growth particularly of A. japonicus erect axes, and species of Gigartina, Rhodomela, Sargassum, and Fucus, prevented a clear view of the crusts and substrate.

A. japonicus was observed to grow over Ralfsia crusts in the field - the reverse was never observed. Both A. japonicus and Ralfsia grew over Petrocelis crusts and again the reverse situation was never seen.

The sculpin, Oligocottus maculosus, was often present in these photographs. The colour and patterning of this fish disguise it well normally, as it lies amongst the

algal cover close to the substrate.

Settlement Plates

Over the time that the plates were in the field, three were lost apparently to storm or log damage, and on some of the plates that remained there was both breakage and loss of individual slides. No crustose phases of A.japonicus were recognised on the ground glass slides. Limpets were found on all plates that remained in the field.

General Observations

In November-December 1975 and 1976, the intertidal and subtidal region of Bath Island were grazed heavily by the green sea urchin Strongylocentrotus droebachiensis (De Wreede, 1980). No urchins were found in the study site over the course of this work although a few very small urchin tests were found washed up in the drift in 1979.

In the summer of 1977 and 1978 large numbers of Pisaster ochraceus were seen in the intertidal region and apparently were feeding on Mytilus as a trail of broken shells lay in their wake as they passed across the shore. Both Mytilus and Pisaster were found only rarely in the summer of 1979.

Limpets (Collisella pelta and Notoacmea scutum) were seen in the quadrats and transects although only infrequently sampled by an intersect point. The isopods

Idotea wosnesenskii and Gnorimosphaeroma oregonensis were both found infrequently on upright axes of A. japonicus.

Discussion

The results presented here - specifically, shifts that were found in the species composition - suggest that during 1977-79, the intertidal community was recovering from the effects of sea-urchin grazing. In the first summer of this study Mytilis edulis was very abundant, covering large areas of the intertidal region. As illustrated in Figure 27, the numbers of M. edulis found in the quadrats and transect dropped dramatically in the second and third summer of the study. Another pronounced shift in species abundance was that of Microcladia borealis (refer Figure 27) which was much reduced in abundance in the 1979 summer from the 1978 records. Even more marked was the virtual disappearance of Cryptosiphonia woodii. From the autumn of 1977 through the summer of 1978, C. woodii was abundant and apparently an important element of the community (refer Figure 28) particularly in the upper portion of the transect and in quadrats QI and QII. This plant was virtually absent, however, in late 1978 and in 1979.

The proportion of species which exhibit annual or perennial life patterns can be used as an indicator of the successional state of the community (e.g. in a study of

succession of algal communities on breakwaters in Japan, Saito, Sasaki and Watanabe (1976) noted that, as a rule, the dominance of most perennial algae increased as the substrata became older; Newell and Tramer (1978), in a study of an herbaceous plant community during succession, found a shift from annual to perennial life strategies i.e. longer life spans; Sousa (1980), in a study of recolonisation and succession in the intertidal, noted that perennial algae were characteristic of later successional stages). When the numbers of plants with either annual or perennial life forms were graphed over the time of the study period at Bath Island (Figures 28 and 29) interesting trends are shown. In the spring and early summer of 1978 (March to June) of all five quadrats, there is apparently a seasonal peak in abundance of annual species. In the same period in 1979, however, this peak in the numbers of annual plants is found only in QIII and QV, the two lowest on the shore. In QI, QII and QIV there is a shift to a predominance of perennial plants. Figure 26 shows that over the study period the annual species Rhodomela larix and Sargassum muticum increase in abundance. These plants are found particularly in QIII and QV and it is the increase in these species that causes the seasonal peak in 1979 in the numbers of annual species in these two quadrats.

The phenological study (reported in Chapter 2) showed that the A. japonicus population at Bath Island exhibited a marked seasonality with respect to the time of maximal

growth, as measured by mean axis height, and the production of plurangia. The plants were at their smallest in late summer, and this can be correlated with highly stressful environmental conditions (as indicated by culture experiments reported in Chapter 3) of lowered salinity, increased temperature and extreme low tides occurring in the middle of the day. The plants collected between July and September have an eroded red/tan appearance and although erect axes are being produced continually, few attain maturity at this time. Figure 32 indicates that the size distribution of erect axes measured in the phenological study is strongly skewed to the lower size classes.

The total number of A. japonicus occurring in all the quadrats (Figure 30) shows a steady and steep increase in the population from October to March in both 1978 and 1979. In 1978 there is a decrease in the numbers of A. japonicus recorded between April and June. In 1979 there is a small decrease in the plant numbers recorded for April. As illustrated in Figure 30, in 1978 and 1979 April/May is the time that the mean axis height (and also the fertility of the population) (Figure 14) are at their peak. In June-August of 1977-1979 a secondary peak and decline is seen in the A. japonicus numbers. Several factors are apparently involved in these population fluctuations reported here. The decline in the A. japonicus population in the spring and early summer coincides with the time of maximal growth.

This suggests the involvement of intraspecific competition - as Harper (1977) states "...one of the high risk states in the life cycle...is the seasonal hazard when the survivors are growing fast. The period of rapid growth is the time at which individuals make their heaviest demands on environmental resources and are most likely to interfere with their neighbour's activities. This would explain the high mortality risk at this time and also the fact that the risk is species specific. Each plant is most likely to suffer from the presence of a neighbour of its own species because their phenologies will be synchronous.".. If intraspecific competition is involved in this decline in A. japonicus numbers at a time when its maximal growth is being recorded it would be expected that there is a relationship between the risk of death and the density of the population. To test this hypothesis, experiments should be carried out on populations of A. japonicus at different densities and data gathered on the longevity/mortality of both crusts and upright axes. It has been reported that plants growing under density stress have a skewed distribution of plant weight (Harper, 1977).. No measurements of biomass were made in this study, but the skewed distribution of axis height (Figure 32) would seem to support the suggestion of the role of intraspecific competition.

Another factor that may be involved in the decline in A. japonicus numbers at this time was the increase in growth

and abundance of other species - that is, interspecific competition. Particularly in 1978, Microcladia was at its peak abundance over the period of decline in the A. japonicus numbers. The other annual plants which increased in importance (as measured by their numbers in the quadrats and transect) at this time were Cryptosiphonia (in 1978 only), Sargassum, Rhodomela, and Fucus (Figure 31). Interestingly, Ralfsia and Petrocelis, the two other crusts occurring in the study site were least abundant over the same period of April-June. This may indicate that the annual plants overgrow the crusts and that the crusts were obscured by a temporary overstorey but were still present in the community. In the case of A. japonicus this is less likely as the presence of the erect axes means that these crusts are less easily obscured. Further experimentation is needed to determine the relative contribution of intra- and interspecific competition to these dynamics of the A. japonicus population.

The peak in A. japonicus numbers occurring in late summer happens 2-4 months after the peak in fertility, and it is probably due to settlement and colonisation by spores and the growth of the new resultant crusts. The sharp decrease in this peak (August/September 1977-1979) coincided with a decrease in the mean axis height of the population. Young plants are unlikely to be able to survive the environmental extremes experienced at this time of the year at Bath Island (- this is supported by the work

reported in Chapter 3), and there are few crevices or shaded areas where new crusts could escape the rigours of the environment. It was hoped that the settlement plates would allow study of earlier stages of crust development in the field and also would indicate the time of year when maximal settlement occurred. The settlement plates need to be redesigned to exclude grazers, in this case limpets, which appeared to be affecting the growth of algae on the plates. Limpets have been reported to control algal growth by consuming young plants and thereby affecting recruitment (Lodge, 1948; Jones, 1948; Southward, 1958). Other materials might also prove more successful substrates for A. japonicus plants. Harlin and Lindbergh (1977) demonstrated substrate surface relief can regulate the development of an algal community: increased algal settlement occurred with an increase in particle size. Although the slides in my study had been roughened, possibly they were still too smooth to allow for the development of the A. japonicus crusts.

The results of the clearing experiment (Table II) support the previous explanation of the quadrat results. The quadrats cleared in August had a dense cover of A. japonicus a year later. Both pairs of quadrats cleared in May and June, however, had only scattered A. japonicus plants occurring on them after a year, despite the fact that there was a much higher percentage of the A. japonicus population fertile in May and June than in August (Figure

14). Apparently any A.japonicus settlement which did occur on these substrates in May-June was unable to survive due to the extremes of the environment. Also they may be unable to compete successfully with ephemeral species which occurred at least in the quadrats T2 and T6 cleared in May. It appears that the reproductive 'peak' of A.japonicus between April and June at Bath Island makes a negligible contribution to its successful settlement and colonisation at this site. Both the results of the clearing experiment and the quadrats suggest that the significant reproductive period in terms of resultant recruitment to the population occurs in the autumn, winter and early spring. In culture work (described in Chapter 1) the crusts were found to spread by forming "satellite-discs" - it would be of considerable value to this plant if this type of vegetative growth can occur in the field and particularly if it is operative in the autumn prior to the onset of increased erect axis development and increased fertility in the population.

The A.japonicus population as a whole increased in size over the study period (Figure 30) although as illustrated by Figures 23, 24, and 25, the same trends are not occurring at all the vertical heights sampled. An increase in A.japonicus numbers was not observed in either QIII or QV, or in PTIV where Sargassum and Rhodomela were becoming increasingly important (both being more numerous and dominating the area in terms of the overstorey cover

they provided). QI, QII, and QIV and the upper three sections of the transect showed an increase in the numbers of A.japonicus recorded in the second year of this study. In mid-1977 and mid-1978, A.japonicus numbers were highest in QV and PTIV - a situation which is virtually reversed by the following year.

At other sites in British Columbia where I have collected A.japonicus, its vertical distribution in the intertidal region is generally much more restricted than at Bath Island. (This is discussed in Chapter 2.) The shift seen here in the vertical position of A.japonicus' maximal abundance over two years may be an indication of differing competitive ability in A.japonicus at different heights. In the lower portion of its range A.japonicus was contending with increasing cover from Sargassum and Rhodomela. The success of these two species and the concomitant decline in A.japonicus numbers may be related to the overstorey formed by Sargassum and Rhodomela. This overstorey could be affecting the A.japonicus plants by severely restricting the incident light. The increase in Sargassum and Rhodomela may also be due to their germling vigour and growth being superior to that in A.japonicus. Experiments, similar to those suggested in Chapter 2, to test if the decline in A.japonicus numbers in the lower intertidal region is due to competition with the overstorey species could be performed by removal of Sargassum and Rhodomela and subsequent observation of the A.japonicus population to see

if it increases in abundance or extends its vertical range.

Predators and herbivores have been shown to play important roles in shaping community structure and in influencing distribution and abundance of intertidal and subtidal plants and sessile animals (e.g. Jones and Kain, 1967; Paine and Vadas, 1969; Paine, 1971; Foster, 1975; Lubchenco and Menge, 1978; Lubchenco, 1978, 1980). Although a period of heavy urchin grazing had preceded this study, urchins were absent from the site over the study period, and thus any effects they had on the community were historical. The dynamics of the populations of Mytilus and Pisaster are far from clear. Whether the settlement of perennial algae in the zones previously occupied by Mytilus prevented successful establishment of the Mytilus, or whether the grazing by Pisaster resulted in a small population of Mytilus that was unable to replace itself, or whether the changes in the population are part of a cycle which spans a longer period than that of this study, is not known. Whilst limpets may be grazing germlings and young crusts of A.japonicus, no herbivores which were affecting the growth of mature A.japonicus crusts and erect axes were seen. Thus the role of both herbivory and predation in this community remains uncertain.

From this study it appears that the mid- to lower-intertidal community at Bath Island was in the process of recovery in 1977-79 from sea-urchin grazing which occurred in 1975 and 1976. Algae dominate the space available in the

intertidal region and also structure the form of the community. Molluscan herbivores apparently have a minor effect on the mature structurally dominant plants in this community although they may be influencing the recruitment of germlings and affecting the growth of ephemeral species. This type of situation has been reported in the lower intertidal zone of shores of New England by Lubchenco and Menge (1978) and by Dayton (1975) on the Washington coast. A discussion of A.japonicus' growth and life history features is presented in the General Discussion.

Figure 21. Map of Bath Island in the Strait of Georgia,
British Columbia.

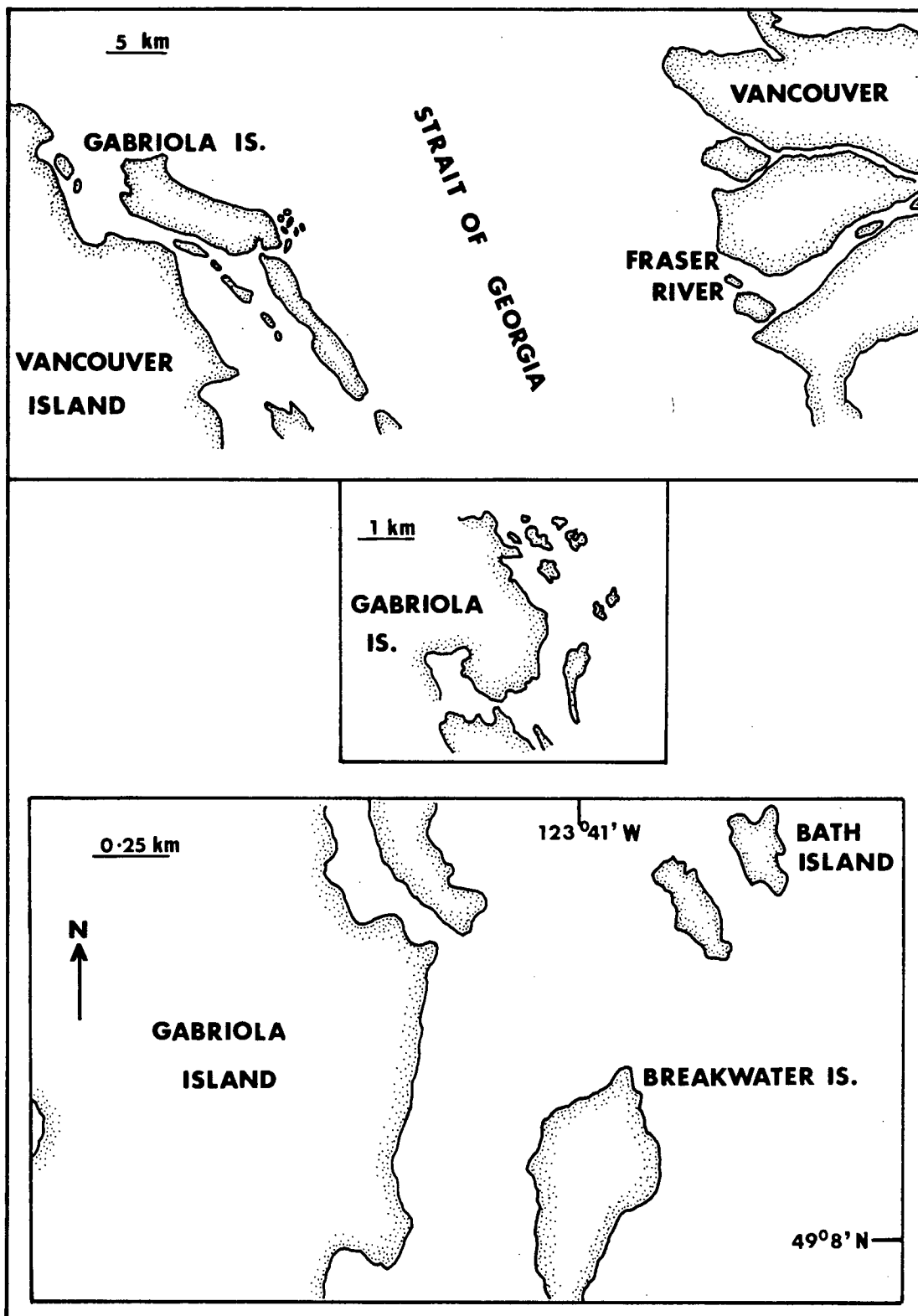


Figure 22. Diagram of field site at Bath Island indicating relative positions in intertidal region of quadrats and transect used in this study. Transect and quadrat points are following heights (in m) above chart datum:-

FT 0 - 1.98	Q I - 1.74
PT 1 - 1.80	Q II - 1.36
FT 2 - 1.66	Q III - 1.02
PT 3 - 1.50	Q IV - 1.14
FT 4 - 1.34	Q V - 0.92
PT 5 - 1.18	
FT 6 - 0.98	
PT 7 - 0.80	
FT 8 - 0.74	
PT 9 - 0.56	
FT 10 - 0.48	

(height of top left hand corner of Q I-V measured)

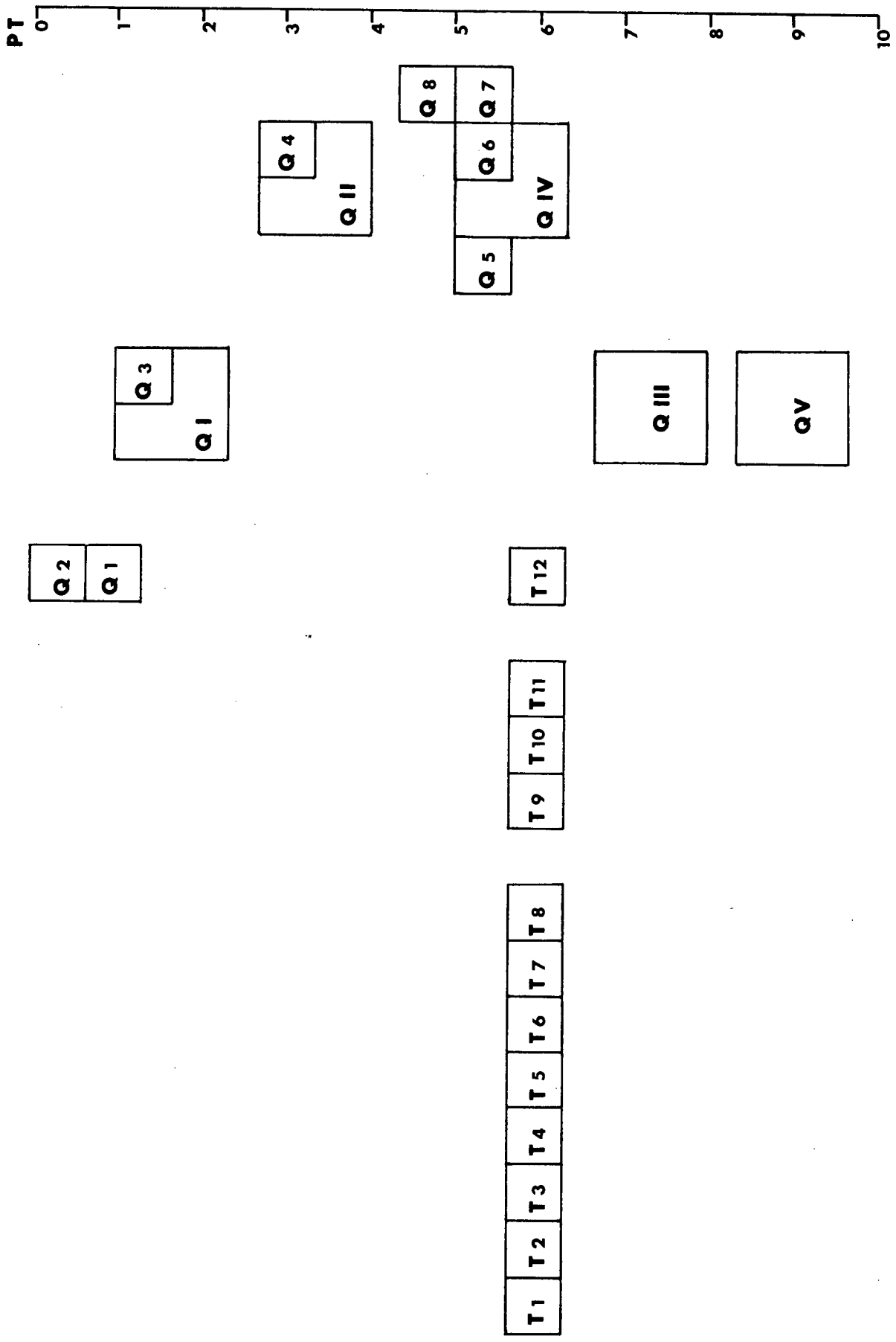


Figure 23. Numbers of A. japonicus plants recorded over study period in QI and QII. (Sample size of 81 points in 0.5 m x 0.5 m quadrat.)

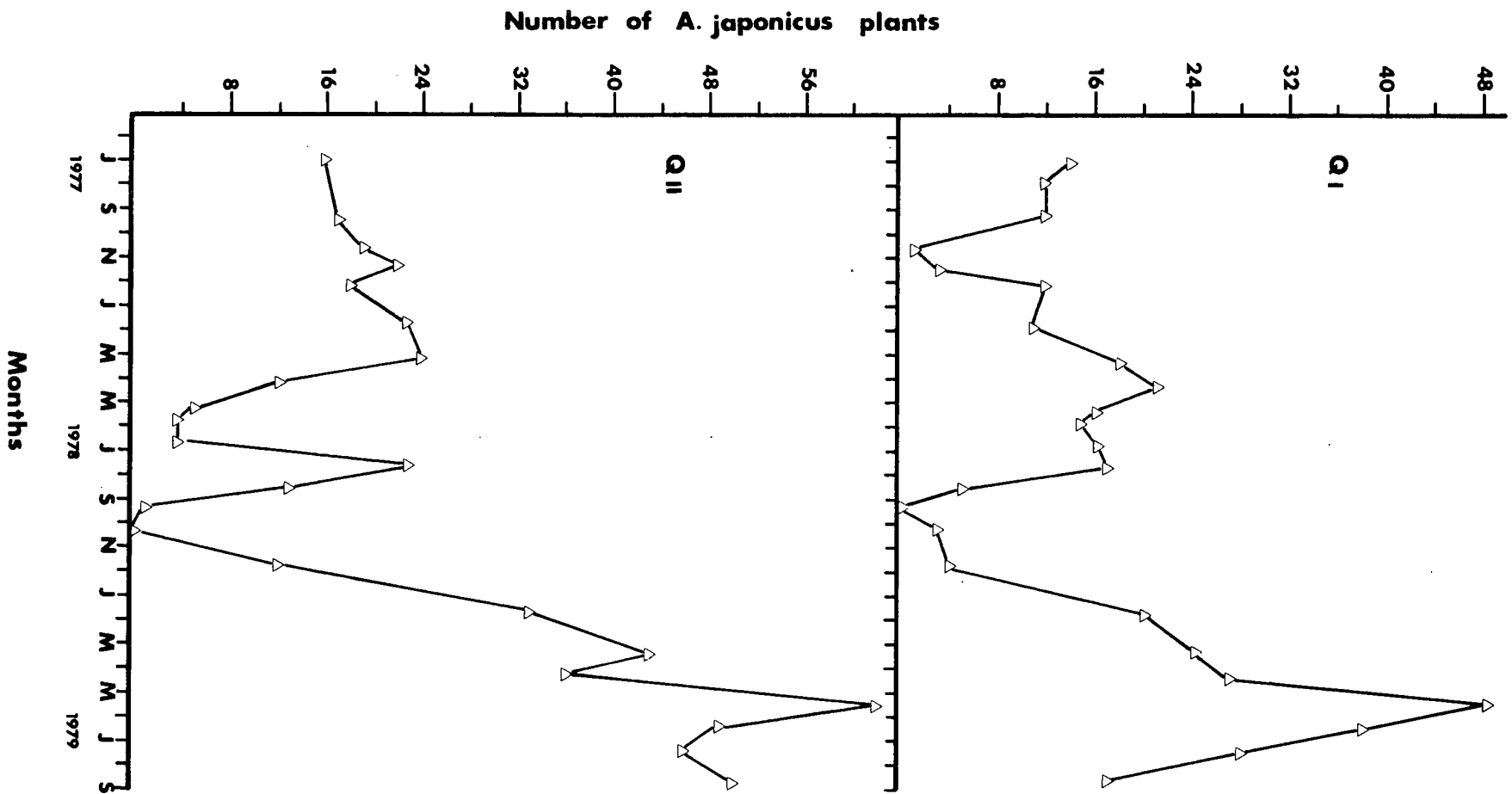


Figure 24. Numbers of A. japonicus plants recorded over study period in QIV, QIII, and QV. (Sample size of 81 points in 0.5 m x 0.5 m quadrat.)

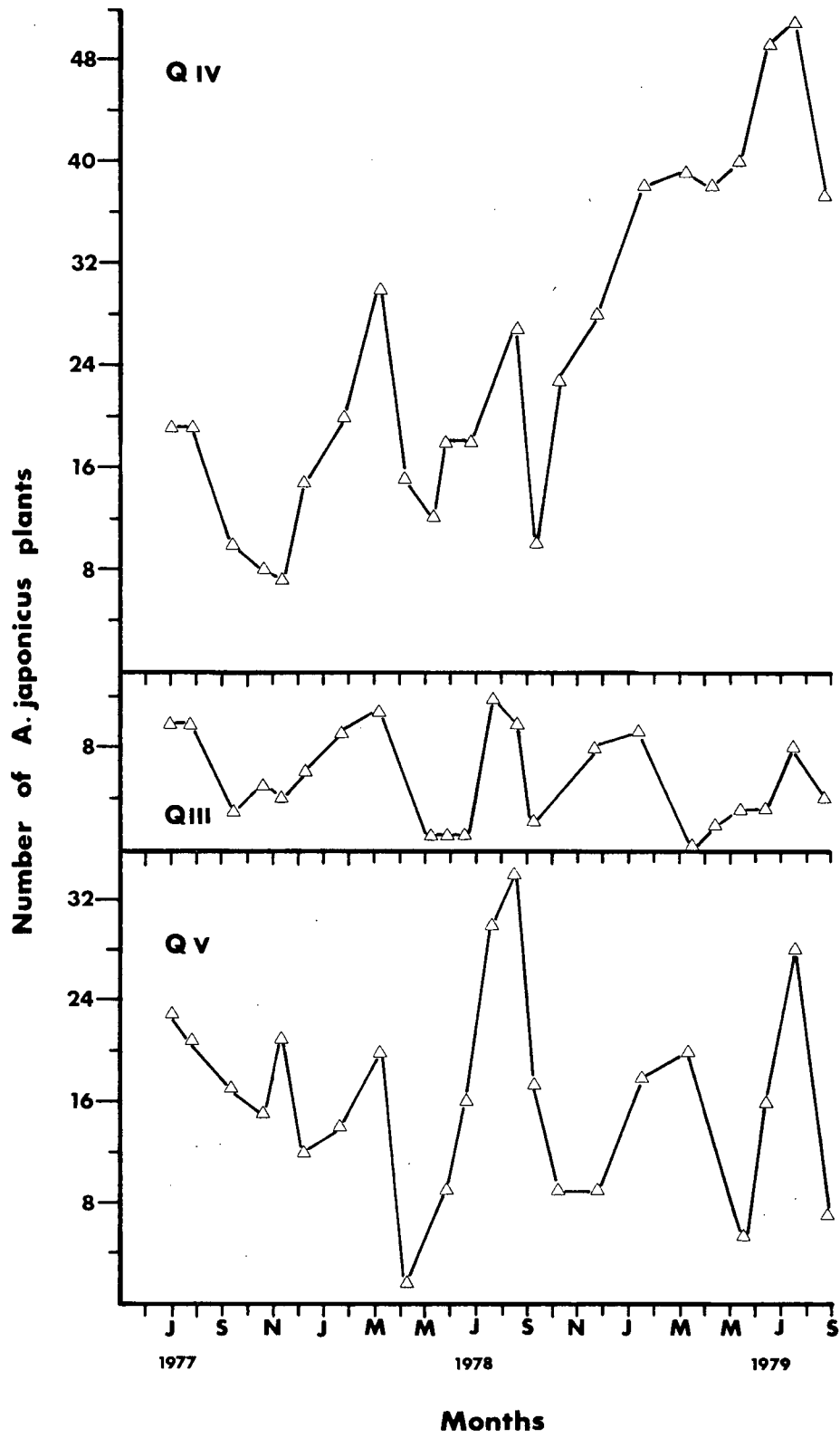


Figure 25. Number of A.japonicus plants recorded in transect over study period. Upper 8 m of transect were divided into four, 2 m lengths and labelled PTI-PTIV, from upper to lower intertidal region respectively. (40 sample points in each 2 m length of transect.)

Number of *A. japonicus* plants

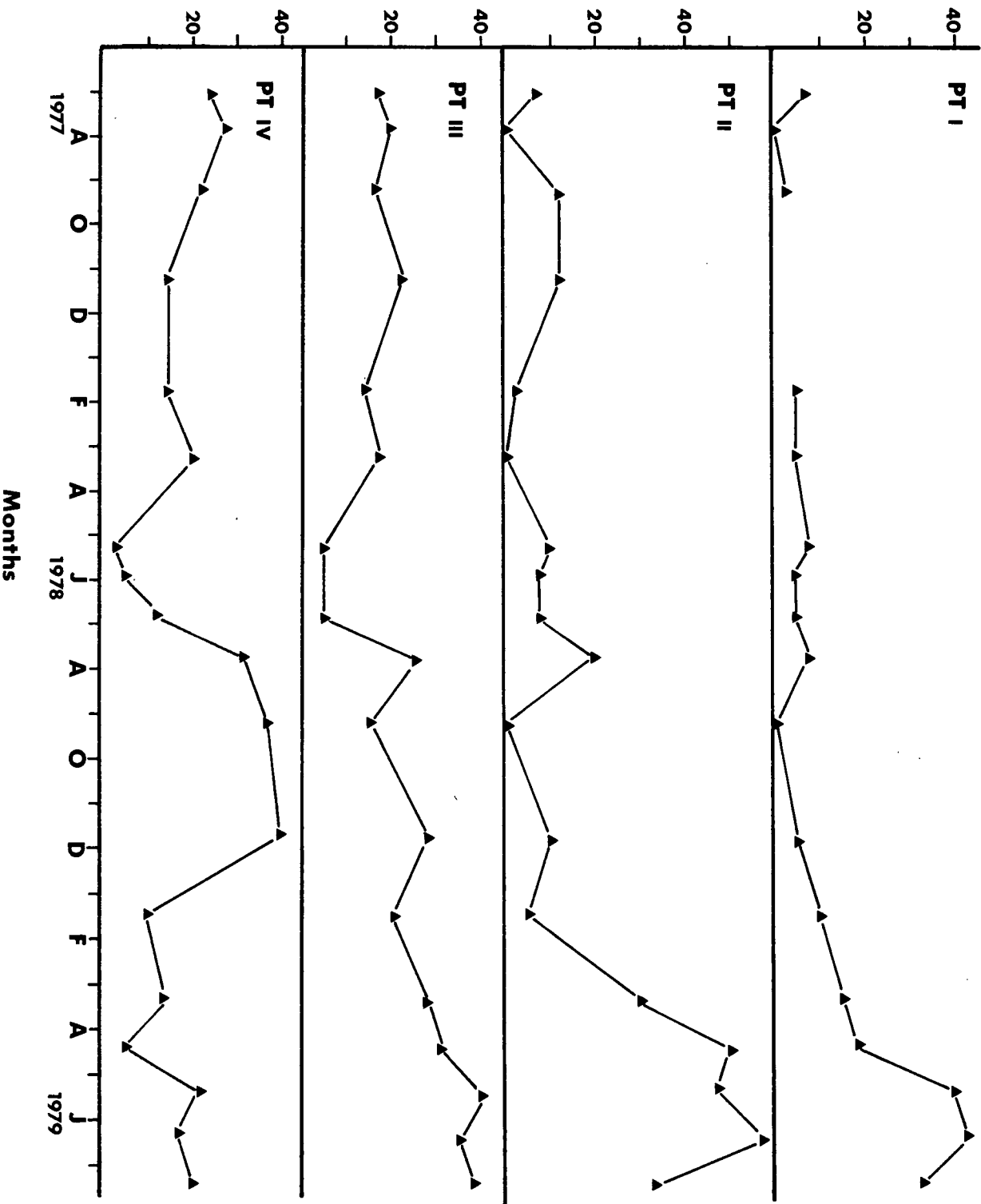


Figure 26. Numbers of Rhodomela, Sargassum, and Cryptosiphonia plants recorded in QI-QV and PT1-PT10 over study period.

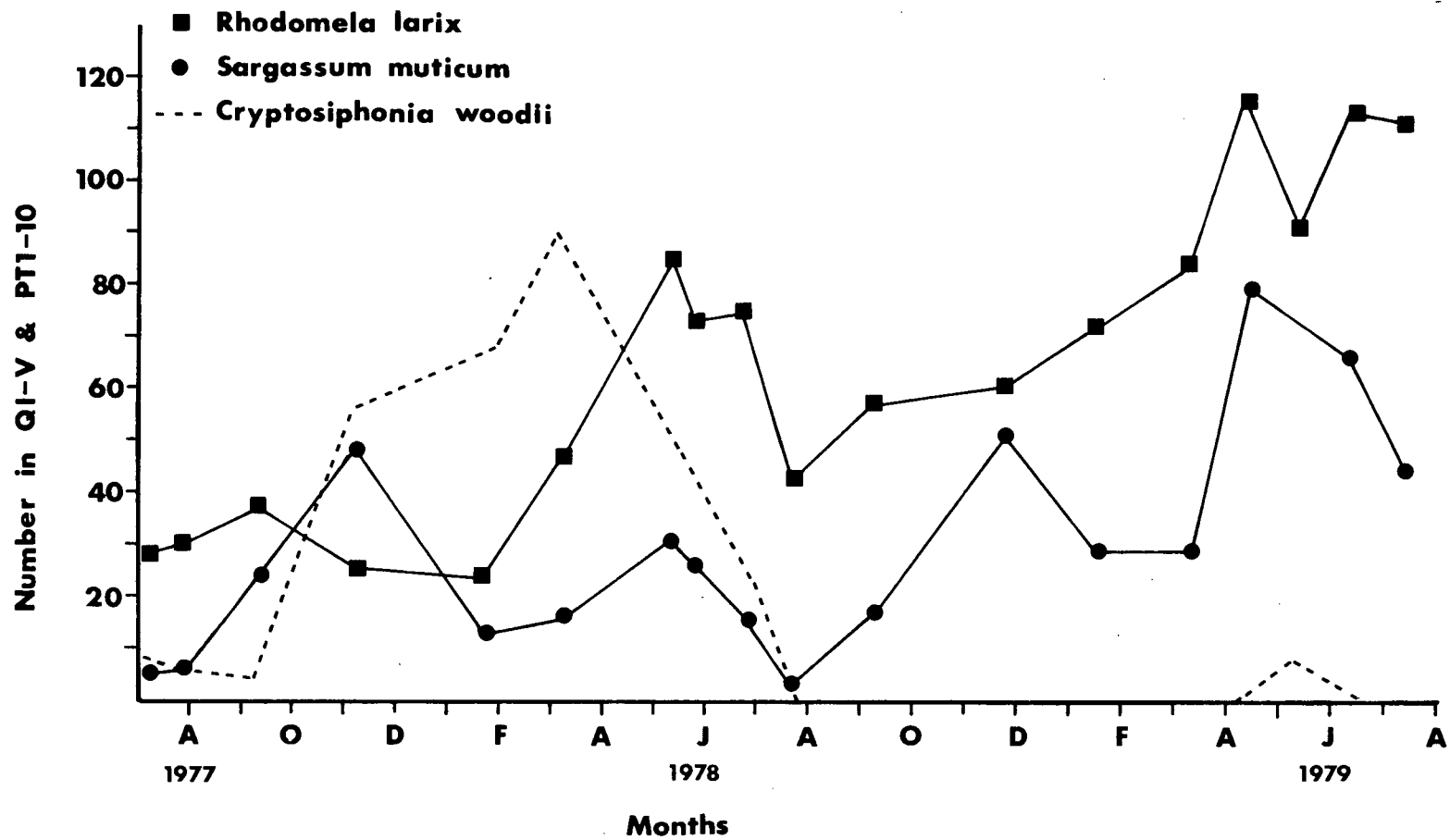


Figure 27. Numbers of individuals of Mytilus and Microcladia recorded in QI-QV and PT1-PT10 over study period.

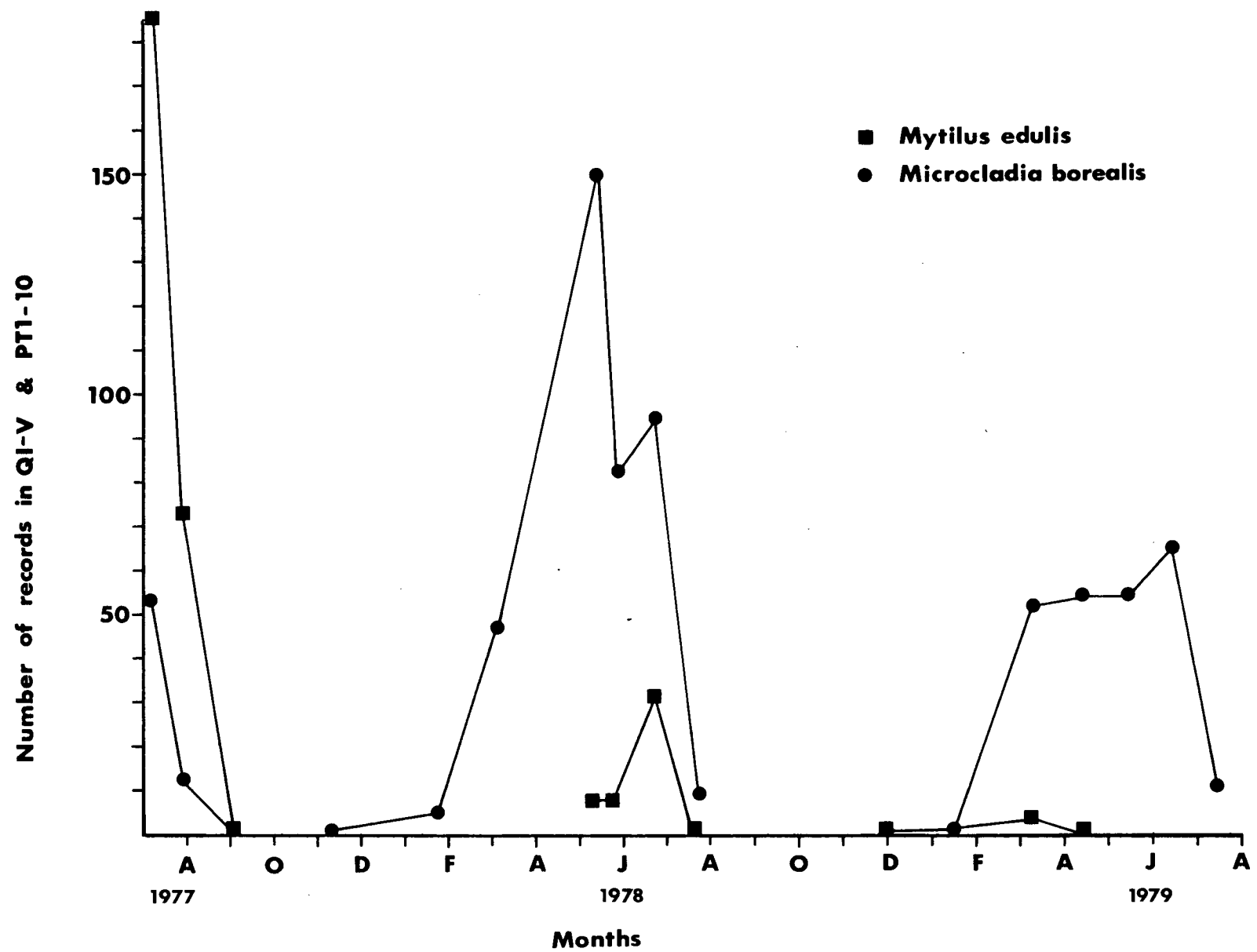


Figure 28. Numbers of plants with either annual or perennial habit recorded from QI, QII, and QIV over study period.

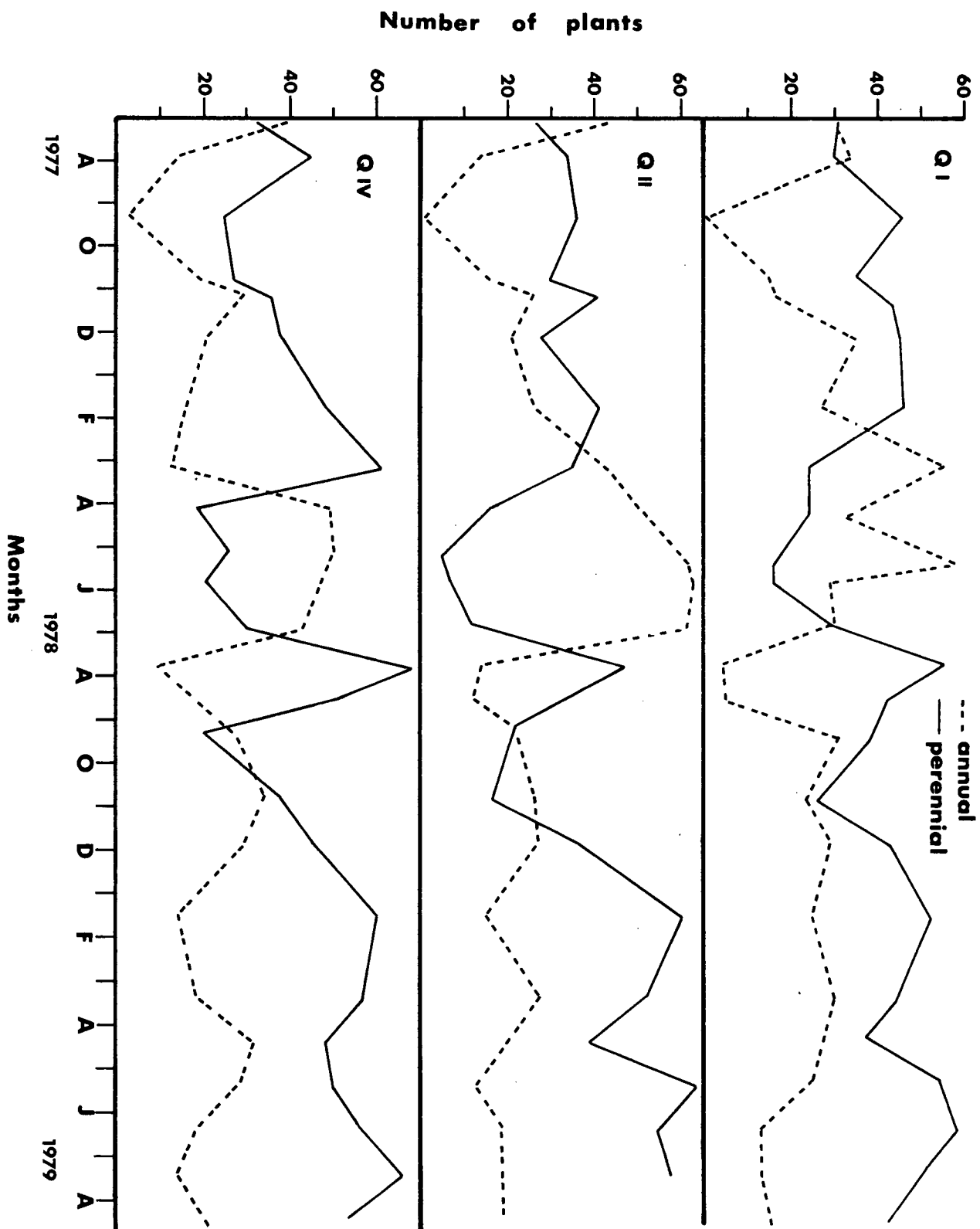


Figure 29. Numbers of plants with either annual or perennial habit recorded from QIII and QV over study period.

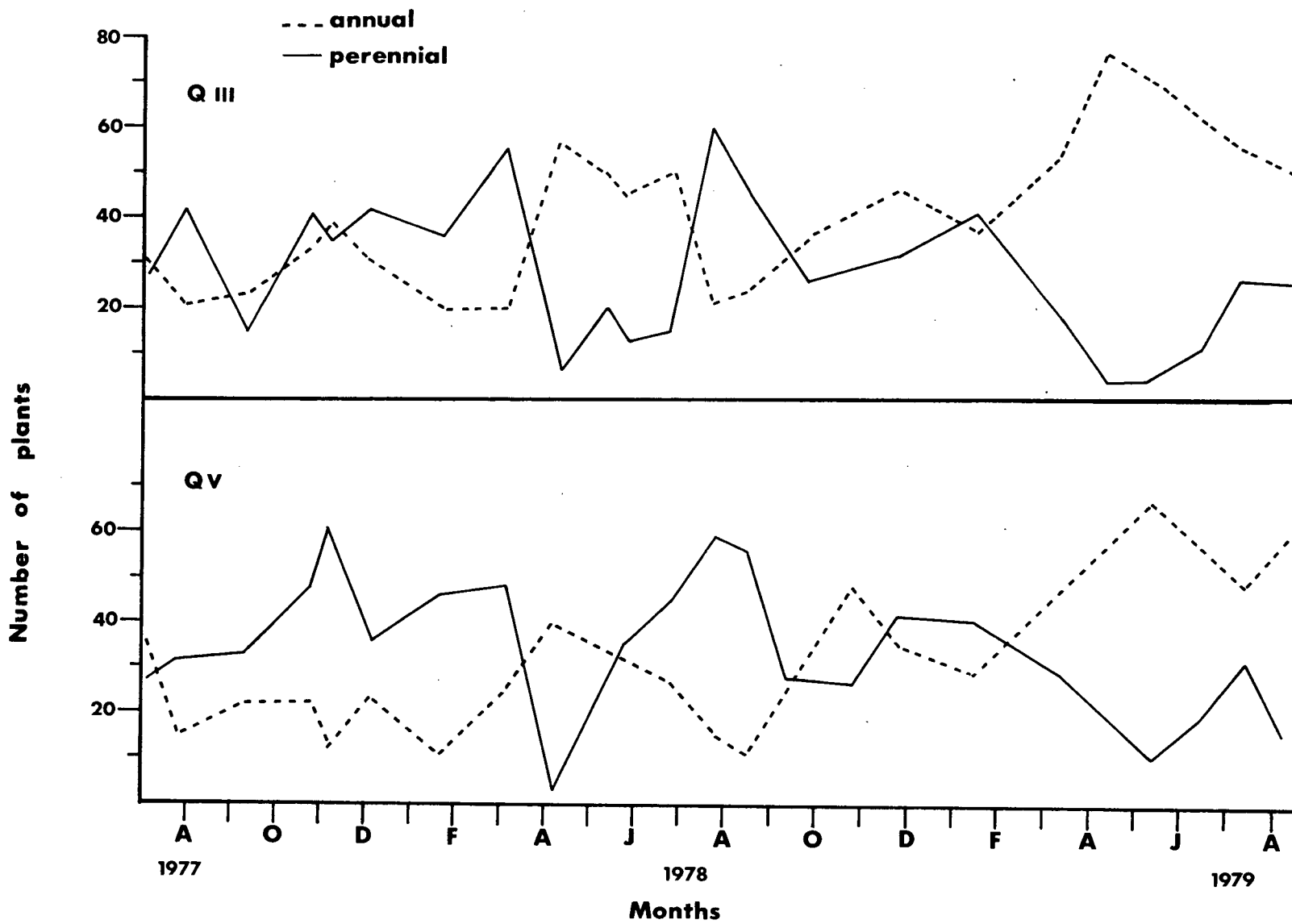


Figure 30. Mean axis height of A.japonicus and numbers of A.japonicus plants recorded from QI-QV over study period.

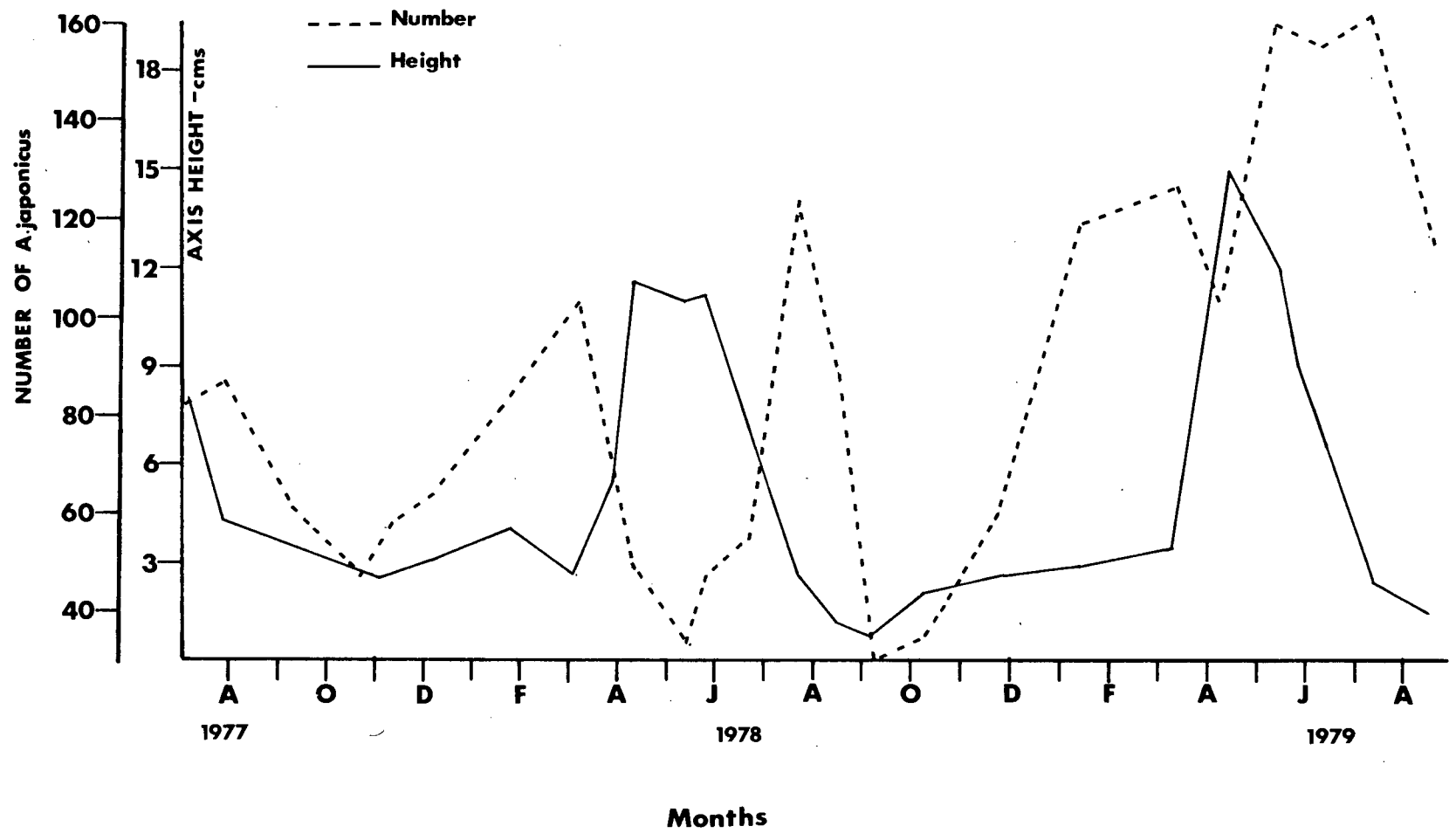


Figure 31. Occurrence of 8 species of algae in QI-V and PT1-10 over study period. Shaded areas represent time of maximal abundance.

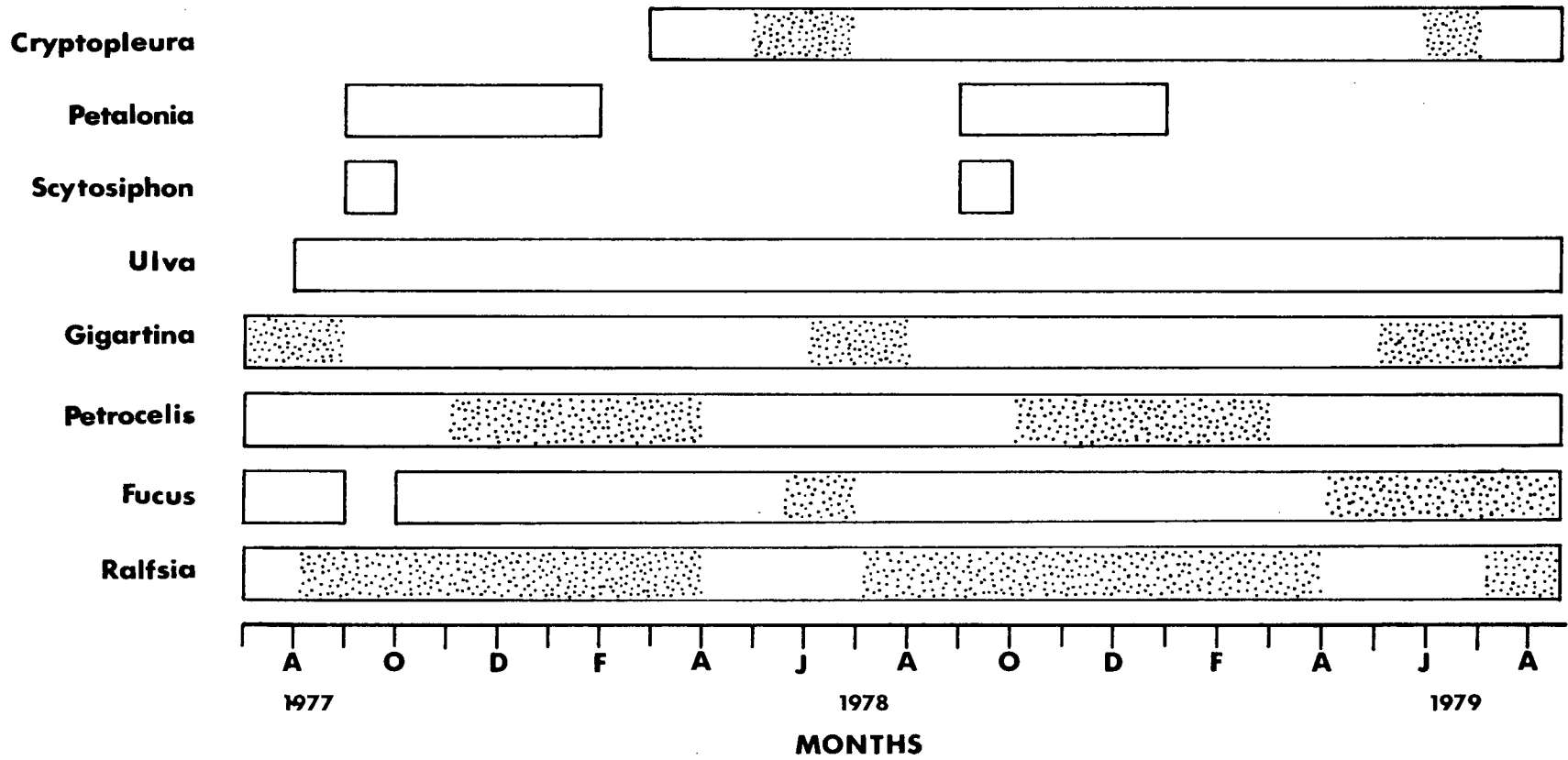


Figure 32. Frequency distribution of plants of A. japonicus collected from August 1978 to July 1979 according to upright axis height. N=250

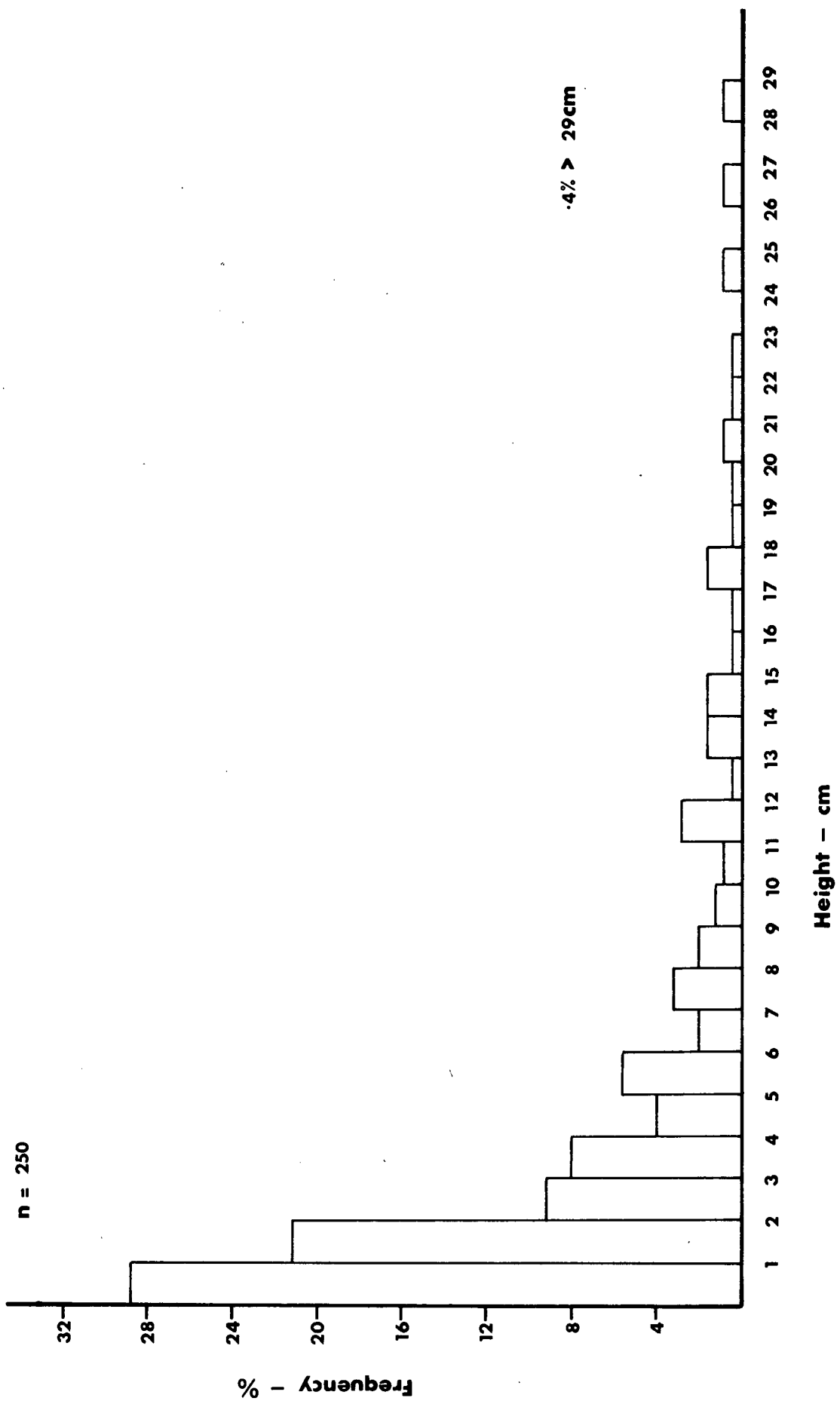


TABLE II.- Summary of quadrat clearing experiment. Macroscopic algae present in the quadrats are listed in order of understorey to overstorey habit.

QUADRAT NUMBERS AND DATE OF CLEARING	OBSERVATIONS			
	JUNE 20, 1978	JULY 18, 1978	DECEMBER 8, 1978	JULY 12, 1979
2	dense green turf	dense cover of <u>Ulva</u> sp.	<u>Ralfsia</u> , <u>Petrocelis</u> , crustose coralline, <u>Petalonia</u> , <u>Ulva</u> , <u>Colpomenia</u> , <u>Fucus</u> , <u>Sargassum</u> , (mussels scattered)	dense overstorey of <u>Fucus</u> and <u>Sargassum</u> (understorey obscured from view)
6 (May 24, 1978)	dense green turf	dense cover of <u>Ulva</u> sp.	<u>Ralfsia</u> , <u>Petrocelis</u> , <u>Ulva</u> , <u>Petalonia</u> , <u>Colpomenia</u> , <u>Fucus</u> , <u>Sargassum</u> , (mussels-scattered)	<u>Petrocelis</u> , <u>Ulva</u> dense overstorey of <u>Fucus</u>
4	-	no macroscopic algae - (limpets and sculpins)	rock virtually bare - <u>Ralfsia</u> , <u>Petrocelis</u> , <u>Ulva</u> , <u>Petalonia</u> , <u>Colpomenia</u>	<u>Ralfsia</u> , <u>Petrocelis</u> , <u>Analipus</u> , <u>Ulva</u> , <u>Gigartina</u>
8 (June 20, 1978)	-	no macroscopic algae - (limpets and sculpins)	mostly bare rock - <u>Ralfsia</u> , <u>Petalonia</u> , <u>Ulva</u> , <u>Fucus</u>	mostly bare rock - <u>Ralfsia</u> , <u>Fucus</u> , filamentous greens
10	-	-	<u>Analipus</u> (abundant), <u>Ralfsia</u> , <u>Petrocelis</u> , <u>Ulva</u>	<u>Analipus</u> , <u>Petrocelis</u> , <u>Fucus</u> , <u>Gigartina</u>
12 (August 15, 1978)	-	-	<u>Analipus</u> (very dense), <u>Petrocelis</u> , <u>Colpomenia</u>	<u>Analipus</u> (very dense), <u>Ulva</u> , <u>Petrocelis</u> , <u>Ralfsia</u> , <u>Gigartina</u> , <u>Fucus</u>
Controls 1,3,5 7,9,11 (not cleared)		<u>Ralfsia</u> , <u>Analipus</u> , <u>Petrocelis</u> , <u>Gigartina</u> , <u>Ulva</u> , <u>Microcladia</u> , <u>Fucus</u> , <u>Rhodomela</u> , <u>Cryptosiphonia</u> , <u>Sargassum</u>	<u>Ralfsia</u> , <u>Analipus</u> , <u>Petrocelis</u> , <u>Gigartina</u> , <u>Ulva</u> , <u>Colpomenia</u> , <u>Fucus</u> , <u>Petalonia</u> , <u>Sargassum</u>	<u>Analipus</u> , <u>Ralfsia</u> , <u>Petrocelis</u> , <u>Gigartina</u> , <u>Ulva</u> , <u>Microcladia</u> , <u>Fucus</u> (major overstorey plant)

CHAPTER 5,

A comparison of Caepidium antarcticum J.Ag. and Analipus
japonicus (Harv.) Wynne - (Phaeophyta)

Introduction

The monotypic genus Caepidium antarcticum J.Ag. is found in subantarctic waters. It has been recorded from Fuegia, the Falkland Islands (the type locality), South Georgia, Iles Kerguelen, and Campbell Island, New Zealand. This brown alga consists of a crustose system from which arise both upright axes and wart- or bladder-like proliferations. The upright Chordaria-like axes are generally unbranched and clothed with short numerous laterals which bear unilocular sporangia. These erect shoots develop from cup-like depressions found at the tips of the branching prostrate thallus. The bladders, which have been described as Colpomenia-like, develop out of the crust and form plurangia in sori. Thus, an individual of this species can possess three morphological phases. This distinctive morphology has posed a problem in interpretation, and Caepidium has been treated by taxonomists in basically two ways; classified on the basis of the upright system into the Chordariaceae (e.g. Agardh, 1882; Skottsberg, 1921, Kylin, 1933, 1940; Lindauer, 1947; Lindauer, Chapman and Aiken, 1961; Papenfuss, 1964; Kim, 1971), or placed in the Encoeliaceae or Punctariaceae on the basis of crust and bladder characteristics (e.g. Skottsberg, 1907; Reinbold, 1908; Levring, 1945, 1960).

Skottsberg (1907, 1921) gives a detailed account of the morphology and anatomy of Caepidium and particularly in the

later paper discusses some features of its ecology and phenology. Cotton (1915) did not accept Skottsberg's (1907) interpretation of this plant as possessing crust, upright and bladder phases, and quotes Dr. Yendo (who had examined the type material of Caepidium) as also disagreeing with Skottsberg. Cotton reports that Yendo considered Caepidium to resemble closely Chordaria abietina (now Analipus japonicus) possessing "creeping branches and upright shoots", and that the bladder-like fronds were quite distinct consisting of "young plants of Colpomenia sinuosa or an allied species". Although Cotton (pp.168-169) gives the impression of agreeing with Yendo that the bladder is not part of the crust and erect system of Caepidium, he states later (under Colpomenia, p.173) "the forked basal branches.....would appear to be part of the Colpomenia plant (and not of the Chordaria) and these are unlike anything found in the ordinary Colpomenia of Europe". Skottsberg (1921) re-iterated that the bladder-bearing Caepidium was quite distinct from Colpomenia, and that the crust, bladders and upright axes were all part of the same species. Skottsberg (1921) placed Caepidium in the Chordariaceae, changing his earlier view of its classification after finding unilocular sporangia in the erect axes (although these had in fact been described by Agardh (1882)).

Skottsberg (1921) speculated on the relationship between the life history of this plant and its morphology,

suggesting that the bladders and erect axes might be alternating generations and occur on different crusts. He noted the difficulty of separating individuals due to the overlapping nature of the crusts. The upright shoots were only found in the summer months (November to February) whilst the bladders and crusts were present year round. Kylin (1933) interpreted Skottsberg's (1921) work slightly differently. He accepted that the three morphological phases were all part of the same species, and considered that they all could be present on the same individual. Kylin suggested that the Chordaria-like uprights are diploid, undergoing alternation of generations with microscopic gametophytes. The bladders were also interpreted to be diploid, and the plurangia were seen as serving as a means of vegetative reproduction.

There are a number of references in the literature to the similarities between Caepidium and the now Analipus japonicus (previously Chordaria abietina and Heterochordaria abietina). Kjellman (1889) noted similarities between Analipus fusiformis (now A. filiformis) and Caepidium as did Kylin (1940). Kylin (1940), as well as Yendo in Cotton (1915) and Skottsberg (1921), commented on the possible relationship between Analipus japonicus and Caepidium. Skottsberg comments on the likeness between these two plants noting that both possess hairs of the same type on the horizontal thallus.

This morphological and anatomical study of

C. antarcticum and comparison with A. japonicus has been undertaken to approach the question of the affinities of these genera raised in the literature.

Materials and Methods

The material of C. antarcticum used in this study came from a variety of sources:-

(1) the type specimen - borrowed from the Botanical Museum, Lund (LD). This is shown in Figure 33. (Yendo in Cotton (1915) considered that the type of this species "resembles very much an old and nearly decayed plant of Chordaria abietina".)

(2) wet preserved material collected by Skottsberg in South Georgia, the Falklands, and Tierra del Fuego - lent by Dr R.B. Searles

(3) wet preserved material from Argentina and Chile - lent by Dr R.B. Searles

(4) wet preserved material from Campbell Island, New Zealand - borrowed from Botany Division, D.S.I.R., New Zealand (CHR)

(5) wet preserved material from Chile - lodged in the U.E.C. herbarium

(6) herbarium specimens from Chile - U.B.C. collections

(7) two specimens from the Kerguelens - borrowed from the British Museum, London (BM).

The material of Analipus japonicus used in this study is listed in Chapter 1. In addition the type specimen and two other specimens of A. japonicus borrowed from Trinity College, Dublin, were examined.

Material was either hand-sectioned or embedded in glycol methacrylate, sectioned with a Sorval JB4 microtome, and mounted on glass slides. Sections were stained with toluidine blue O (TBO) or periodic acid-Schiff's (PAS) (sometimes with a counterstain of aniline blue - PAS/AN) following the methods given in Chapter 1.

Results and Discussion

Morphology

The morphology of the crust and erect axis of C. antarcticum, as figured by Skottsberg (1921 - figure 12a), is very similar to that of Analipus japonicus. Both plants have a basal system consisting of cylindrical, branching axes forming a lobed and multilayered crustose system. The erect axes of C. antarcticum arise from the tips of the lobed crust and, as in A. japonicus, are described as being Chordaria-like (e.g. Skottsberg, 1921; Kylin, 1933; Lindauer, 1947), consisting of cylindrical, percurrent axes which become clothed in laterals. It is at this point that the morphological similarities between these two species

end as C. antarcticum possesses bladders or wart-like proliferations which also arise out of the crust.

Crustose System

The crust of Caepidium has a pseudoparenchymatous construction although in cross-section (Figure 34 and 35a), the axes of the crust may appear to be parenchymatous. However, the filamentous construction of the crust can be clearly seen in longitudinal sections of these axes (Figures 35b and d). The filaments within the medulla of the crust consist of large, elongated cells which are loosely packed. These filaments curve out obliquely to the surface layers of the crust. The cortical layers (2-4 cells deep on the upper surface and 1-2 cells deep on the lower surface) consist of closely arranged small cells.

As presented in Chapter 1, A. japonicus also possesses a filamentous crust. It differs from C. antarcticum in the relative dimensions of the cortical and medullary cells, as in A. japonicus there is much less difference in size between these cell types (compare Figures 4 and 35b).

When in contact with another part of the crust or with the substrate, the cortical cells of the lower surface can form peg-like extensions (Figure 35a) which develop into either uni- or multicellular rhizoids. Rhizoids of A. japonicus and C. antarcticum are very similar in development and appearance.

Hairs were found in C. antarcticum (Figure 35c), and as

described by Skottsberg (1921), these are very similar to those found in A.japonicus. In both species they arise from a pit below the cortex and are borne singly or in twos, but do not appear in clusters or in cryptostomata.

The growth of the crust, as in A.japonicus, results from divisions of cortical cells at the margins of the crust, particularly at the tip of each lobe. Elongation and expansion of cells internal to the meristematic zone occurs, and in C.antarcticum, unlike A.japonicus, one can find hollowed portions where medullary cells have been pulled apart by the extension of the crust (Figure 38).

Interspersed with the small and closely packed cells of the cortex are cells of varying size, frequently very large, which are distinguishable by their contents. The material in these cells when unstained is golden-brown and has a bead-like, almost granular, appearance. When stained with TBO this material becomes bright green indicating the presence of phenolics (refer to Chapter 1 for a discussion of the TBO staining reaction). It is unstained by PAS. These cells were noted by Skottsberg (1907, 1921), Fritsch (1945) and Lindauer et al. (1961) as occurring with the plurilocular sporangia on the bladders. As shown in Figures 34, 35a, 35b, 37 and 38, these structures are fully immersed in the tissue and they occur on the solid crusts as well as the sterile bladders (Figures 36a and b). I have designated these cells to be 'physode-cells' although they are not the only cells in which phenolic materials are

located in C. antarcticum (as can be seen in Figures 34, 35 and 36). Within the crust of A. japonicus there also is a considerable amount of phenolic material present, but there is no cell type equivalent to these physode-cells.

Erect Axis

The erect axes of C. antarcticum arise from the lobetips of the crust. An axis is surrounded by crust tissue in a cup-like depression (Figure 37 and 38). In the zone of transition between the crust and the upright axis, the medulla cells become more closely packed and narrower as they rise in filaments of elongate, cylindrical cells (Figure 37b and 38). The cortex of the crust gives way to a Chordaria-like cortex in the dip of the depression (arrowed in Figure 37a and 38), consisting of filaments 2-4 cells in length terminated by a larger somewhat club-shaped cell (Figure 40a). These terminal cells are filled with golden-brown contents which stain green with TBO indicating their phenolic composition (Figures 40a-c). Scattered in the cortex are colourless hairs arising from the sub-cortex (Figure 39b and 40c). As in A. japonicus, the sub-cortex of C. antarcticum can be understood as consisting of 2-3 cell layers of rounded cells, arising from the medulla, and which give rise to the cortical filaments and the unilocular sporangia.

The growth of the erect axis follows a pattern similar to that of A. japonicus with meristematic activity located

apically at the base of a 'fan' of cortical filaments (Figure 39c). As in A.japonicus, the growth of C.antarcticum could be described as fitting the multiple strand model of Parke (1933). The axis of C.antarcticum becomes hollowed as the plant grows (Figure 39a), in a similar fashion to that described for A.japonicus (Chapter 1). I found secondary filaments in the medulla of the erect axis of C.antarcticum, as in A.japonicus, but no indication of secondary systems in the cortex or subcortex.

The erect axis of C.antarcticum produces thin lateral branches similar to those formed in A.japonicus. Divisions occurring in the sub-cortex result in the cortical filaments being pushed outward. The meristematic activity continues at the base of the apical cortical 'fan' (Figures 39a-c). As with the main axis, when the lateral continues grow, the medulla is split apart.

The erect axis of C.antarcticum produces unilocular sporangia immersed in the cortical filaments. The sporangia are cut off from sub-cortical cells (Figures 39b, 40b and c). Although the anatomy of the erect axes of these two species is very similar, the sporangia differ in size. The unilocular sporangia in A.japonicus are usually at maturity $60-90\text{ }\mu\text{m} \times 25-40\text{ }\mu\text{m}$, and in C.antarcticum apparently mature sporangia are $30-45\text{ }\mu\text{m} \times 10-15\text{ }\mu\text{m}$. The cortical filaments of A.japonicus surrounding the sporangia are usually 4-7 cells in length, whereas in C.antarcticum these sterile filaments are only between 2 and 3 cells long.

Although the erect axes of C. antarcticum which I have seen fall within the size range of A. japonicus plants, they are smaller than most A. japonicus plants, with thinner lateral branches and main axes.

Bladders

The bladders of C. antarcticum, which initially are small, wart-like structures, become folded and convoluted bladders as they extend. They arise from the surface of the crust. When the bladders are sectioned they can be seen clearly to be part of the basal system of C. antarcticum, and as Skottsberg (1921) observed, there is no difference in the anatomy of the crust or the bladder. The bladder becomes hollowed out as it develops, and the filamentous construction of the thallus is obscured as the cell files are broken. The bladder has a tightly packed cortex just as the crust does, and below this are large rounded cells which clearly show signs of being pulled apart with the growth of the plant (Figures 36a, c and e).

Although the bladders have been interpreted as being 'Colpomenia-like' in construction (e.g. Cotton, 1915; Levring, 1945, 1960; Lindauer, 1947), their development is not parenchymatous. Thus, the placement of this species in the Punctariaceae (Levring, 1945, 1960) is inappropriate. Fritsch (1945) correctly noted the resemblance of these vesicles to Leathesia and makes the following comment - "In view of the close relationship between Mesogloiaceae and

Leathesiaceae there is nothing very surprising in the erect growths from the basal stratum repeating the structural characteristics of the two families.". The bladders differ from Leathesia in not possessing a filamentous cortex. They are also less mucilaginous, and the cells more tightly bound than in Leathesia. The bladders grow out of the crust and can form rhizoids to attach themselves to other parts of the crust and to the substrate. The rhizoids of the bladder (as well as those of the crust) issue from the lower cortex and can be uni- or multicellular.

The plurangia which occur on the bladders are formed by transformation of the surface cells into filaments with usually 4-6 cells per file. The surface of the thallus becomes pushed up where the plurangia develop - raised by the development of these filaments. The surface of the bladder possesses a cuticle-like layer (staining purple with TBO) and this becomes more pronounced over the scri (Figures 36a-d). On release of the swarmers, the cuticle-like layer is no longer present, and the empty cell walls of the basal plurangia remain (Figures 36e and f).

The systematic affinities of C. antarcticum and its relationship to A. japonicus

Although these two species share a pseudoparenchymatous construction and similar morphologies, this comparative study supports the separation of these plants in distinct genera. In particular, the possession of

bladders bearing plurangia and the characteristic physode-cells are distinguishing features of C. antarcticum. C. antarcticum has also been reported to possess pyrenoids (Asensi, Delepine, and Guglielmi, 1977), a feature which according to some workers has phylogenetic significance (Evans, 1966; Hori, 1972; Magne, 1978) and which is not shared by A. japonicus (Hori, 1972).

As outlined in the introduction to this chapter, Skottsberg (1921) and Kylin (1933) put forward different interpretations of the life history of C. antarcticum using the information available from the morphology of the plant. This study cannot resolve which of these interpretations is correct as no life history studies of C. antarcticum were possible.

If, as suggested by Skottsberg (1921), the bladder-bearing and erect axis-bearing crusts are alternating generations, the placement of C. antarcticum within the Chordariales cannot be continued. Such an alternation of partially heteromorphic generations with a pseudoparenchymatous construction would make this genus very unusual in the brown algae. It could be accommodated within the Ectocarpales in a new family. Although Müller (1972) reported that the gametophyte and sporophyte generations of Ectocarpus siliculosus could be morphologically distinguished, the differences between these generations were not nearly as substantial as those between upright-bearing and bladder-bearing crusts.

If Kylin's (1933) suggestion is correct, with C. antarcticum possessing a microscopic gametophyte stage alternating with diploid bladders, erect axes, and crusts, this species can be left within the Chordariales. It would seem, however, that a separate family would be warranted for this genus on the basis of the elaborated crustose system and bladders bearing plurangia functioning in asexual reproduction.

Before the affinities of C. antarcticum can be fully appreciated, information on the life history and caryological states of the morphological phases is needed. A microspectrophotometric analysis of DNA content of crusts, bladders and erect axes, is now being pursued to further this end.

Figure 33. Type specimen of Caepidium antarcticum J.Ag.



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Capricornia antarctica

collected by William Steadman, 1901

BOTANISKA
MUSEET
LUND

Figure 34. Montage of a cross-section of crustose thallus of C. antarcticum. UC = upper cortex; LC = lower cortex; M = medulla; arrows point to 'physode-cells'. (200x)

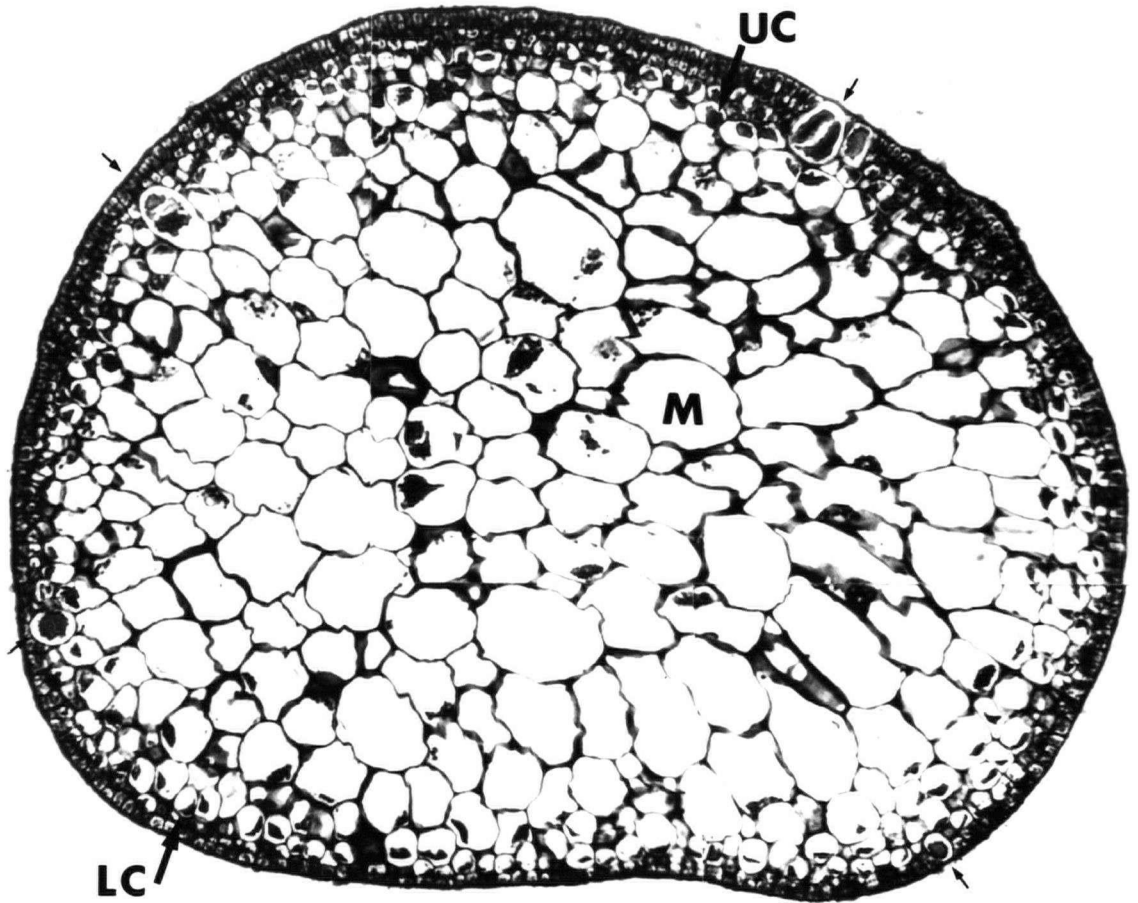


Figure 35a. Cross-section of two layers of C. antarcticum crust. Arrows indicate peg-like extensions of lower cortical cells developing into rhizoids. . (165x)

Figure 35b. Longitudinal section of crust of C. antarcticum. (125x)

Figure 35c. Cross-sectional view of crust of C. antarcticum showing hair(h). . (375x)

Figure 35d. Medulla of crust of C. antarcticum in longitudinal section. Arrow indicates direction of growth. (385x)

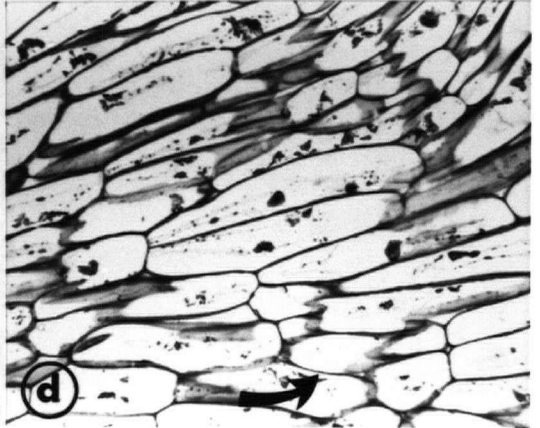
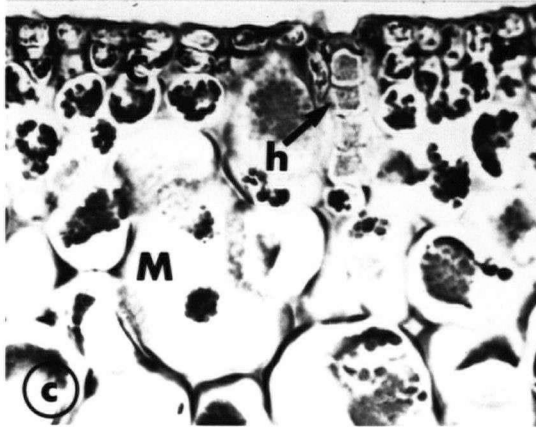
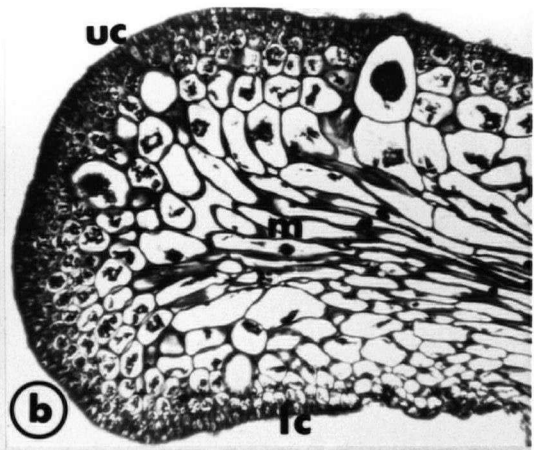
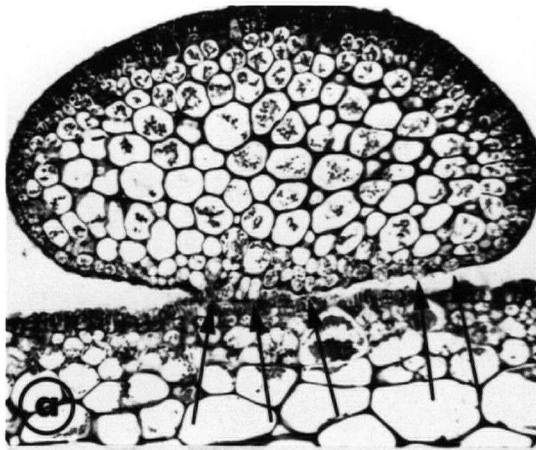


Figure 36a. Cross-section of sterile bladder of C. antarcticum. Arrow points to wall breakdown and hollow central region (hc). (200x)

Figure 36b. Sterile bladder in cross-section. Arrow points to cuticle-like material on surface of plant. (675x)

Figure 36c. Plurilocular bladder. Arrows point to wall breakdown in medulla near hollow central region. (200x)

Figure 36d. Plurangia (P) with large 'physode-cell'. Cuticle-like material on thallus surface arrowed. (675x)

Figure 36e. Bladder with plurangia which have released their contents. Arrow indicates wall breakdown in medulla. (200x)

Figure 36f. Released plurangia (RP) in C. antarcticum bladder. Note absence of cuticle-like layer. Arrows point to empty locules of plurangia. (675x)

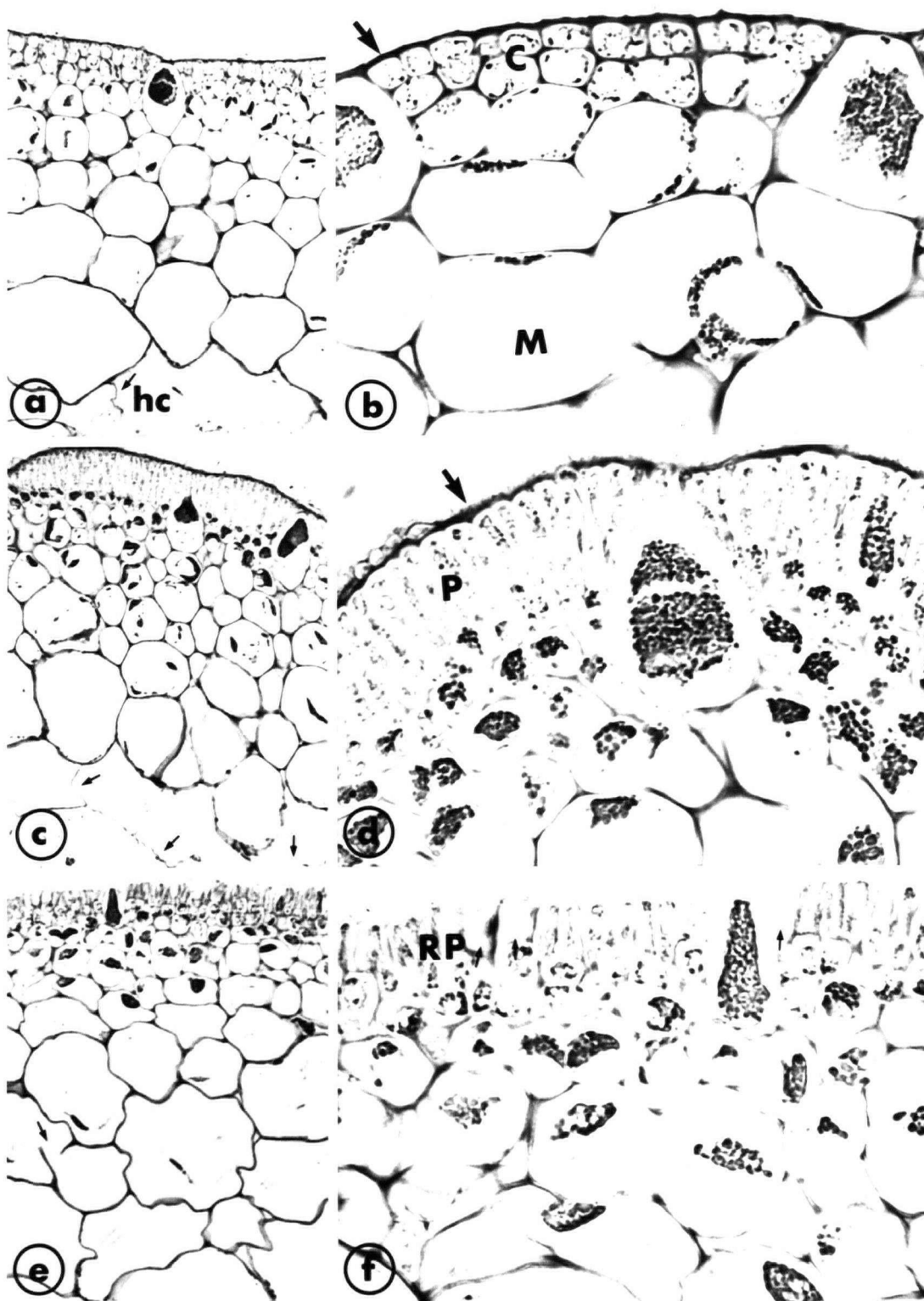


Figure 37. Longitudinal section through crust at base of an erect axis.

(a) section through crustose portion in cup-like depression. Arrows point to zone of transition of cortex.

(b) section through base of erect axis and crust depression.

CM = crust medulla; EM = erect axis medulla (190x)

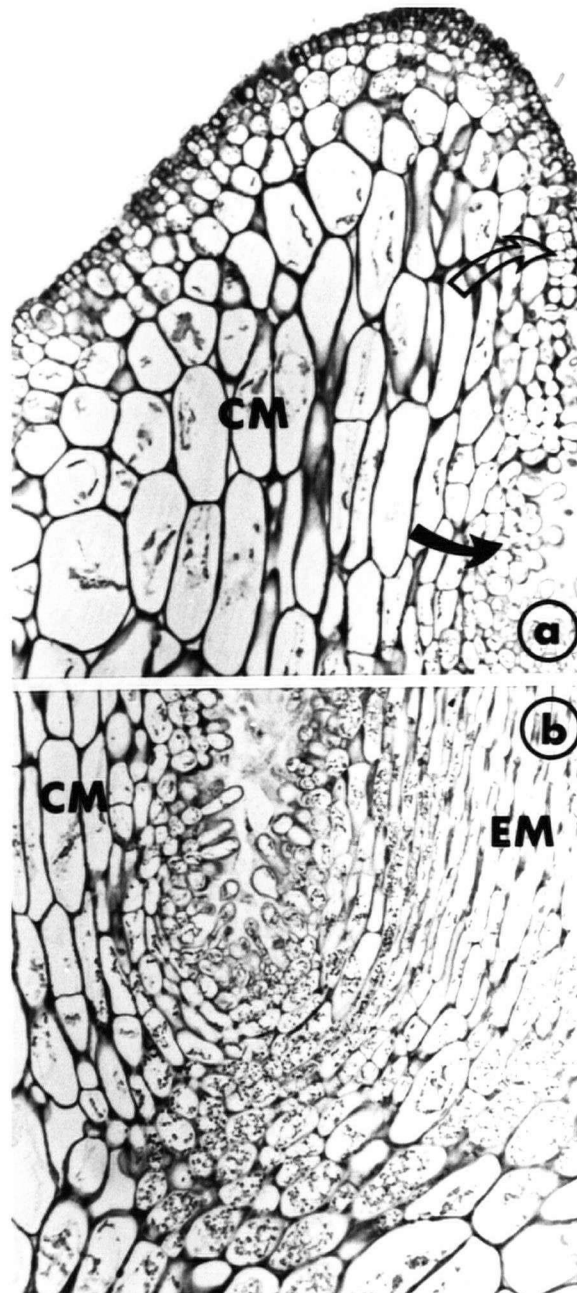


Figure 38. Line drawing of a longitudinal section at point where erect axis of C. antarcticum grows out of crust. Arrows (T) indicate point of transition in the cortex of crust. Arrow (EA) indicates direction of growth of erect axis. (140x)

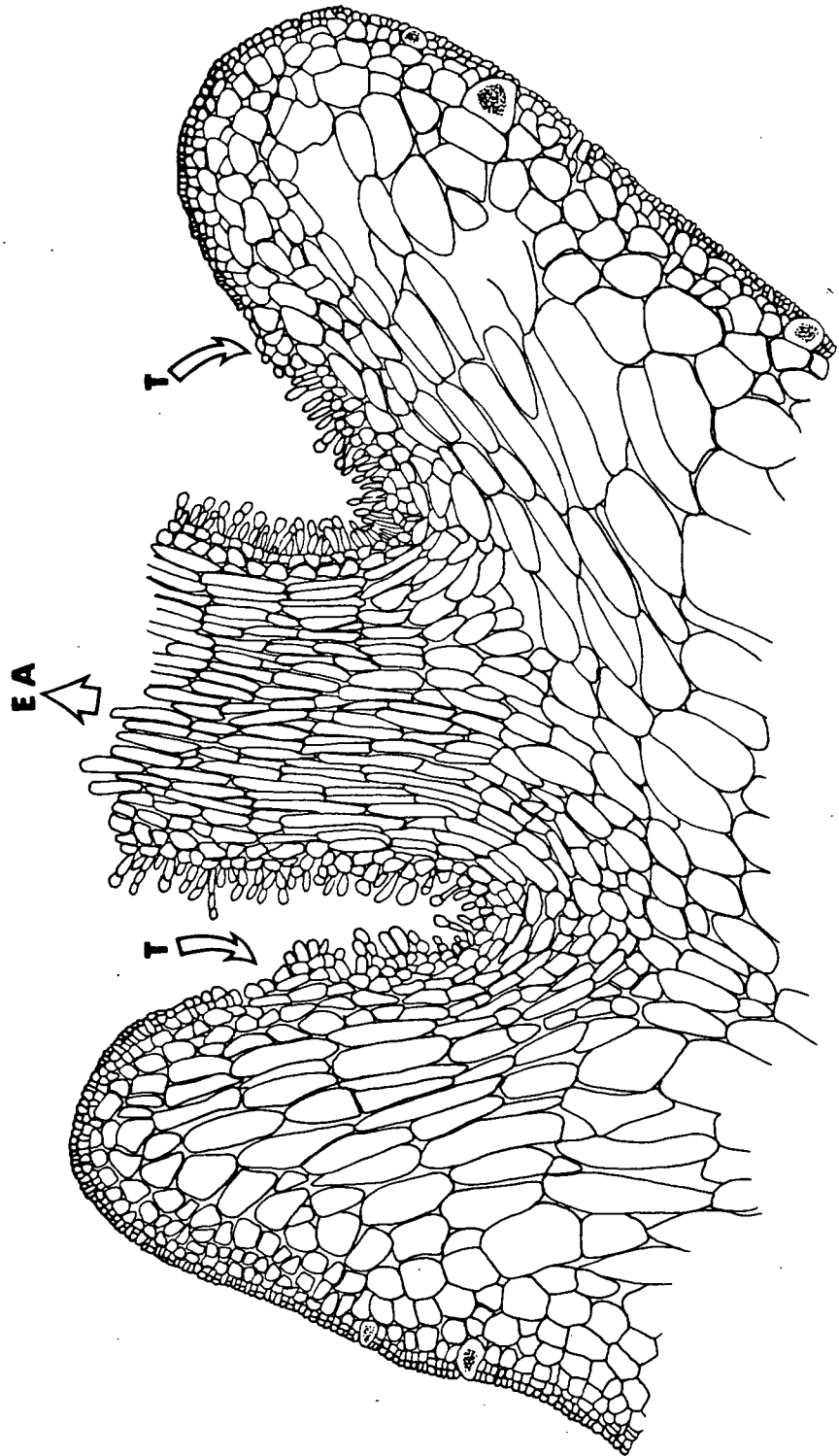


Figure 39a. Longitudinal section of upright axis of C.antarcticum showing developing lateral branch (DL - curved arrow). Centre of axis is becoming hollow (hc). Solid arrow points to enlarged cell lumen resulting from crosswall breakdown. (175x)

Figure 39b. Developing lateral of C.antarcticum showing hairs (h) and unilocular sporangia (u). Growth occurs at the base of the cortical filaments. (460x)

Figure 39c. Longitudinal section through apex (A) and lateral (L) of C.antarcticum. (190x)

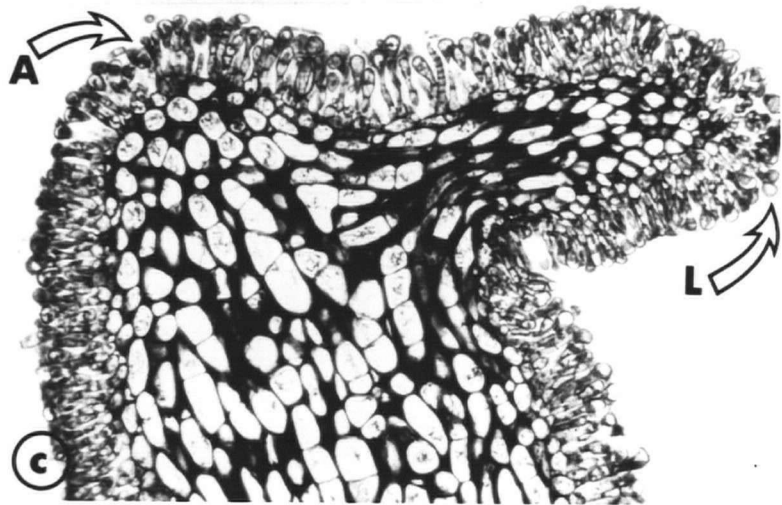
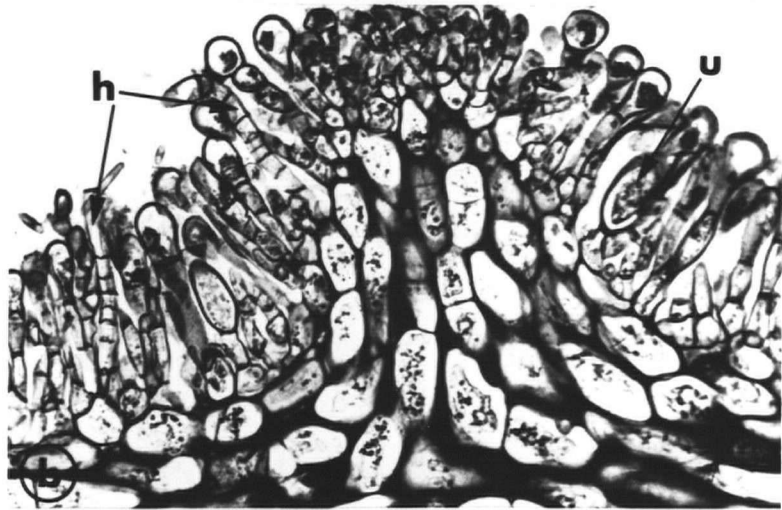
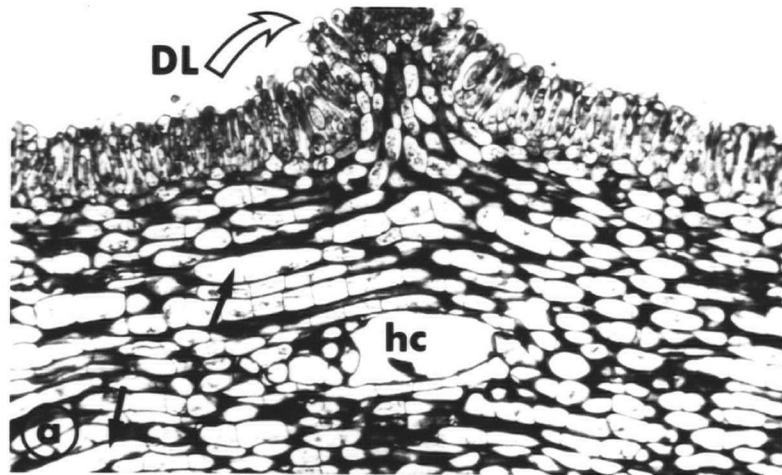


Figure 40a. Longitudinal section of sterile erect axis of C.antarcticum. C = cortex; SC = sub-cortex; M = medulla; arrows point to cells developing from sub-cortex. (675x)

Figure 40b. Longitudinal section of erect axis of C.antarcticum showing unilocular sporangia. (arrows to u = unilocular sporangia) (675x)

Figure 40c. Unilocular tissue of C.antarcticum. H = hairs; u = unilocular sporangia. (675x)



CHAPTER 6,

An evaluation of the Ralfsiales, Ralfsiaceae, and the
taxonomic position of Analipus japonicus (Harv.) Wynne

The Ralfsiales

The order Ralfsiales was established by Nakamura (1972) "on the basis of Ralfsia especially because of peculiarities in the early development of the thallus, and in the structure of cells and reproductive organs". Nakamura gave the following criteria as distinguishing features of this order:- (1) a single, parietal, plate-like chloroplast without pyrenoid per cell, (2) discal-type of development of the thallus, and, (3) an Ectocarpus-type of life cycle. He recognised three families in the order separating them entirely on the basis of the placement of reproductive organs. Thus, in Nakamura's arrangement, in the Lithodermataceae are placed plants with terminally borne unilocular and plurilocular organs, in the Nemodermataceae are those with intercalary unilocular and lateral plurilocular organs, and in the Ralfsiaceae are the plants with lateral unilocular and intercalary plurilocular organs. Although recognising these families, Nakamura does state "... the possibility cannot be excluded that these three families should be united into a single family Ralfsiaceae."

In describing the Ralfsiales, Nakamura refers in particular to Ralfsia verrucosa and Analipus japonicus (Heterochordaria abietina). Prior to Nakamura's treatment, A. japonicus had been placed in the Chordariaceae and

Chordariales (Wynne, 1971) (and previously as Heterochordaria abietina in the Heterochordariaceae, Chordariales). Nakamura maintains that the genus Analipus should be included in the Ralfsiaceae because of its discal-type development and anatomy, particularly of its reproductive organs. This taxonomic judgement was supported by Wynne (1972) who considered that the affinity between Ralfsia and Analipus should be recognised by these genera being classified in the same family.

Although Wynne and Loiseaux (1976) note that "many problems remain to be resolved before this order can be maintained safely", the order Ralfsiales as described by Nakamura (1972) has been accepted by Bold and Wynne (1978) in their recent algal textbook, and in a slightly modified form by Tanaka and Chihara (1980). The order has not been used by a number of workers (e.g. John and Lawson, 1974; Russell and Fletcher, 1975; Abbott and Hollenberg, 1976; Pedersen, 1976; Rueness, 1977) and their treatments of crustose brown algae have varied.

There are fundamental objections to this order established by Nakamura as can be realised when one examines the three distinguishing criteria of the order:-

(1) a single, parietal, plate-like chloroplast, without pyrenoid, per cell

If the criterion of one chloroplast per cell is enforced, a number of crustose brown algae, classified in

either the Ralfsiaceae or Lithodermataceae are excluded from the order e.g. Basispora africana, B. saxigena (John and Lawson, 1974); Diplura simulans, Endoplura aurea (Hollenberg, 1969); Lithoderma fatiscens (Waern, 1949); Pseudolithoderma paradoxum (Sears and Wilce, 1973), P. roscoffensis (Loiseaux, 1968), P. rosenvingii (Lund, 1959, as described in Waern, 1949).

Pyrenoids have also been reported in a number of these crustose plants e.g. Ralfsia borneti and R. clavata (Edelstein, Chen and McLachlan, 1970), R. clavata (Loiseaux, 1968), R. verrucosa (Fletcher, 1978), Ralfsia and Petroderma (Russell and Fletcher, 1975); Sorapion kjellmanii, Symphycarpus longisetus, S. strangulans, (Lund, 1959). (Although Tanaka and Chihara (1980) accept the order Ralfsiales, they do not consider chloroplast features -e.g. number, shape or absence of pyrenoids - to be one of the criteria for membership in this order.)

(2) discal-type of development

Nakamura (1972) discussed the early development of brown algal thalli and distinguished three developmental patterns:- (a) creeping-type (exhibited by members of the Ectocarpaceae and Myrionemataceae), (b) erect-type (as found, for example, in the Dictyotales and Fucales), and (c) discal-type. In describing the discal type, Nakamura used as examples Ralfsia verrucosa and Analipus japonicus. He stated "This discal-type of development has been described by Kuckuck (1897) in Nemoderma tingitana, by

Kylin (1934) and Loiseaux (1968) in Ralfsia clavata and by Sauvageau (1907) in members of the Sphacelariales.". In fact, Kuckuck (1897) as cited by Nakamura, does not refer to Nemoderma, although Kuckuck (1912) does describe and figure a discal morphogenesis for Nemoderma Schousbe. While Kylin (1934) shows discal development for spores from unilocular spcrangia of Ralfsia clavata, there is a considerable amount of work contradicting this. Loiseaux (1968), Edelstein et al. (1970), and Fletcher (1978) have described and illustrated the unispores of R. clavata developing initially into a uniseriate filament and later forming a disc structure. It would appear that the non-discoidal early development of R. clavata unispores is well documented, and that the material cultured by Kylin was unlikely to have been the same entity.

Other developmental studies on crustose brown algae have shown filamentous development (e.g. Petroderma maculiforme, (Wynne, 1969; Wilce, Webber and Sears, 1970), Pseudolithderma nigra (Hollenberg, 1969), P. paradoxum (Sears and Wilce, 1973), P. roscoffensis (Loiseaux, 1968; Fletcher, 1978); Ralfsia borneti (Edelstein et al., 1970), R. confusa, R. integra (Hollenberg, 1969)), and fewer with discoidal development (e.g. Ralfsia pacifica (Wynne, 1972), R. verrucosa (Loiseaux, 1968; Edelstein, Chen and McLachlan, 1971; Fletcher, 1978); Analipus japonicus (Wynne, 1972; Chapter 1 (in the case of the latter two species, confirming the work of Nakamura, 1972)). Although

the pattern exhibited by a species seems to be a constant feature, at least within one genus, namely Ralfsia on which this order is based, both types of germination-development patterns occur. This may be a reflection of the state of Ralfsia taxonomy rather than of the value of this character at the generic level. In my opinion, however, the developmental pattern or type, as outlined by Nakamura, does not constitute a good ordinal criterion for this group of crustose brown algae.

(3) Ectocarpus-type life cycle

Nakamura was referring here to the cycle classically understood as the Ectocarpus-type with a alternation of isomorphic generations. In their review of life history studies in the Phaeophyta, Wynne and Loiseaux (1976) note that the classical interpretation of Ectocarpalean life histories has been subjected to modifications over the past twenty years. The work of Müller (1967, 1972) on Ectocarpus siliculosus reveals a complex life history which involves different growth forms for the gametophyte and sporophyte generations as well as a number of possible cytological pathways. It would appear that the description of a life history pattern by the term 'Ectocarpus-type' is not particularly clear, given the variation now known to occur in this genus.

Although life history studies have been carried out on a number of members of this group of brown algae (e.g. Loiseaux, 1968; Wynne, 1969, 1972; Edelstein et al.,

1970, 1971; Fletcher, 1974, 1978), only 'direct' or apomeiotic life histories (e.g. Petroderma maculiforme, Wynne, 1972), or life histories involving the crusts in the life histories of members of the Scytosiphonales (e.g. Ralfsia clavata and R. borneti, Edelstein et al., 1970) have been reported, and no completed life history with evidence of sexuality has been shown. Kuckuck (1912) gives a convincing description of anisogamy in Nemoderma tingitana and the fusion of settled female and swimming male gametes. A reproductively mature plant was not grown from the resulting zygotes, however, and no cytological evidence was given. It would appear that throughout this group there has occurred a reduction in life history stages and possibly in some, a loss of sexuality. The life histories of these organisms need further work, however, in both culture and, particularly, in cytological studies, before useful generalisations can be made about them.

It is my opinion that the Ralfsiales cannot be recognised for the reasons outlined above; to continue to use this taxon will only further confuse the already troubled state of the taxonomy of the so-called 'lower' brown algae.

The Ralfsiaceae

As already mentioned, Nakamura (1972) recognised three families in his treatment of the crustose brown algae, and these families were separated by him entirely on the basis of the placement of the reproductive structures. This treatment was also followed by Bold and Wynne (1978). Although both Hcllenberg (1969) and Fletcher (1978) make passing reference to chloroplast numbers being used generally as a character to distinguish the Ralfsiaceae and the Lithodermataceae, in fact in those works where family descriptions are provided (e.g. Taylor, 1957, 1960) chloroplast characters are not used but rather the descriptions are based on the placement of reproductive structures - in keeping with the original descriptions of these families. Tanaka and Chihara (1980) correctly observe that chloroplast number cannot be used as a criterion to distinguish these families.

Feldmann (1937) established the monotypic family, the Nemodermataceae, for Nemoderma tingitana Schousbe. Members of this genus possess intercalary unilocular sporangia, and lateral plurilocular organs which are clearly distinguishable by size as oogonial or antheridial in nature, and produce anisogametes. Feldmann considered that these reproductive structures were sufficiently distinct from those found in the Ralfsiaceae (which he used in its wider sense to include Lithodermataceae) to warrant

familial status. Nemoderma is restricted in its distribution to the Canary Islands, Morocco, Algeria, and Tunisia, and for this reason is often not discussed or dealt with in descriptions of crustose brown algae. Papenfuss (1951, 1955) includes Nemoderma in the Ralfsiaceae in his taxonomic treatments of the Phaeophyta. Fletcher (1978) in speculating on the phylogeny of crusts mentions Nemoderma as a possible example of an advanced plant in an evolutionary line of soft crusts starting from a Petroderma-like ancestor. As the only crustose brown alga with clearly oogonial and antheridial plurilocular structures which are laterally borne and also the only one with intercalary unilocular organs, Nemoderma is very distinctive. In my opinion, the continued use of this family is justified.

The Ralfsiaceae was described by Hauck (1885) and was also used, but in a somewhat different sense, by Kjellman (1891-93). The Lithodermataceae was first suggested by Kjellman (1883), described by Hauck (1885), and again by Kjellman (1891-93). As understood by these two workers, the primary difference between the members of these two families was the position in which sporangia were produced. Thus, the Lithodermataceae were seen as being distinctive because the unilocular sporangia were formed by transformation of surface cells rather than being found immersed among sterile cortical filaments, as in the Ralfsiaceae. The plurilocular organs in the

Lithodermataceae were described by Kjellman (1883) as 'arranged in specific stands issuing from the surface-cells of the frond', and by Kjellman (1891-93) as being lateral outgrowths from surface cells. In the Ralfsiaceae, the plurilocular sporangia were described as being formed by the transformation of the cortical filaments (Hauck, 1885). When these families are recognised as separate entities today, the unilocular sporangia are understood as first represented by Hauck and Kjellman, although the understanding of the plurilocular organs is somewhat different, with those in the Ralfsiaceae being described as intercalary and those in the Lithodermataceae as terminal in origin (e.g. Bold and Wynne, 1978).

In the literature there has been considerable variation in the treatment of crustose Phaeophyta, both in terms of how many families are recognised and, at a higher taxonomic level, with which order they are to be placed. Some workers do not consider the Lithodermataceae to be a distinct family recognising only the Ralfsiaceae (e.g. Setchell and Gardner, 1925; Feldmann, 1937; Papenfuss, 1951, 1955; Lund, 1959; Hollenberg, 1969; John and Lawson, 1974; Russell and Fletcher, 1975; Lindstrom, 1977; Rueness, 1977). Other workers recognise both families (e.g. Taylor, 1957, 1960; Wilce, 1959; Bold and Wynne, 1978; Tanaka and Chihara, 1980), and rarely, only the Lithodermataceae is recognised (e.g. Tokida, 1954). A number of workers (e.g. Setchell and Gardner, 1925; Russell and Fletcher, 1975;

Fletcher, 1978) have discussed the similarities between the Myrionemataceae and the Ralfsiaceae/Lithodermataceae (Nemodermataceae is sometimes also mentioned) and there are those who have carried this understanding further and classified these plants together in one family, the Myrionemataceae (e.g. Skottsberg, 1921; Borgesen, 1926; Hamel, 1939; Loiseaux, 1967a and b, 1968) ..

Sometimes the Myrionemataceae and the other families of crustose brown algae are all classified in the same order, this being either the Ectocarpales (e.g. Setchell and Gardner, 1925; Feldmann, 1937; Russell and Fletcher, 1975; Kornmann and Sahling, 1977) or the Chordariales (e.g. Kylin, 1947; Lindauer, Chapman and Aiken, 1961; Taylor, 1957, 1960; Abbott and Hollenberg, 1976; Lindstrom, 1977). Other workers, however, have placed the Myrionemataceae in the Chordariales and the Ralfsiaceae/Lithodermataceae into the Ectocarpales (e.g. Kylin, 1933; Papenfuss, 1951, 1955), separating at an ordinal level what other workers consider to be very closely related plants. Pedersen (1976) while recognising both the Myrionemataceae and the Lithodermataceae in the Ectocarpales, has placed the genus Ralfsia into the Scytosiphonales - not only those species of Ralfsia which have been shown to be involved in the life histories of members of the Scytosiphonales but also those species which in culture studies have shown no connection as yet to scytosiphonalean life histories.

The crusts vary anatomically, in particular, in the

origin, development, and degree of cohesion of the erect filaments e.g. tightly packed assurgent and downwardly curving cell rows from a central layer in Ralfsia fungiformis cf. loosely associated erect filaments arising from 1-several layers of prostrate filaments in Basispora africana. Other vegetative features such as a cuticle layer, hairs, and rhizoids, may be absent or present. As noted earlier, germination and early development in these plants takes place in at least two different ways - either in a discoidal fashion e.g. Ralfsia pacifica, R. verrucosa, or filamentously e.g. Petroderma maculiforme, Pseudolithoderma nigra. Chloroplast characters such as number, shape and the presence or absence of pyrenoids vary considerably amongst these plants.

The classical character used to differentiate these plants - namely, the placement of reproductive structures - causes some problems e.g. Ralfsia lucida possesses unilocular sporangia which may be in a terminal, lateral, or intercalary position (Lund, 1967); Edelstein, Chen and McLachlan (1968) note that in the early stages of development of Ralfsia fungiformis the unilocular sporangia are terminal on erect filaments and it is only once a paraphysis develops on the same filament that the unilocular organ can be said to be laterally positioned; Fletcher (1978) records the production of terminal unilocular sporangia which are unaccompanied by paraphyses in cultures of R. spongiocarpa; Lithoderma fatiscens

Aresch. emend. Waern (the type species of a family now understood to have terminal plurilocular structures) possesses laterally inserted plurilocular sporangia (Waern, 1949).

As previously noted, the life histories of Ralfsiaceae/Lithodermataceae which have been examined to date show two basic patterns - either a direct type without evidence of sexuality, or one which shows the crusts to be stages in the life history of plants in the Scytosiphonales. A number of workers consider that the Ralfsia crusts involved in scytosiphonalean life histories belong to the subgenus Stragularia (e.g. Wynne, 1969; Edelstein et al., 1970; Roeeveld, Duisterhof and Vroman, 1974). Stragularia was first described as a genus by Strömfelt (1886) and later given sub-generic rank with Euralfsia by Batters (1890). Stragularia was distinguished from Euralfsia by having a looser vegetative structure and diffuse sporangia or in the words of Batters (1890) "The species belonging to this subgenus resemble Myrionemata in fructification and Lithodermata in structure." Wynne (1969) voices the opinion that Stragularia is quite distinct from Ralfsia sensu stricto and much of the culture evidence available supports this view. There are, however, reports which show Stragularia-type Ralfsia species to have direct life histories in culture e.g. R. clavata (Loiseaux, 1968), R. confusa (Hollenberg, 1969), R. spongiocarpa (Fletcher, 1978). As Wynne (1969) notes, even Stragularia

may in fact be a heterogeneous collection of species and warrant further subdivision. Fletcher (1978) considers that a subdivision of the Euralfsia species may be justified. The complexity of the situation is further shown by culture studies of members of the Scytosiphonales which showed no crustose phase in their life histories (e.g. Fletcher, 1974; Sauvageau, 1927), and studies of other members of the Scytosiphonales which showed the involvement of myrionematoid stages in their life histories (e.g. Loiseaux, 1970; McLachlan, Chen and Edelstein, 1971b; Fletcher, 1978).

It can be seen from the foregoing that there is currently no consensus on the taxonomic affinities either within the crustose brown algae, or between these plants and other members of the Phaeophyta. Although these crusts share a similar habit and a pseudoparenchymatous construction, the many differences which exist suggest that they do not form a natural assemblage. At present, however, there is no clear grouping of characters which allows for taxonomic segregation above the level of genus. Even at the generic level there have been some longstanding and frequently discussed problems in the literature, such as, for example, the circumscription of the genera Lithoderma (e.g. Lund, 1938; Waern, 1949) and Ralfsia. (In the case of the latter this still is unresolved.) Neither the division of the crustose brown algae into the two families Ralfsiaceae and Lithodermataceae, nor the grouping of these

plants with the Myrionemataceae, in my opinion, seems to reflect a natural grouping, nor does it appear to help us more easily conceptualise the relationships and affinities of these plants. Thus, I favour the recognition of the Myrionemataceae, the Ralfsiaceae (to include the Lithodermataceae) and also the Nemodermataceae, and consider that at present they are most appropriately placed in the Ectocarpales. As more is learnt about the members of the Ralfsiaceae this family will probably be divided into groupings based more on relationships and affinities and less on the current basis of habit and pseudoparenchymatous construction.

The taxonomic position of *Analipus japonicus* (Harv.) Wynne

Early taxonomic treatments of *Analipus japonicus* (Harv.) Wynne (as *Chordaria abietina* Rupr. ex Farlow and later as *Heterochordaria abietina* (Rupr. ex Farlow) S. and G.) dealt exclusively with characteristics of the erect axes and placed this plant either in the Chordariaceae (e.g. Farlow, 1875; Okamura, 1910, 1933; Kylin, 1940; Scagel, 1957; Inagaki, 1958) or the Heterochordariaceae (a monotypic family erected by Setchell and Gardner, 1925) (e.g. Nagai, 1940; Smith, 1944). Nakamura (1972) placed *A. japonicus* in the Ralfsiaceae and this treatment has been followed by Wynne (1972) and Bold and Wynne (1978) although

Abbott and Hollenberg (1976) classify this plant in the Chordariaceae.

As discussed at the beginning of this chapter, Nakamura's justification for the placement of Analipus with the Ralfsiaceae was its discoidal early development, and anatomy, particularly that of its reproductive structures. The review of the literature here has shown that these criteria are not shared by many other members of the group of plants generally known as the Ralfsiaceae. Although the anatomy of the A. japonicus crust is similar to that of Ralfsia verrucosa, it is very different from that of some other Ralfsia species and other members of the Ralfsiaceae (e.g. Petroderma, Symphycarpus, Basispora, R. clavata, R. spongiocarpa). Although the unilocular sporangia in Analipus are terminal/lateral and immersed in paraphyses, and the plurilocular organs are intercalary, they are borne on the upright thallus system and the crust is never fertile. I consider that Analipus is quite distinct from the majority of crusts now recognised in the Ralfsiaceae.

The genus Analipus does not seem to be appropriately placed in the Chordariaceae. At the moment there are a number of different understandings of the order Chordariales. Some treatments of this order include the Chordariales within the Ectocarpales (e.g. Russell, 1964; Parke and Dixon, 1964) as originally proposed by Oltmanns (1922) and followed by Fritsch (1945). Bold and Wynne

(1978) consider, however, that the Chordariales should be maintained as a distinct order "on the basis of the heteromorphic life history in which the haploid microthallus alternates with a diploid macrothallus". If the separation of the Ectocarpales and Chordariales is understood to be primarily on life history characteristics, Analipus should not be placed in the Chordariales as it has been shown to possess an alternation of isomorphic generations.

In my opinion the genus Analipus should not be placed in either the Ralfsiaceae or Chordariaceae but warrants placement in a separate family. The monotypic family Heterochordariaceae was erected by Setchell and Gardner in 1925 for Heterochordaria abietina (Rupr. ex Farlow) S. and G. Heterochordaria was merged with Analipus by Wynne (1971). The family is legitimate as it was based on a legitimate genus Heterochordaria S. and G. 1924 and "Because its merger with Analipus is a matter of taxonomic opinion, it was not nomenclaturally superfluous when published." (Dr P.C. Silva - pers. comm.). Setchell and Gardner's (1925) description of the Heterochordariaceae included 1) vegetative characters of the upright axes, and 2) the occurrence of isomorphic plants bearing either unilocular or plurilocular structures. It was this second feature which was the primary basis for separation of this genus from the Chordariaceae. When Smith (1944) used the Heterochordariaceae (placed in the Ectocarpales) the family

characteristics given included mention of the crustose system, and the position of the unilocular and plurilocular sporangia. In order to accommodate both A. filiformis (Rupr.) Papenfuss and A. japonicus in this family the original description needs to be emended.

Two other species possibly fall within this family but require closer examination before this can be effected. The species Chordaria gunjii Yendo (Yendo, 1913) (or Heterochordaria gunjii (Yendo) Tokida - Tokida, 1938; Nagai, 1940) was discussed by Wynne (1971). I have examined the type material of this species and on the basis of the anatomy of the erect system (of both plurilocular and unilocular plants) it would appear to fall within the genus Analipus. As I have only seen the erect axes of this species and none of the crustose portion which has been reported, I am not able to make any judgement on its taxonomic position. It deserves close attention, however, as a likely third species in this genus.

The other species of interest here is the sub-antarctic plant Stereocladon rugulosum (Skottsberg, 1921; Papenfuss, 1964) which possesses a crustose ralfsioid system from which arise erect axes with a "Chordaria"-like construction. This plant has not received attention since Skottsberg's treatment and needs further examination to determine its taxonomic affinities.

GENERAL DISCUSSION,

A variety of approaches have been used in this study of the biology of Analipus japonicus. Data obtained from life history, anatomical and cytological studies have enabled a re-evaluation of the taxonomic affinities of this alga. A morphological and anatomical comparison of A. japonicus and Caepidium antarcticum was made because of reports in the literature of their similarity. Whilst certain differences and similarities of these two were distinguished, life history and cytological data are needed for C. antarcticum before its affinities can be fully understood. This general discussion is an attempt to integrate the data derived from the various approaches I employed, and to point to questions which remain outstanding.

The culture and cytological studies have confirmed an alternation of isomorphic generations for A. japonicus. However, the phenological and ecological studies show that for A. japonicus, in the eastern Pacific, the haploid plurilocular phase predominates and sexual reproduction and unilocular plants are rare. In a review of the Phaeophyta, Russell (1973) observed that in those brown algae which have been studied, a number have been shown to possess solely asexual life histories, reproducing either by plurispores or unispores. It has been suggested that in some of these species the reports may be artifacts of culture conditions (Edelstein, Chen and McLachlan, 1971). It has also been found that different populations or

isolates of the same species exhibit different life histories (e.g. Hecatonema maculans - Loiseaux, 1969; Edelstein et al., 1971). Russell (1973) states, however, "the incidence of genuinely asexual life histories, particularly in the Ectocarpales, seems likely to be high". In this study, potentially sexual populations of A. japonicus were identified only in Japan and in one site in California.

The generally accepted wisdom in biology is that sexual reproduction is a positive factor in the evolutionary potential of a population and in its survival (Mayr, 1963; Williams, 1975). As Williams (1975) notes, if these benefits are important, the relative inferiority of asexual forms should be demonstrable in terms of ecological success and genetic potential. There is little evidence, however, to support this idea. Amongst weeds both asexuality and selfing are common (Allard, 1965; Baker, 1974). Williams (1975) observes that the success of asexual or selfed weeds may be due to a number of possible reasons - "the ability to establish themselves with single propagules, their lack of reproductively wasteful male functions, their frequent polyploidy, or freedom from recombinational load". Apparently asexuality in A. japonicus is a derived feature, with at least one remnant sexual population remaining in California. No information is available on the distribution of unilocular and plurilocular populations of A. japonicus in the western

Pacific. It would be most interesting to have more information on the relative contributions of sexual and asexual reproduction to population maintenance and variation in these regions.

Although the generations of A.japonicus are isomorphic, within each generation the thallus can be regarded as consisting of two quite different parts - the crust and the upright axes borne on the crust. The crust is perennial and appears to play a significant role in the survival and success of this species. It is the colonising portion of the plant. Although it doesn't bear sporangia, the crust is able to spread vegetatively holding onto settlement space - the resource which is at a premium in the intertidal region. It appears to be resistant to environmental stress and also to grazing. The erect axes, however, are relatively shortlived and are constantly being replaced by growth from the crust. Particularly in the plurilocular plants there is a very high investment in reproductive tissue. Thus, the two parts of the thallus have very different mortality rates and adaptations to their intertidal existence.

The life histories of a number of algae include both a crust and an erect phase in separate generations (e.g. Ralfsia and Petalonia; Petrocelis and Gigartina) and it has been considered that these distinct morphological phases have evolved under different selective pressures (Littler and Littler, 1980; Lubchenco, 1980). Studies on

Petrocelis middendorffii have shown it to be slow growing, with both low recruitment and mortality rates (Paine, Slocum and Duggins, 1979). Its alternate generation Gigartina papillata has a significantly different growth strategy and productivity (Littler and Littler, 1980). A. japonicus differs from these plants, however, in possessing two morphologically and functionally different parts within the same caryological phase.

There are many examples of algae such as A. japonicus in which an erect axis or blade is borne on a crustose or perennating structure (e.g. Apophloeoa lyallii, Iridaea cordata, Chondrus crispus, Phaeostrophion irregulare, Caepidium antarcticum). The plants have been termed Hemicryptophyceae by Feldmann (1937) on the basis of their "life-form". This is equivalent to the term hemicryptophyte used in terrestrial plants in the terminology of Raunkiaer 'plant life-forms' (Mueller-Dombois and Ellenberg, 1974). From studies in Brittany, Ernst (1968) suggests that there is a vertical zonation of life-forms in marine algal communities, with the sequence from upper intertidal to subtidal regions being phanerophyceae, hemiphanerophyceae, hemicryptophyceae, and chamaephyceae. In a study of Iridaea cordata (Hansen, 1977) growth of upright thalli was found to occur mainly from the perennating crusts. Hansen likened the role of the crust to that of the laterally spreading root system in some vascular plants. The majority of the blades developed tetrasporangia and therefore the

population should have had a high potential for sexual reproduction. However, Hansen found that gametangial thalli were only a minor component of the population and apparently the contributions of sexual reproduction and sporeling development were minimal to the population growth and maintenance. Lubchenco (1980) found that in New England shores, perennation from the holdfast/crust of Chondrus crispus was a significant feature in its ecological success and long term persistence. Mathieson (1965) carried out an autecological and life history study of Phaeostrophion irregulare, a brown alga which lives in habitats which are seasonally buried in sand. He observed that the blades are ephemeral and do not survive the seasonal submergence in sand. The crust, however, was found to be an important element in the survival of Phaeostrophion in its rigorous habitat. The crust is much more resistant than the blades to sand burial and is capable of initiating new blades once it is uncovered. Mathieson regarded the life history of Phaeostrophion - a direct life history with no evidence of sexuality - to have considerable adaptive value in the plant's success. Although vegetative reproduction and perennation have been discussed (Knight and Parke, 1931; Dixon, 1965) as important features in the growth of perennial algae, in the opinion of Hansen (1977) generally these features have been underestimated or not considered.

Differences in the crust and erect portions of the A. japonicus thallus deserve further attention to understand

more fully the relationship between these phases and their relative contributions to the plant's competitive ability. It would be interesting to study photosynthetic activities of the crust and of erect axes, and their relative importance to growth and reproduction - that is, whether crusts are subsidising growth and reproductive activity of uprights or whether uprights are exporting photosynthates to the crust for storage, or growth and expansion of the crustose system. The seasonal pattern of growth and allocation of photosynthetic products may well vary, with the crust contributing significantly to growth of erect axes after a period of stress or grazing, when erect axes have been removed or substantially reduced.

Evolution of life history strategies or life history traits has received considerable attention in recent years and was recently reviewed by Stearns (1976, 1977). Models have been developed to explain adaptation of life history traits to stable or to fluctuating environments. Stearns (1977) reviewed deterministic (e.g. MacArthur and Wilson, 1967; Pianka, 1970) and stochastic (e.g. Murphy, 1968; Schaffer, 1974) models of life history traits. He concluded that neither type of model is empirically sufficient - "their predictions are not consistent with much of the evidence. We do not yet have a general and reliable theory of life history evolution.". Searles (1980) discusses the evolution of the triphasic life history of red algae. He considers that this complex life history pattern can be

related to the lack of motility of the gametes and the apparently infrequent occurrence of syngamy. However, Searles was addressing the generalised life history rather than the adaptation strategy of an individual species or population. The strategy of an individual alga is not going to be solely defined by its potential mechanisms for sexual and asexual reproduction but is also the result of its morphology or "life-form" and its physiology. There is very little information available for seaweed species on basic population characteristics such as natality and mortality, fecundity, reproductive effort, age of first reproduction (Chapman, 1974). Before the understanding of algal life history strategies can be extended and generalisations of any creative value arrived at, more data are needed on these basic population processes.

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APPENDICES,

Appendix I

List of collection sites and dates of collections .

BRITISH COLUMBIA

Aguilar House Point - 48°50'N, 125°08'W

18 August 1978, 16 October, 1978, 4 December 1978, 25 February 1979, 25 April 1979, 10 June 1979, 11 July 1979, 23 August 1979

Bath Island - 49°09'N, 123°40'W

29 June 1977, 30 July 1977, 8 September 1977, 21 October 1977, 4 November 1977, 2 December 1977, 20 January 1978, 3 March 1978, 28 March 1978, 7 April 1978, 12 May 1978, 24 May 1978, 21 June 1978, 18 July 1978, 13 August 1978, 6 September 1978, 6 October 1978, 19 November 1978, 8 December 1978, 13 January 1979, 10 March 1979, 10 April 1979, 12 May 1979, 11 June 1979, 10 July 1979, 24 August 1979

Botany Beach - 48°32'N, 124°27'W

10 March 1978, 22 June 1978, 3 February 1979, 10 March 1979, 17 March 1979, 13 May 1979, 9 July 1979, 15 March 1980

Brady's Beach - 48°49'N, 125°09'W

5 February 1978, 17 August 1978, 2 December 1978, 25 February 1979, 26 April 1979

Breakwater Island - 49°08'N, 123°40'W

7 April 1978, 19 July 1978

Diana Island - 48°50'N, 125°11'W

16 October 1978, 2 December 1978, 10 June 1979, 12 July 1979

Haines Island - 48°50'N, 125°10'W

28 May 1979

Helby Island - 48°51'N, 125°10'W

26 April 1979, 27 May 1979, 12 July 1979, 23 August 1979

Pachena Bay - 48°51'N, 125°20'W

26 April 1979

Ross Islets - 48°52'N, 125°09'W

21 May 1978

Second Beach - 48°49'N, 125°10'W
 28 May 1978, 17 August 1978, 15 October 1978, 2 December
 1978, 27 April 1979, 27 May 1979, 9 June 1979, 12 July
 1979, 23 August 1979

Serrings Island - 48°51'N, 125°12'W
 28 May 1979

Sidney - 48°38'N, 123°25'W
 20 July 1978

Sombrio River - 48°30'N, 124°17'W
 14 May 1979

Sooke - 48°21'N, 123°44'W
 18 February 1978, 1 May 1978, 21 June 1978, 20 July 1978, 3
 March 1979, 1 April 1979

Victoria Breakwater - 48°24'N, 123°24'W
 1 April 1979, 19 August 1979

Wizard Rock - 48°51'N, 125°09'W
 26 April 1979, 27 May 1979, 9 June 1979, 12 July 1979, 23
 August 1979

WASHINGTON

Cattle Point - 48°27'N, 122°58'W
 6 July 1978

Kalaloch - 47°55'N, 124°38'W
 8 September 1978

Mar Vista - 48°28'N, 123°01'W
 6 July 1978

OREGON

Boiler Bay - 44°50'N, 124°05'W
 21 June 1978, 29 March 1979

Cape Arago (south cove) - 43°19'N, 124°24'W
 1 13 June 1979

Devil's Punchbowl - 44°47'N, 124°05'W
 30 March 1979

Fogarty Creek - 44°49'N, 124°05'W
 24 June 1978

Harris Beach - 43°10'N, 124°20'W
28 March 1979

Marine Gardens - 44°48'N, 124°05'W
20 June 1978, 30 March 1979, 7 August 1979

Sunset Beach - 43°18'N, 124°23'W
29 March 1979

Yaguina Head - 44°40'N, 124°04'W
20 June 1978

CALIFORNIA

Anchor Bay - 38°40'N, 123°30'W
27 March 1979

City (Mission) Point - 36°33'N, 121°55'W
22 August 1979, 6 September 1979

Davenport Landing - 37°00'N, 122°11'W
25 March 1979, 8 September 1979

Montana de Oro - 35°20'N, 120°50'W
23 March 1979

Moss Beach - 37°30'N, 122°30'W
5 September 1979

Pebble Beach - 37°20'N, 122°30'W
5 September 1979

Pescadero Beach - 37°25'N, 122°28'W
5 September 1979

Piedras Blancas - 35°39'N, 121°17'W
24 March 1979, 6 September 1979

Pigeon Point - 37°15'N, 122°30'W
26 March 1979, 8 September 1979

Point Arena - 38°55'N, 123°50'W
27 March 1979

Point Joe - 36°37'N, 121°57'W
7 September 1979

Point Pinos - 36°37'N, 121°57'W
7 September 1979

Stillwater Cove - 36°36'N, 121°56'W
24 March 1979, 7 September 1979

Trinidad Point - 41°05'N, 124°10'W
28 March 1979

Westport - 39°38'N, 123°46'W
28 March 1979

Appendix II

Representative herbarium specimens of Analipus japonicus

(Harv.) Wynne

PLURILOCULARJapan

Onagawa, Rikuzen - 37°53'N, 140°04'E - UC 315269, FEB 1926
Murcran, Hokkaido - 42°20'N, 140°58'E - UBC A56339, 1 JUNE 1949; UBC A45981, 9 AUG 1971

Alaska

Rocky Points, Massacre Bay, Attu Island - 52°49'N, 173°11'W - UC M61586, 3 JULY 1953
Trapper's Cove, Adak - 51°48'N, 176°50'W - UBC A8421, 14 JUNE 1960
Cape Muzon, Dall Island - 54°40'N, 132°41'W - UBC A20564, 13 JUNE 1965
Klokachef Island - 57°25'N, 135°53'W - UBC A22598, 30 JUNE 1965
St. Makarius Bay, Amchitka Island - 51°23'N, 179°12'E - MICH, 16 MARCH 1968

British Columbia

Glacier Point, Vancouver Island - 48°24'N, 123°59'W - UBC A32537, 23 MARCH 1968
Kyuguot, Garden Island - 50°01'N, 127°21'W - UBC A4074, 27 MAY 1959
Orlebar Point, Gabriola Island - 49°11'N, 123°39'W - UBC A28922, 27 APRIL 1967
Muir Creek - 48°22'N, 123°52'W - UBC A 3400, 24 JUNE 1957
Kelsey Bay - 50°24'N, 125°58'W - UBC A4076, 4 JUNE 1959
Aguilar House Beach - 48°50'N, 125°08'W - UBC A41766, 29 JULY 1969
North Kalanawa River, Vancouver Island - 48°42'N, 124°59'W - UBC A7400, 10 JULY 1959
Sandstone Creek - 48°24'N, 125°00'W - UBC A3393, 10 AUG 1957
Maude Island, Strait of Georgia - 50°07'N, 125°21'W - UBC A7401, 15 AUG 1959
Triangle Island - 50°52'N, 129°05'W - UBC A19199, 7 SEPT 1964
Brooks Peninsula - 50°07'N, 127°42'W - UBC A19734, 6 OCT 1964
Roller Bay, Hope Island - 50°55'N, 127°56'W - UBC A43856, 13 SEPT 1970
Point-No-Point, Vancouver Island - 48°23'N, 123°59'W - UBC A39096, 22 NOV 1968

Washington

West Beach, Whidbey Island - 48°13'N, 122°46'W - WTU
244774, 21 MAY 1966

Turn Point, San Juan Island - 48°32'N, 122°59'W - WTU
244770, 18 JUNE 1955; UBC A2379 6 JULY 1956

Mukkaw Bay - 48°20'N, 124°40'W - WTU 244771, 21 AUG 1949

Waadah Island - 48°23'N, 124°36'W - UBC A2771, 15 AUG 1958

Oregon

Middle Bay, Cape Arago - 43°29'N, 124°23'W - UC 751284, 30
JUNE 1942

Harris Beach - 43°10'N, 124°20'W - UBC A5051, 19 MAY 1958

Sunset Beach - 43°18'N, 124°23'W - UBC A60700, 29 March
1979

California

Point Sal, Santa Barbara Co. - 34°40'120, 45°W - UC
M200208, 14 MAY 1949

Punta Gorda, Humboldt Co. - 40°15'N, 124°20'W - UC 1-
462021, 13 JUNE 1968

Eagle Glen Canychn, Santa Cruz Co. - 37°05'N, 122°05'W - UC
M316643, 10 JULY 1964

Moss Beach, San Mateo Co. - 37°N, 122°30'W - UC M106575,
5 SEPT 1925

Piedras Blancas - 35°25'N, 121°20'W - UBC A60650, 23 MARCH
1979

Tomales Head, Marin Co. - 38°14'N, 122°59'W - UBC A19985,
24 JUNE 1964

Mission Point - 36°32'N, 121°56'W - GMS 2507, 26 APRIL 1967

Fort Ross - 38°32'N, 123°15'W - MICH, 2 NOV 1945

Point Lobos, Monterey Co. - 36°32'N, 121°55'W - MICH, 3
DEC 1965

UNIIOCULARJapan

Ayukawa, Honshu - 38°15'N, 141°30'E - UBC A5503, 5 FEB 1957

Muroran, Hokkaido - 42°20'N, 140°58'E - UC 360340, 31 MARCH
1926; UBC A56338, 6 JUNE 1951

Appetsu, Hokkaido - 42°27'N, 142°40'E - UC 360338, 4 JUNE
1925

Oshoro, Hokkaido - 42°53'N, 140°35'E - UC 360339, 10 JUNE
1926

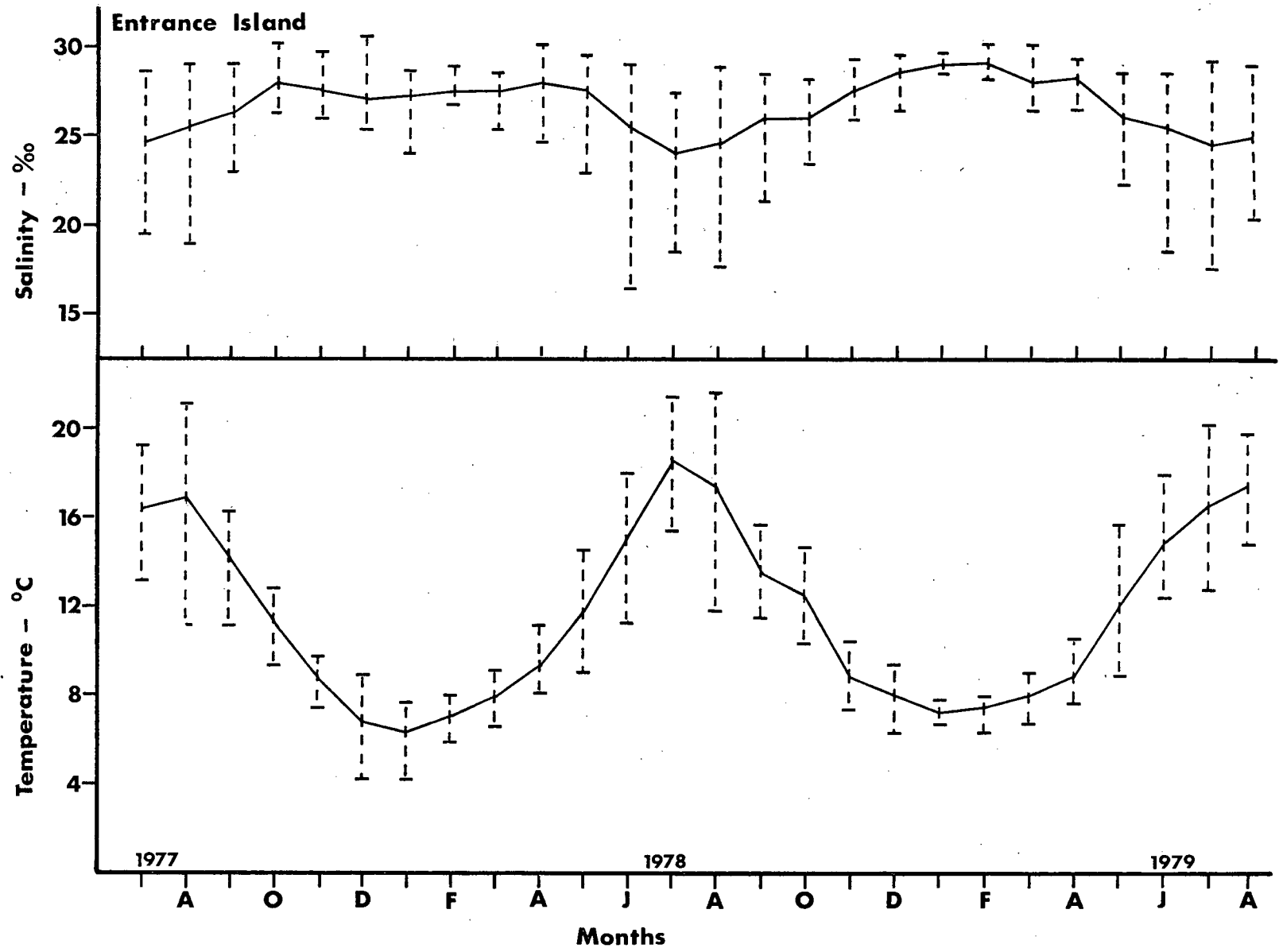
California

Point Lobos, Monterey Co. - 36°32'N, 121°55'W - UC 74617,
15 JULY 1892

City Point, Carmel - 36°33'N, 121°55'W - GMS 1694, 19 JULY
1958

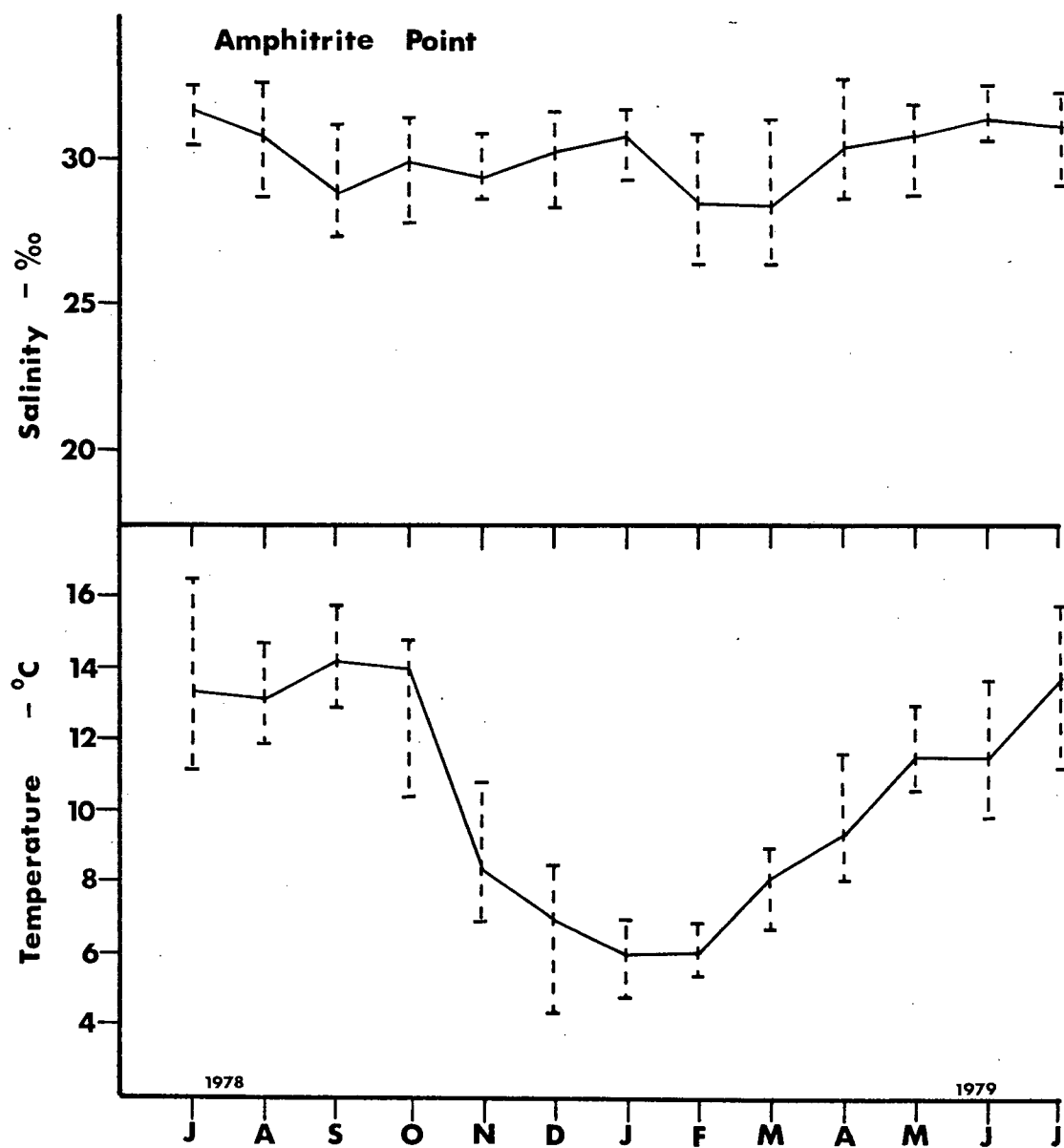
Appendix III

The mean monthly salinity and temperature of surface water at Entrance Island ($49^{\circ}12'N$, $123^{\circ}48'W$) for July 1977 to August 1979. Bars indicate the recorded monthly maxima and minima. (Giovando, 1977, 1978, 1979)



Appendix IV

The mean monthly salinity and temperature of surface water at Amphitrite Point (48°54'N, 125°33'W) for July 1978 to July 1979. Bars indicate the recorded monthly maxima and minima. (Giovandc, 1978, 1979)



Appendix V

Composition of enrichment solutions added to artificial seawater. The volume added to 1L of seawater base is in brackets.

Nutrients and Trace Metals (10ml)

NaNO ₃	4.667 g
Na ₂ SiO ₃ .9H ₂ O	3.000
Na glycerol P ₀₄	0.667
Na ₂ EDTA	0.553
Fe (NH ₄) ₂ (SO ₄) ₂ .6H ₂ O	0.234
FeCl ₃ .H ₂ O	0.016
MnSO ₄ .H ₂ O	0.041
ZnSO ₄ .7H ₂ O	0.007
CoSO ₄ .7H ₂ O	0.0016
glass distilled water	1000 ml

Vitamins (1ml)

Thiamine HCl	0.5 g
Inositol	0.5
Nicotinic acid	0.03
Calcium pantothenate	0.03
p-aminobenzoic acid	0.003
Vitamin B ₁₂	0.002
Biotin	0.001
Folic acid	0.0006
glass distilled water	1000 ml

Appendix VI

Sample dates of quadrats (QI-V) and transect (PT1-10)

Quadrat

1. VII.77
 29. VII.77
 8. IX.77
 21. X.77
 5. XI.77
 2. XII.77
 20. I.78
 2. III.78
 7. IV.78
 11. V.78
 23. V.78
 20. VI.78
 19. VII.78
 14. VIII.78
 6. IX.78
 6. X.78
 26. XI.78
 13. I.79
 9. III.79
 11. V.79
 12. VI.79
 9. VII.79
 24. VIII.79

Transect

2. VII.77
 29. VII.77
 8. IX.77
 5. XI.77
 20. I.78
 3. III.78
 12. V.78
 24. V.78
 20. VI.78
 19. VII.78
 7. IX.78
 26. XI.78
 13. I.79
 9. III.79
 9. IV.79
 12. V.79
 12. VI.79
 9. VII.79

Appendix VII

List of species recorded from quadrats and transects at
Bath Island study site

CHLOROPHYTA

Ulva sp.

PHAEOPHYTA

Analipus japonicus

Colpomenia peregrina

Colpomenia bullosa

Fucus distichus

Petalonia fascia

Sargassum muticum

Scytosiphon lomentaria

Ralfsia sp.

RHODOPHYTA

Bossiella orbigniana

Ceramium sp.

Corallina vancouveriensis

crustose corallines

Cryptopleura violacea

Cryptosiphonia woodii

Gigartina agardhii

Iridaea sp.

Lithothrix aspergillum

Microcladia borealis

Petrocelis franciscana

Plocamium tenue

Porphyra sp.

Rhodomela larix

CHRYSTOPHYTA

Navicula sp.

INVERTEBRATES

Collisela pelta

Notoacmea scutum

Idotea wosnesenskii

Gnocrimosphaeroma oregonensis

Pisaster ochraceus

VERTEBRATES

Oligocottus macululosus