EXPERIMENTAL STUDIES ON THE SPORES AND SPORELINGS OF THE RED ALGA *MASTOCARPUS STELLATUS*.

by

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PLATE CXCIX.

GIGARTINA MAMILLOSA, *J. Ag.*

GEN. CHAR. *Frond* cartilaginous, either filiform, compressed, or flat, irregularly divided, purplish-red; the axis, or central substance, composed of branching anastomosing longitudinal fibres; the periphery of dichotomous filaments, laxly set in pellucid jelly; their apices moniliform, strongly united together. *Fructification* double, on distinct plants; 1, external *tubercles*, containing, on a central placenta, dense clusters of *spores*, scattered among the filaments of the periphery. GIGARTINA (*Lamour*),— from γιγαρτον, a *grape stone*; which the tubercles resemble.

GIGARTINA *mamillosa*; frond flabelliform, dichotomous, plane, channelled; segments wedge-shaped, cleft; tubercles roundish or ovate, pedicellate, scattered over the disc of the frond.

GIGARTINA mamillosa, *J. Ag. Alg. Medit.* p. 104. *Endl. 3rd Suppl.* p. 42.

MASTOCARPUS mamillosus, *Kütz. Phyc. Gen.* p. 398.

CHONDRUS mamillosus, *Grev. Alg. Brit.* p. 127. *Hook. Br. Fl.* vol. ii. p. 302. *Wyatt, Alg. Danm.* no. 117. *Harv. in Mack. Fl. Hib.* part 3. p. 201. *Harv. Man.* p. 77.

SPHÆROCOCCUS mamillosus, *Ag. Syn.* p. 29. *Lyngb. Hyd. Dan.* p. 14. t. 5. *Ag. Sp. Alg.* vol. i. p. 260. *Ag. Syst.* p. 220. *Hook. Fl. Scot.* part 2. p. 102. *Grev. Fl. Edin.* p. 295. *Spreng. Syst. Veg.* vol. iv. p. 336.

FUCUS mamillosus, *Good. and Woodw. in Linn. Trans.* vol. iii. p. 174. *Turn. Syn.* p. 237. *Turn. Hist.* t. 218. *E. Bot.* t. 1054.

FUCUS polymorphus (*fourth series*) *Lam. Diss.* p. 3. t. 17. f. 37. t. 18. f. 38.

FUCUS echinatus, *Stack. Ner. Brit.* p. 65. t. 12.

FUCUS canaliculatus β., *Huds. Fl. Ang.* p. 583.

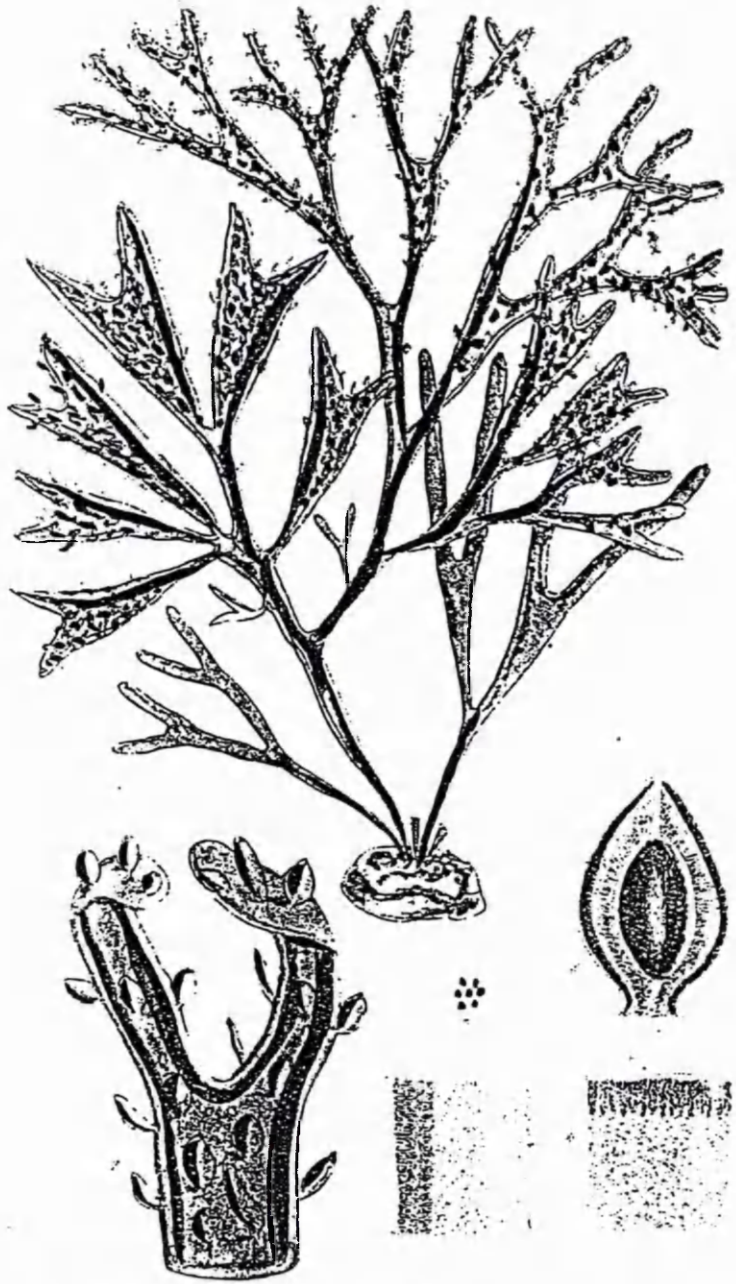
FUCUS ceranoides, vars. *Lightf. Fl. Scot.* p. 916. *Gmel. Hist.* p. 115. *With. Arr.* vol. iv. p. 99.

FUCUS alveolatus, *Esper. Ic.* p. 139. t. 70.

HAB. On rocks near low-water mark. Perennial. Winter. Common on all our rocky shores.

GEOGR. DISTR. Atlantic shores of Europe and North America.

DESCR. *Root*, a membranous expansion. *Fronds* tufted, from four to eight inches long or more, rising with an undivided stem or stipes, which is filiform at base, but almost immediately becomes compressed, and then flattened, widening gradually upwards till it attains from an eighth to a quarter of an inch in breadth. At an inch or two above the base, the stipe forks; and this mode of branching, repeated again and again, results in a many times dichotomous, flabelliform frond. The branches are more or less channelled by the introflexion of the margin; they are very commonly twisted, often in a spiral manner; and the upper ones are gradually more and more



Frontispiece: Photocopy of W.H. Harvey's drawing and description of Mastocarpus stellatus (as Gigartina mamillosa) from his

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CONTENTS

List of Tables

LIST OF FIGURES

SUMMARY

Young sporelings of *Mastocarpus* regenerated faster than old sporelings following induced severe damage. The tendency was always to return to a discoid shape. Survival of 'islands' of living cells in dead sporelings allowed some partial recovery.

The interactions between *Mastocarpus* sporelings with other juvenile algae showed that there was a some effect on growth, development and survival, especially with *Ulva* and *Enteromorpha* when sporelings of *Mastocarpus* died after 6 weeks. Germlings of *Fucus* on *Mastocarpus* sporelings showed close bonding of rhizoids to the *Mastocarpus* 'cuticle', and some evidence of penetration.

Diatom mucilage material on the substratum had some effect on the morphogenesis and growth rate of *Mastocarpus* sporelings and *Fucus* germlings and on interactions between the two.

Measurements of photosynthetic rates have shown that for *Mastocarpus* sporelings there was sensitivity to water movements, illumination levels and temperature and salinity change. Varying degrees of recovery of photosynthetic activity were observed after protracted storage of sporelings under cold and dark conditions. Juvenile erect fronds were more active than the attaching discs.

The photosynthetic rates of both fertile and non-fertile papillae were evenly affected by exposure to dry air. Both types of papillae and the spore masses were affected to salinity change. Recovery was observed after short exposure periods and re-immersion in sea water.

1. General introduction

Mastocarpus stellatus (Stackh. in Wither.) Guiry (=*Gigartina stellata* Stackh. in Wither.) Batt. is a member of the Family Petrocelidaceae, Order Gigartinales, Class Florideophyceae. It inhabits the lower littoral area of rocky shores in north temperate regions. It is particularly abundant on the shores of the Firth of Clyde (Marshall, Newton and Orr, 1949; Khfaji, 1978). Guiry *et al* (1984) reinstated the genus *Mastocarpus* Kutzing (Rhodophyta) using morphological, life history and biochemical characters. They concluded that *Mastocarpus* should be reinstated to include four widely distributed species of *Gigartina* (Gigartinaceae). The separation was based on clear-cut characters, viz., *Gigartina* species do not have specially formed papillae, have isomorphic gametophytes and sporophytes, and tetrasporangia which are produced in branched or unbranched chains. *Mastocarpus* species have channelled thalli on which the female reproductive structures and carposporophytes are produced in specially formed papillate structures, usually have a heteromorphic-type of life history, involving an alternation including tetrasporophytes previously referred to the genus *Petrocelis*, and with tetrasporangia which are formed singly.

Setchell and Gardner (1933) subdivided the genus *Gigartina* of the Northeast Pacific into seven subgenera. They found a *Polysiphonia*-type life history in most of the species, but in some including *Mastocarpus stellatus* (as *Gigartina stellata*), gametophytic and cystocarpic phases only were recognised with an apparent absence of a tetrasporophyte. Lindauer (1939) described the tetrasporic plants of *G. alveota* (Turn.) J. Ag. in New Zealand as resembling the cystocarpic ones very closely with the branching being strictly dichotomous. Marshall *et al* (1949) obtained only basal discs from carpospores of *M. stellatus* (as *G. stellata*) in Britain. They were unsuccessful in associating a crustose stage, and therefore speculated that the gametophyte was formed directly from the carpospores. Taylor (1957) observed some members of the order Gigartinales to have a triphasic cycle in which the gametophyte is morphologically similar to the tetrasporophyte (i.e a *Polysiphonia*-type life history).

Chen and McLachlan (1972) studied the life history of *Chondrus. crispus* Stackh. which was completed in culture in 20 months. Plants established from tetraspores formed male and female gametophytes and subsequently carposporophytes, and the carpospores gave rise to plants with mature tetrasporangia. They found that tetraspores from these formed second generation gametophytes. Carpospores of plants from nature developed into tetrasporophytes and gametophytes derived from the tetraspores became sexually mature within a year. Sporophytic and gametophytic plants were as expected, morphologically similar. Edelstein *et al* (1974) stated that the carposporophyte of *Mastocarpus stellatus* (as *G. stellata*) from nature developed without any evidence of fertilization. Chen *et al* (1974) found that the carpospores produced by plants in nature gave rise directly to gametophytic plants without the intervention of tetraspores. Kim (1976) Found that tetrasporelings of *Gigartina corduta* (Setch. and Gard.) Kim produced tetraspores. In *G. heterocorpa* (Rupr.) Kim and *Gigartina rosea* (Kylin) Kim, plants grown from single tetraspores produced apomictically spores resembling carpospores. Masuda *et al* (1987) studied the life history in culture of *Mastocarpus* species from central Japan. *Mastocarpus* plants were collected at 9 localities covering its whole geographical range in Japan. They found two types of life history among the strains: a heteromorphic type with the alternation of foliose dioecious *Mastocarpus*-phase gametophytes and crustose *Petrocelis*-phase tetrasporophytes, and a direct type involving only cystocarpic *Mastocarpic* plants. The former type was found throughout the range. They observed in a single locality that the carposporelings from a cystocarpic plant developed into either *Petrocelis*-like crusts or *Mastocarpus* blades. The tetraspores grew into dioecious *Mastocarpus* gametophytes. Hybridization experiments with 14 female and 9 male plants from 8 localities showed that interbreeding was potentially free among the populations studied. Heteromorphic type plants showed a short day response in reproduction, but direct type plants did not show such a photoperiodic response.

West (1972) studied the development of tetraspores from *Petrocelis franciscana* Setch. and Gardn. at Rockaway Beach, San Mateo County, California. He found that sporelings gave rise to a fertile gametophyte which resembled *Mastocarpus jardinii* (J.

Agardh) West (as *G. agardii* Setch. and Gardn). The germlings developed as crustose discs with marginal meristems until about 300-500 μm in diameter. The erect plants grew to reproductive maturity at 15 °C but not at 10 °C. West and Polanshek (1972) obtained the same results as Chen *et al* (1974), in that the carpospores of *Mastocarpus papillatus* (C. Agardh) Kutzing [as *G. papillata* (C. Ag.) J. Ag.] gave rise directly to the gametophytic phase. Polanshek and West (1975) reported that the culture of tetraspores of *P. middendorfii* (Rupr.) Kjellm. from Alaska gave rise to crustose plants, and the fertilized plants formed cystocarps and carpospores which gave rise to crustose plants anatomically similar to field-collected *Petrocelis* sporophytes. West *et al* (1977) cultured the tetraspores of *P. cruenta* J. Agard. from Ireland. They found the tetraspores gave rise to foliose dioecious gametophytes, resembling *M. stellatus* (as *G. stellata*) as naturally occurring gametophytes. Polanshek and West (1977) found that *P. middendorfii* gave rise to crustose *Petrocelis*-like plants that reproduced by tetraspores. Hence there is evidence for the occurrence of a *Petrocelis*-phase in the life history of some *Mastocarpus* species.

Chen and Taylor (1976) stated that the sporelings of *Chondrus crispus* developed as discoid or filamentous forms and that coalescence could take place between adjacent sporelings. West and Guiry (1982) observed the life history of *Gigartina johnstonii* Dawson from California. The life history was rapid when plants were grown in culture. Some 24-25 weeks were required for a complete cycle. Sylvester and Waaland (1984) studied the early development of sporelings derived from tetraspores of *G. exasperata* (Hav. and Baily). Two development types were observed, discoid and filamentous. The proportion of filamentous sporelings increased as spore density increased. Discoid sporelings produced upright blades approximately one week earlier than filamentous sporelings.

Guiry and Cunningham (1983) found tetraspores of *G. acicularis* from the British Isles once in November, and carpospore-bearing plants have only been found in January. Tetraspores collected from France gave rise to male and female gametophytes at 15 °C, under daylengths of 12 h and less, not in different regimes at 15 °C. When transferred to 10 °C, carpospores gave rise to plants which formed

tetrasporangia at daylengths of 16, 12, 10, and 8 h at 15 °C and an 8:7.5⁻:1:7.5⁻ h photoregime was ineffective in inhibiting reproduction. Guiry (1984) studied the life cycle of *G. teedii* (Roth) Lam. from subtidal habitats in the River Yealm, South Devon, England. He found that gametangial plants are always dioecious, and reproduced rapidly when unattached. *G. teedii* from England, France and Brazil were interfertile and fertile tetrasporophytes developed carpospores. Plants started after the same time to reproduce at 20 °C,16:8⁻ h; 15 °C, 16:8⁻ h and 15 °C, 8:16⁻ h, but the morphology of the plants at the time of reproduction varied. Plants grown at 10 °C, 16:8⁻ h daylength did not reproduce.

Meer *et al* (1983) studied several cultures of male plants of *Chondrus crispus* from widely separated geographical locations. These were observed to produce large masses of spores in structures resembling cystocarps, but the origins did not appear to be sexual. The spores released from these masses, were viable and gave rise to plants closely resembling normal tetrasporophytes complete with tetrasporangial sori. These sori did not release spores. Guiry and West (1983) studied the life history and hybridization of *M. stellatus* (as *G. stellata*) and *P. cruenta* from the North Atlantic. Fourteen of the crustose plants of *P. cruenta* were isolated from various localities in the British Isles, France, Spain, and Portugal. These gave rise in culture to dioecious foliose plants identifiable as *M. stellatus* (as *G. stellata*). They found two basic types of life history, a direct type of life history involving foliose plants from carpospores, and a heteromorphic-type life history in which only crustose plants resembling *P. cruenta* formed carpospores. The heteromorphic type life history was only found from Spain and Portugal. Both life history types were found in plants from U.S.A., the British Isles and Northern France. The only direct type life history was found in plants from Iceland and Denmark. Some *Petrocelis*-like crusts derived from field collections of *M. stellatua* (as *G. stellata*) carpospores formed tetrasporangia in 8:16 h LD, 10 °C but not in 8:16 h LD, 15 °C; 16:8 LD, 10 °C or 15 °C; and 10:6.5:1:6.5 h LD, 10 °C. The spores formed were viable and produced normal dioecious male and female gametophytes. Short day and low temperature conditions appear necessary

for tetrasporogenesis. The crossing experiments with isolates of *M. stellatus* (as *G. stellata*) and *P. cruenta* showed that two virtually non-interbreeding populations with a high degree of geographical separation exist in the northeastern Atlantic. Zupan and West (1988) studied the geographical variation in the life history of *Mastocarpus papillatus* (C. Agardh.) Kutzing. Carpospores were isolated from 377 female gametophytes collected from eight localities on the Pacific coast of Baja California, Maxico, and California, U.S.A., and grown in laboratory culture. All carpospores from a single female gave rise either to basal discs with gametophyte-like uprights or crustose plants formerly referred to the genus *Petrocelis*. Early stages in the development of each type of germling were observed, and the environmental factors affecting development. All females from the two southernmost locations in Baja California exhibited the sexual life history. Based on carpospore germlings, females from each location were scored as having either. 1- sexual life history (crustose germlings). 2- direct-development life history (discoid germlings with uprights). The relative ecological advantages and disadvantages of life histories were unknown as are the environmental factors that produced the ratios of sexual to direct development females observed at each locality.

Chen *et al* (1974) cultured *M. stellatus* (as *G. stellata*) through two generations starting from carpospores. Carpospores gave rise directly to plants. West and Polanshek (1975) isolated five *M. stellatus* (as *G. stellata*) strains from several localities in the North Atlantic and one isolate of *P. cruenta*, the presumed tetrasporophyte, originating from Ireland. They found that sexual reproduction occurs in some populations of *P. cruenta* and *Mastocarpus stellatus* (as *G. stellata*). West and Guiry (1982) observed that the life history of *G. johnstonii* was rapid when plants were grown in culture. Guiry and West (1983) isolated the crustose plants of *P. cruenta* from various localities in the British Isles, France, Spain and Portugal. These gave rise in culture to dioecious foliose plants identifiable as *M. stellatus* (as *G. stellata*). Guiry (1984) found that gametangial plants of *G. teedii* are always dioecious, and tend to reproduced more rapidly when unattached. Gametangial plants of *G. teedii* from England, France and Brazil are interfertile and fertile

tetrasporophytes developed from the resulting carpospores.

Burns and Mathieson (1972a) studied the development of carpospores of *Chondrus crispus* and *Mastocarpus stellatus* (as *Gigartina stellata*) when grown under different light intensities, temperatures, and salinities. *Chondrus* showed rapid increase in growth concurrent with increased light intensities up to 4708 Lux. *Mastocarpus* exhibited a slow increase in cell production with light intensity through 8239 Lux. *Chondrus crispus* spores germinated and grew rapidly under increasing temperatures and salinities (15-45‰ at 19 ^{o}C). *Mastocarpus* exhibited tolerance to reduced salinity (20‰ at 19 ^{o}C). Rueness (1978) studied plants of *Mastocarpus stellatus* (as *G. stellata*) from the Norwegian west coast. These gave rise directly to carpospore-bearing plants through two successive generations in culture. He found the carpospores germinated to form crustose discs at 12 ^{o}C and 17 ^{o}C, at light intensities of 200-300 Lux and 1500 Lux under photoperiods of L/D 16:8, and in the higher temperature and light intensities the erect axes grew to reproductive maturity in about 12 months. There was a slight increase in disc diameter with the establishment of the erect axes. Under low temperatures and light intensities the basal disc increased in diameter through marginal growth.

Khfaji (1978) carried out experimental studies on carpospores of *M. stellatus* (as *G. stellata*) collected from the Firth of Clyde. Under the experimental conditions used they formed disc-like sporelings which grew healthily for 5 months. The carpospores of *Chondrus crispus* were fast growing. The effects of laboratory induced environmental factors on the growth of sporelings of *Mastocarpus* were also investigated. He found that the growth of sporelings was very slow at salinities of 4‰ and 8‰. The sporelings became pale in colour and many became detached and lost into the surrounded medium after the first few weeks. Growth at salinities of 24‰ and 32‰ produced healthy sporelings, whilst a salinity of 16‰ produced healthy growth of sporelings but a slightly slower growth rate than at 24‰. Sporelings of *Mastocarpus* showed healthy growth at an illumination of 1200 Lux. Sporelings of *Mastocarpus* in fact showed healthy growth in most illumination levels used, with the growth rates gradually increasing from 800 to 3000 Lux. Illuminations of 400 Lux and

600 Lux produced very slow sporeling growth rates and many became pale in colour and detached from the substratum. Growth of sporelings at 1200 Lux was good. They showed healthy growth, but at illuminations of 2400 Lux and 3000 Lux was slightly slower than at 1200 Lux. He stated that when sporelings of *M. stellatus* (as *G. stellata*) were cultured under three daylength regimes 8:16", 12-12" and 16:8" at 12 °C, 2400 Lux and at a salinity of 32‰, maximum growth rate was obtained in the long day regime of 16 h light in 24 h and minimum growth rate was observed in the shortest day regime (8:16"). Rueness (1978) stated that the diameter of *M. stellatus* (as *G. stellata*) sporelings reached about 25 mm after 4 years. Khfaji (1978) cultured the carpospores of *M. stellatus* (as *G. stellata*) under laboratory conditions, and found the average diameter of sporelings reached 0.5 mm after 3.5 months whilst germinated carpospores of *C. crispus* reached 0.8 mm diameter in the same time.

Mathieson and Burns (1971) studied the photosynthesis and respiration of *Chondrus crispus* and *M. stellatus* (as *G. stellata*) under different light intensities, temperatures, salinities and degrees of desiccation. The photosynthesis of *C. crispus* was light saturated at 186 μE m^{-2} s^{-1}, that of *M. stellatus* at 224 μE m^{-2} s^{-1}. They found that the optimal temperature for growth in both species was 20 °C. Maximum photosynthesis and minimum respiration was obtained at a salinity of 24‰ with *Chondrus crispus*. But *M. stellatus* (as *G. stellata*) exhibited maximum photosynthesis and reduced respiration at 40‰. The apparent photosynthesis and respiration of *Chondrus crispus* were adversely affected by high degree of dehydration, whilst *M. stellatus* (as *G. stellata*) was more flexible. Burns and Mathieson (1972b) reported the growth of *Mastocarpus stellatus* (as *G. stellata*) in nature. Rapid growth coincided with increasing summer temperatures, but the maximum carpospore release occurred during the period of decreasing temperatures. Waaland (1973) studied the growth of *Iridaea cordata* (Turner) Bory and *G. exasperata*, and found that the optimum growth rates were obtained at temperatures of 10-14 °C,and light intensities of 8.9-16X10^3 ergs cm^{-2} sec^{-1}.

Mathieson and Burns (1975) confirmed that *C. crispus* growth coincided with increased temperatures and daylength during summer. Cystocarpic plants were

abundant during the late summer-winter.

Neish *et al* (1977) studied the cultivation of *Chondrus crispus* in flowing sea water in tanks in a greenhouse. In one method individual plants were tethered to the fixed support, and in the other plants were allowed to float freely in circulating sea water. They showed that nitrate or ammonia and phosphate increased the growth rate, but Iron did not. Nourished plants had relatively high nitrogen and water contents and were richly pigmented. Growth under continuous illumination was superior to that under normal illumination with alternating day and night periods, the variations in growth rates closely correlated with mean temperature of the sea water.

Pybus (1977) studied the ecology of *Chondrus crispus* and *M. stellatus* (as *G. stellata*) in the neighbourhood of Galway Bay, Ireland. The growth of both species was continuous throughout the year with same variations in rate. Khfaji (1978) cultured *Mastocarpus* plants from different localities under the same conditions. Plants from Loch Long had slower growth rates than plants from other localities. Plants from Cumbrae Island and Portencross did not show any significant differences in growth rate. Plants from all localities grew healthily for the whole period of experiment. Khfaji (1978) also described the rates of oxygen liberation in plants of *Mastocarpus* from the 3 localities in the Firth of Clyde after drying for 12 hours. Plants from Cumbrae Island showed slightly higher rates of oxygen liberation than Loch Long. Plants from Portencross showed much higher rates of oxygen liberation when dried for 12 h compared with plants from the other localities. He also studied the rates of oxygen liberation of plants of *Mastocarpus* at different salinities. At the very low salinities 4-8‰ the rate of oxygen liberation was very low. At a salinities 24-32‰ there were no significant differences in the rates of oxygen liberation.

Previous experimental evidence points to the *Mastocarpus stellatus* life history sequences as being linked with environmental conditions and geographical factors. It is also evident that within the Gigartinales there are variable expressions of life history events, which with certain species enables different strategies in relation to habitat conditions. For northern 'strains' of *M. stellatus*, however, the 'direct' type of life

history applies, and so the sporeling stages form the next foliose generation. In the present work the sporeling stages, spores and spore-bearing structures, have been studied experimentally in order to learn more about their responses to conditions simulating environmental factors.

2. Materials and Methods

2.1. Collection and storage of fertile plant material.

Fertile fronds of *Mastocarpus stellatus* , *Fucus serratus*, *Fucus spiralis*, *Porphyra umbilicalis*, *Ulva lactuca* and *Enteromorpha intestinalis* were collected from the eulittoral zone on Great Cumbrae Island in the Firth of Clyde and from Ardmore Point in the Clyde Estuary (Figure 2.1) and brought to the laboratory within 1-2 hours of collection. After collection, the excised fronds were stored in polythene bags for 1-2 days in a cold room at 5 ^{o}C in the dark before the release of reproductive cells.

2.2. Culture studies.

2.2.1. Spore release from red, green and brown algae.

With *Mastocarpus stellatus*, the papillae bearing fertile carposporangia (Figure 2.2) were picked from the fronds and placed in a sterile flask with sterile seawater. Washing methods were carried out by shaking the flask at middle speed on a laboratory shaker. The seawater was changed and replaced several times with fresh sterile seawater to eliminate contamination. The washed papillae were put in sterile seawater in a dish under the culture conditions to be described. After the carpospores were released from the papillae, they were collected and placed on glass slides which were submerged in the sterile enriched seawater medium in petri dishes. The dishes were placed in the growth chamber at 10 ^{o}C $\pm$ 2 ^{o}C and a quantum irradiance of 17.5 μmol m^{-2} s^{-1} (12.5- 21.75 μmol m^{-2} s^{-1}). The cultures were illuminated from above with light 40 watt fluorescent tubes at a distance of 23 cm from the culture vessels. The quantum irradiance was measured with Skye Instrument Ltd. SKP 200, calibrated for sensor SKP. The fluorescent tubes were changed whenever necessary to keep the illumination as required. The day length was 16 hours. The carpospores then became attached to glass slides and formed crustose discs after germination and growth.

With the brown algae *Fucus serratus* and *Fucus spiralis*, the excised fertile

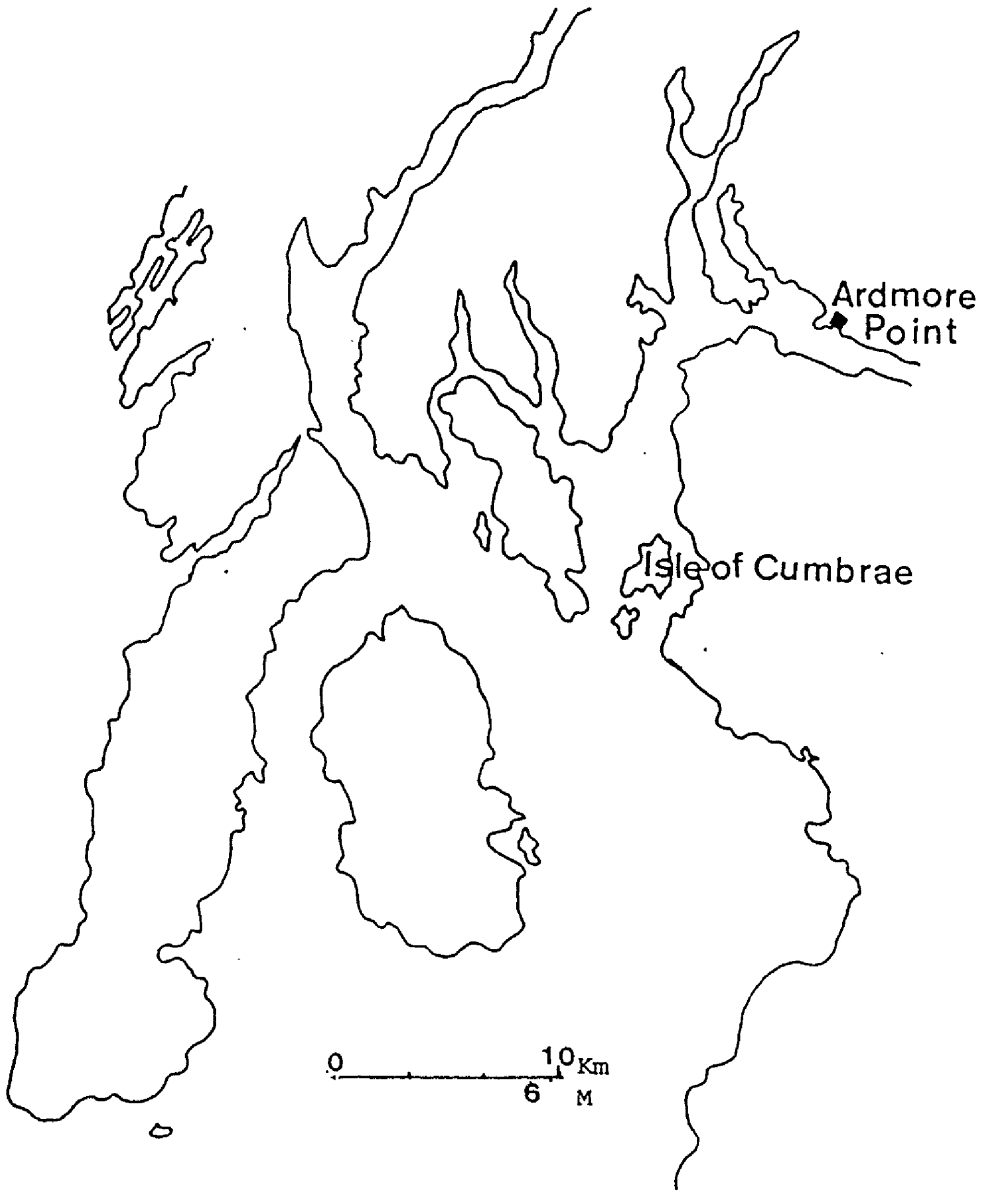


Fig. 2.1. The collecting sites at Cumbrae Island and Ardmore Point in the Firth of Clyde and Clyde Estuary.

Fig. 2.2. Plant of *Mastocarpus stellatus* with the morphological features.

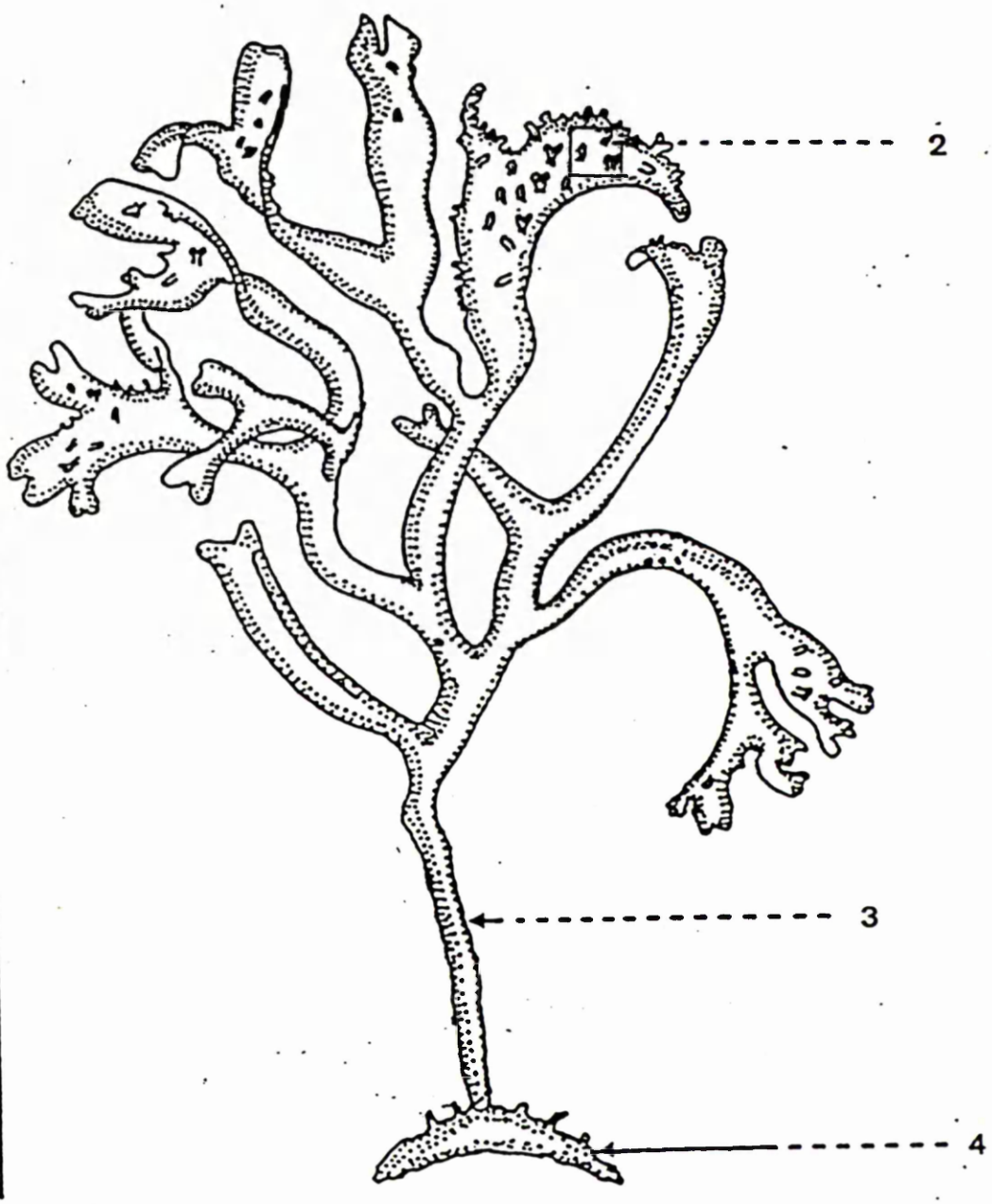
1- frond

2- papillae

3- stalk

4- holdfast

1



receptacles were washed in cool tap water and sterile seawater many times, then dried slightly at room temperature. Dehydration and rehydration as a means of obtaining release of gametes was carried out as described by Pollock (1970). Male and female receptacles of *F. serratus* were put seperately into specimen jars of sterile seawater under conditions of light and temperature described above and left overnight (separation is not necessary with bisexual conceptacles of *F. spiralis*). Oospheres and antherozoids were released after several hours and were then collected and mixed together for 30-45 minutes to ensure fertilization. The zygotes were then collected in small petri dishes and allowed to settle for 1-2 minutes. This procedure was repeated several times. This method of cleaning zygotes was described by McLachlan *et.al* (1971). The cleaned zygotes were finally placed in sterile seawater, then 2-3 drops of zygote suspension were added to the glass slides which were then submerged in sterile enriched seawater medium in petri dishes. The dishes were placed in a growth chamber under the conditions already described. The zygotes attached and grew on glass slides as described by Reynolds (1950).

With *Porphyra umbilicalis* , fertile plants were collected in winter and early spring at Ardmore Point. The fronds were washed several times in sterile seawater, and then were put in a petri dishes containing sterlized seawater and incubated overnight in the growth chamber. The released α-spores were collected onto cover glasses in petri dishes containing enriched seawater medium, and the petri dishes were transfered into growth chamber under the conditions as described above.

With *Enteromorpha intestinalis* and *Ulva lactuca* , release of zoospores from freshly collected plants was achieved readily in the laboratory during spring and early summer. Fertile fronds of *Enteromorpha* and *Ulva* were recognised by the presence of yellowish area along the margins of the blade. Fertile fronds were removed from several different plants and washed alternately in distilled water and sterile seawater. Fronds were then placed in petri dishes containing enriched seawater medium and clean microscopic slides. The dishes were illuminated in growth chamber overnight, and examined periodically for release of zoospores. Fronds were removed and the released

zoospores were allowed to settle overnight. After zoospore settlement, the enriched seawater medium was changed. (This method is as described by Watson,1983).

2.2.2. Media and culture vessels

All algae were cultured in an enriched seawater medium, based on the formula of Boney and Burrows (1966). Aged seawater collected from the Firth of Clyde was further aged for at least two weeks, filtered through a double layer of Whatman filter paper No. 1 and then pasteurized by heating twice to 75 ^{o}C at room temperature. The formula of the medium is as follows:

To 1 litre of filtered and pasteurized seawater were added:

60 ml of solution A, which contained:

50 ml of 0.4% $NaNo_3$ in distilled water.

2 ml of each of the following:

1.47 g/l $MnSO_4.4H_2O$

0.0023 g/l $CuSO_4.5H_2O$

0.064 g/l $CoCl_2.6H_2O$

0.23 g/l $Na_2MoO_4.2H_2O$

0.005 g/l $LiCl.H_2O$

2 ml of solution B, which contained:

4.98 g/l $ZnSO_4.7H_2O$

15 ml of solution C, which contained:

2.6 g/l tetrasodium salt of EDTA

0.12 g/l $FeSO_4.7H_2O$

1.5 ml of solution D, which contained:

15 g/l $Na_2HPO_4.12H_2O$

Each of the above solutions was autoclaved separately and stored in a refrigerator until used.

2.2.3. Control of contamination

Contamination was successfully controlled by:

1- Using the carefully washed papillae and fertile fronds and receptacles.

2- Changing the medium once a week.

3- Using Germanium dioxide (2 ml of 5 mg/l solution) to control any diatom growth.

4- Removal of other algal sporelings.

2.2.4. Measurement of growth of sporelings and germlings

Growth of sporelings and germlings were measured by increase in diameter of disc-like sporelings of *Mastocarpus*, and lengths, width, rhizoids lengths, and terminal hairs length of germlings of *Fucus* (Figure 2.3), and length of primary filaments of *Ulva* and *Enteromorpha.*

2.2.5. Measurements of surface area of sporeling of *Mastocarpus* and *Porphyra*

The increases in surface area were measured by a Gillett and Sibert conference microscope with Projector Head (Viewing Head) with 14 cm diameter hooded screen. Over the glass screen was placed a glass disc divided into squares. The size of each square on the screen was calibrated by a stage micrometer. The projected images in the squared glass screen were then copied onto graph paper with the same size squares as on the screen. The drawings were then cut from the graph paper and weighed. The surface area of the specimen was then calculated from the weight of graph paper, the relationship between surface area and weight having been previously determined with allowance made for the magnification of the specimens in the projector head.

2.2.6. Regeneration studies

Mastocarpus sporelings were cultured as above and after several weeks the disc-like of sprelings were cut into different shapes, then left to regenerate under laboratory conditions. Photomicrographs were obtained at the start of the experiment, the size of sporelings being determined with a calibrated eyepiece scale. By tracing the outlines of the photographed sporelings and placing the tracings on squared paper it was possible to equate the area of squared paper covered to the actual measurements made via the

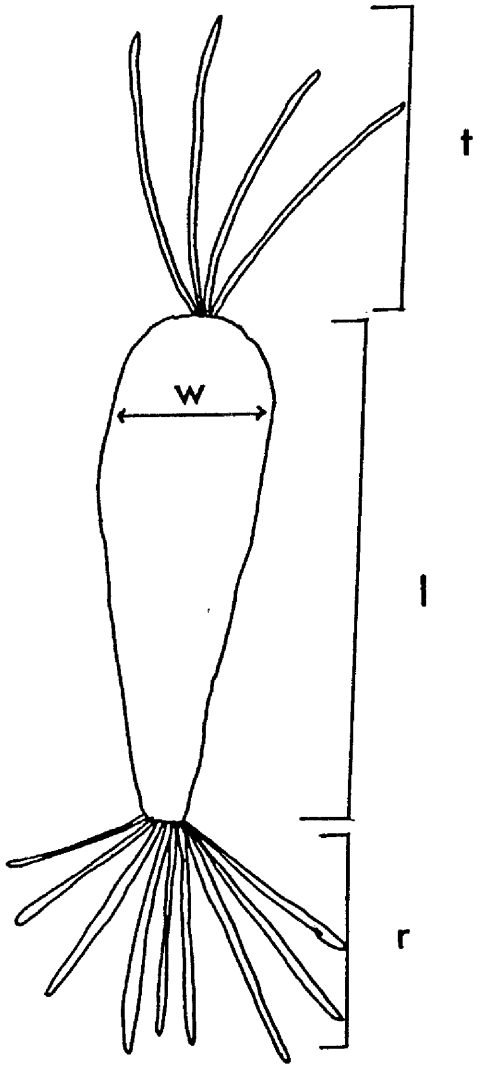


Fig. 2.3. Germlings of Fucus
t = terminal hairs
l = length of germling
w = width of germling
r = rhizoids

eyepiece micrometer (in μm^2). When a suitable amount of regenerative growth had been obtained, further tracings were made from the photomicrographs, and by placing these tracings on the squared paper the area of regenerated tissue could be calculated. The new tissue percentage was calculated as follows:

$$\% \text{ of new tissue} = \frac{\text{new surface area - original surface area}}{\text{original surface area}} \times 100$$

2.3. Processing specimens for transmission and scanning electron microscopy

2.3.1. Processing of vegetative tissue.

1. Fixation

The disc-like *Mastocarpus* sporelings, *Fucus* and *Enteromorpha* germlings were fixed in 5% glutaraldehyde in 0.1 cacodylate buffer containing 0.25 M sucrose at pH 7 on ice for 24 hours (Mandura, 1981).

2. Washing

The specimens were then given 3 washes for 30 minutes in 0.1 M cacodylate buffer with decreasing sucrose concentrations:

0.25 M sucrose on ice

0.125 M " " "

zero " " "

3. Post-fixation

The specimens were post-fixed in 1% OsO_4 in 0.1 M cacodylate for 5 hours on ice.

4. Dehydration

The specimens were dehydrated in a graded series of ethyl alcohol concentrations on ice.

30% ethyl alcohol for 30 minutes.

50% " " " " "

70% " " " " "

85% " " " " "

95% ethyl alcohol for 30 minutes.

100% " " " " " at room temperature.

100% propylene oxide for 30 minutes, two changes at room temperature.

5. Infiltration.

Samples for infiltration in resin were placed in a 1:1 mixture of resin : propylene oxide at room temprature. The amount of propylene oxide in the mixture was gradually decreased through evaporation by leaving the vials open overnight in the fume cupboard.

6. Embedding.

When infiltration of the tissues was completed, the samples were dried by paper tissue and embedded in a pure resin in small aluminum dishes. The formula of the resin was as follows:

Epon (Epicote) 812	40 cm^3		
DDSA (Dodecenyl Succinic Anhydride)	40 cm^3	Mixed well	Mixed well
MNA (Methyl Nadic Anhydride)	20 cm^3		
BDMA (Benzyldimethylamine)	2 cm^3		

The resin was prepared during the infiltration and stored in a deep freeze to be used the following day.

7. Polymerization.

Polymerization was carried out in ovens of 3 diffrent temperatures:

35 ^oC for 12 hours

45 ^oC " " "

60 ^oC " " "

2.4. Sectioning and staining for light microscope and transmission electron microscope

Semi-thin sections (one micrometer) for the light microscope were cut with LKB ultratome III and dried down in a drop of water on a gelatin coated slide. Serial sections of specimens were obtained by mounting each section in order on the slides.

Permanent preparations were made (following staining) by using a drop of resin as mounting medium and sealing with a cover slips. The following stain was used for staining the section for the light microscope:

Toluidine blue: All sections were routinely stained for about 10 seconds on a hot plate with 1% solution of toluidine blue in 1% borax.

Ribbons of ultra-thin sections for transmission electron microscope were cut with LKB ultratome III using glass knives made with knife maker. The ribbons were floated on to the surface of water in the bath made from silver self-adhesive tapes attached to the knife. The ribbons were floated with chloroform and collected on copper grids.

A twice staining procedure with uranyl acetate and lead citrate was used for transmission electron microscopy.

A. The grids were floated downwards side on the meniscus of solution of saturated uranyl acetate in water 7.5% at room temperature for 30 minutes. The grids were washed with distilled water and dried on velin tissue.

B. The grids were floated downwards on drops of lead citrate (Reynolds, 1963) in a petri dish. The lid of this dish was lined with dental wax to make an air-tight seal and pellets of KOH were included to absorb CO_2.

2.5. Preparation of algal material for scanning electron microscopy

Mastocarpus stellatus sporelings, *Fucus* and *Enteromorpha* germlings were fixed and dehydrated using the following procedures.

1- Fixation:

Placed in 5% glutaraldehyde in 0.1 M Sodium cacodylate and 0.25 M sucrose overnight, then washed in 0.1 M cacodylate and 0.2 M sucrose for 30 minutes. Washed in 0.1 M cacodylate and 0.125 for 30 minutes. Post-fixed in 1% OsO_4 in 0.1 M cacodylate for 2.5 hours.

2. Dehydration: (as in Hayat 1978)

25% acetone 5-10 minutes

50% " " "

70% acetone 5-10 minutes

90% " " "

95% " " "

100% " " "

100% " " "

The dehydrated algal material was then critical point dried in a drier type Polaron E 3000 and then fixed on stubs and gold coated using SEM coating unit E5000 (Polaron Equipments Limited).

2.6. Microscopes

For light microscopic observations an Ortholux (Leitz) microscope was used.

The transmission electron microscopic (TEM) studies were made on a Phillips EM 301 in the Botany Department and scanning electron microscopy (SEM) on a Phillips SEM 500 in the Zoology Department.

2.7. Photomicrographs

The TEM was used to examine the growth of *Fucus* germlings on *Mastocarpus* sporelings and recovered sporelings. Photographs of the specimens in thin sections were obtained. The SEM studies were of *Fucus* egg and *Enteromorpha* growth on *Mastocarpus* sporelings.

2.8. Dissolved oxygen.

The principle of Winkler's method (1888) is that when a concentrated solution of divalent manganese and alkaline potassium iodide are added to the water sample, white manganous hydroxide is first formed and then oxidised to manganic hydroxide by the molecularly dissolved oxygen. The brown mangenic hydroxide settles to the bottom of the bottle. Sulphuric acid is then added and this dissolves the manganic hydroxide and iodine is liberated. The liberated iodine takes up the excess iodine and forms I_3^-. This solution is then titrated against thiosulphate and the end-point of the titration is

indicated by starch solution. This method can be used for pure clean waters which contain no iron, nitrates or organic matter. Waters with high concentrations of the substances indicated give significant errors. Rideal and Stewart's (1901) modification of Winkler's method was advised to be used as a regular procedure for all kinds of water.

The photosynthesis rates of plants of *Mastocarpus* was determined by the rates of oxygen evolution.

The bottles (280 ml) were cleaned and filled with filtered seawater . Some bottles filled with seawater were used as controls to determine the initial oxygen concentration of the seawater. Five branches of plants of *Mastocarpus* (fronds with and without papillae) were collected seasonally (from winter 1985 - 1986). The fronds of *Mastocarpus* were cleaned and placed in the bottles, hung on a thread passing between the bottle neck and the stopper. The bottles were incubated at the room temperature for 6 hours. All bottles were illuminated by 244 μmol m^{-2} s^{-1} fluorescent light. Immediately after incubation, the algae samples were withdrawn and dried to a constant weight at 75 ^{o}C, then the oxygen concentration of the seawater in each bottle was determined by Winkler's method as described by Stricland and Parsons (1968).

The oxygen concentration found in the control bottles was subtracted from that found in each sample bottle to obtain the net change due to combined photosynthesis and respiration of the algal sample.

Net photosynthesis rate mg O_2/g dry weight/hour was calculated as follows:

$$\text{Rate} = \frac{\text{(net change in } O_2 \text{ conc.in mg/l) X (bottle volume in litres)}}{\text{(plant weight in gram) X (length of incubation period in hour)}}$$

Reagents

1) Manganous sulphate: 240 gm of $MnSO_2.4H_2O$. was dissolved in distilled water and made up to 500 ml.

2) Alkaline potassium iodide: 250 gm of sodium hydroxide (KOH) in 250 ml of

distilled water and 150 gm of potassium iodide in 225 ml of distilled water and mix the two solution and made up to 500 ml. This was solution cooled and stored in a glass bottle.

3) Concentrated sulphuric acid. Analar standard reagent.

4) Starch solution (indicator): 2 gm soluble starch was suspended in 400 ml of distilled water and 20% potassium hydroxide was stirred until clear and then allow to stand for 1-2 hours. Hydrochloric acid was added until solution was acid (with litmus paper) and then 2 ml of glacial acetic acid was added for preservation. The solution was diluted to 1 litre and stored in the refrigerator.

5) Sodium thiosulphate: 2.9 gm of $Na_2S_2O_3$ was dissolved in distilled water and made up to 1 litre in volumetric flask and stored in an amber bottle and renewed every 4-5 weeks.

Procedure.

1- Removed stopper from the bottle and added 1 ml of manganous sulphate reagent with an automatic pipette followed at once by 1 ml of alkaline iodide solution. Re-stoppered the bottle immediately and mixed the contents thoroughly by shaking until the precipitated manganous hydroxide was evenly dispersed. No air bubbles should be trapped in a bottle.

2- When the precipitate had settled slightly (2-3 minutes) the bottles were shaken again. Finally samples were allowed to stand until the precipitate had settled.

3- Added 1 ml of concentrated sulphuric acid, re-stoppered the bottle and mixed. All the precipitate then dissolved.

4- Within an hour of acidification, transfered 50 ml of solution into a conical flask. Titrated against 0.01 N thiosulphate solution until a very pale straw colour was obtained. Added 5 ml of starch indicator and continued the titration until the blue colour disappeared.

2.9. Determination of organic carbon (wet oxidation by acid dichromate)

This method was used in examine the amount of organic material in different parts of *M. stellatus* (holdfast, stalk, basel of fronds, frond without papillae, fronds with papillae, non-fertile papillae, fertile papillae, and discs of sporelings) were collected seasonally (from winter 1985 - winter 1986) in Great Cambrae Island in Firth of Clyde. The method involved the wet oxidation of carbon by acid dichromate. (This method as described by Johnson, 1949).

Reagents

1- $K_2Cr_2O_7.2H_2O$: 5 gm of potassium dichromate was dissolved in 20 ml of distilled water and diluted to 1 litre with 95% H_2SO_4 and stored in glass stoppered bottle.

2- $Na_2S_2O_3$: 1.24 gm of 0.005 N sodium thiosulphate in 1 litre, prepared 2 hours before the titration.

Procedure

1- 1 mg samples of different parts of *Mastocarpus* were dried by freeze drier.

2- Placed 1 mg samples in three tubes each tube containing 5 ml of sodium sulphate solution (22.5 gm/500 ml distilled water) and shaken for 30 seconds.

3- Transfered to another 5 ml of sodium sulphate solution and shaken for 30 seconds to prevent the chloride ion interfering.

4- Rinsed in small volume of distilled water.

5- Placed sample in 1 ml of distilled water and 1 ml of 70% phosphoric acid.

6- Heated for 30 minutes in a boiling water bath.

7- Cooled all the tubes.

8- Placed 1 mg samples in 3 test tubes.

9- Add 2 ml of oxidizing reagent and heated for 20 minutes in boiling water bath.

10- At the same time placed 1 mg of glucose in 1 ml distilled water in separate tube.

11- 2 ml of $K_2Cr_2O_7.2H_2O$ with 1 ml of H_2O in a separate tube as the blank heated for 20 minutes.

12- Cooled immediately and add 10 ml of distilled water to each tube.

Titration procedure

The blank, glucose and sample tube were rinsed into a conical flask with

approximately 20 ml of H_2O, and a small crystals of KI (potassium iodid) were added and mixed well, then titrated in the presence of starch indicator with sodium thiosulphate. The amount of organic matter in the sample was calculated between the blank, glucose and sample.

2.10. Measurement of calorific value of *Mastocarpus* by Microbomb Calorimeter

Mastocarpus stellatus were collected seasonally (from winter 1985 - winter 1986). After collection the plants were cleaned with filtered seawater several times and cut into different parts (holdfast, stalk, basal of fronds, fronds without papillae, fronds with papillae, non-fertile papillae, fertile papillae, tissue of papillae and spore masses), and then put all material in deep freeze for an hour, and then in liquid nitrogen for several minutes. All parts of *Mastocarpus* were left in freeze drier for 3-4 days before measurements. The parts of *Mastocarpus* were ground to homogenize the material, and stored in a desiccator until used for combustion in a microbomb calorimeter (model A.H 9 Micro-bomb Calorimeter Newham Electronics Ltd.). A diagram of assembly bomb is shown in figure 2.4 and a diagram of firing system is shown in figure 2.5.

Firing technique

1. Prepared dried sample in pellet maker and kept in a desiccator.
2. Clamped bottom half of bomb in vice. Placed 0.025 ml distilled water in bottom of bomb.
3. Placed top half of bomb in clamp at eye level. Using 4 cm of 0.15 mm platinum wire, connected firing wire to terminal and pull out to taut V-shape.
4. Placed tray with pellet onto pan, and bend the v-shaped firing wire so that point touched the pellet (but not the tray) so holding it in position.
5. Screwed top onto bottom and tightened until metal meets metal using correct spanner.
6. Fitted quick release connection from oxygen cylinder, slowly fill to 25 atomospheres.
7. Connected calorimeter to recorder, and switched on the recorder to establish room

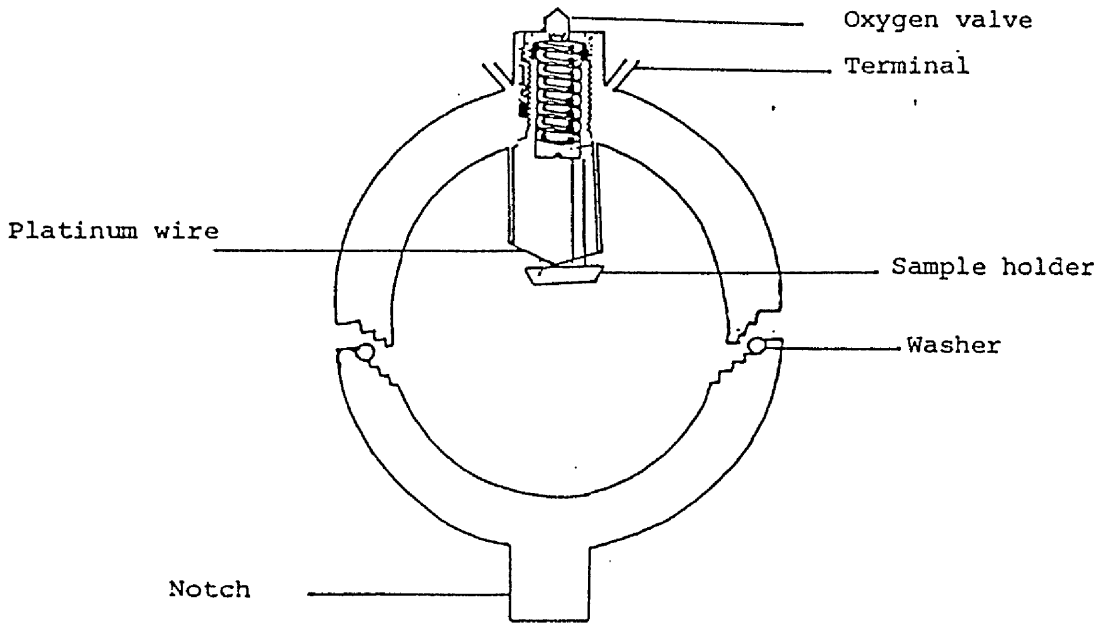


Fig. 2.4. The Phillipson's Micro-bomb.

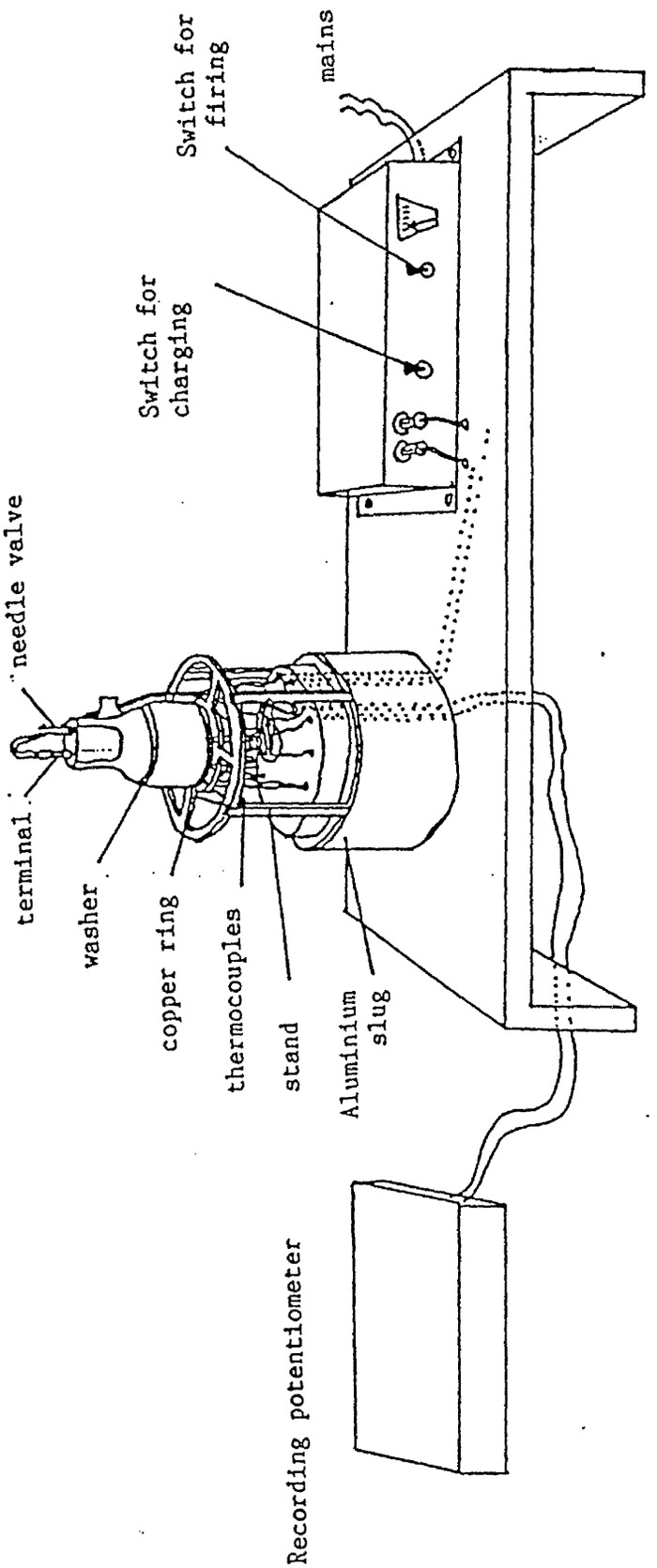


Fig. 2.5. The firing assembly.

temperature trace (base line).

8. Placed bomb on thermocouple ring. Waited until steady trace, with a very slow increase or decrease, which was acceptable.

9. Connected leads and replaced both covers.

10. Switched on calorimeter, check charge (35 volts). Switched to fire, returned to charge and then switched off calorimeter. If the material has combusted the pen will rise smoothly.

11. Observed the time for the pen recorder to rise and peak on recorder. Waited until there is a linear cooling rate.

12. Switched off recorder.

Analysis of results

a) Calibration: Prepared a series of pellets of pure dry benzoic acid, varying in weight from (5 mg - 20 mg) are used to provide a number of different points. A calibration line is then obtained by plotting the heat output from the calorimeter against the weight of benzoic acid oxidized.

b) Calculation: For each sample we need to calculate **ΔH**, the amount of heat given out during the combustion of the sample and convert that into calories using the calibration line constructed by oxidizing benzoic acid pellets in the calorimeter. The calorific value of the sample should be expressed as calories per mg ash-free material.

1- For each trace determine the peak of the curve representing the maximum temperature detected by thermocouple (as shown in Fig. 2.6)

apparent **ΔH** = peak (units) - Baseline (units)

2- To correct for heat loss, draw the line AB. From the peak of the curve (point c) draw the line CD parallel to AB.

3. Calculate 60% the value of the apparent **ΔH.**

ΔH value = apparent **ΔH** X 60%

4. Find the point at which the curve crosses the point **ΔH** value on the chart paper and draw the vertical line EF through this point.

5. Draw the line GH to extend the original baseline, to account for the gradual change in

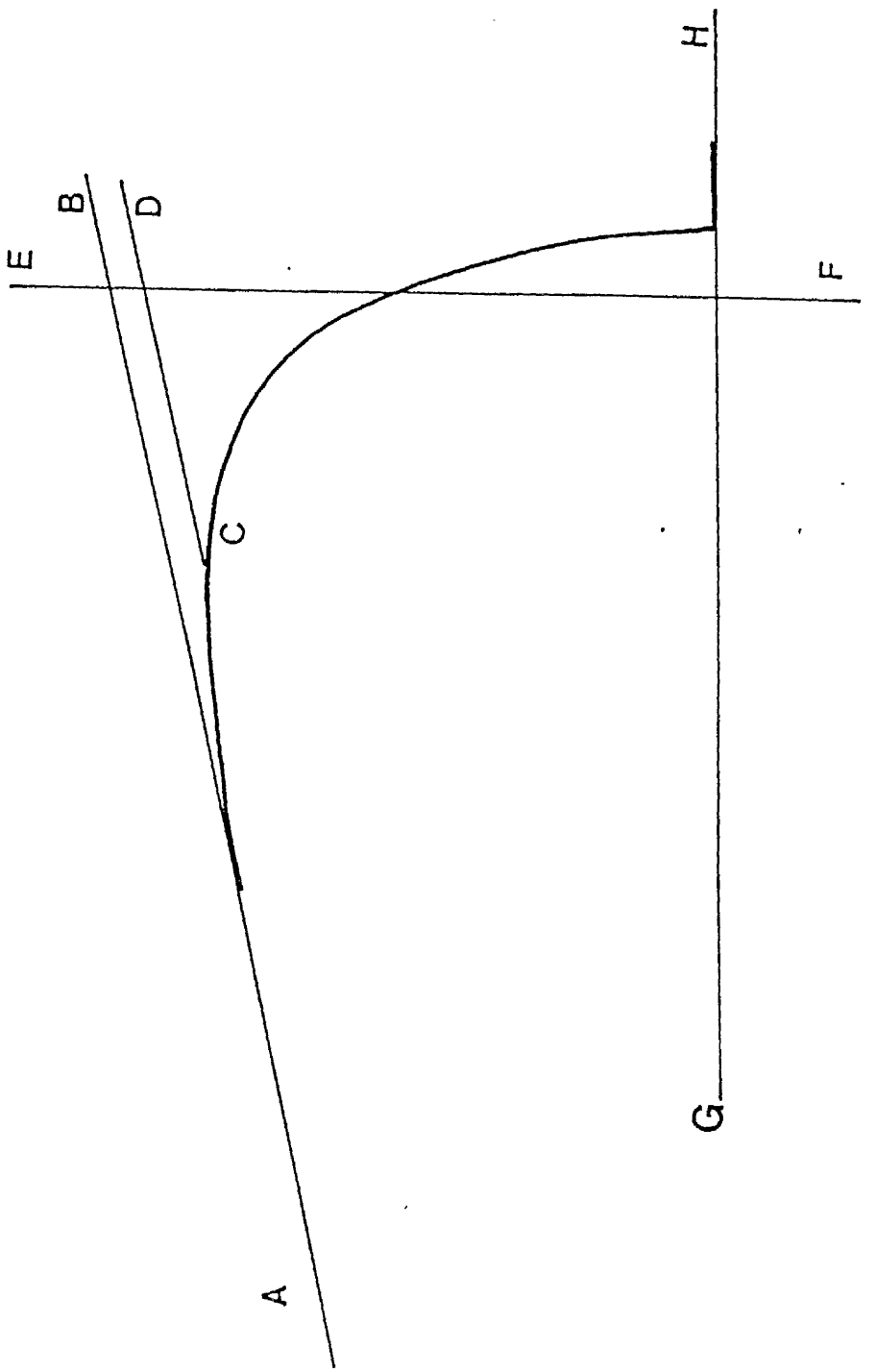


Fig. 2.6. The typical curve obtained with combustion of benzoic acid.

the baseline.

6. Read off the values of the points at which the lines CD and GH intersect the line EF.

7. Calculate the true **ΔH.**

True **ΔH** = true peak (line CD intersect EF) - base line (line GH intersects EF).

8. From benzoic acid calibration line, determine the weight of benzoic acid which this **ΔH**. Converted to calories (1 mg of benzoic acid has a calorific value of 6.332 calories). Finally calculate the sample as calories per mg ash-free dry weight.

2.11. Measurement of photosynthesis by the oxygen electrode.

The oxygen evolution was measured in a modified Clark oxygen electrode (Hansatech) of the type described by Delieu and Walker (1972).

The apparatus consists of a platinum wire sealed in plastic as the cathode, and a circular anode of silver wire bathed in a saturated potassium chloride (KCl) solution. The electrodes are separated from the reaction mixture in the plastic container which was stirred constantly with a small magnetic 'flea' stirring rod. When a voltage is applied across the two electrodes using the polarising meter, the platinum electrodes became negative with respect to the reference electrode and the oxygen in the solution is thought to undergo electrolytic reduction at the cathode:-

$$4H^- + O_2 + 4e^- = 2H_2O$$

The flow of current in the circuit when the polarising voltage is set between 0.5 and 0.8V has a linear relationship to the partial pressure of the oxygen in solution. The instrument was usually operated at a polarising voltage of about 0.65V. The current following was measured by connecting the electrode to a sensitive potentiometer chart recorder.

The reaction chamber was kept at a constant temperature by circulating water from a temperature controlled water bath and illuminated with light source (desk lamp) when needed and was concentrated on the reaction chamber by flask full of water. A diagram of the layout of the apparatus is shown in figure 2.7 and a diagram of the electrode is shown in figure 2.8.

The oxygen electrode was used to compare rates of photosynthesis (by O_2 release)

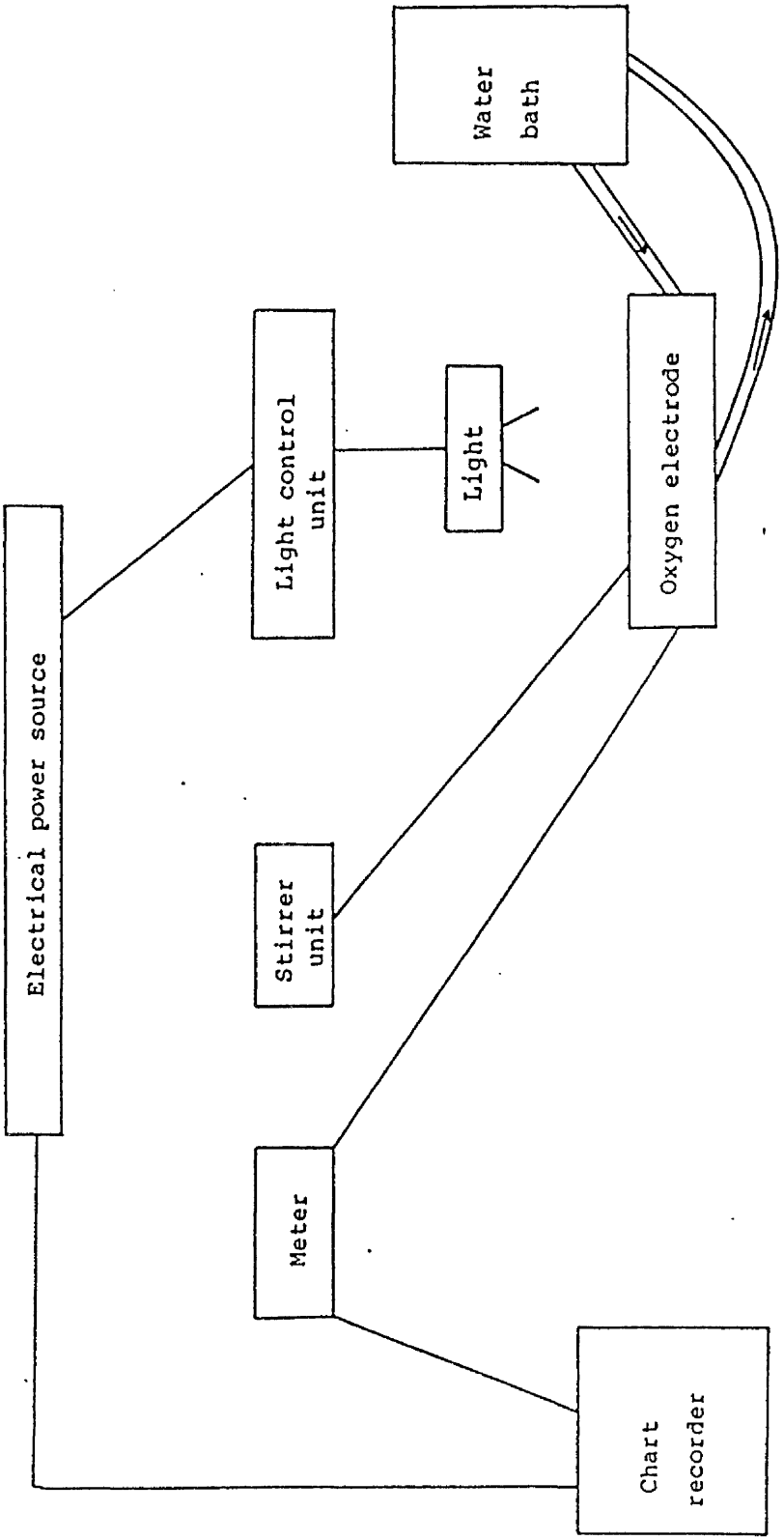


Fig. 2.7. The layout of the apparatus used in the oxygen electrode experiments.

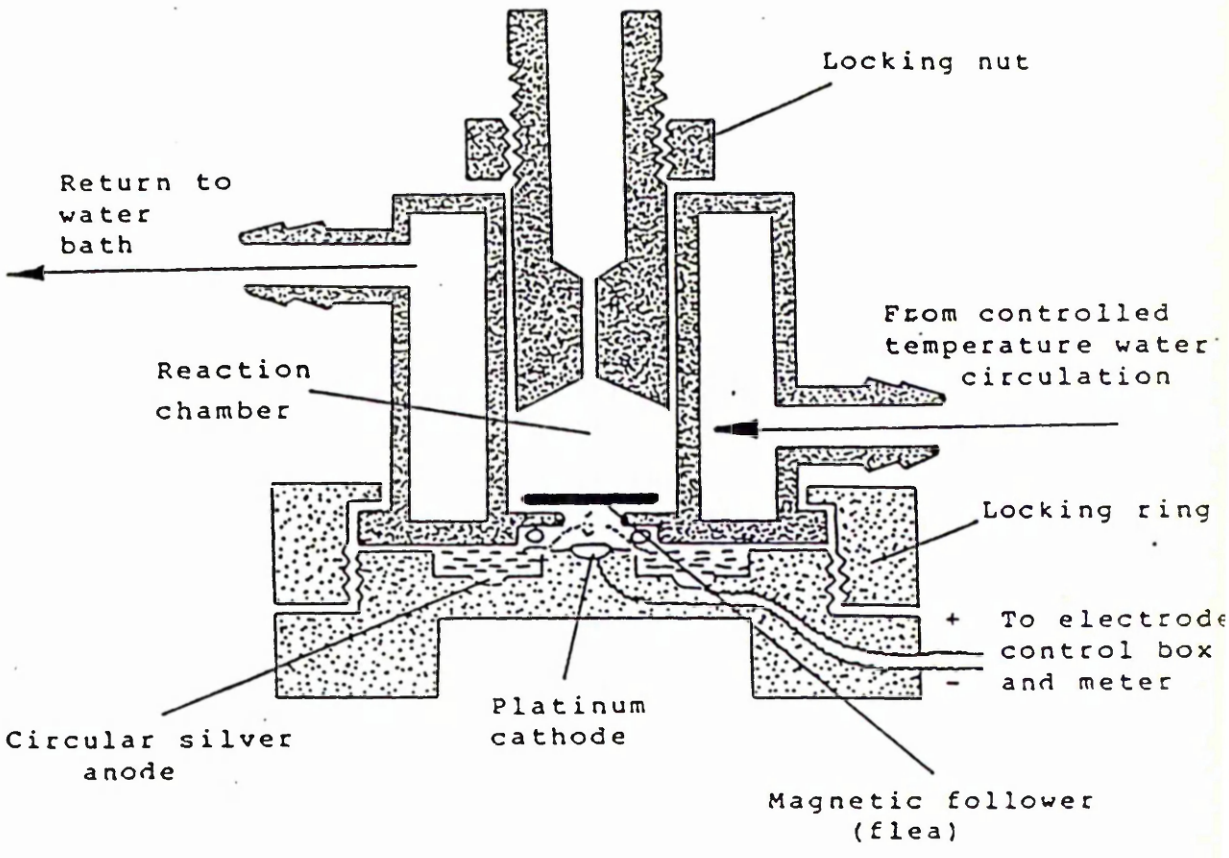


Fig. 2.8. Diagram of the oxygen electrode.

over suitable time intervals. The O_2 content of sea water in the electrode cell in equilibrium with air was measured with the trace set at maximum level on the chart recorder. After the addition of sodium dithionite (which absorbs the dissolved oxygen) the trace then falls to be zero level on the chart. From this the quantity of dissolved oxygen can be equated to chart recorder units. With a fresh sea water sample at equilibrium the trace was checked at maximum level on the recorder, then the recording pen was brought down to the zero level previously obtained. Any change in O_2 content due to photosynthetic activity of test material was then recorded by the trace, and the quantity of O_2 assessed from the chart recorder units.

The oxygen electrode was found to be very suitable for measuring the photosynthetic activity of discs of sporelings, and of papillae and spores of *Mastocarpus stellatus*.

The quantity of O_2 (mg) released (or absorbed) was measured in relation to mm^2 of sporeling surface area, gram fresh weight of papillae, or number of spores ml^{-1}.

Sporeling surface area were measured as described on p. 15, and spore numbers determined by mean of a haemocytometer.

3. Observation on the regeneration and recovery growth of *Mastocarpus* sporelings after injury.

3.1. Regenerative growth of *Mastocarpus* sporelings.

3.1.1. Introduction.

Regenerative growth of *Mastocarpus* sporelings is not a well known phenomena. Previous studies on the regenerative growth of red algae have been mainly carried out with filamentous or frondose forms, and there is little information regarding the crustose habit. Marshall *et.al* (1949) stated that the damaged plants *Mastocarpus stellatus* (as *Gigartina stellata*) have been found to be capable of forming new branches, regeneration normally taking place from the wounded surface if parts of the thallus are removed by injury or by harvesting. They also found that if the apex of a thallus with papillae is wounded, new growth develops from meristems that would normally produce reproductive papillae immediately behind the growing point. Chen and McLachlan (1972) in preliminary experiments with *Chondrus crispus* showed that, in culture, regeneration can occur from both the erect frond and the basal disc. In the latter, erect fronds may arise from any point on the dorsal surface of the disc. Waaland (1975) found that with plants of *Griffithsia* spp. the dead intercalary cells were often replaced by the process of cell repairs. The rhizoids of three different species of *Griffithsia* were tested in the plant repair system showed that the rhizoids of one species could induce repair shoot formation and cell fusion in the filaments of the other two species.

Perrone and Felicini (1976) cultured segments of the fronds of *Gigartina acicularis* (Wulf.) Lamour., the segments produced two types of buds, proliferative and regenerative. Either one type or both may be found on cut surfaces. Suitable trophic conditions increased the relative frequency of regeneration buds in comparison with proliferations. Segments of the distal part of the fronds show a greater tendency to proliferate, while segments of the basal part show more regeneration. Boney (1975a) observed that excised pseudolaterals of limited growth of *Heterosiphonia plumosa*

(Ellis) Batt. showed rhizoid formation either from the region of the rhizoids in close proximity to a basal cell or directly from the basal cell. Khfaji (1978) found the plants of *Mastocarpus stellatus* (as *Gigartina stellata*) regenerated very slowly in nature and the new growth on the cut surface of fronds reached about 2 cm. in length in 7 months from the date of cutting. Duffield *et al* (1972) studied the regeneration of *Griffithsia pacifica* Kylin from single cells. Regeneration could start from any cell and was triggered by the removal of an abutting cell. The single shoot cell formed a shoot and rhizoid cells within one day . The shoot then added new cells by apical division at the rate of 1-2 cells/day. Branches were formed at predictable but not fixed location by the budding of subapical cells. The plants consisted of uniseriate, pseudodichotomously branched filamentous shoots with filamentous multicellular rhizoids at the base.

Perrone and Felicini (1972) stated that when cut into segments the fronds of *Petroglossum nicaeense* (Duby) Schotter gave rise to adventitious buds produced distally by the proliferation activity of the erect fronds but the creeping axes are formed from the regenerated buds. These facts clearly indicate a polarization in the development of the alga and suggest that correlative mechanisms are operative in this species. Felicini and Perrone (1972) and Perrone and Felicini (1974) found that the older plants of *Petroglossum nicaeense* tend to produce numerous proliferations arising from the margins of the leafy fronds. They observed that removal of the distal portion of the erect fronds induced outgrowths of adventitious branches. Cessation of apical growth in older fronds is characterized by the outgrowth of marginal plagiotropic proliferations. The generation and proliferation were affected by nitrogen starvation and Indole-3-acetic acid (IAA).

Felicini and Perrone (1986) stated that cultured frond segments regenerate buds which develop as either erect or creeping axes. They confirmed that hyphae were a feature of the erect thallus. Paine *et.al* (1979) studied the growth and longevity in the crustose red algae *Petrocelis middendroffii* . They found the growth was slow and that the surface area increased about 4% per year. Other plants were observed to decrease in total surface coverage, fragment and eventually disappear. Konrad-

Hawkins (1964) studied the development and regeneration of *Callithamnion roseum* Harvey. Individually they increased in cell number in an arithmetic order, while the total cell output of the whole shoot grows exponentially with time. Branches appeared to have two growth phases. The decreased growth rate in the second phase was probably correlated with the structural and functional differentiation of the branch. Dixon (1973) stated that the cell on the apical side of the dead cell produced a downgrowing rhizoids of *Griffithsia corallinoides*, while the cell on the basal side produced new apex. When the cell size is small, then the regenerating spices and rhizoids external to the filamentous. The ingrowing apices and rhizoids from a series of filaments which may fill completely the old cell lumen.

Discoid, encrusting sporelings of *Mastocarpus* can suffer damage from grazing molluscs, or by attrition from suspended stones and gravel. Since the filamentous and macroscopic red algae possess the abilities to regenerate rapidly from cut or damaged surfaces with often new regions of meristematic tissue being formed, experiments were carried out to examine the regenerative capacities of *Mastocarpus* sporelings of different ages.

3.1.2. Materials and Methods

Mastocarpus sporelings were obtained from fresh spore settlements, and cultured on cover slips with enriched sea water medium and incubated in a growth chamber. Sporelings of *Mastocarpus* of different age (39-129 days) were cut into different shapes and incubated in growth chamber for several weeks, and also the segments of sporelings of different shapes were left several weeks in growth culture at 10 ^{o}C±2 ^{o}C and light intensity 17.5µmol m^{-2} s^{-1} for 16 hours day^{-1}. For calculation of surface area see chapter 2 section 2.2.6.

3.1.3. Results

Young sporelings of *Mastocarpus* regenerated very rapidly within 11 days of removal of the tissue, and the surface area of sporelings after removal the tissue showed evidence of regeneration (Table 3.1.3.1). Fig. 3.1.3 a,b shows a sporeling at

Table 3.1.3.1. Regeneration and growth of attached *Mastocarpus* sporelings after removal of tissue. Age of sporelings at start was 39 days, and the regeneration period 12 days. Temperature 10 $^{o}C \pm 2\ ^{o}C$ and light intensity 17.5 µmol $m^{-2}\ s^{-1}$.

Surface area at start (μm^2)	Surface area after removal of tissue (μm^2)	% of original surface area removed	Surface area after 12 days (μm^2)	% increase in surface area after 12 days
50342.57	29401.4	41.60	72438.60	146.38
46246.19	26319.15	43.09	44319.83	68.39
43957.95	29984.05	31.79	85698.41	185.81
22661.70	18794.56	17.06	54286.92	188.84
----	14212.05	---	49565.55	248.76
----	13561.13	---	32577.72	140.23

Fig. 3.1.3. Regenerative growth of attached *Mastocarpus* sporelings. All sporelings 39 days old at time of experimental treatments.

a, semicircular residual mass. Scale bar= 63 μm

b, appearance of same sporeling after 12 days. Scale bar= 64 μm

c, "tear- drop" like residual mass. Scale bar= 61 μm

d, appearance of same sporeling after 12 days. Scale bar= 51 μm

e, sporelings after removal of V-shape mass of tissue. Scale bar=59 μm

f, appearance of the same sporeling 12 days. Scale bar= 64 μm

g, sporeling after removal of two V-shaped segments. Scale bar=43 μm

h, appearance of the same sporeling after 12 days. Scale bar= 55 μm

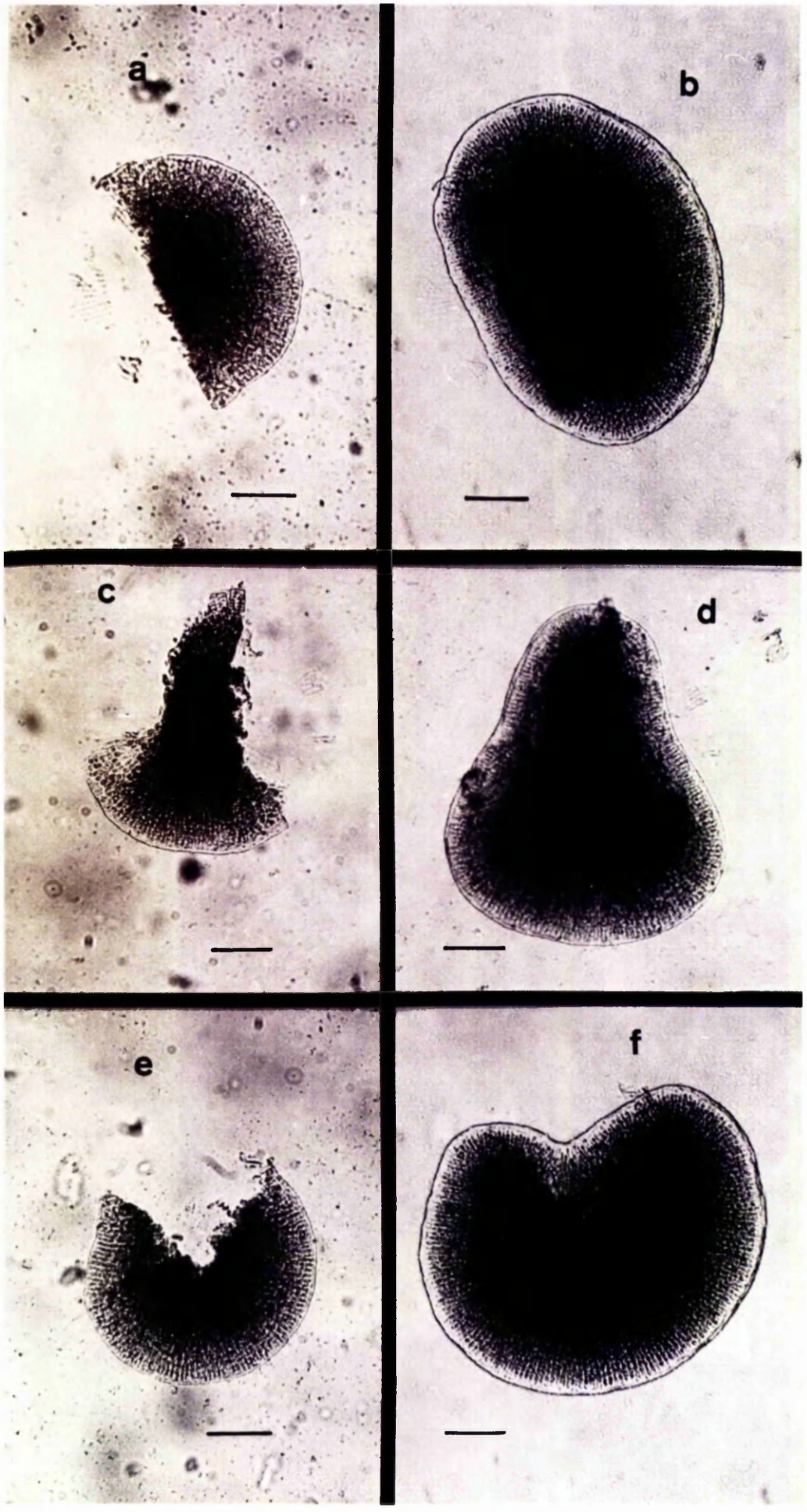
i, sporeling of rectangular shape after removal of peripheral tissue.

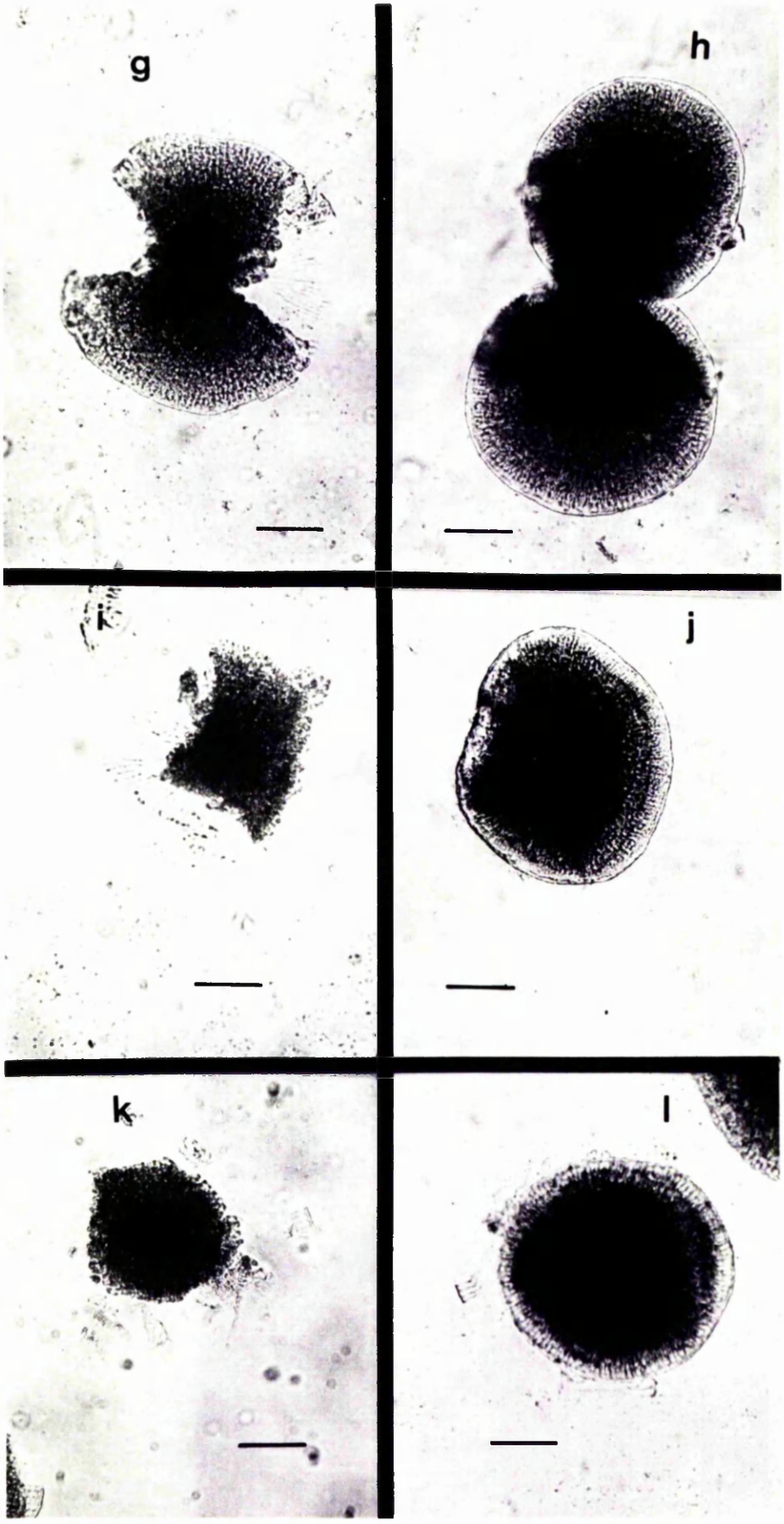
Scale bar= 58 μm

j, the same sporeling after 12 days. Scale bar= 73 μm

k, sporeling with angular shape after removal of peripheral tissue. scale bar= 59 μm

l, the same sporeling after 12 days growth. Scale bar= 60 μm


a
b
c
d
e
f


g
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l

semicircular shape with 41% of tissue removed. Combined regenerative growth and continuing growth of the marginal meristem resulted in new tissue formation equalling 146% increase in surface area over the residual sporeling material (Table 3.1.3.1). New meristematic cells formed on the cut surface do not quite compensate for the growth of the remaining marginal area, but there was an attempt to return to the discoid shape. Fig. 3.1.3 c, d shows a sporeling with 43% of its tissue removed leaving a 'tear-drop' like shaped sporeling mass. The combined regenerative and marginal growth here resulted in a 68% increase in surface area over the residual mass. Most of the growth came from the original marginal meristem, whilst that from the cut surface was slower in formation, so that the 'tear drop' shape remained. Fig. 3.1.3 e,f shows a sporeling with 31% of the tissue removed. New tissue formation was 186% compared with the residual mass. In this case almost 3/4 of the original sporeling remained, so that continued growth was possible from 3/4 of its peripheral meristem. New tissue formation following new meristematic activity in the v-shaped cut region showed a 'filling' in process with almost a return to a discoid shape. In Fig. 3.1.3 g,h 17% of tissue was removed from opposite sequents of the disc. In this case the new tissue increased 186% over the residual mass, with the result that within 12 days two almost discoid masses were obtained. Here it would seen that the remaining peripheral meristem areas were able to compensate for loss of tissue, but some meristematic activity most have came from the two v-shaped cut surfaces. In all proceeding cases the removal of sporeling tissue had left some of the original peripheral meristematic region. In Fig. 3.1.3 (i, j) and (1 k, l) the results are shown of a complete removal of all peripheral tissue. Regrowth in both cases was from new meristimatic activity on all cut surfaces, with 248.8% increase in surface area with the rectangular shaped residue Fig. 3.1.3 i, j and 140% with the angled shape (Fig. 3.1.3 k, l). Both have retained something of their residual tissue shapes, with that of the rectangular form showing some approach to a disc,whilst the angled, more regular shape returned to a discoid form. The probable patterns of regrowth of the sporelings are shown in Fig. 3.1.3a.

Sporelings at age 69, and 129 days regenerated new tissue within period 69 days after removal of the tissue, and surface area of sporelings after tissue removal showed evidence of regeneration (Table 3.1.3.2).

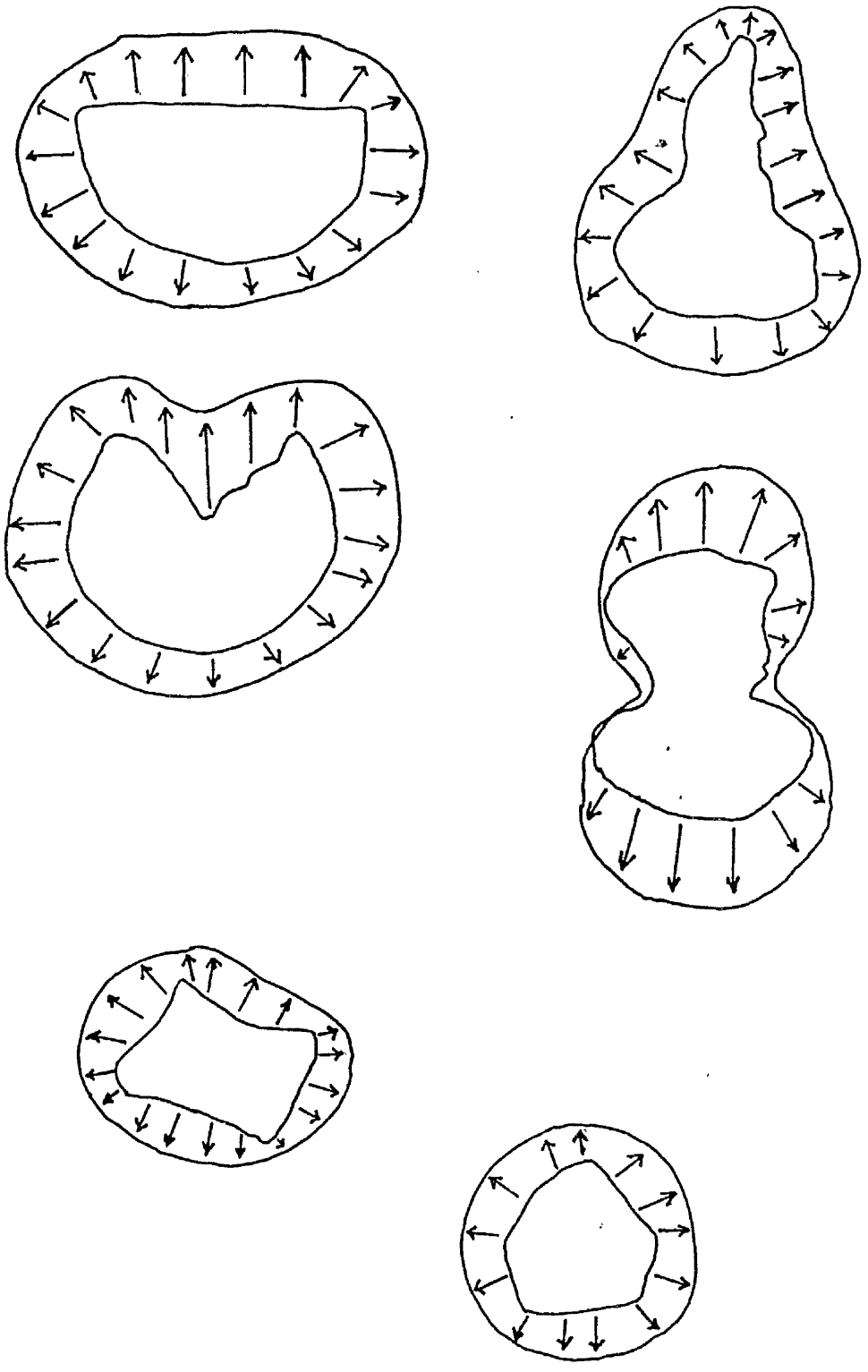


Fig. 3.1.3a. Diagramatic representation of probable growth patterns of sporelings shown in Fig. 3.1.3.

Table 3.1.3.2. Regeneration and growth of attached *Mastocarpus* sporelings after removal of tissue. Age of young sporelings at start was 69 days, old sporelings 129 days,regeneration and growth period 69 days. Temperature 10 $^{o}C \pm 2\ ^{o}C$ and light intensity 17.5 $\mu mol\ m^{-2}\ s^{-1}$.

Age of sporelings (days)	Surface area at start (μm^2)	Surface area after removal tissue (μm^2)	% original surface area removed	Surface area after 46 days (μm^2)	% increase in surface area after 46 days	Surface area after 69 days (μm^2)	%increase in surface area after 69 days
69	132403.27	77936.63	41.14	455548.50	484.51	784728.96	906.9
	132403.27	71941.50	45.66	425778.54	491.84	838429.33	1065.4
69	180639.02	48052.83	73.40	161202.25	235.47	538806.34	1021.3
	180639.02	42940.83	76.23	284768.44	563.16	511569.98	1091.3
	260120.19	53164.83	79.56	271887.76	411.41	434264.53	716.8
69	262269.57	109170.49	58.37	442889.85	305.69	738934.42	576.9
	262269.57	76791.09	70.72	461681.19	501.22	1231695.8	1054
	192677.29	60493.04	68.60	496236.48	720.32	671435.60	1010
129	941534.38	441391.1	53.12	537954.17	21.88	810497.32	83.6

Fig. 3.1.3.1 a, b shows sporelings of semicircular shape after removal of 41.1% and 45.7% of tissue. Growth of the marginal meristem and new tissue formation from the cut surface over 46 days produced new growth equal to 484.5% and 491.8% over the residual sporeling material, and after a further 3 weeks new tissue was about 906.9% and 1065.4% (respectively) of the residual sporeling material. New tissue formed on the cut area tended to compensate for the growth of the remaining marginal region, and there was a partial return to a discoid shape. In Fig. 3.1.3.2 a, b, c 73.4%, 76.2% and 79.6% (respectively) of the tissue was removed, new tissue formation was 235.5%, 563.2% and 411.4% after 46 days, but after a further 3 weeks regrowth new tissue was equal to 1021.3%, 1091.3% and 717% of the residual sporeling tissue, while (Fig. 3.1.3.2c) increased 716.8% over the residual sporeling material. In this case about 1/4 of original sporeling remained, so that continued growth was possible from 1/4 of its peripheral meristem. New tissue formed on the cut surfaces made a significant contribution to its return to normal discoid form. In Fig. 3.1.3.3 a, b, c 58.4%, 70.7%, and 68.6% of tissue was removed from opposite segments of the disc. New tissue showed increases of 305.7%, 501.2%, and 720.3% after 46 days, but after a further 3 weeks regrowth new tissue was 576.9%, 1504%, and 1009.9% over the residual mass. The results showed that within 69 days there was a return to a discoid form, and the peripheral meristem regions were here much reduced. New tissue thus came mainly from meristematic activity on the cut surfaces. Fig. 3.1.3.4 shows a sporeling 129 days old and of semicircular shape with 53.1% of the tissue was removed. Regenerative growth of the marginal meristem and new tissue formation in 46 days increased the surface area by 21.9%, and after further 3 weeks increased 83.6% over residual mass. In this case almost 1/2 of original sporeling remained, new tissue being formed on the cut surface to compensate for the growth of the remaining marginal area. New tissue growth came from marginal meristem but could not return to the normal discoid shape, and regrowth of new tissue from underneath of old tissue was of irregular shape.

Segments of sporelings regenerated very slowly within 69 days after removal of

Fig. 3.1.3.1. Regeneration growth of 69 day old attached sporelings of *Mastocarpus stellatus* after removal of tissue; a_1, 41% tissue removed; b_1, 45.7% tissue removed (see Table 3.1.3.2). Scale bar= 274 µm. a_2, b_2, appearance of sporelings after 46 days. Scale bars= 277 µm, and 282 µm. a_3, b_3, appearance of sporelings after a further 23 days. Scale bars= 240 µm and 243 µm.

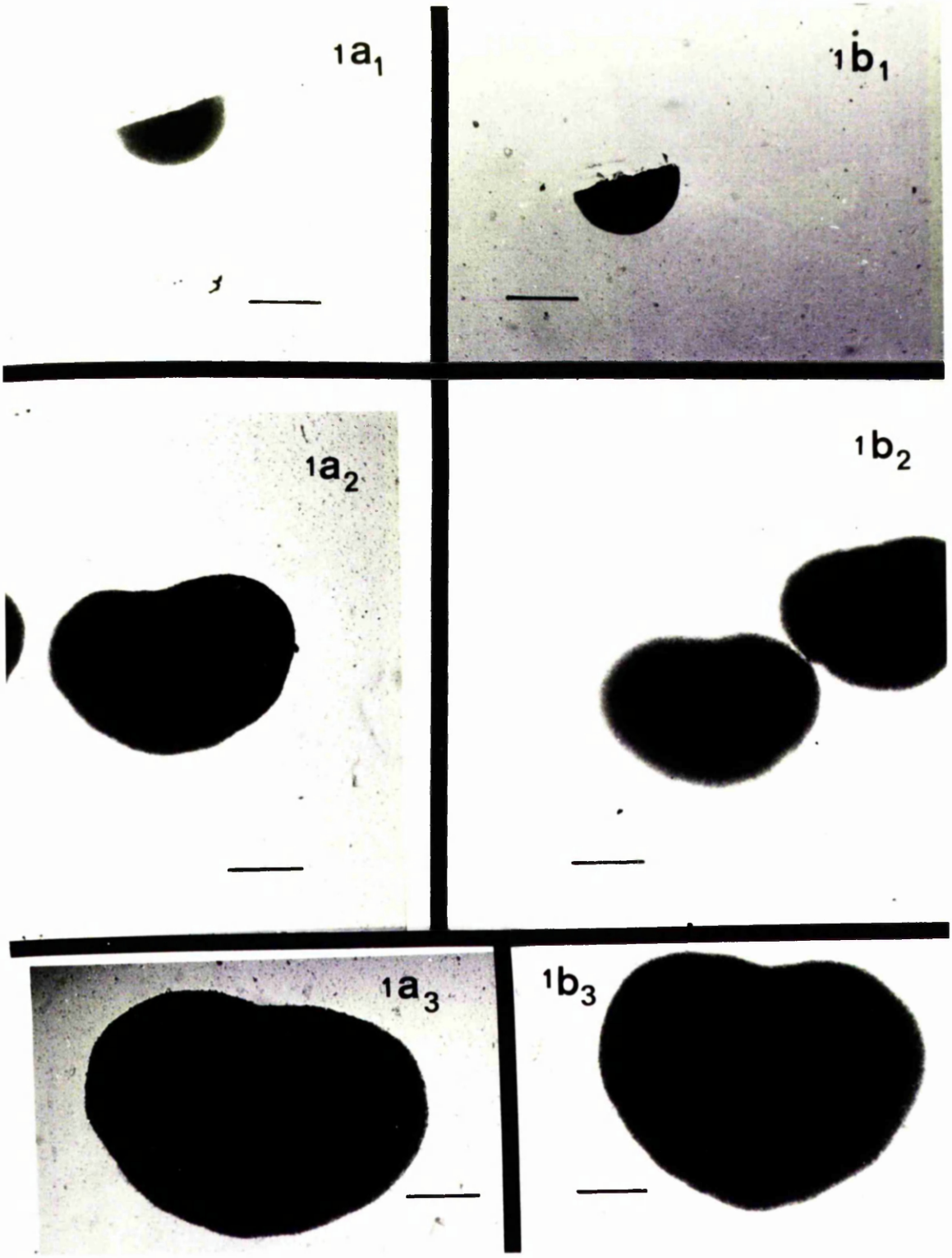

$^1a_1$
$^1b_1$
$^1a_2$
$^1b_2$
$^1a_3$
$^1b_3$

Fig. 3.1.3.2. Regeneration growth of 69 day old attached sporelings with most of tissue removed.

a_1 , b_1, and c_1 at start (see Table 3.1.3.2). Scale bar= 320 μm.

a_2, b_2, and c_2 after 46 days. Scale bar= 201 μm.

a_3, b_3, and c_3 after a further 23 days. Scale bars= 243 μm, 247 μm, and 229 μm.

Fig. 3.1.3.3. Regeneration growth of 69 day old attached sporelings of *M. stellatus* with tissue removed from opposite sides of disc (see Table 3.1.3.2).

a_1, b_1, and c_1, residual masses at start. Scale bars= 304 μm, 304 μm, and 261 μm..

a_2, b_2, and c_2, appearance of sporelings after 46 days. Scale bars= 274 μm, 288 μm, and 292 μm..

a_3, b_3, c_3, appearance of sporelings after 69 days. Scale bars= 243 μm, 315 μm, and 243 μm.

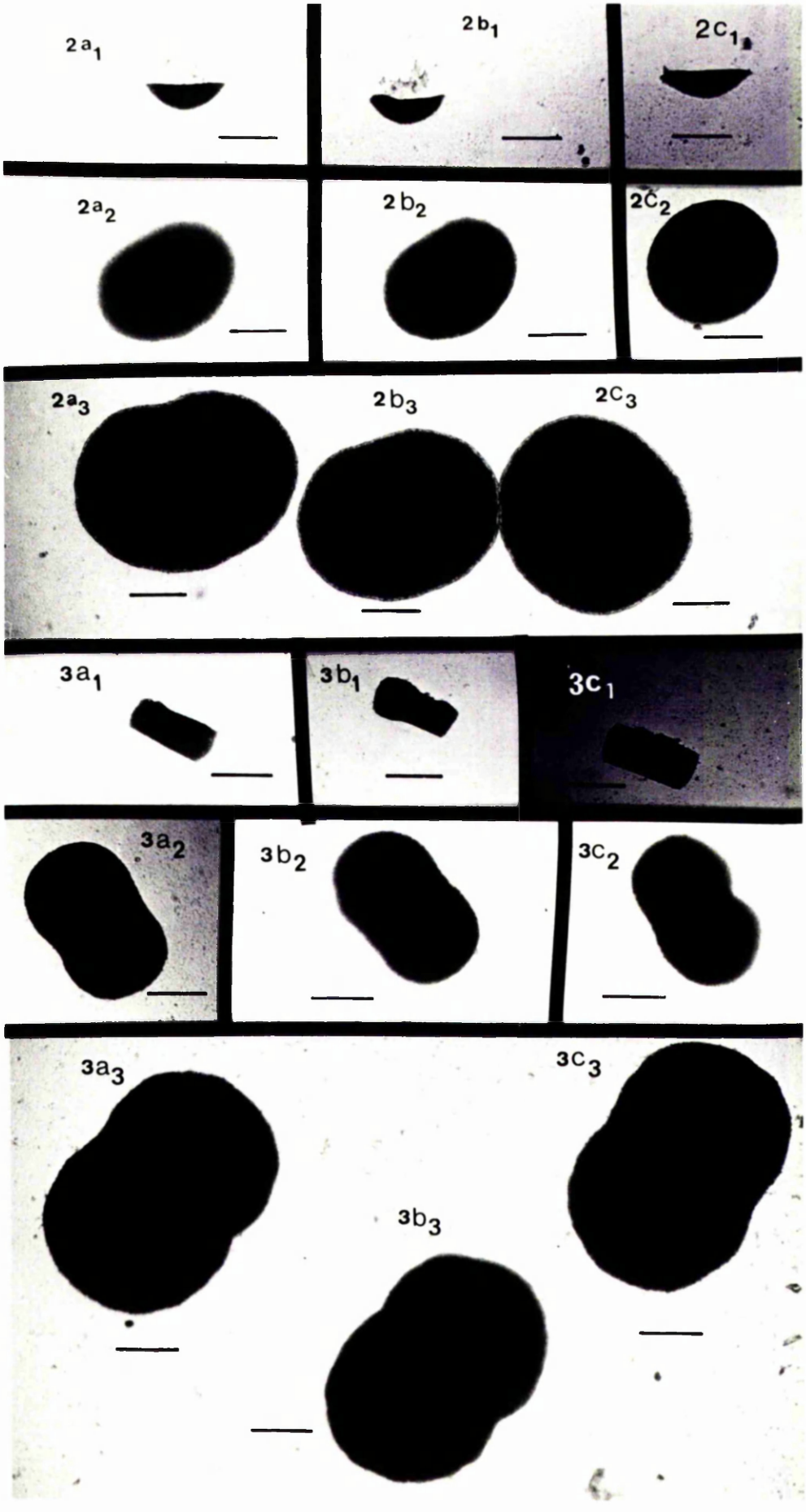
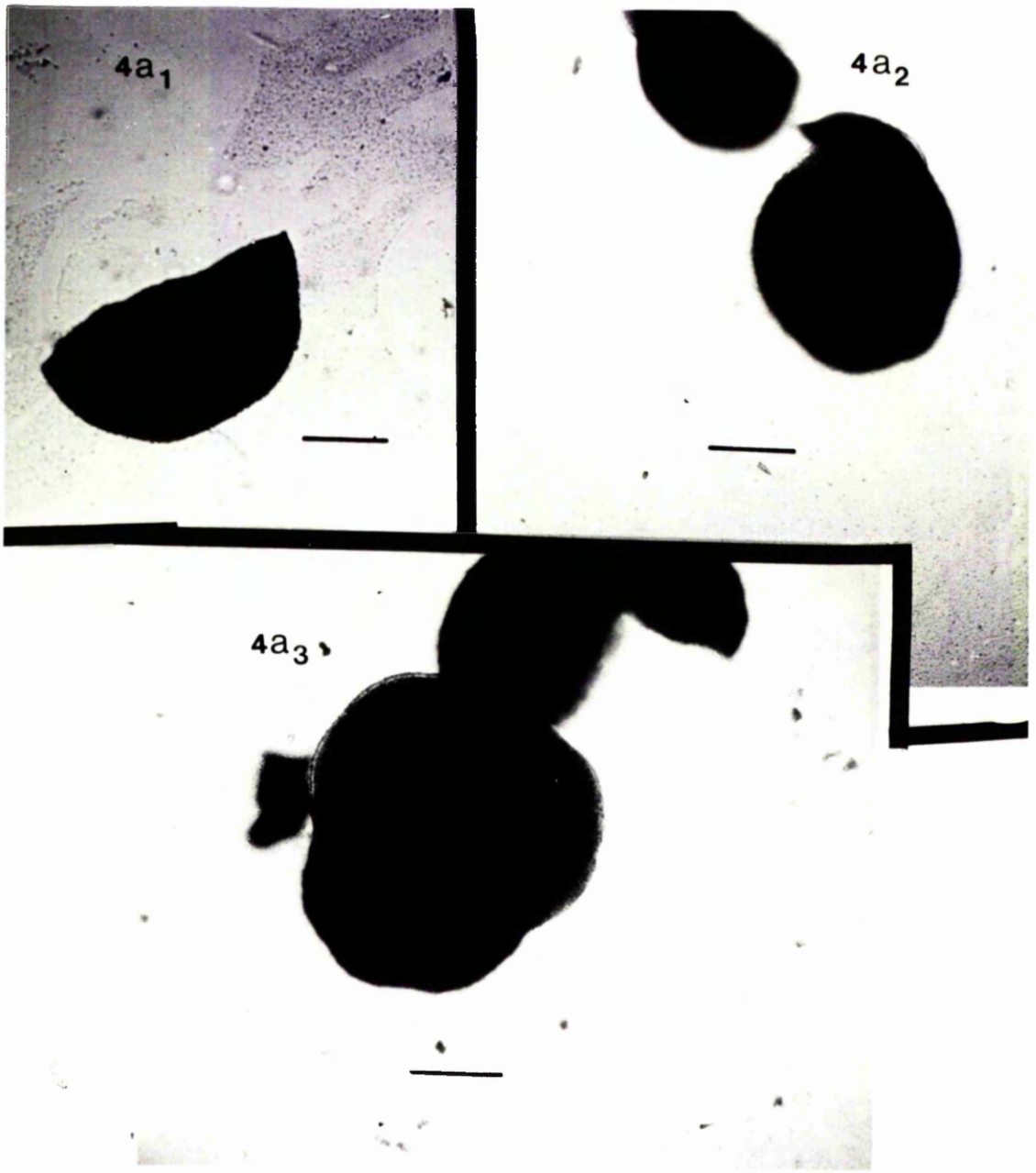

2a1
2b1
2c1
2a2
2b2
2c2
2a3
2b3
2c3
3a1
3b1
3c1
3a2
3b2
3c2
3a3
3b3
3c3

Fig. 3.1.3.4. Regeneration growth of 129 day old attached sporeling of *M. stellatus* of semicircular shape after removal of tissue (see Table 3.1.3.2).

a_1, immediately after removal of tissue. Scale bar= 274 μm.

a_2, appearance after 46 days. Scale bar= 280 μm.

a_3, appearance after a further 23 days. Scale bar= 257 μm.


4a1
4a2
4a3

tissue, and the surface area of sporelings after cut surface showed little regeneration (Table 3.1.3.3).

In all proceeding cases the removal of tissue from the sporelings were carried out on attached sporelings. In Figs. 3.1.3.5-8 the results are shown of new tissue formation on sporelings detached from the glass surface. Regrowth in all cases was by new meristematic activity from all cut surface with resulting irregular shapes, but increased 148.9%, 176% in surface area at different irregular shapes after 46 days, after additional 3 weeks increased 177.8%, 267.62% (respectively) (Fig. 3.1.3.5 a, b). Fig. (3.1.3.6 a, b) 73%, 73.89% of the tissue was removed. New tissue increased 401.9%, and 309% in 46 days, but within 69 days the new tissue increased about 600.7%, and 477.8% with curved shape and irregular shapes as well. In Fig. (3.1.3.7 a,b) 75.5%, and 73.6% of the tissue was removed. New tissue formation was 380.5%, and 241.64%, but after further 3 weeks of incubation was increased 531.6%, 463.5%. In this case about 3/4 of orginal sporeling was removed, but regrowth resulted in irregular shapes with proliferation of segments. Fig. (3.1.3.8) 53% of the tissue was removed combined regenerative growth and regrowth new tissue formation equalled 43%, and after further 3 weeks of incubation a increase in surface area was 334% obtained. New growth showed same attempt to a return to discoid form, with a circular shape.

Results of these experiments showed that regeneration of damaged sporelings when attached produced an attempt to return to the normal discoid form, but detached and damaged sporelings could not reestablish the normal discoid form.

3.2. Observations on the "recovery growth" of *Mastocarpus* sporelings

3.2.1. Introduction and Methods

It was earlier shown that young sporelings could recover after the removal of large quantities of tissue. This treatment left living cells as the sources of regeneration.

The question whether in such a compact cell mass as with *Mastocarpus* sporelings there may be isolated cell survival after the apparent death of the sporelings was investigated with a batch of sporelings which appeared bleached and dead after subjection to a temperature of 35-40 ^{o}C overnight when the thermostatic control in a

Table 3.1.3.3. Regeneration and growth of detached *Mastocarpus* sporelings after removal of tissue. Age of young sporelings at start was 69 days, old sporelings 129 days, regeneration and growth period 69 days. Temperature 10 $^{o}C \pm 2\ ^{o}C$, and light intensity 17.5 $\mu mol\ m^{-2}\ s^{-1}$.

Age of spore-lings (days)	Surface area at start (μm^2)	Surface area after remo-val tissue (μm^2)	% of or-iginal surface area	Surface area after 46 days (μm^2)	% increase in surface area after 46 days	Surface area after 69 days (μm^2)	% increase in surface area after 69 days
69	169454.92	98727.32	41.74	245700	148.87	274223.46	177.76
	150641.37	85853.76	43.01	236955.68	176	315613.14	267.62
69	185977	49735.97	73.26	254086.72	410.87	348480	600.66
	185977	48551.78	73.89	198588.52	309.02	280512	477.76
69	267814.22	65653.28	75.49	315484.12	380.53	414687.11	531.63
	289000.55	76367.54	73.58	260904.68	241.64	430335.68	463.51
129	279167.32	130845.51	53.13	187509.55	43.31	568014.68	334.11

Fig. 3.1.3.5. Regeneration growth of 69 day old sporelings of *M. stellatus* regeneration after removal of about half sporeling tissue and detachment from substratum (see Table 3.1.3.3).

a_1, b_1, immediately after removal of tissue. Scale bars= 332 µm, and 243 µm.

a_2, b_2, detached sporelings after 46 days. Scale bars= 300 µm, and 277 µm.

a_3, b_3, detached sporelings after a further 23 days. Scale bars= 231 µm, and 238 µm.

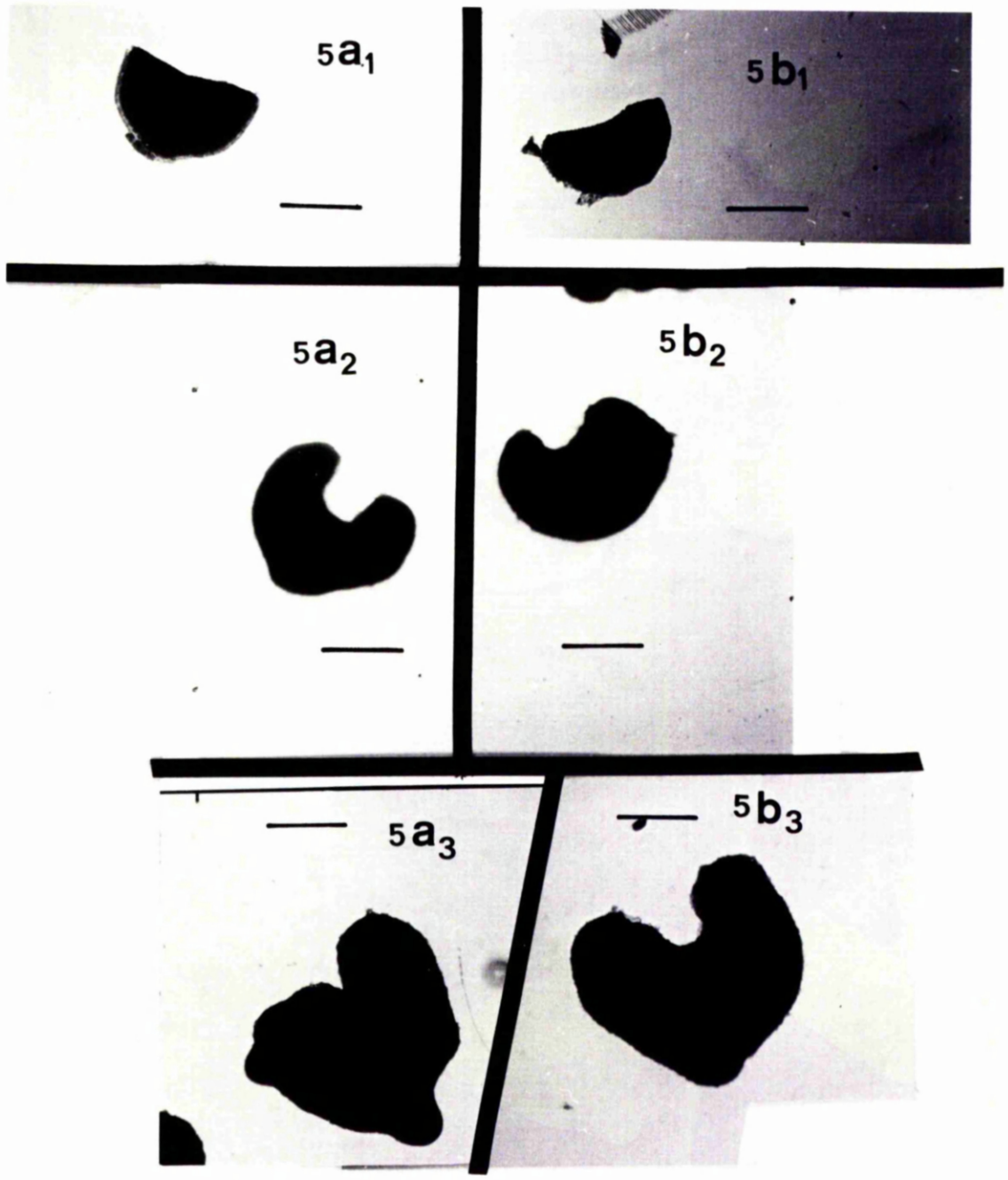

$^5a_1$
$^5b_1$
$^5a_2$
$^5b_2$
$^5a_3$
$^5b_3$

Fig. 3.1.3.6. Regeneration growth of 69 day old sporelings of *M. stellatus* after removal of most sporeling tissue and detachment from substratum (see Table 3.1.3.3).

a_1, b_1, immediately after removal of tissue. Scale bar= 243 μm.

a_2, b_2, detached sporelings after 46 days. Scale bars= 229 μm, and 274 μm.

a_3, b_3, detached sporelings after a further 23 days. Scale bars= 246 μm, and 240 μm.

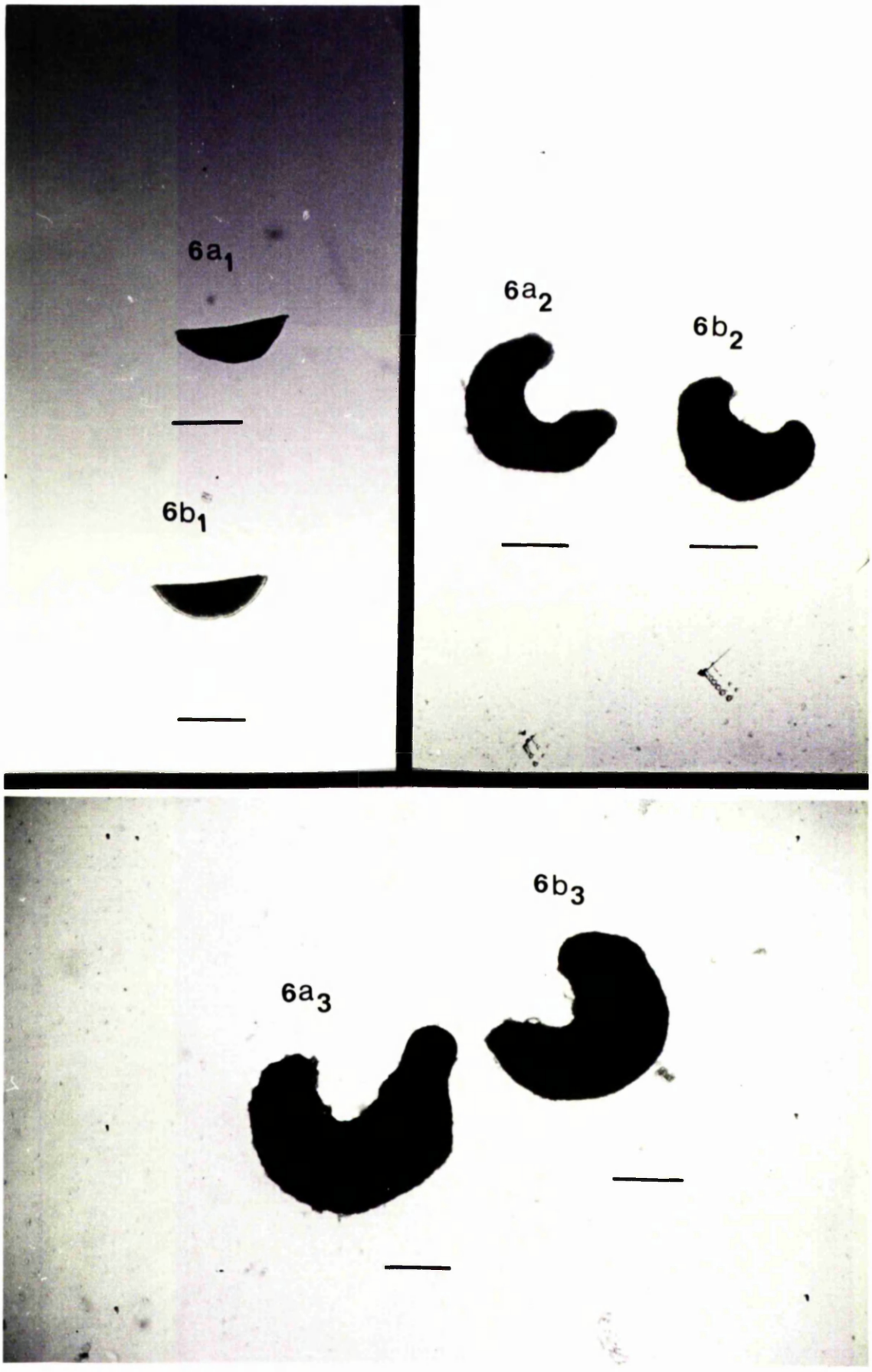

6a1
6b1
6a2
6b2
6b3
6a3

Fig. 3.1.3.7. Regeneration growth of 69 day old sporelings of *M. stellatus* regeneration after removal of opposite sequents of disc and detached from substratum (see Table 3.1.3.3). a_1, b_1 immediately after removal of tissue. Scale bars= 292 μm, and 303 μm.

a_2, b_2, detached sporelings after 46 days. Scale bar= 304 μm.

a_3, b_3, detached sporelings after a further 23 days. Scale bar= 245 μm

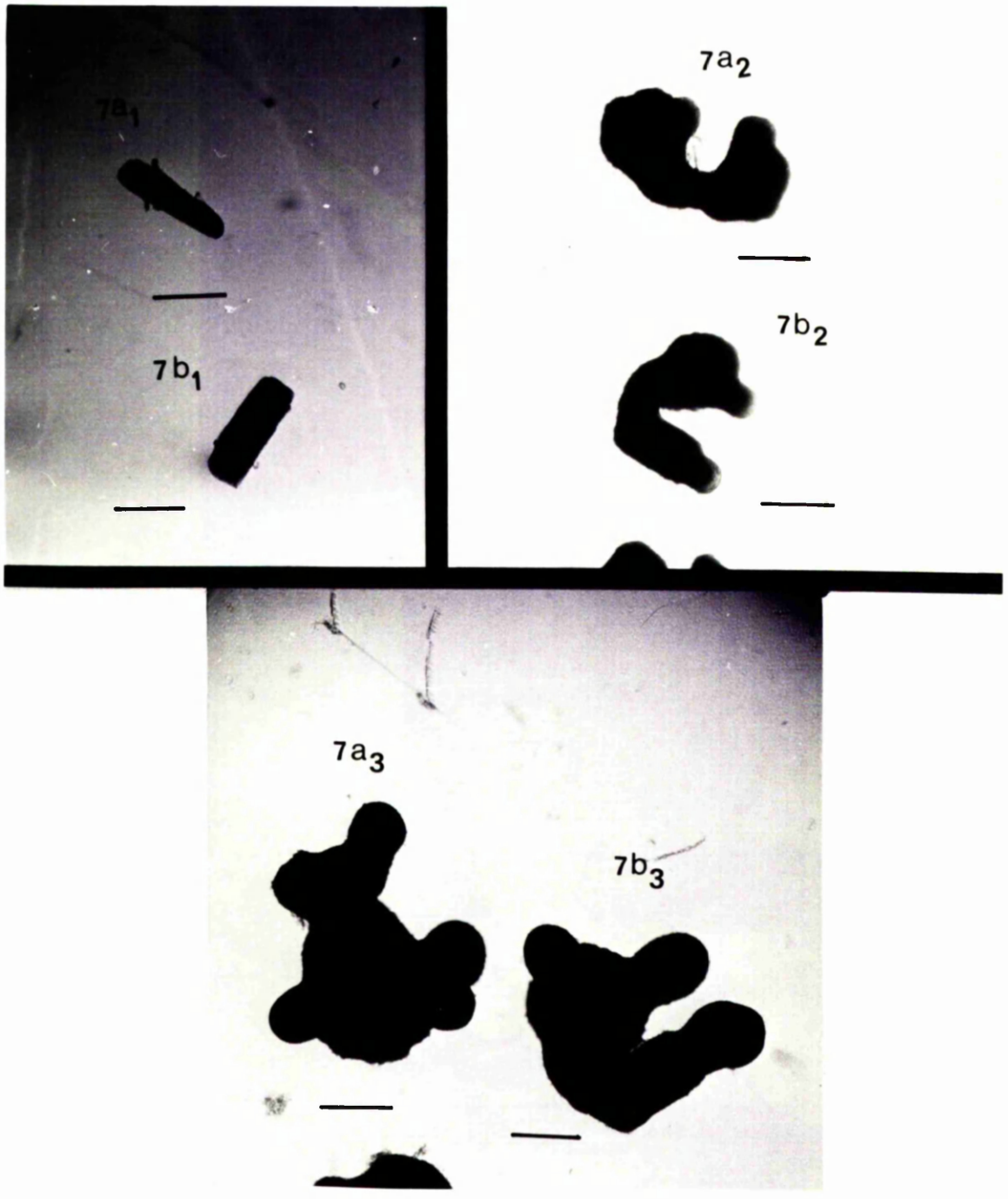
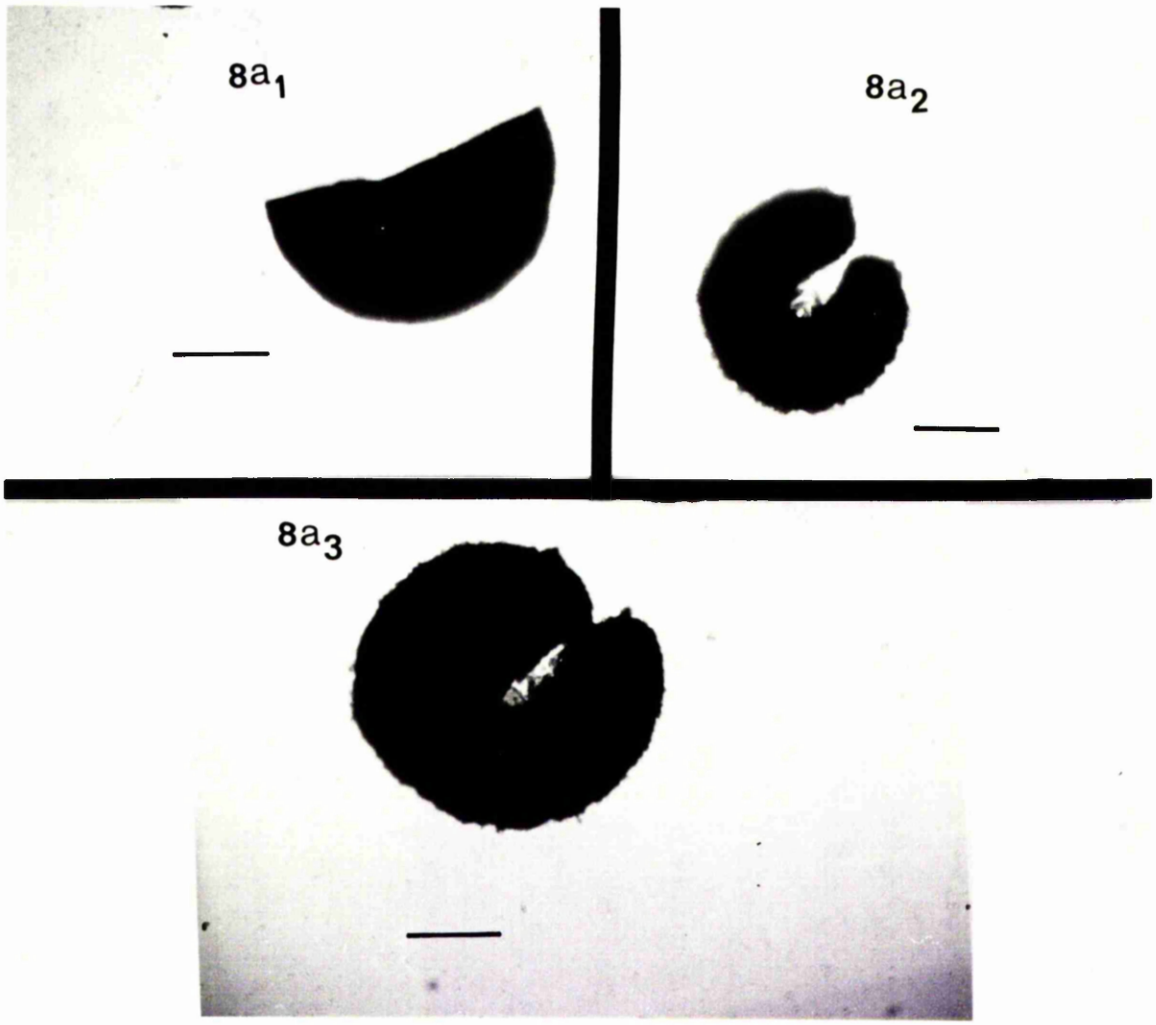

7a1
7b1
7a2
7b2
7a3
7b3

Fig. 3.1.3.8. Regeneration growth of 129 day old sporeling of *M. stellatus* regeneration growth after removal of about half of its tissue and detachment from substratum (see Table 3.1.3.3).

a_1, immediately after removal of tissue. Scale bar= 128 μm.

a_2, detached sporeling after 46 days. Scale bar= 290 μm.

a_3, detached sporeling after a further 23 days. Scale bar= 240 μm.


8a1
8a2
8a3

growth chamber failed. The bleached and apparently dead sporelings were placed in fresh medium and kept under the normal culture conditions at 10 ^{o}C, with 16 hours illumination periods. 15 bleached sporelings which remained intact were kept under observation for any signs of recovery. Recovery was regarded as taking place when the normal 'healthy' red colour was detected in any region of a bleached sporeling. Measurements of the recovery 'areas' were carried out as described on page 15.

3.2.2. Results

Of the 15 sporelings, 8 showed sings of recovery (i.e the development of areas normal red colour) after 20 days. These sporelings are shown in Fig. 3.2.2.1. Observations and measurements on these 8 sporelings were continued over 10 weeks (Table 3.2.2.1 and Fig. 3.2.2.2).

As shown in Table 3.2.2.1 and Figs. 3.2.2.3-5 sporeling number 1 showed a recovery area of $1.1X10^{-4}$ mm^2 at 20 days. This area had doubled over 3 weeks, and doubled again after 7 weeks. At the end of the period of observation the recovery region has increased over sevenfold. Sporeling number 2 showed a recovery area of $1.7X10^{-4}$ mm^2 at 20 days. This region had doubled over 3 weeks, and doubled again after 8 weeks, and at end of the period of incubation the recovery area has increased by a factor of about eight times. Sporeling number 3 showed a recovery area of $3.4X10^{-4}$ mm^2 at 20 days, and the area had doubled over 6 weeks, and the area increased approximately 8 times after 8 weeks, and at end of the observation period the recovery area increased almost tenfold. Sporeling number 4 showed a recovery area of $2.1X10^{-4}$ mm^2 at 20 days. This area had doubled after 3 weeks, and the area increased 5 times after 6 weeks, and the area of recovery increased approximately 14 times after 8 weeks. At the end of the period of observation the recovery area had increased over twentyfold. Sporeling number 5 showed a recovery area of $0.69X10^{-4}$ mm^2 at 20 days, and the area of recovery had about doubled over 3 weeks, and had more than doubled after 6 weeks, and after 10 weeks the red area had increased by a factor of about 47 over the area at the start. Sporeling number 6 showed a recover area of $3.6X10^{-4}$ mm^2 at 20 days. This area had almost doubled over 4 weeks, and had increased fivefold after 7 weeks, and after 10 weeks showed a

Fig. 3.2.2.1. The 8 sporelings kept observation, shown at start of measurements. The recovery area in black; dead tissue in white (see Table 3.2.2.1). The recovery growth surface area shown for sporeling 7 can be compared with that of the others.

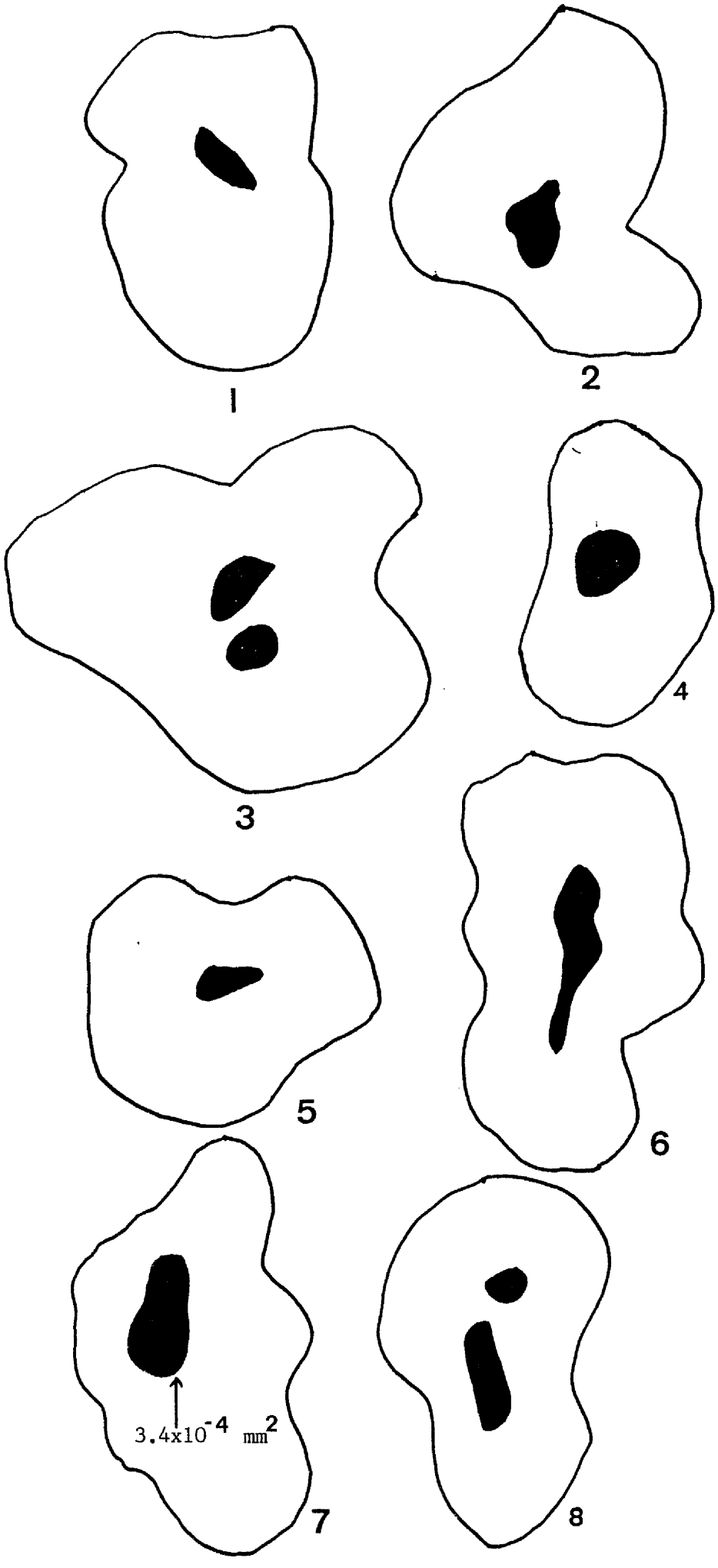

1
2
3
4
5
6
7
3.4x10-4 mm2
8

Table 3.2.2.1. Showing recovery growth of sporelings of *Mastocarpus* . 8 sporelings measured. Temperature 10 $^{o}C \pm 2\ ^{o}C$, and light intensity 17.5 µmol m^{-2} s^{-1}.

Time in days	Surface area of recovery (i.e red) region of sporelings (mm^2)							
	1	2	3	4	5	6	7	8
20	$1.1X10^{-4}$	$1.7X10^{-4}$	$3.4X10^{-4}$	$2.1X10^{-4}$	$0.69X10^{-4}$	$3.6X10^{-4}$	$3.4X10^{-4}$	$2.1X10^{-4}$
27	$1.8X10^{-4}$	$1.9X10^{-4}$	$3.8X10^{-4}$	$2.8X10^{-4}$	$0.83X10^{-4}$	$4X10^{-4}$	$3.9X10^{-4}$	$3.4X10^{-4}$
34	$2.1X10^{-4}$	$2.2X10^{-4}$	$4.1X10^{-4}$	$3X10^{-4}$	$1.1X10^{-4}$	$4.8X10^{-4}$	$4.1X10^{-4}$	$3.6X10^{-4}$
41	$2.2X10^{-4}$	$3.9X10^{-4}$	$4.4X10^{-4}$	$4.8X10^{-4}$	$1.7X10^{-4}$	$6.3X10^{-4}$	$4.5X10^{-4}$	$3.9X10^{-4}$
48	$2.5X10^{-4}$	$3.9X10^{-4}$	$4.7X10^{-4}$	$5.8X10^{-4}$	$2.3X10^{-4}$	$6.9X10^{-4}$	$4.8X10^{-4}$	$3.9X10^{-4}$
55	$2.8X10^{-4}$	$4.4X10^{-4}$	$5X10^{-4}$	$6.1X10^{-4}$	$2.3X10^{-4}$	$8X10^{-4}$	$5.1X10^{-4}$	$4.1X10^{-4}$
62	$4.1X10^{-4}$	$5.8X10^{-4}$	$7.3X10^{-4}$	$10X10^{-4}$	$3.9X10^{-4}$	$12X10^{-4}$	$14X10^{-4}$	$4.3X10^{-4}$
69	$4.4X10^{-4}$	$7X10^{-4}$	$8.3X10^{-4}$	$12X10^{-4}$	$5.5X10^{-4}$	$18X10^{-4}$	$19X10^{-4}$	$7X10^{-4}$
76	$5.8X10^{-4}$	$8.3X10^{-4}$	$24X10^{-4}$	$28X10^{-4}$	$24X10^{-4}$	$29X10^{-4}$	$30X10^{-4}$	$7.5X10^{-4}$
83	$6.3X10^{-4}$	$9.8X10^{-4}$	$27X10^{-4}$	$34X10^{-4}$	$28X10^{-4}$	$30X10^{-4}$	$37X10^{-4}$	$8.9X10^{-4}$
90	$8.3X10^{-4}$	$13X10^{-4}$	$30X10^{-4}$	$44X10^{-4}$	$33X10^{-4}$	$38X10^{-4}$	$44X10^{-4}$	$12X10^{-4}$

Fig. 3.2.2.2. Recovery growth as described in text (expressed as increase in surface area) of sporelings of *Mastocarpus* over 10 weeks (see Table 3.2.2.1).

- Sporeling number 1.
- Sporeling number 2.
- Sporeling number 3.
- Sporeling number 4.
- Sporeling number 5.
- Sporeling number 6.
- Sporeling number 7.
- Sporeling number 8.

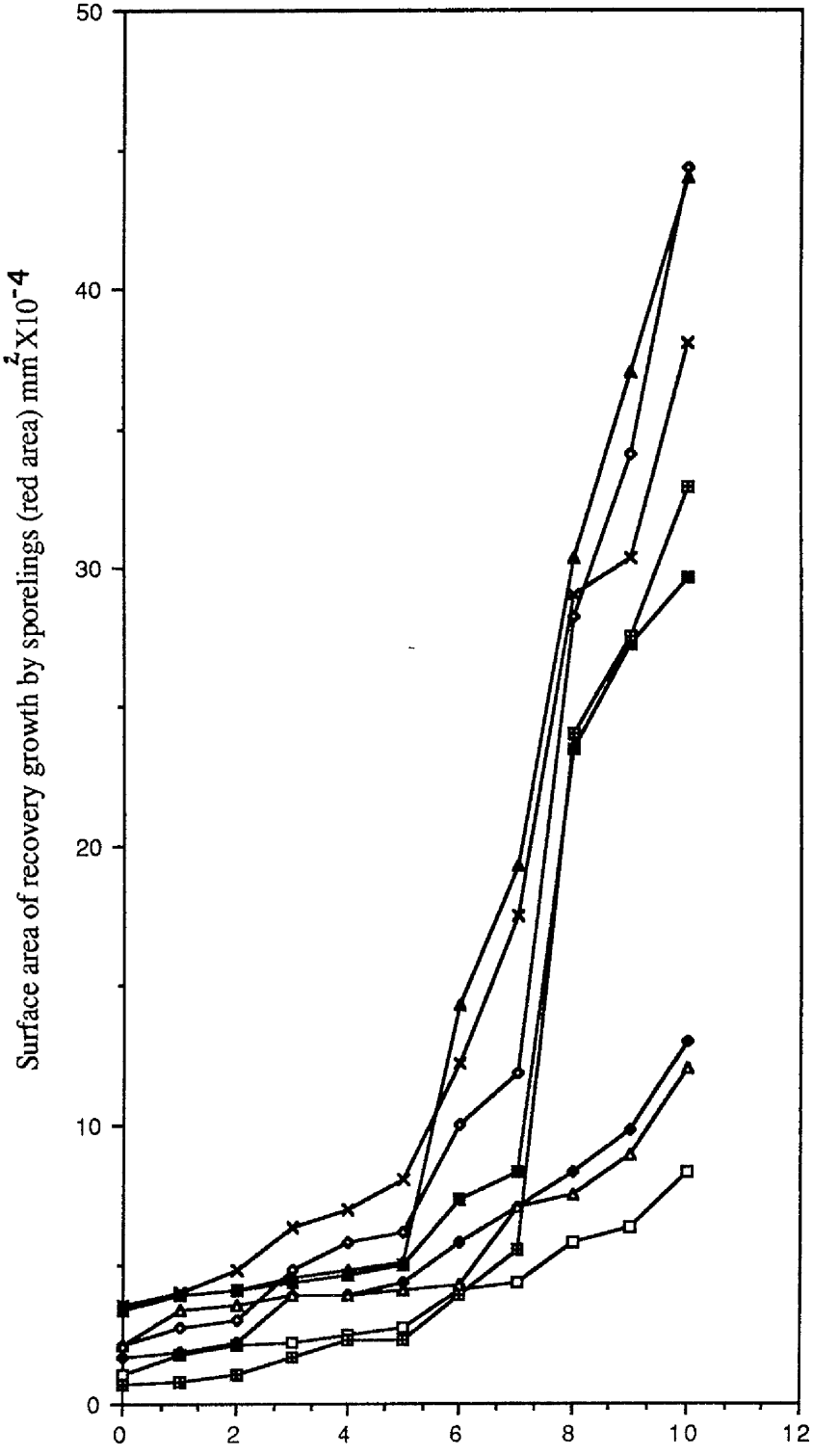

50
40
30
20
10
0
0
2
4
6
8
10
12
Surface area of recovery growth by sporelings (red area) mm^{2}X10^{-4}
Time in weeks

Fig. 3.2.2.3. Sporeling number 1 (Table 3.2.2.1) recovery with discoid shape of red area over 8 weeks period , the numbers refer to time in weeks .

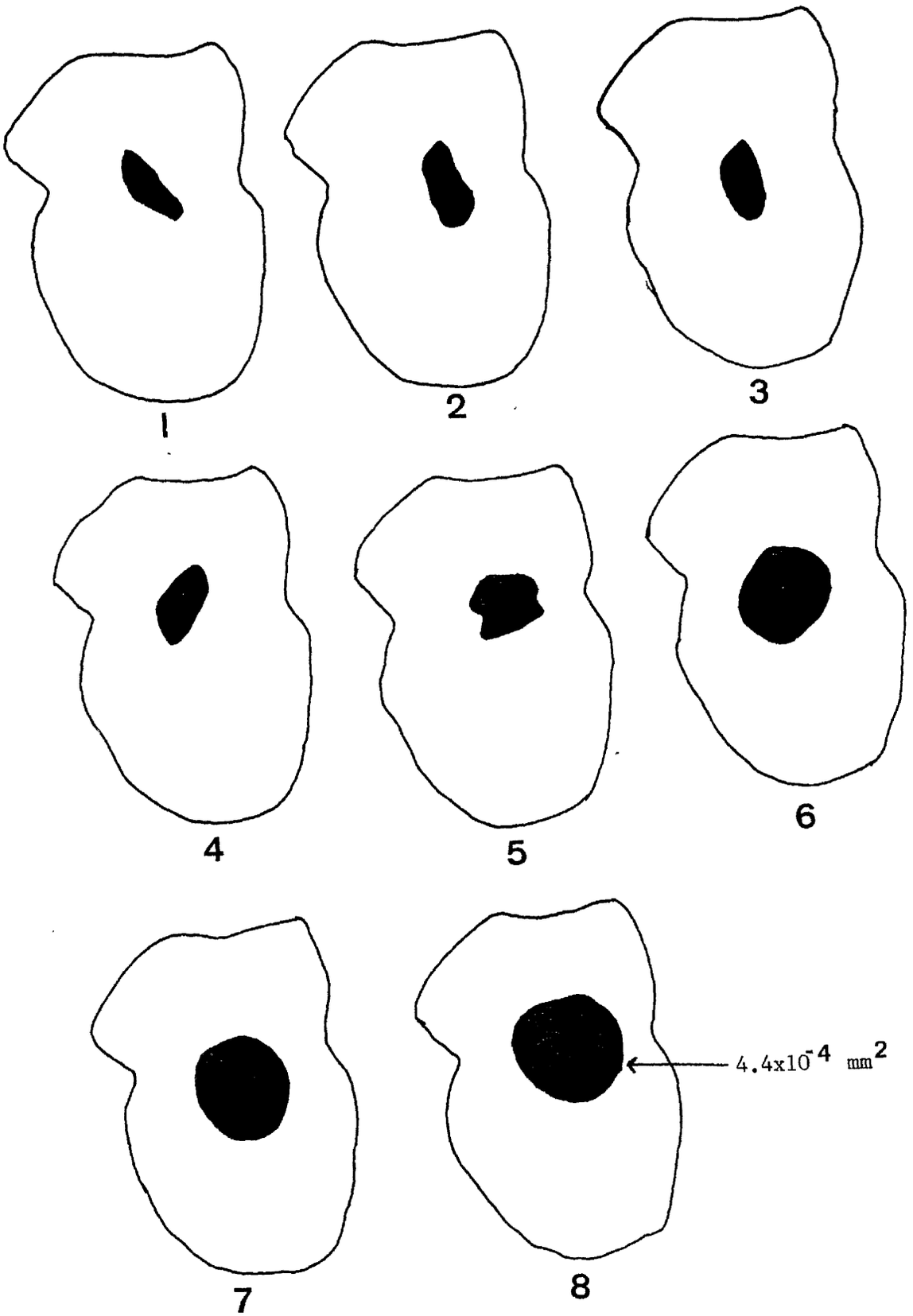

1
2
3
4
5
6
7
8
4.4x10⁻⁴ mm²

Fig. 3.2.2.4. Sporeling number 2 (Table 3.2.2.1) recovery with discoid shape of red area over 8 week period.

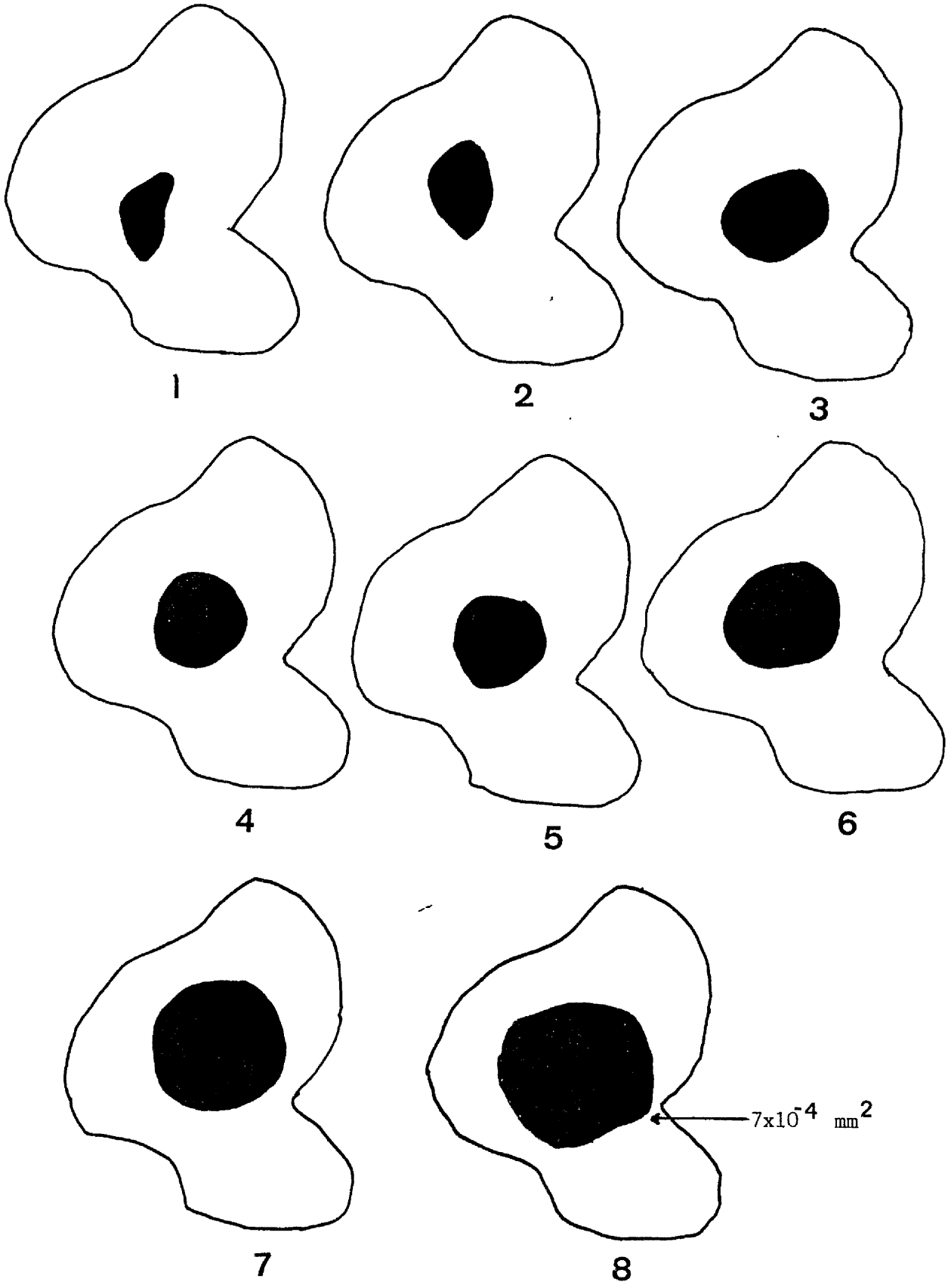
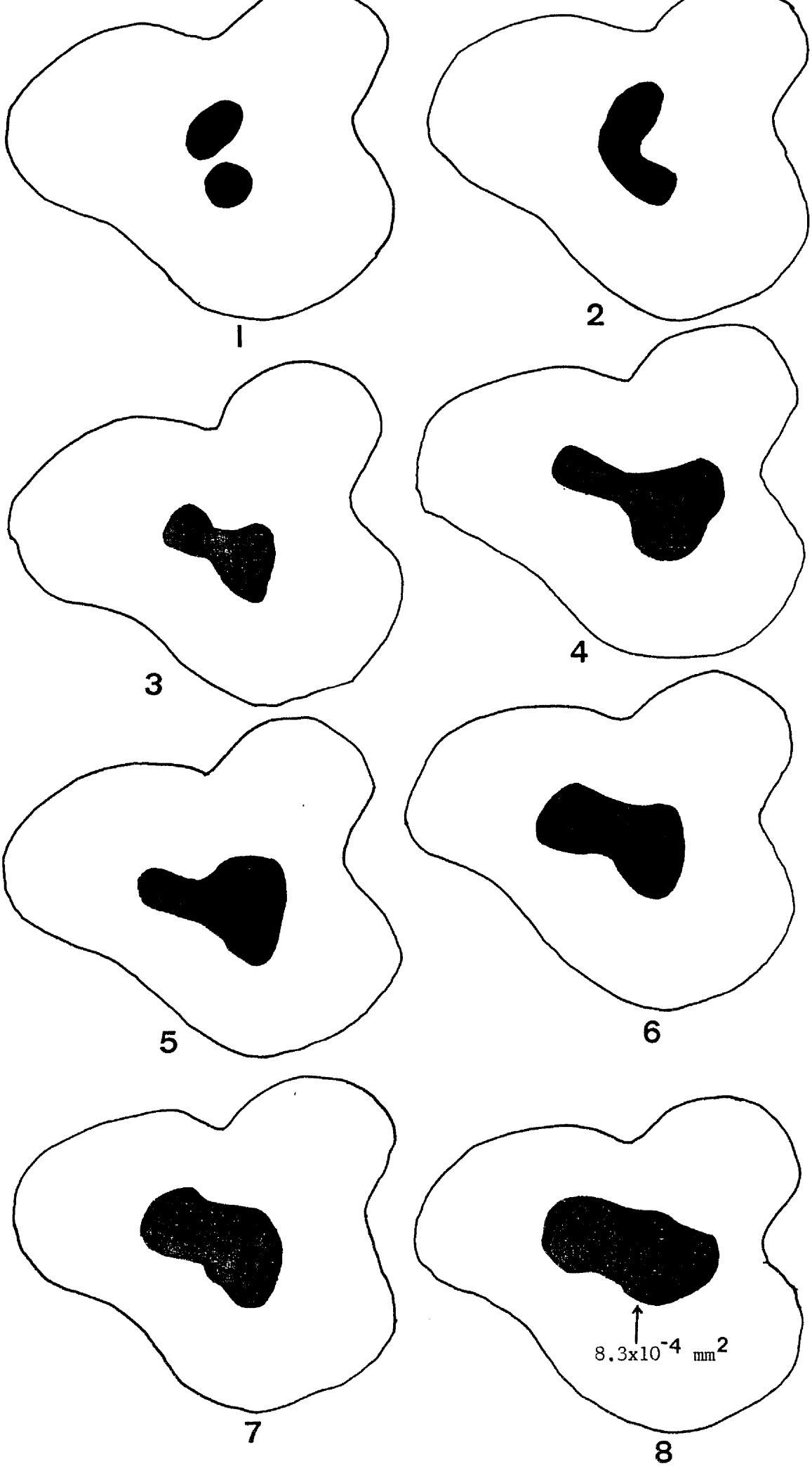

1
2
3
4
5
6
7
8
$7x10^{-4}$ mm^2

Fig. 3.2.2.5. Sporeling number 3 (Table 3.2.2.1) recovery with irregular shape of red area over 8 week period.


1
2
3
4
5
6
7
8
8.3×10^{-4} mm^2

tenfold increase in size. Sporeling number 7 showed a recovery region of 3.4 $X10^{-4}$ mm^2 at 20 days, and showed a slow recovery up to 5 weeks, but thereafter the change was dramatic, showing almost a tenfold increase in area after 8 weeks, and after 10 weeks over 12 times. Sporeling number 8 showed a recovery area of $2.1X10^{-4}$ mm^2. This area doubled over 5 weeks, and doubled again after 9 weeks. At end of the period of observation the recovery area had increased by almost sixfold. Whilst the rates of recovery growth were variable, in all cases the most noticeable changes took place between 5-7 weeks. After 10 weeks, however, recovery growth ceased, and the recovery areas on all of the sporelings examined were losing colour. After 11-12 weeks all areas were colourless and dead.

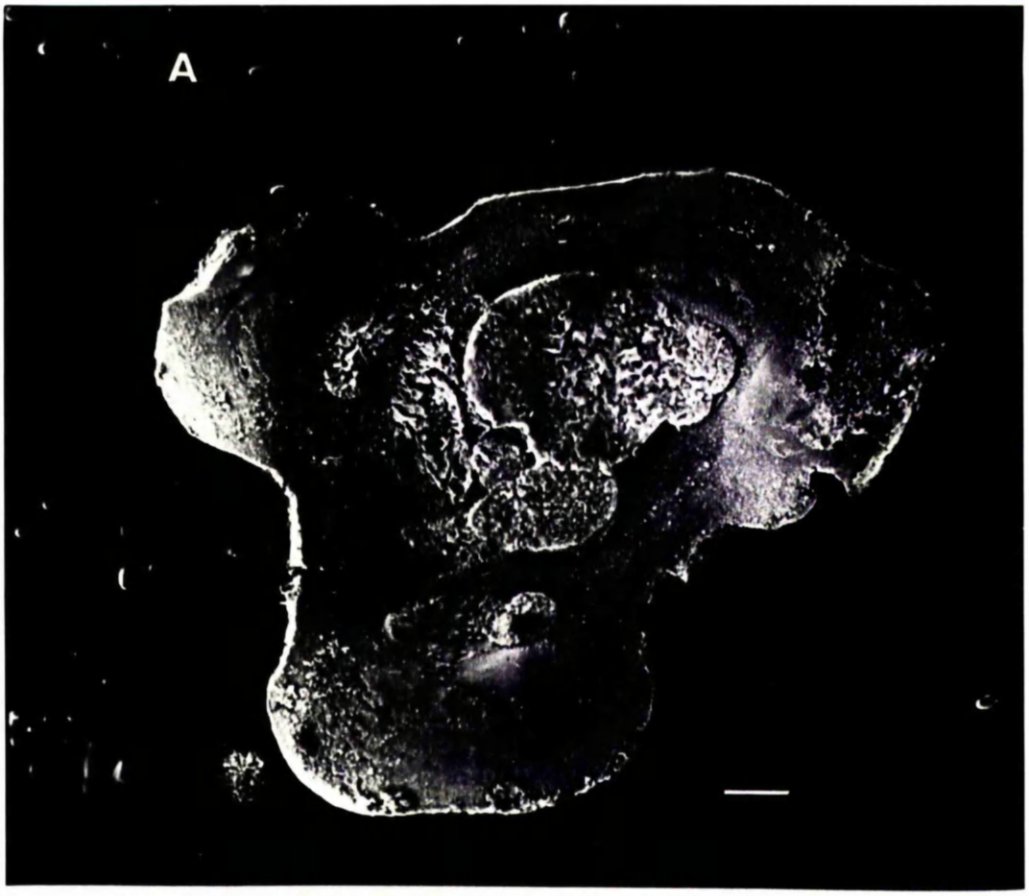
The areas of recovery tended to remain the middle region of the otherwise dead sporelings, in many cases assuming a discoid shape, irrespective of their appearances at the start (Figs. 3.2.2.3-4) , although this was not always so (Fig. 3.2.2.5). All recovery areas lay on the surface of the dead sporeling material (Fig. 3.2.2.6). The surviving cells lay deep in the tissue (Fig. 3.2.2.7). If there are sufficient of these cells, usually near the base of the sporelings (Fig. 3.2.2.8), these cells grow out through the dead tissue, and come lie on the surface. The probable growth of these surviving cells is shown in (Fig. 3.2.2.9).

3.3. Discussion

Young sporelings of *Mastocarpus* (39 days) of different shapes after removal of tissue and incubated for 12 days showed regenerative growth from the cut surface as well as continuing growth from the remaining marginal meristem. Irrespective of the shape of the residual mass, regrowth was a rapid process. Young sporelings (69 days) showed more rapid regenerative growth than with old sporelings (129 days). Any part of a cut surface of any shape can form new meristematic tissue. Complete removal of all the marginal meristimatic tissue is followed by formation of entirely new meristem layers.

With attached sporelings the tendency is always to return to discoid form irrespective of the shapes to which they are cut. With detached sporelings there is no

Fig. 3.2.2.6. S.E.M. photomicrographs showing the recovery tissues lying on the surface of the dead sporelings. Scale bar= 100 μm.


A

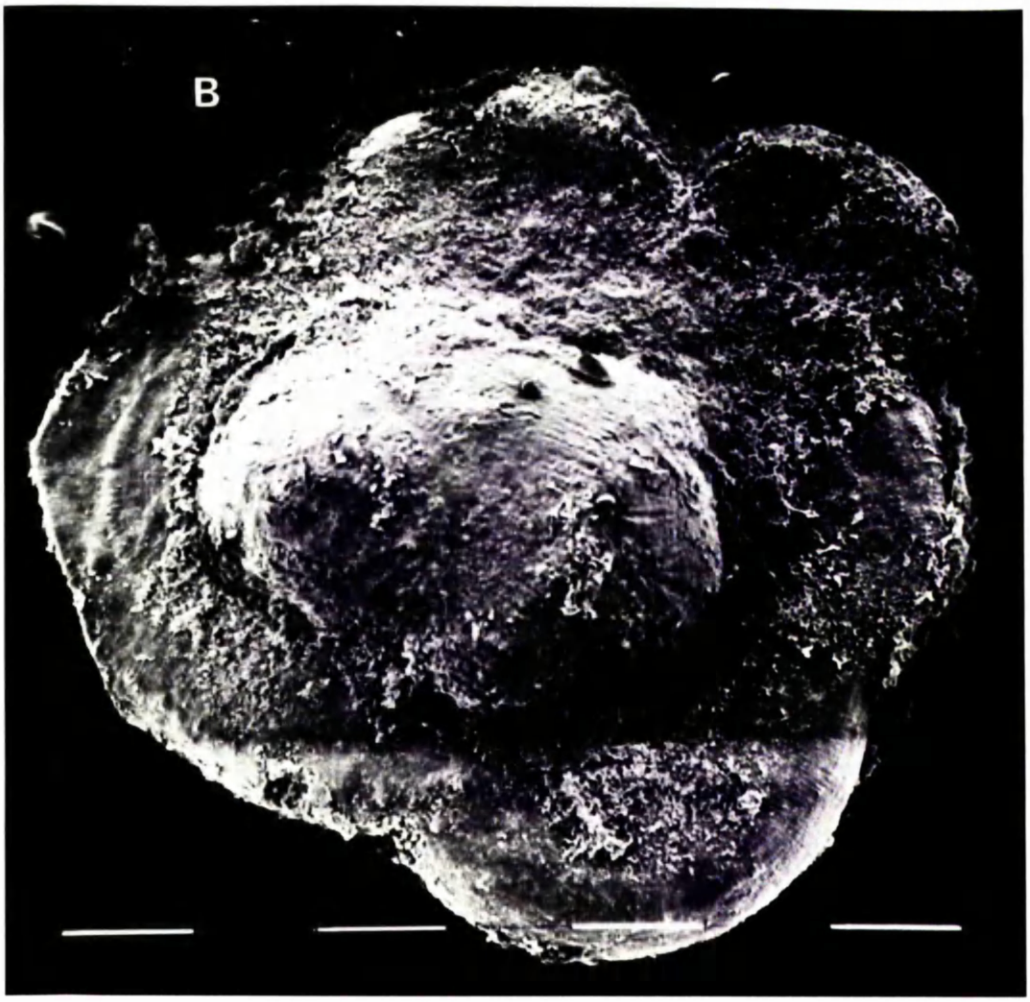

B

Fig. 3.2.2.7. T.E.M. photomicrographs of isolated living cells in dead tissue. Magnification = X38,500

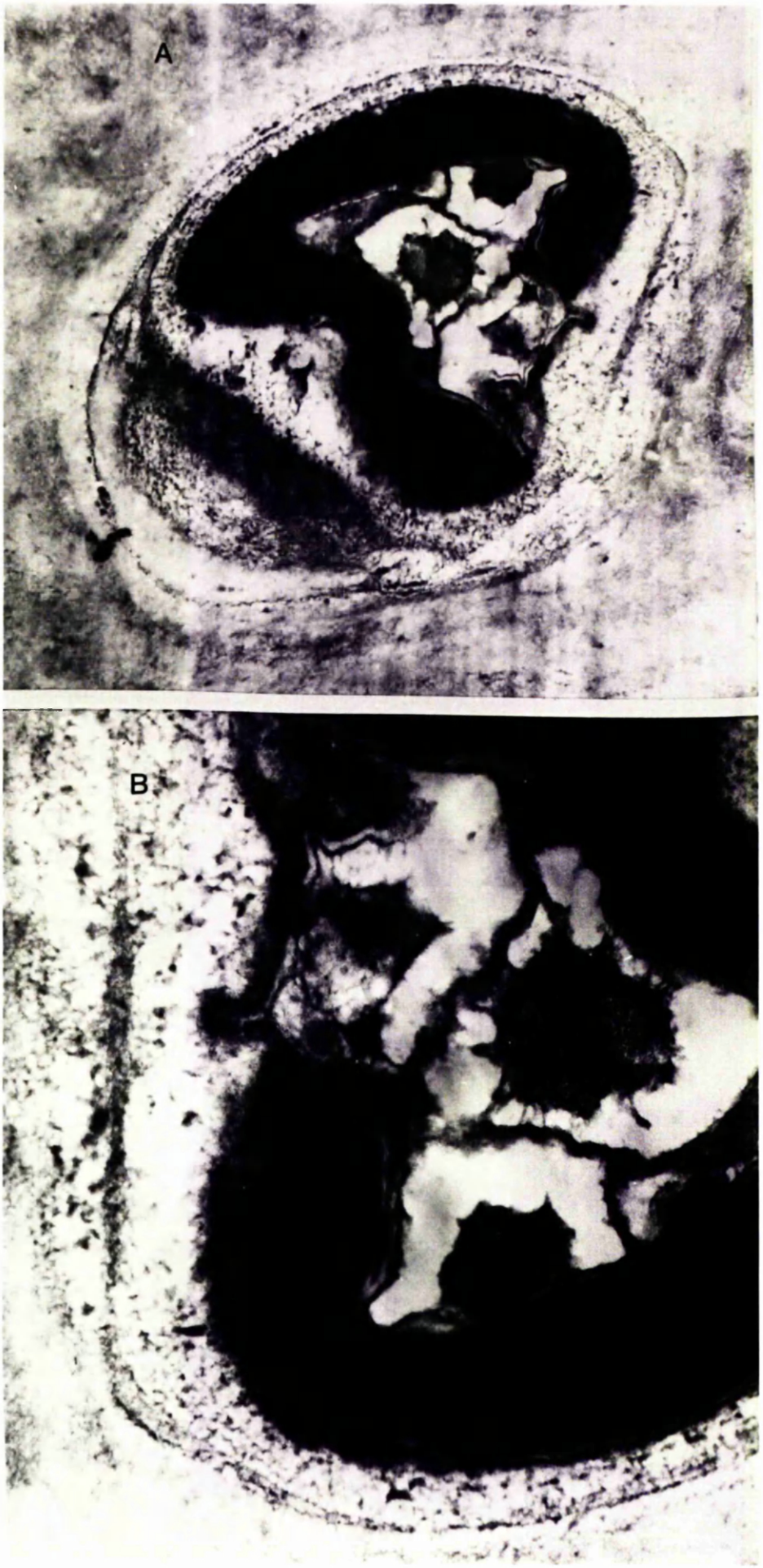
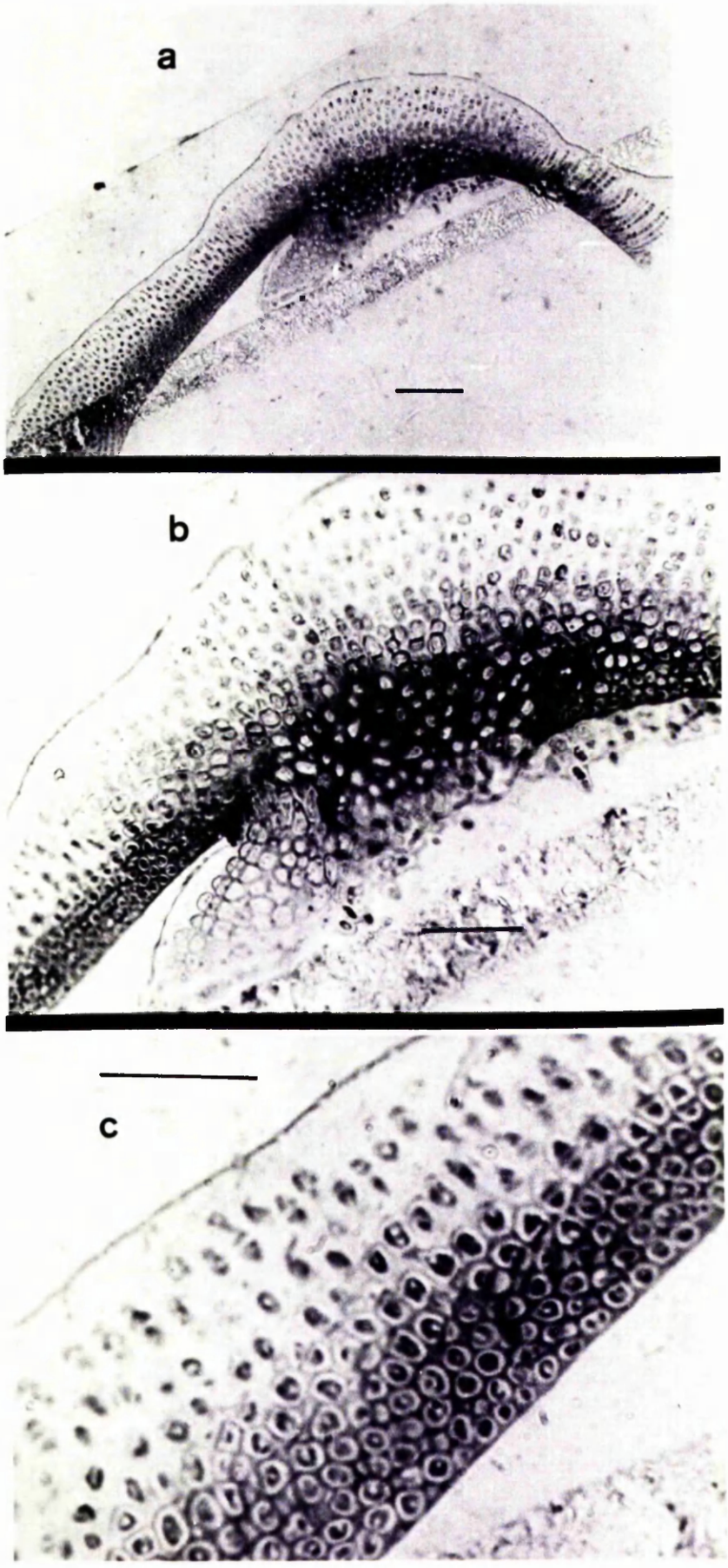

A
B

Fig. 3.2.2.8. Light microscope photomicrographs showing the living cells (dark colour) near the base of the sporelings and dead tissue (light colour).

a, Low magnification of photomicrograph showing living cells near the basal region. Scale bar= 9 μm.

b, Higher magnification of same photomicrograph showing living cells near the basal region. Scale bar= 6 μm.

c, Very high magnification of same photomicrograph showing basal living cells in (dark colour). Scale bar= 6 μm.


a
b
c

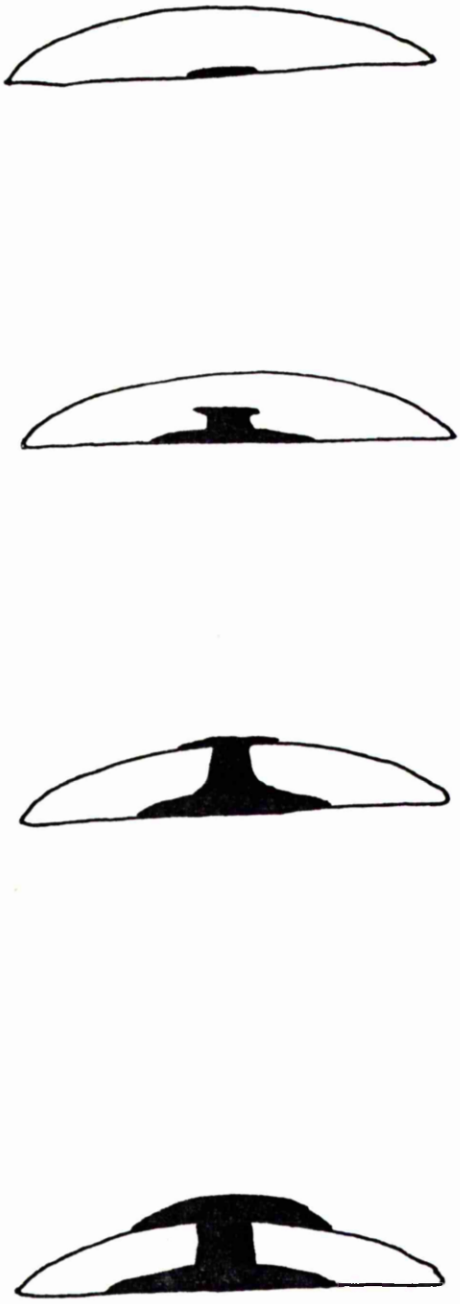


Fig. 3.2.2.9. Diagramatic representation of likely pathway of recovery growth from basal surviving cells (shown in black) onto the surface.

reformation of the discoid shape, and sporelings of irregular forms developed and their shape after further growth often depends on the shape left after tissue removal. Chen and McLachlan (1972) observed that the regeneration of segments of *Chondrus crispus* may occur from any portion of the basal disc, and after 4-5 weeks of incubation the segments increased considerably through marginal growth and became reattached to the substrata in the region of new growth. After this growth proceeded as of plants had not been detached. The normal even centrifugal growth from a marginal meristem ensures the discoid sporeling form, at least in the early stages. This shape combines the advantage of attachment over the whole under surface, and room for the spacing of erect branches at a later stage. As shown above, any growth control mechanism seems to be dependent on attachment, since sporelings once detached and free floating with tissue removed assume irregular growth forms and fail to return to the discoid shape.

Up to 3/4 of a sporeling can be removed and still in time the discoid form or a shape near it is obtained. In such cases much of the energy in the sporeling must be directed at regenerative growth from the cut (or damaged) surface, since the residual marginal meristems cannot entirely compensate for the lost tissue.

Of those sporelings which showed 'recovery' cell growth, in all cases the new tissue came to lie on the surface of the dead sporeling, and usually in the middle region. Subsequent growth of these 'recovery areas' was by spreading over the dead tissue, and for up to 10 weeks all those under examination showed varying degrees of cell multiplication of this new tissue, after following a centrifugal pattern of growth. The quantities of new tissue formed were variable and seemed not to be dependent on the initial size of the recovered cell areas. In some cases there was a significant increase in surface area in the 5-7 week intervals, and the rate of new cell formation increased dramatically.

With the apparent success of 'recovery' cell growth over the proceding weeks, the death of all new tissue in weeks 11-12 was unexpected. The possible explanation may lie in the decomposition products of the underlying dead cells, and /or bacteria and fungi, may have been responsible. In the examples under observation there may well have been too much dead tissue for the recovery growth to be successful.

4. Interactions of *Mastocarpus* sporelings with juveniles stages of other marine algae

4.1. Introduction

A number of studies on spore settlement and attachment have been recently reported. Ogata (1953) found better growth of spores of coralline red algae which were attached more securely on roughened glass than on smooth slides. He showed that more spores of coralline red algae became attached on surfaces with microscopic roughness (unevenness of ca 500-750 μm) than to surfaces smooth or slightly roughened (when unevenness was less than the spore diameter). He also observed that with undulated glass surfaces larger numbers of spores were found in the sunken areas. Nienhuis (1969) studied the significance of the substratum for intertidal algal growth on artificial rocky shores in the Netherlands. He found that the significance of different kinds of surface texture increased from the eulittoral to the supralittoral area. The soft and rough substrata showed a more complete zonation pattern than hard and smooth surfaces. Harlin and Lindbergh (1977) cemented acrylic discs to the surfaces of the shore quadrants; 25 plates were bolted directly to basement rock in the low intertidal area. *Chondrus crispus*, *Ulva lactuca* and *Polysiphonia harveyi* grew on large surfaces (1-2 mm diameter); whilst *Corallina officinalis* L. grew best on the small sized particles (0.5-1.0 mm diameter). Norton and Fetter (1981) studied the settlement of *Sargassum muticum* (Yendo) Fensholt propagules in still water and flowing water. They found in stationary water that the germlings sink at velocities from 0.2-0.9 mm s^{-1}. Sinking velocity increased with germling size until the development of protruding rhizoids increased the drag and slowed down the rate of descent. Smooth substrata were the least favourable for propagule settlement. Norton (1983) studied the attachment capabilities of *Sargassum muticum* germlings in the laboratory on smooth and rough substrata and in moving water. Attachment tenacity was measured by the germlings, ability to withstand dislodgement by water flow. A film of bacteria and micro-algae on the surface of the substratum was not a requirement for germling adhesion, but its presence enhanced the tenacity of adhesion. Moss (1974) studied the

attachment of the young embryos of *Pelvetia canaliculata* to a substratum using scanning electron microscopy. The mesochiton of the oogonia persists for a long time and has an adhesive role, anchoring the pairs of zygotes to the substratum until attaching rhizoids develop. Zygotes of *Halidrys siliquosa* (L.) Lyngb. become attached by a rapidly secreted wall with associated adhesive mucilage production. Rhizoid formation occured after a lapse of several days (Hardy and Moss 1978). Quatrano (1972) showed that with zygotes of *Fucus* about 70% of the population lacked the ability to orientate rhizoid outgrowths when exposed to unilateral light for 14 hours. The rhizoids of *Bifurcaria bifurcata* Ross grew across the substratum and penetrated into holes or cavities (Hardy and Moss 1979a). Hardy and Moss (1979b), using scanning electron microscopy, showed that germination of species of *Fucus* on artificial substrata resulted in young plants whose morphology differed obviously from that encountered on more natural rocky substrata. The spores became attached in areas where rounded particles of the substratum protected them from wave action.

Many studies have been carried out on the effects of physical and chemical conditions on the growth of algal sporelings and germlings (Mathieson and Burns, 1971; McLachlan *et.al*, 1971; Chen and Mclachlan, 1972; Khfaji, 1978; Terry and Moss, 1981). The effects of irradiance , temperature and nutrients on the growth of germlings of species of Fucales were studied by (McLachlan, 1974, 1977; Stromgren, 1977; McLachlan and Bidwell, 1983). McLachlan and Graigie (1964, 1966) observed that the yellow extracellular phenolic substances yielded by *Fucus vesiculosus* were toxic to unicellular algae.

Nutrient transference from living supporting plants to their epiphytes was described by Cattaneo and Kalff (1979). Community interactions on marine rocky intertidal shores in terms of physical and biological factors were studied by Connell (1972). It is known that interactions take place between diatom species in culture (Sharp *et al* 1979).

The structure and development of rhizoids of *Polysiphonia lanosa* (L.) Tandy was studied by Rawlence and Taylor (1972) by the light and electron microscope. The rhizoids of *Polysiphonia lanosa* digested their way into the supporting host

Ascophylum nodosum (Rawlence and Taylor 1970). The ultrastructure of the *Polysiphonia lanosa* rhizoids was described by Rawlence (1972). Kazama and Fuller (1970) studied the fine structure of host-parasite interaction of *Porphyra perforata* infected with the marine fungus *Pythium marinum.* Their studies showed that the during penetration the fungus caused very litlle modification of the host cell boundary and the fungal hyphae also were not modified during the penetration process. Boney (1978) observed the penetration of a number of algal thalli by *Conchocelis* filaments which developed when α-spores of *Porphyra schizophylla* Hollenberg were germinated on red algae from low intertidal and sub-tidal habitats.

The growth of two red alga crusts, *Porphyrodiscus simulans* Batt. and *Rhodophysema elegans* (Crouan frat, et J. Ag) Dixon were inhibited by ectocrines on the sides adjacent to *Ralfsia spongicocarpa* Batt., a brown algae, in mixed cultures (Fletcher 1975). Khfaji and Boney (1979) described anti-diatom activity of sporeling discs of the red alga *Chondrus crispus* with formation of inhibition zones. Huang and Boney (1985) observed the interactions between *Gigartina stellata* (*Mastocarpus stellatus*) sporelings and various littoral diatoms at different temperatures. They found the survival of *Mastocarpus* was 100% at 15 ^{o}C and 10 ^{o}C, and that there were no diferences in sporeling diameters of *Mastocarpus* when cultured singly or mixed with the diatoms. However Huang and Boney (1984) observed that one diatom *Stauroneis constricta*, caused both physical damage to *Mastocarpus* sporelings, and that young sporelings of *Mastocarpus* sometimes died in presence of *Stauroneis* without suffering any physical damage.

Hence from a number of sources there is evidence of interactions between red algae and other organisms. Experimental studies with *Mastocarpus* sporelings so far carried out have been mainly concerned with life history sequences. In the present work the development of germlings of *Fucus* species with sporelings of *Mastocarpus stellatus* was examined. Extensive growths of *Mastocarpus* are to be found near *Fucus serratus* in the Clyde Sea area at lower shore levels. Experimental studies were also carried out with *Mastocarpus* sporelings and the green algae *Ulva lactuca* and *Enteromorpha intestinals* and the red alga *Porphyra umbilicalis.*

4.2. Materials and Methods

Fertile plants of *Mastocarpus stellatus* and *Fucus* species (*F. spiralis* and *F. serratus*) were collected from the littoral area of the Firth of Clyde. Spores of *Mastocarpus* were cultured on cover slips for 2-3 months in a growth chamber. Zygotes were obtained from fertile receptacles of *Fucus spiralis* and *Fucus serratus* as described in Materials and Methods (page 10) and were then settled on *M. stellatus* sporelings. The *F. spiralis* zygotes were allowed to grow on *Mastocarpus* to obtain different stages of germination

F. serratus germling measurements (lengths, widths, and length of rhizoids) were made on glass as control, and germlings grown in close proximity and at a distance to the *Mastocarpus* sporelings, and germlings grown on same slide but separate from *Mastocarpus*. The diameter of disc-like *Mastocarpus* sporelings were measured after growing at a distance and in close proximity to *Ulva*, *Enteromorpha*, and *Porphyra*. The growth of *Porphyra* when free and in close proximity to *Mastocarpus* sporelings was also measured.

4.3. Results

4.3.1. The attachment of eggs and germlings of *Fucus spiralis* to *Mastocarpus* sporelings.

The newly settled zygote of *Fucus spiralis* on *Mastocarpus* quickly produced mucilage which appeared as thread-like exudations (Fig4.3.1 a,b). 2 day old *Fucus* zygotes showed the early stage of primary rhizoid formation on *Mastocarpus* sporelings with more mucilage release (Fig 4. 3.1c). 3 day old zygotes became attached with thread-like mucilage exudations (Fig. 4.3.1d). Zygotes of *Fucus* of age 5 days had developed long primary rhizoids with terminal 'pads' of mucilage (Fig. 4.3.1e, f). The germlings of *Fucus* after 12 days on *Mastocarpus* sporelings showed short secondary attaching rhizoids with long primary rhizoids (Fig. 4.3.1.g). The larger rhizoids were almost enclosed within attaching mucilage (Fig. 4.3.1h, i). Germlings of *Fucus* after 15 days produced many short rhizoids (Fig. 4.3.1j).

Fig.4.3.1. Showing attachment of eggs and germlings of *Fucus spiralis* on *Mastocarpus* sporelings. (S.E.M.).

a,b zygote of *Fucus* on sporelings at age one day showing thread like exudations. Scale bar= 10 μm.

c, zygote of *Fucus* on sporelings at age 2 days showing primary rhizoid. Scale bar= 100 μm.

d, zygote of *Fucus* on sporelings at age 3 days showing exudations of mucilage. Scale bar= 10 μm.

e, zygote of *Fucus* on sporelings at age 5 days showing primary rhizoid. Scale bar= 10 μm.

f, diagrammatic representation of Fig. e.

g, germlings of *Fucus* on sporelings at age 12 days showing short and long secondary rhizoids. Scale bar=100 μm.

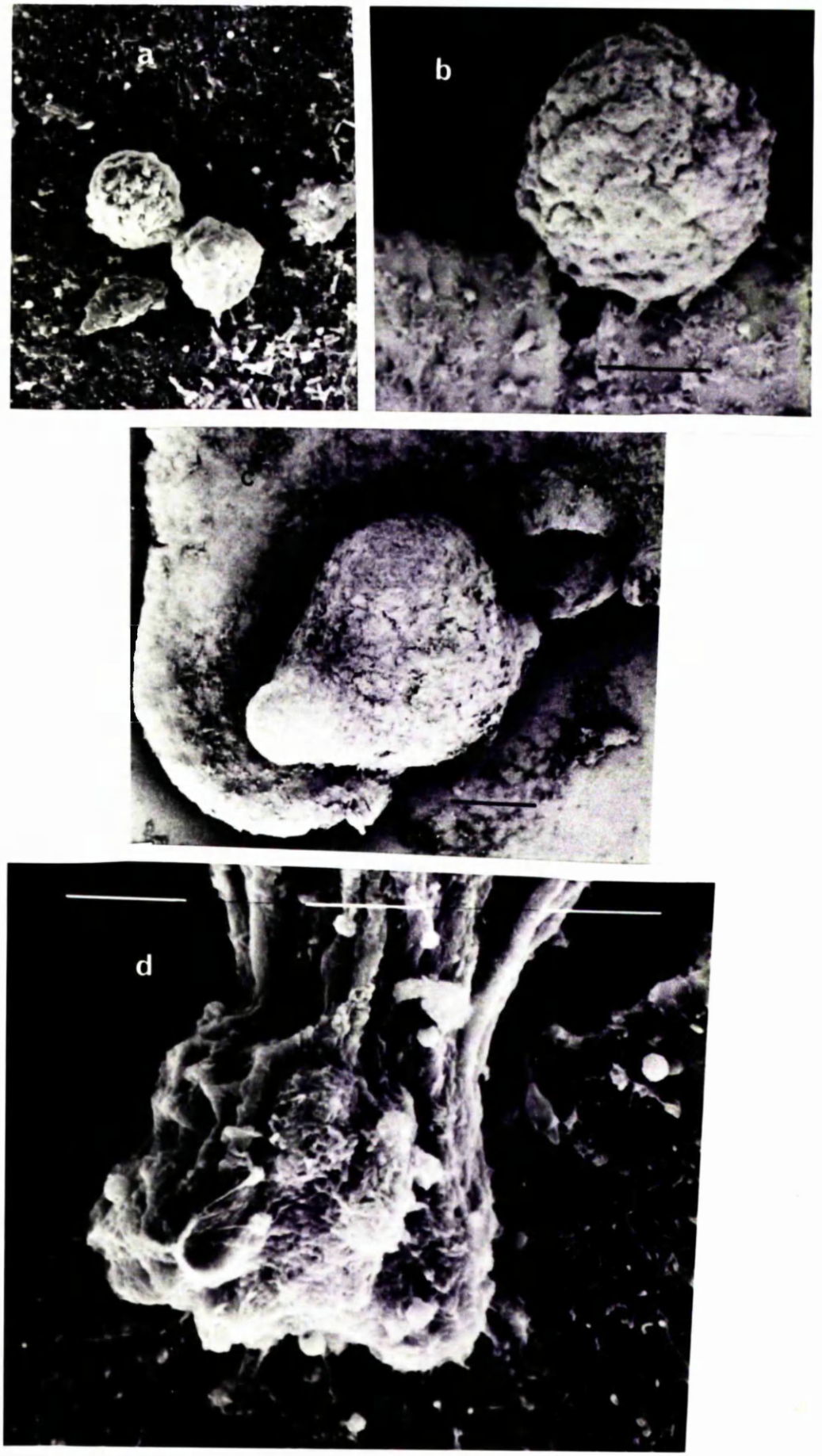
h, the rhizoids of *Fucus* germlings on sporelings at age 12 days showing large rhizoids attached with mucilage. Scale bar= 10μm.

i, diagrammatic representation of Fig. h.

j, germlings of *Fucus* on sporelings at age 15 days showing short rhizoids. Scale bars= 160 μm, and 10 μm.

k, germlings of *Fucus* on sporelings at age 20 days showing attachment with short rhizoids. Scale bar= 100 μm.

l, tip of a rhizoid of *Fucus* on sporelings at age 15 days showing some growth of bacteria. Scale bar= 10 μm.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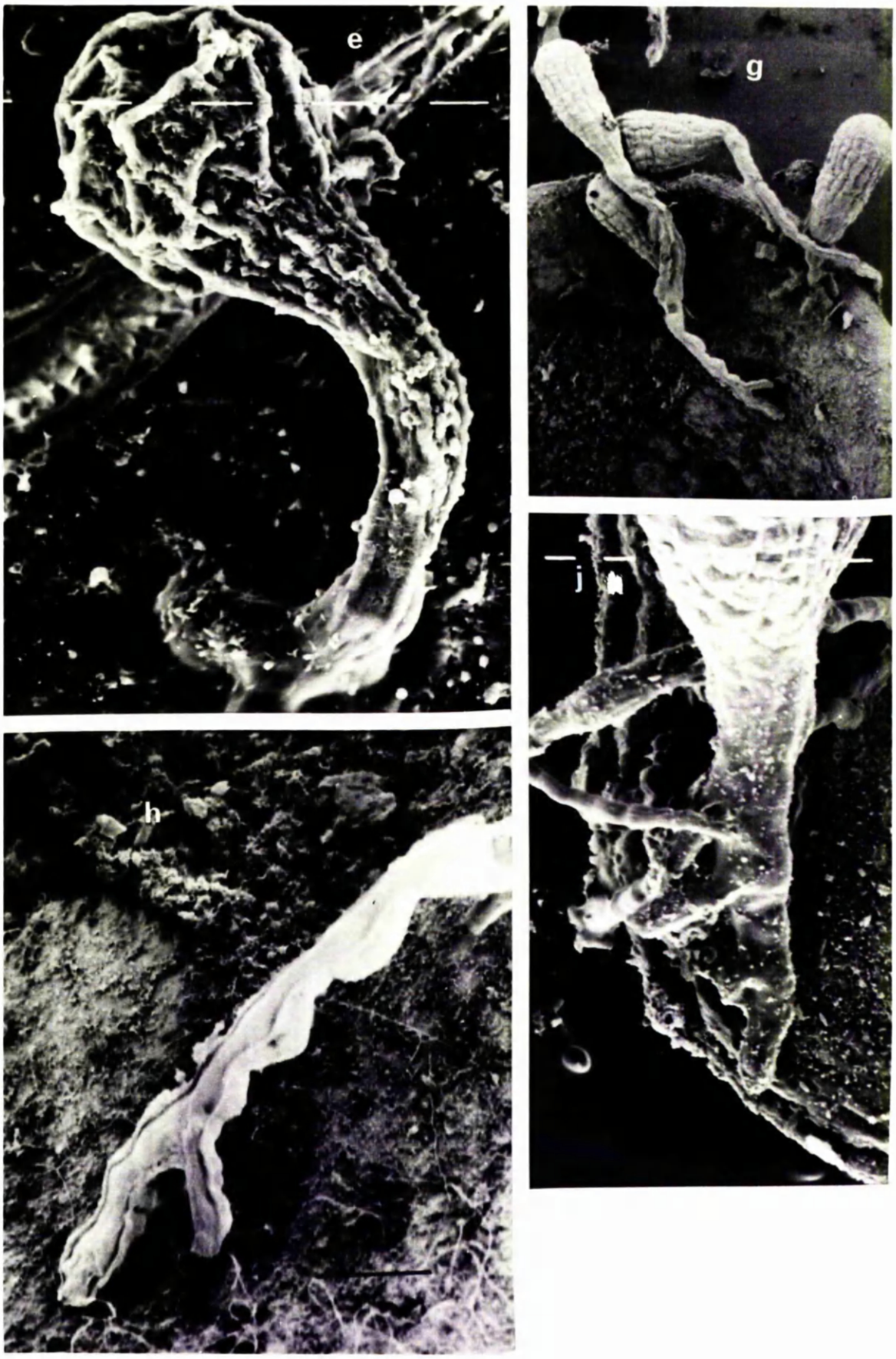
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Fig. 4.3.1.

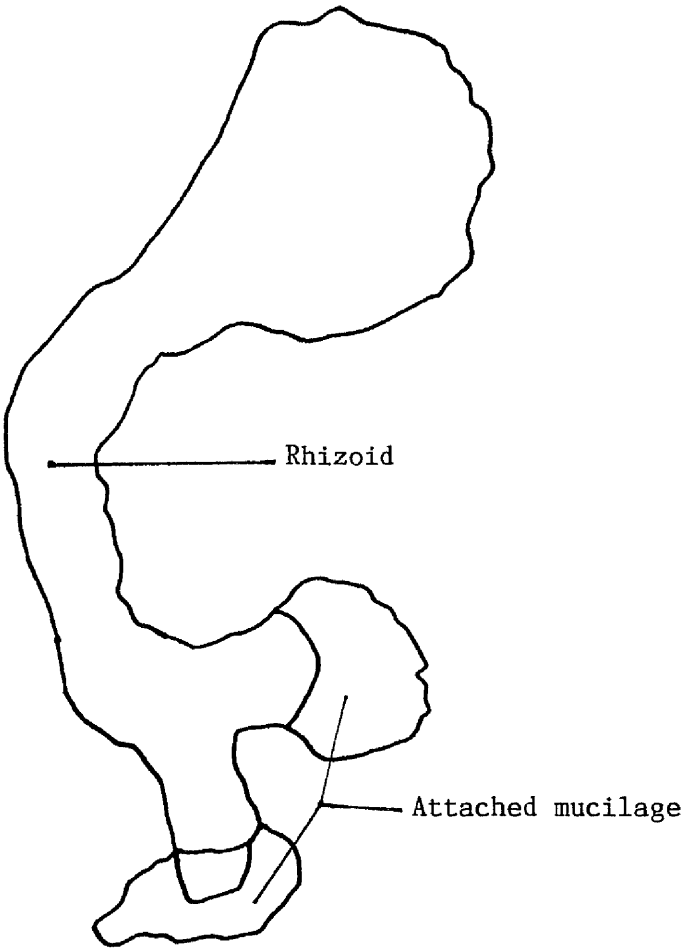
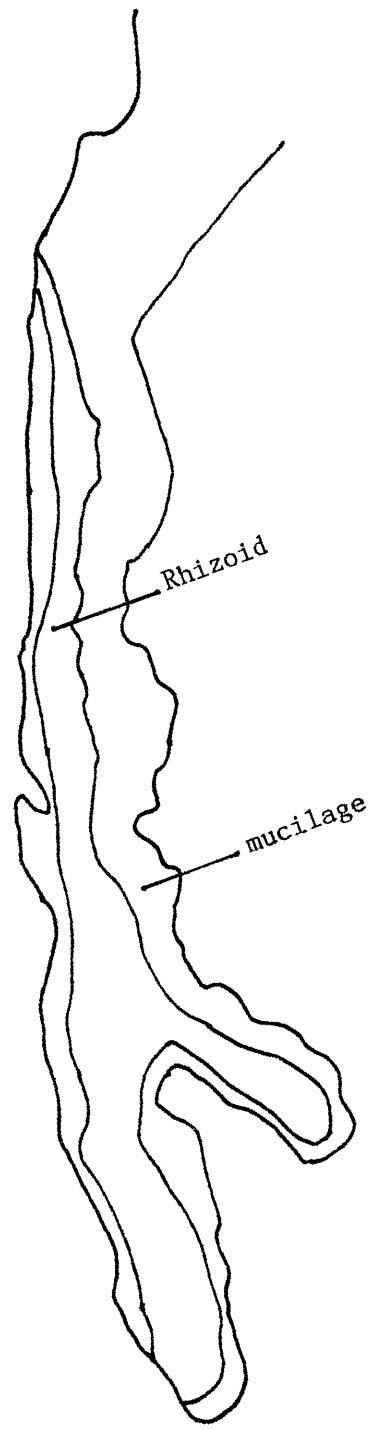
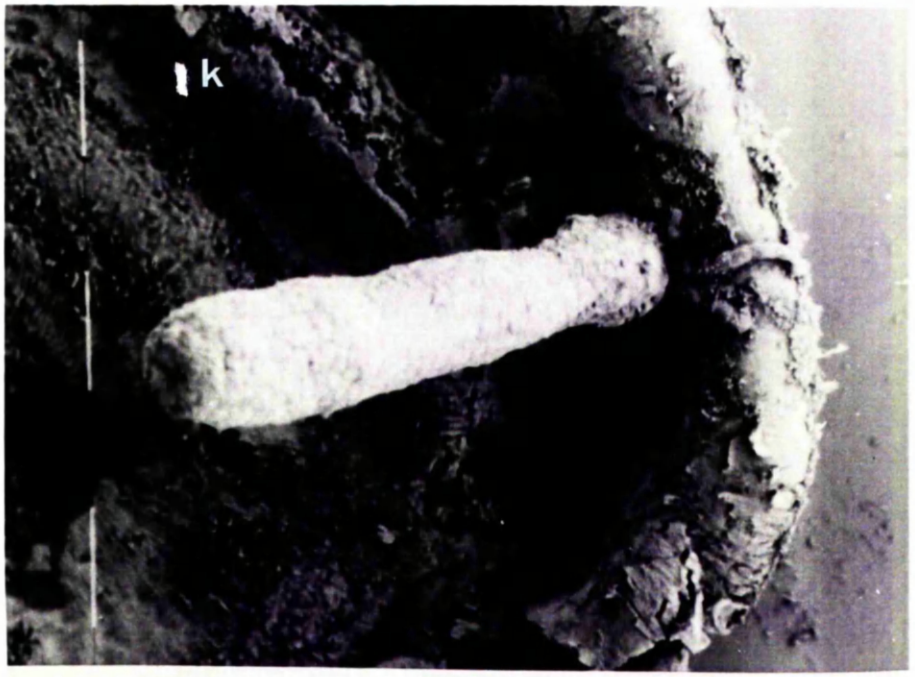
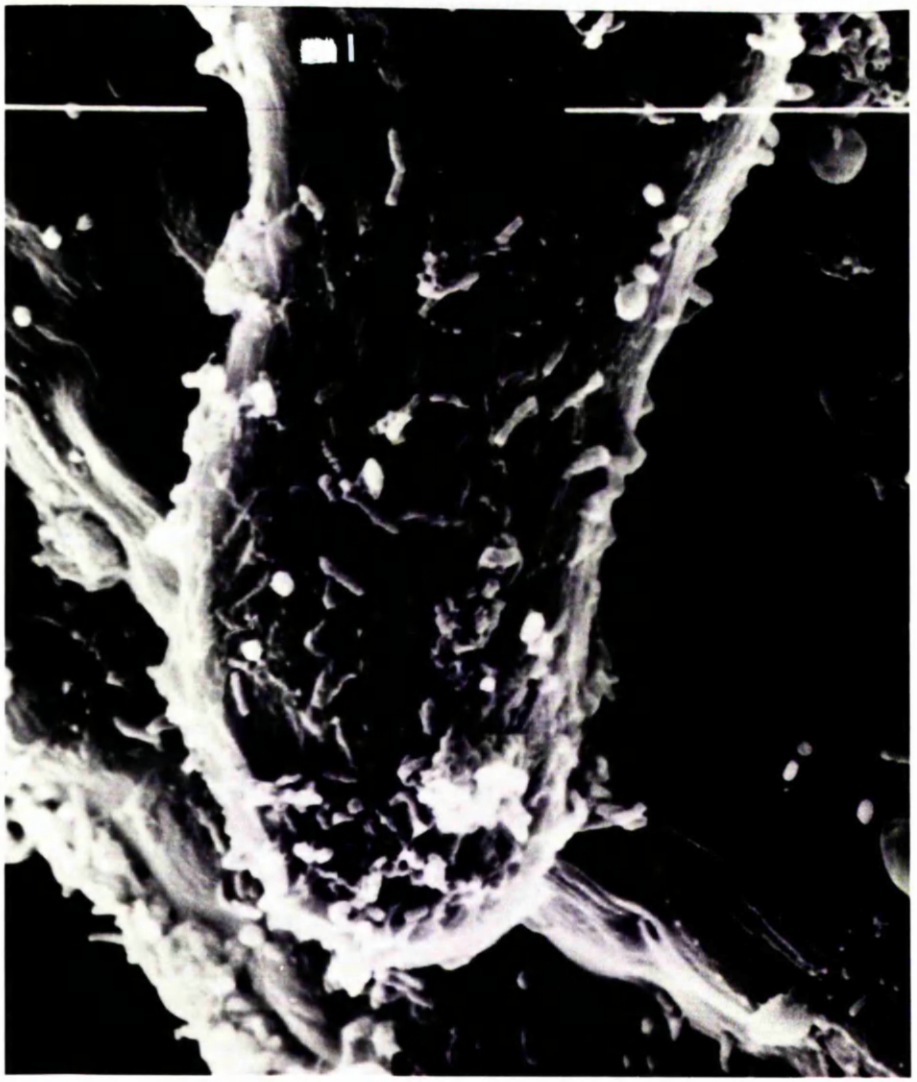


Fig. 4.3.1.




k


l

Fucus spiralis germlings were firmly attached with short rhizoids on *Mastocarpus* after 20 days (Fig. 4.3.1k). The abundance of mucilage at the rhizoid tip appears to encourage the growth of bacteria (Fig. 4.3.1l).

4.3.2. Growth of *Fucus* germlings attached to and free from *Mastocarpus* sporelings

Sporelings of *Mastocarpus* were cultured on cover slips, and after various time intervals between 10 -140 days from germination newly formed zygotes of *Fucus serratus* were added. The growth and development of *Fucus* germlings on *Mastocarpus* sporelings and on glass were measured, and generally the germlings on *Mastocarpus* were slower growing compared with those attached to glass. In a preliminary investigation it was found that the percentage inhibition of *Fucus* germlings on *Mastocarpus* varied between 25.1 - 83% (see Table 4.3.2.1). Whilst the results were variable they indicate that *Fucus* germlings growing attached to *Mastocarpus* could be significantly inhibited. In Fig. 4.3.2.1(a-f) the photomicrographs show the inhibition of germlings of *Fucus serratus* when grown on *M. stellatus*. The of rhizoids of *Fucus* germlings grown on glass were larger than those of germlings on sporelings, and similar differences in rhizoid length were observed at all ages of *Fucus* and *Mastocarpus* studied. Fig. 4.3.2.1(g-n) similarly illustrates the inhibition of growth of germlings of *Fucus* when grown on *Mastocarpus*. 20 day old germlings on *Mastocarpus* grew many short rhizoids and the inhibition of growth of the germling 'body' was 57%. The rhizoids of germlings on *Mastocarpus* could not be clearly seen. They probably grew underneath the sporelings, but the length of germlings on the sporelings was shorter than with germlings free on glass Fig. 4.3.2.1 (g, h). Rhizoids of 35 day old germlings on sporelings again were short, whilst germlings on glass developed longer rhizoids than those on sporelings, and the lengths of germlings on *Mastocarpus* were less than germlings attached on glass. The germlings on sporelings showed an 84% growth inhibition Fig. 4.3.2.1 (i, j). Germlings on old sporelings (131 days) formed many short rhizoids when compared with rhizoids of germlings attached to glass, but the

Table 4.3.2.1. Showing growth of *Fucus serratus* germlings on *Mastocarpus* sporelings and germlings attached to glass surface. Temperature 10 $^{o}C \pm 2\ ^{o}C$, light intensity 17.5 $\mu m/m^{-2}\ s^{-1}$. These data show the measurements for germlings shown in Figures 4.3.2.1 a-n.

Time of growth of *Fucus* germlings (days)	Age of *Mastocarpus* sporelings at start (days)	length of *Fucus* germlings growing on *Mastocarpus* (μm)	length of Fucus germlings growing attached to glass (μm)	% inhibition of growth of *Fucus* germlings o n *Mastocarpus*
15	120	218.5	409.5	46.6
15	140	263.9	409.5	35.5
17	125	255.5	474.5	46.1
20	90	328.5	766.5	57.1
35	75	365	2372.5	84.6
43	131	547.5	730.0	25.1
33	10	547.5	912.5	40

Fig. 4.3.2.1. Showing growth of *Fucus serratus* germlings on *Mastocarpus* sporelings at different ages, and germlings attached from glass surface (see Table 4.3.2).

a, 15 day old *Fucus* germling on 140 day old *Mastocarpus* sporelings. Scale bar= 55 µm.

b,15 day old *Fucus* germling attached to glass . Scale bar= 68 µm.

c,15 day old *Fucus* germling on 125 day old *Mastocarpus* sporelings. Scale bar= 66 µm.

d, 15 day old *Fucus* germling attached to glass. Scale bar= 59 µm.

e,17 day old *Fucus* germling on 125 day old *Mastocarpus* sporelings. Scale bar= 256 µm.

f, 17 day old *Fucus* germling attached to glass. Scale bar= 137 µm.

g, 20 day old *Fucus* germling on 90 day old *Mastocarpus* sporelings. Scale bar= 183 µm.

h, 20 day old *Fucus* germling attached to glass . Scale bar= 256 µm.

i, 35 day old *Fucus* germling on 75 day old *Mastocarpus* sporelings. Scale bar= 268 µm.

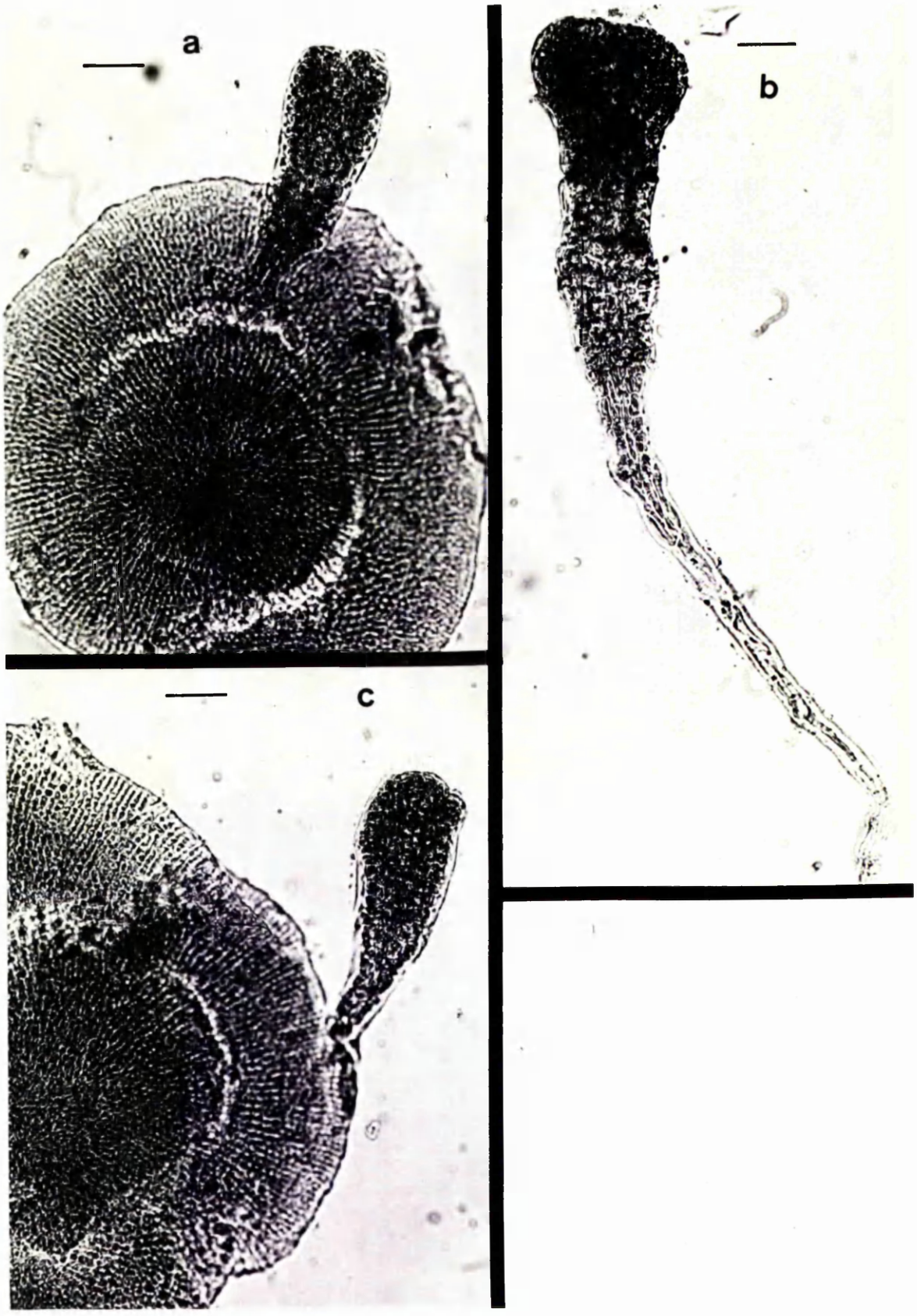
j, 35 day old *Fucus* germling attached to glass. Scale bar= 253 µm.

k, 43 day old *Fucus* germling on 131 day old *Mastocarpus* sporelings. Scale bar= 274 µm.

l, 43 day old *Fucus* germling attached to glass . Scale bar= 183 µm.

m, 33 day old *Fucus* germling with 10 day old *Mastocarpus* sporelings. Scale bar= 274 µm.

n, 33 day old *Fucus* germling attached to glass . Scale bar= 261µm.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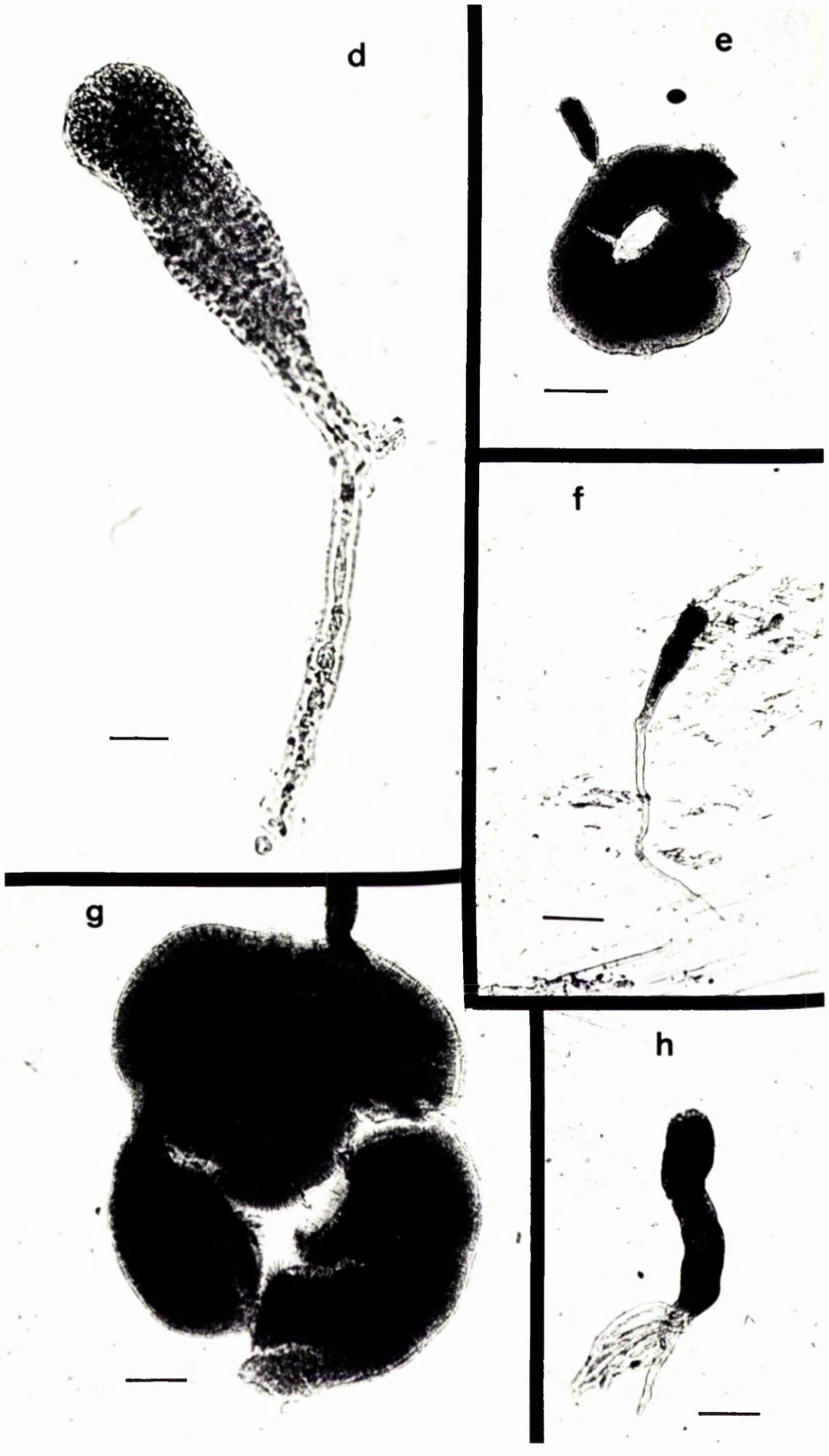
d
e
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g
h

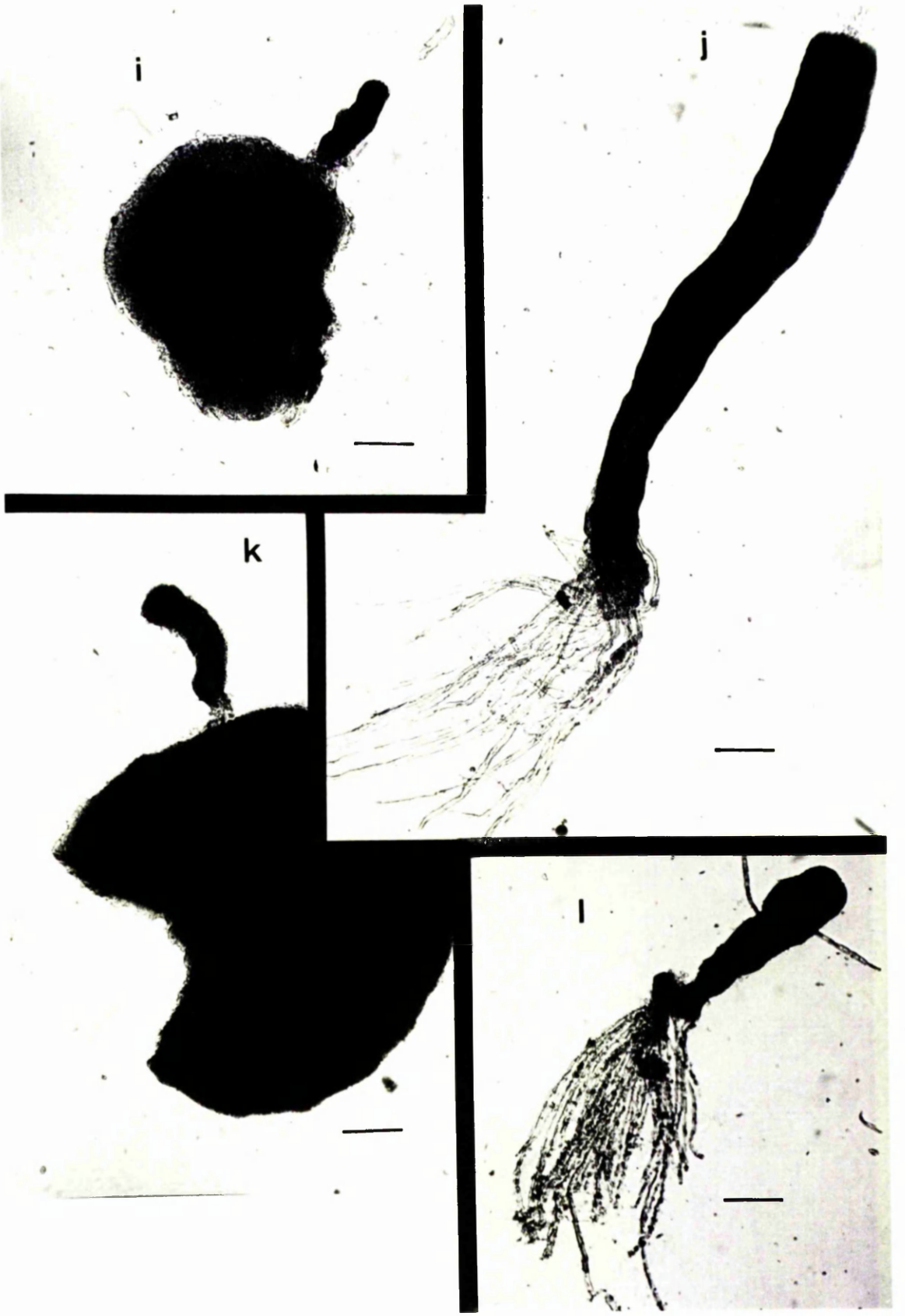
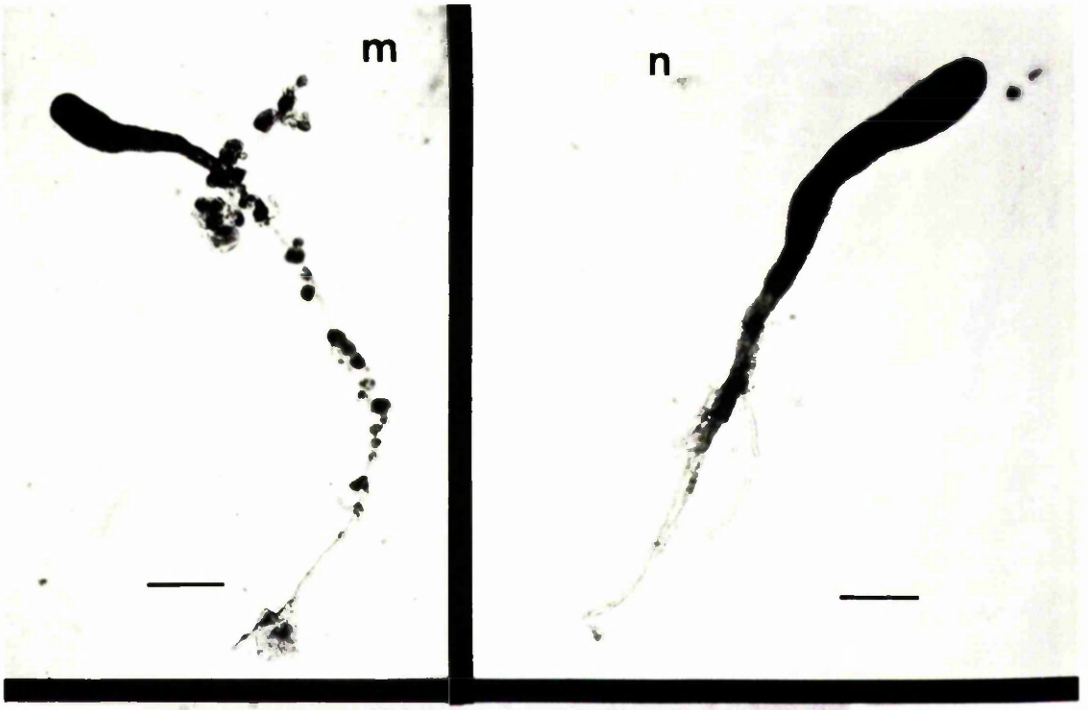
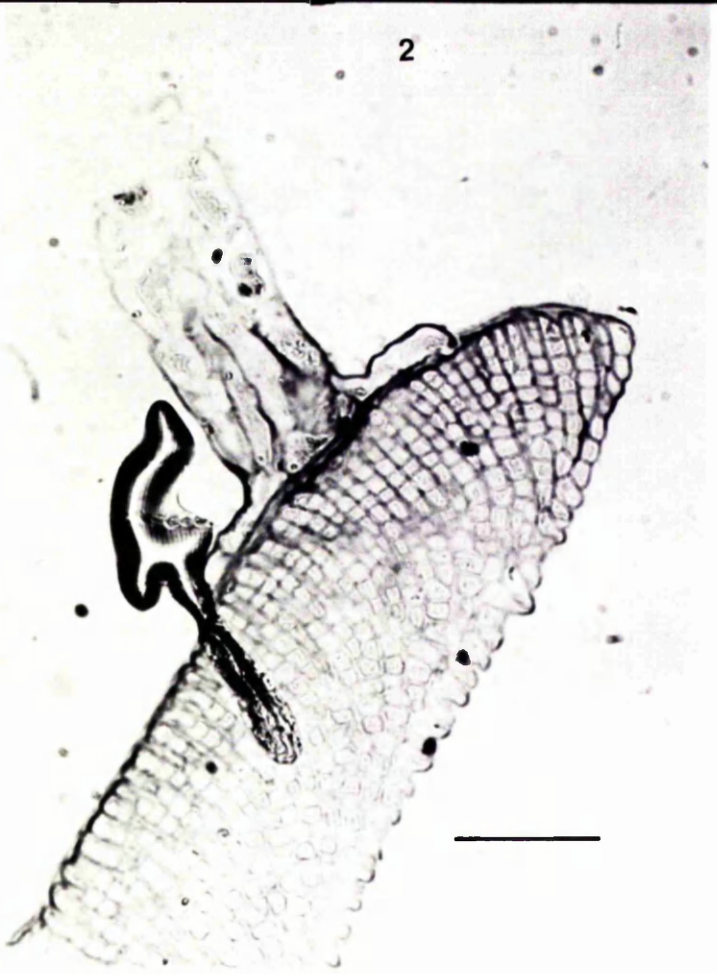

i
j
k
l

Fig. 4.3.2.2. Photomicrograph showing *Fucus serratus* germlings (one month old) attached to *Mastocarpus* sporelings (age of *Mastocarpus* at start 3 months). Scale bar= 48 μm.



2



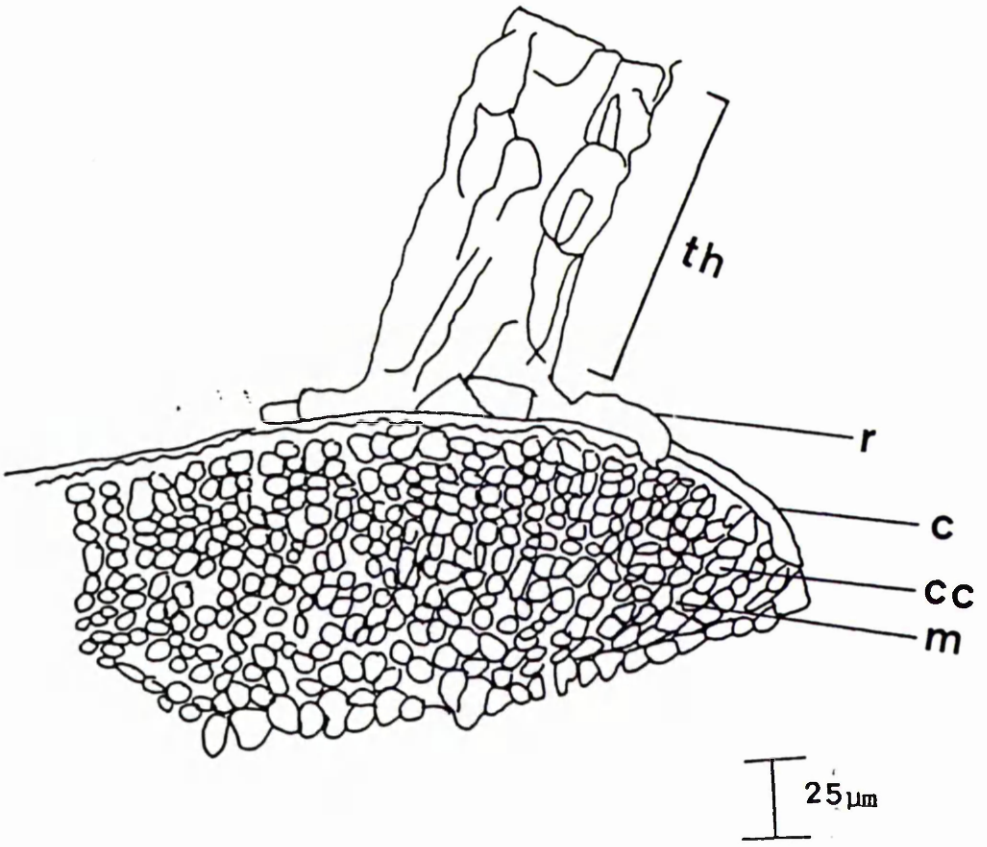


Fig. 4.3.2.3. Diagrammatic representation of the photomicrograph. (Fig. 4.3.2.2).

c = cuticle

cc = cortical cell

m = mucilage

th = thallus of Fucus germlings

r = rhizoids of Fucus germlings

inhibition of growth of germlings on *Mastocarpus* was 25% Fig. 4.3.2.1 (k, l) less than with younger *Mastocarpus* sporelings. Germlings age 33 days with very young sporelings developed single long rhizoids which seemed to be attracted to the scattered sporelings when compared with germlings attached to glass without nearby sporelings which developed many rhizoids. The inhibition of growth of *Fucus* germlings associated with *Mastocarpus* was 40% Fig. 4.3.2.1(m, n).

The attachment of *F. serratus* zygotes to *M. stellatus* sporelings was further studied. Sections were made of *Fucus* germlings growing on *Mastocarpus*. Primary rhizoids of the young *Fucus* showed a close bonding with the 'cuticle' surface of the *Mastocarpus* sporelings (Figs. 4.3.2.2-3), and some indication of penetration of this 'cuticle' was obtained. With the older germlings there was production of numerous secondary rhizoids (Fig. 4.3.2.4), and for these similarly there was evidence of both a close bonding with the *Mastocarpus* 'cuticle', and some slight penetration. This penetration may be part of the bonding and attachment process. Convincing evidence of rhizoid penetration into the *Mastocarpus* tissue was not forthcoming.

4.3.3. Growth of sporelings of *Mastocarpus* and other juvenile marine algae in single and mixed cultures.

Mastocarpus sporelings were grown for 7 weeks singly or in the presence of juveniles stages of marine algae under the same culture conditions. The sporelings and germlings were measured at 7 day intervals.

4.3.3.1. *Mastocarpus* sporelings grown with *F. serratus* germlings at a distance and in close proximity.

Table 4.3.3.1.1. and Fig. 4.3.3.1.1 shows the growth of *Mastocarpus* sporelings with *Fucus serratus* germlings. Sporelings growing at a distance from the germlings and in close proximity did not show any significant growth inhibition after 1 week. The sporelings in close proximity to the germlings showed a 21% growth inhibition

Fig. 4.3.2.4. Sequences of photomicrographs showing 2 month old germling of *Fucus serratus* attached to *Mastocarpus* sporeling.

a, L.S. of total rhizoidal mass of *Fucus* and of *Mastocarpus* sporelings. Scale bar= 82 μm.

b, Part of the above at higher magnification showing close bonding of *Fucus* rhizoidal mass and some penetration of *Mastocarpus* cuticle. Scale bar = 46 μm.

c, Another part of the *Fucus* rhizoidal mass, showing close bonding and some penetration of the cuticle. Scale bar= 74 μm.

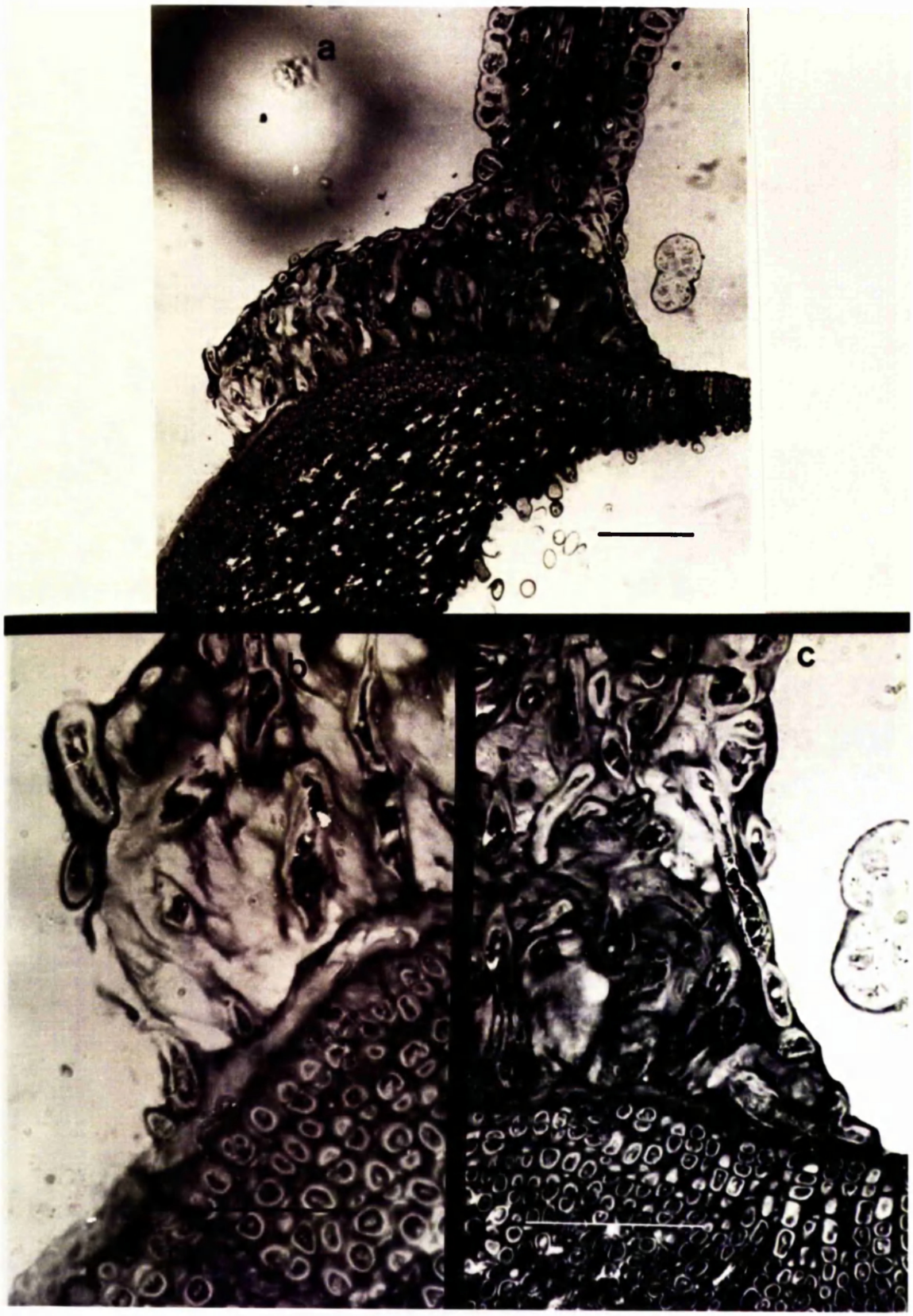
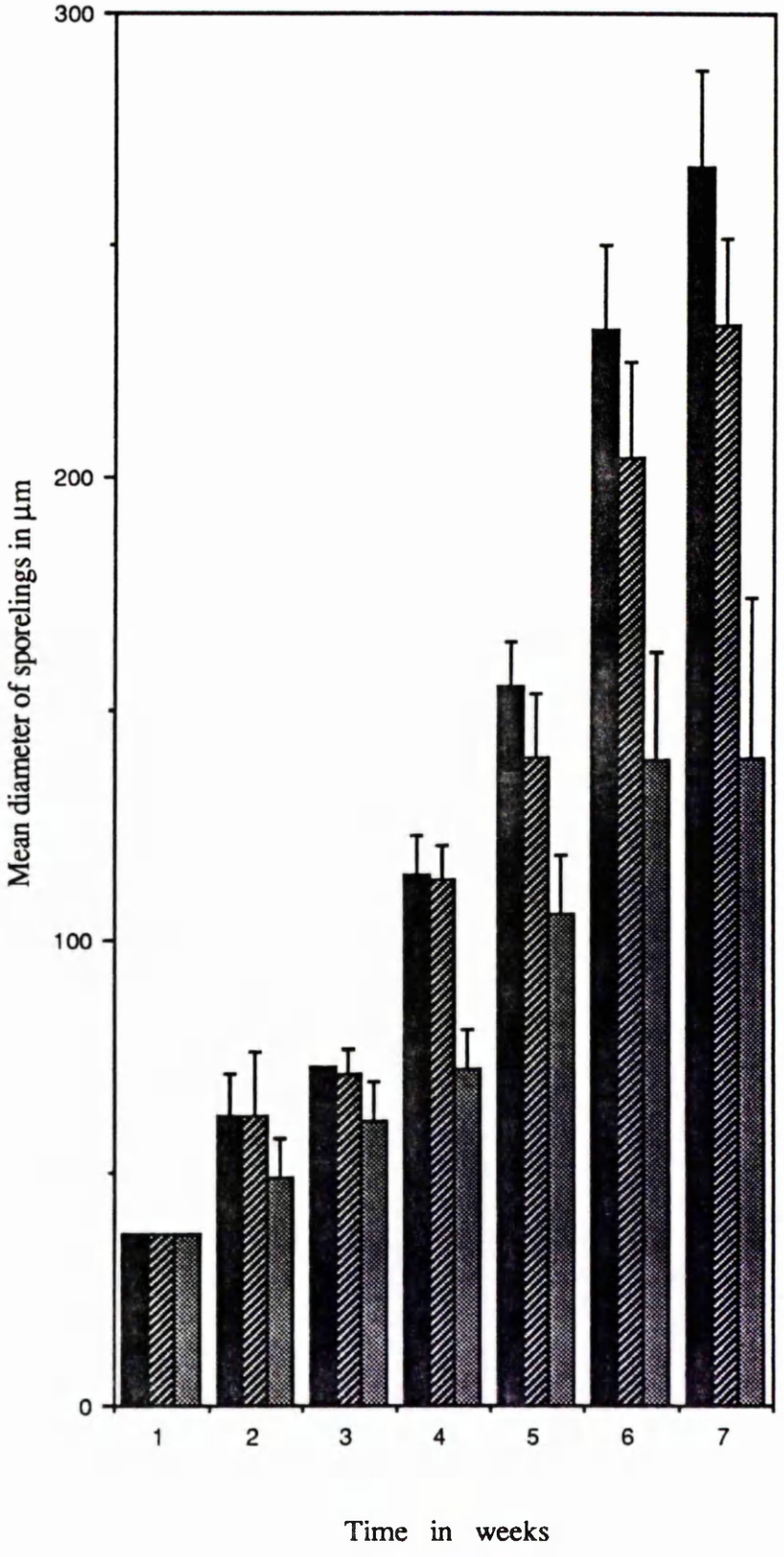

a
b
c

Table. 4.3.3.1.1. Showing growth of *Mastocarpus* sporelings with germlings of *Fucus serratus*. 25 sporelings measured, selected at random. Temperature 10 ^{o}C ± 2 ^{o}C, light intensity 17.5 µmol m^{-2} s^{-1}. (Mean ± S.D).

Time/ weeks	Mean diameter control spore-lings (µm)	Mean diam-eter of spor-eling grown at a distance from germ-lings (µm)	% growth inhibition	Mean diame-ter of spore-lings in close proximity to germlings (µm)	% growth inhibition
1	36.5	36.5	0	36.5	0
2	62.1±9.1	62±14	0.16	48.9±8.7	21.3
3	73	71.5±5.1	2.1	61.3±8.4	16
4	114.6±8.4	113.2±7.5	1.3	72.3±8.3	36.9
5	155.5±9.3	140.1±13.5	9.9	105.9±12.9	31.9
6	232.1±17.9	204.4±20.4	11.9	139.4±23.5	39.9
7	267.2±20.3	232.9±18.5	12.8	139.9±34.7	47.6

Fig. 4.3.3.1.1. Growth of *Mastocarpus* sporelings with *Fucus serratus* germlings.

- ■ Mean diameter of control sporelings
- ▨ Mean diameter of sporelings at a distance from germlings.
- ▩ Mean diameter of sporelings in close proximity to germlings.


300
200
100
0
Mean diameter of sporelings in µm
1
2
3
4
5
6
7
Time in weeks

after 2 weeks, whilst the sporelings at a distance from the germlings showed no significant inhibition at all. Sporelings in close proximity from germlings showed a 16% growth inhibition after 3 weeks. Sporelings at a distance from germlings did not show any significant growth inhibition after 4-7 weeks, but sporelings in close proximity to the germlings showed a 31.9%-47.6% growth inhibition after 4-7 weeks.

Table. 4.3.3.1.2 and Fig. 4.3.3.1.2 show the growth in length of *Fucus* germlings with *Mastocarpus* sporelings, and germlings grown at a distance from the sporelings. The growth of germlings separated from the sporelings was signifigantly affected, but germlings with sporelings in close proximity showed no significant effects on growth after 1 week. Results were variable, germlings growing at a distance from the sporelings did not show any significant growth inhibition after 2-3 weeks, whilst the germlings in close proximity to the sporelings showed a 21% growth inhibition after 2 weeks, but did not show any significant growth inhibition after 3 weeks. Germlings at a distance to sporelings showed a little significant growth inhibition (17%-24%) after 4-7 weeks, and germlings in close proximity to *Mastocarpus* sporelings showed similar growth inhibitions (21.5%-25.5%) after 4-7 weeks. Whilst some effects were observed on germling length, the width of these growing at a distance were only slightly influenced, and the germlings in close proximity showed only slight effects (see Table 4.3.3.1.3 and Fig. 4.3.3.1.3).

Table. 4.3.3.1.4 and Fig. 4.3.3.1.4 shows the growth in length of rhizoids of germlings with sporelings and germlings at a distance from the sporelings. The growth in length of rhizoids of germlings at a distance from the sporelings did not show any significant growth inhibition after 1 week, and the length of rhizoids of germlings in close proximity to the sporelings showed little significant growth inhibition a (16%) after the first week. In the second week the growth inhibition of rhizoids at a distance from sporelings was 23%, but rhizoids in close proximity to the sporelings showed significant growth inhibition (41%) after 2 weeks. The length of rhizoids of germlings growing at a distance from the sporelings appeared not to be significantly affected after 3-5 weeks, and the length of rhizoids of germlings in close proximity showed much significant growth inhibition (32%) after 3 weeks. After

Table. 4.3.3.1.2. Showing growth in length of *Fucus serratus* germlings when grown with *Mastocarpus* sporelings and when free. 25 germlings measured, selected at random. Temperature 10 ^{o}C ± 2 ^{o}C, and light intensity 17.5 µmol m^{-2} s^{-1}. (Mean ± S.D).

Time/ weeks	Mean germling length control (µm)	Mean germling length grown at a distance from *Mastoc-arpus*(µm)	% growth inhibition	Mean germling length in close proximity to *Mastocarpus* (µm)	% growth inhibition
1	188±44.7	142.4±25.6	24.3	180.7±17.2	3.9
2	408.8±66.9	365±67.3	10.7	321.2±58.4	21.4
3	657±62.3	631.5±115.5	3.9	595±140.5	9.4
4	1033±173.6	828.6±49.1	19.8	810.3±127.3	21.6
5	1664.4±102.2	1376.1±196.6	17.3	1303.1±98	21.7
6	1919.9±116.8	1573.2±187.2	18.1	1507.5±131.7	21.5
7	2288.6±385	1722.8±173.4	24.7	1704.6±199.3	25.5

Fig. 4.3.3.1.2. Growth in length of *Fucus serratus* germlings with *Mastocarpus* sporelings.

■ Mean length of control germlings.

▨ Mean length of germlings at a distance from sporelings.

▩ Mean length of germlings in close proximity to sporelings.

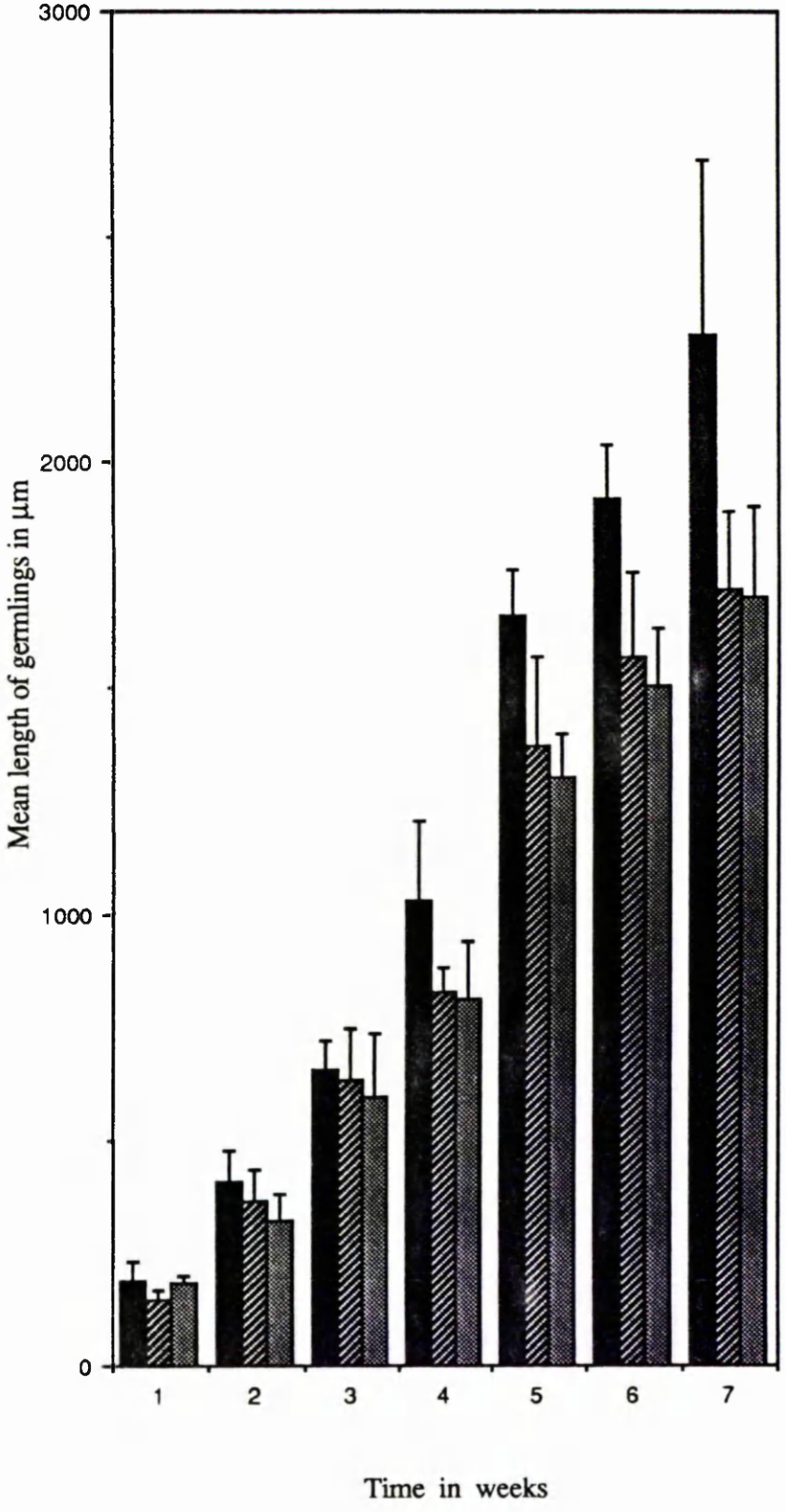

3000
2000
1000
0
Mean length of germlings in µm
1
2
3
4
5
6
7
Time in weeks

Table. 4.3.3.1.3 Showing growth of width of *Fucus serratus* in close proximity to *Mastocarpus* sporelings and at a distance. 25 germlings measured, selected at random. Temperature 10 ^{o}C ± 2 ^{o}C, and light intensity 17.5 µmol m^{-2} s^{-1}. (Mean ± S.D).

Time/ weeks	Mean germling width control (µm)	Mean germling width grown at a distance from *Mastocarpus* (µm)	% growth inhibition	Mean germling width in close proximity to *Mastocarpus* (µm)	% growth inhibition
1	102.2±14.6	84±16.7	17.9	39.1±10.2	8.9
2	149.7±19.7	138.7±20.3	7.3	109.5±15.3	26.8
3	208.1±16.7	195.2±16.2	6.4	178.9±29.2	14
4	244.6±27.3	215.4±19.7	11.9	228.7±11	6.5
5	361.4±41.5	300.3±39.3	16.9	292±23.1	19.2
6	434.4±52.1	346.8±44	20.2	324.9±34.4	25.2
7	434.4±87.1	368.7±30.3	15.1	346.8±61.6	20.2

Fig. 4.3.3.1.3. Growth of width of *Fucus serratus* germlings with *Mastocarpus* sporelings.

- Width of germlings control
- Width of germlings at a distance from sporelings.
- Width of germlings in close proximity to sporelings.

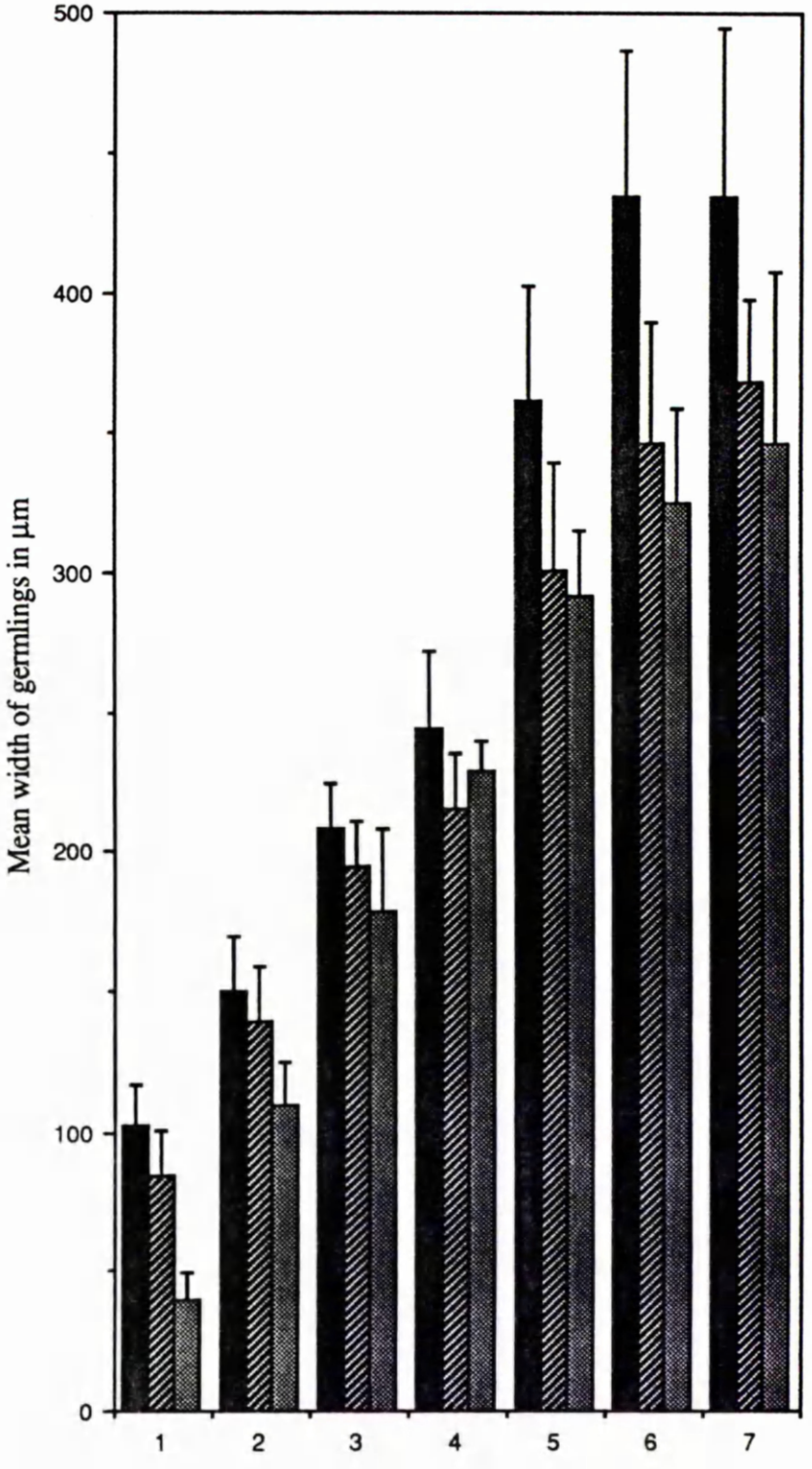
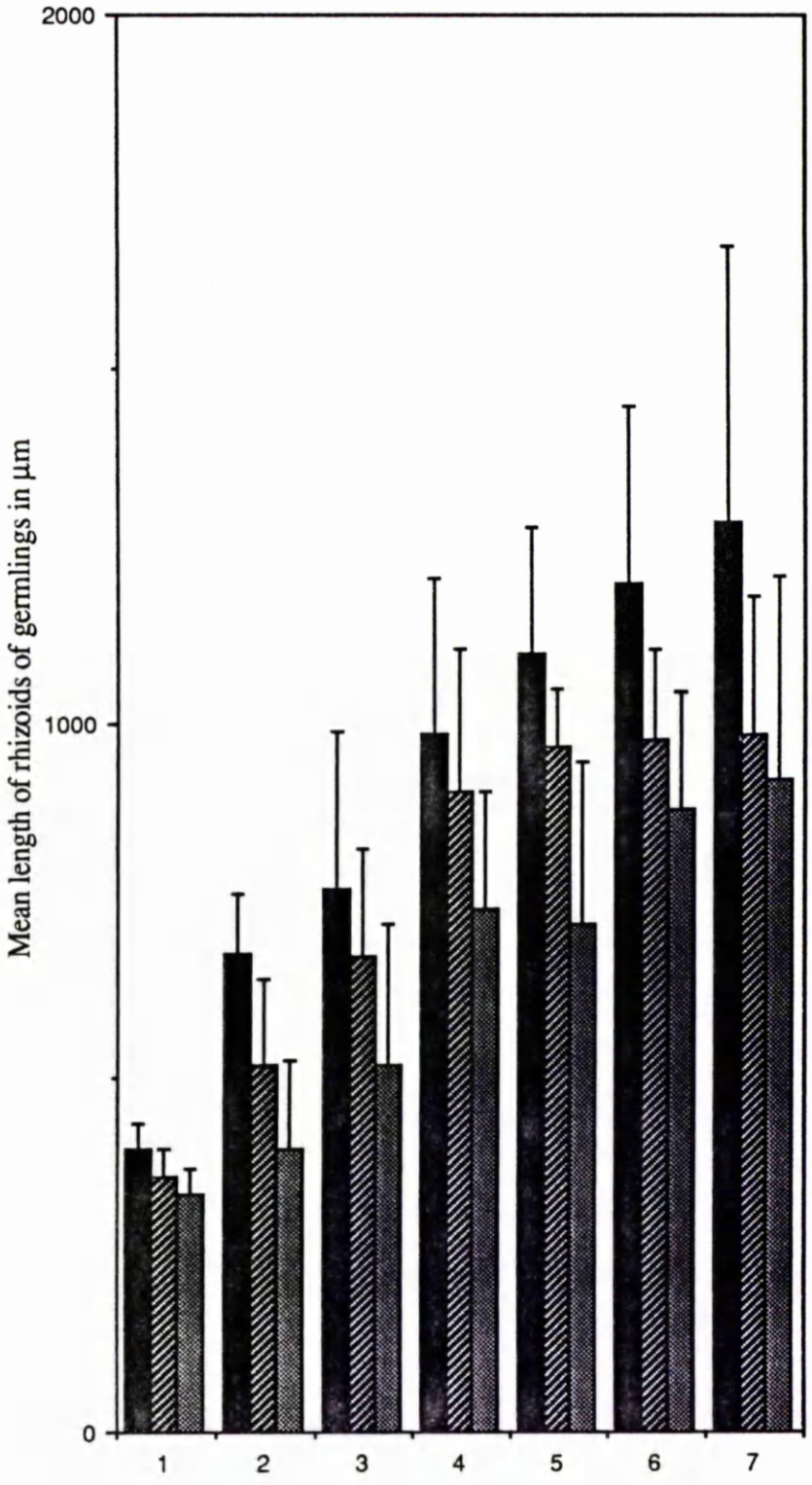

500
400
300
200
100
0
Mean width of germlings in µm
1
2
3
4
5
6
7
Time in weeks

Table. 4.3.3.1.4. Showing growth in length of rhizoids of *Fucus serratus* in close proximity to *Mastocarpus* sporelings and at a distance. 25 germlings measured, selected at random. Temperature 10 ^{o}C ± 2 ^{o}C, and light intensity 17.5 µmol m^{-2} s^{-1}. (Mean ± S.D).

Time/ weeks	Mean length of germling rhizoids control (µm)	Mean length of germling rhizoids at a distance from *Mastocarpus* (µm)	% growth inhibition	Mean length of germling rhizoids in close proximity to *Mastocarpus* (µm)	% growth inhibition
1	400±37.2	360.6±35	9.9	335.8±39.3	16.1
2	675.3±82	519.1±120.5	23.1	400±126.4	40.8
3	765±223.8	671.6±196	20.9	519.1±150	32.1
4	985.5±219	905.2±201.1	8.1	737.3±167.1	25.2
5	1100±176.4	967.3±83.6	12.1	715.4±231	26.6
6	1200±245.5	978.2±127.3	18.5	880.3±164.3	26.6
7	1285.3±390	985.7±193.5	23.31	920.4±288.9	28.39

Fig. 4.3.3.1.4. Growth of rhizoids of *Fucus serratus* germlings, with *Mastocarpus* sporelings.

- ■ Mean length of rhizoids of control germlings.
- ▨ Mean length of rhizoids of germlings at a distance from sporelings.
- ▩ Mean length of rhizoids of germlings in close proximity to germlings.


2000
1000
0
Mean length of rhizoids of germlings in µm
1
2
3
4
5
6
7
Time in weeks

4-7 weeks germlings in close proximity less significant growth inhibition than 3 weeks was 25%-28%, whilst the length of rhizoids of germlings growing at a distance from the sporelings showed little significant growth effects after 6-7 weeks.

Photomicrographs show that some of the germlings rhizoids grew around the disc-like sporelings and some seemed to grow underneath the sporelings (Fig. 4.3.3.1.5).

4.3.3.2. *Mastocarpus* sporelings grown at a distance and in close proximity to *Ulva* and *Enteromorpha* sporelings and free from them.

In this experiment only the growth of *Mastocarpus* sporelings was measured at a distance and in close proximity to the sporelings of *Ulva* and *Enteromorpha*. Table 4.3.3.2.1 and Fig. 4.3.3.2.1 shows the *Mastocarpus* sporelings growing at a distance and in close proximity to *Ulva* did not show any significant growth inhibitions up to 2 weeks. The sporelings at a distance from the *Ulva* were not significantly affected, but sporelings in close proximity to the sporelings of *Ulva* showed a slight growth inhibition (17%) after 3 weeks. After 4-5 weeks sporelings at a distance and in close proximity showed significant growth inhibitions, and after 6 weeks the *Mastocarpus* sporelings with *Ulva* both at a distance and in close proximity were dead (Fig. 4.3.3.2.2).

Table 4.3.3.2.2 and Fig. 4.3.3.2.3 shows that the growth of *Mastocarpus* sporelings with *Enteromorpha* sporelings and sporelings at a distance and in close proximity to the sporelings of *Enteromorpha* were not significantly affected after 1 week, and sporelings growing at a distance and in close proximity did not show any significant growth inhibition over 2-4 weeks. Sporelings at a distance showed 16%-44% growth inhibition after 5-6 weeks, whilst the sporelings of *Mastocarpus* in close proximity to the sporelings of *Enteromorpha* showed high significant growth inhibition of 29%-57% after 4-6 weeks. After 7 weeks all the *Mastocarpus* sporelings died both with *Enteromorpha* at a distance and in close proximity (Figs. 4.3.3.2.4, 4.3.3.2.5).

Fig. 4.3.3.2.5. Showing growth and germination of *Fucus serratus* germlings with *Mastocarpus* sporelings.

a, Rhizoids of *Fucus* showing curved growth around sporelings. Scale bar= 41μm.

b, Rhizoids of *Fucus* showing growth around and probably underneath *Mastocarpus* sporelings. Scale bar= 41μm.

c, Rhizoids of *Fucus* germlings which seem to be growing underneath *Mastocarpus* sporelings. Scale bar= 33 μm.

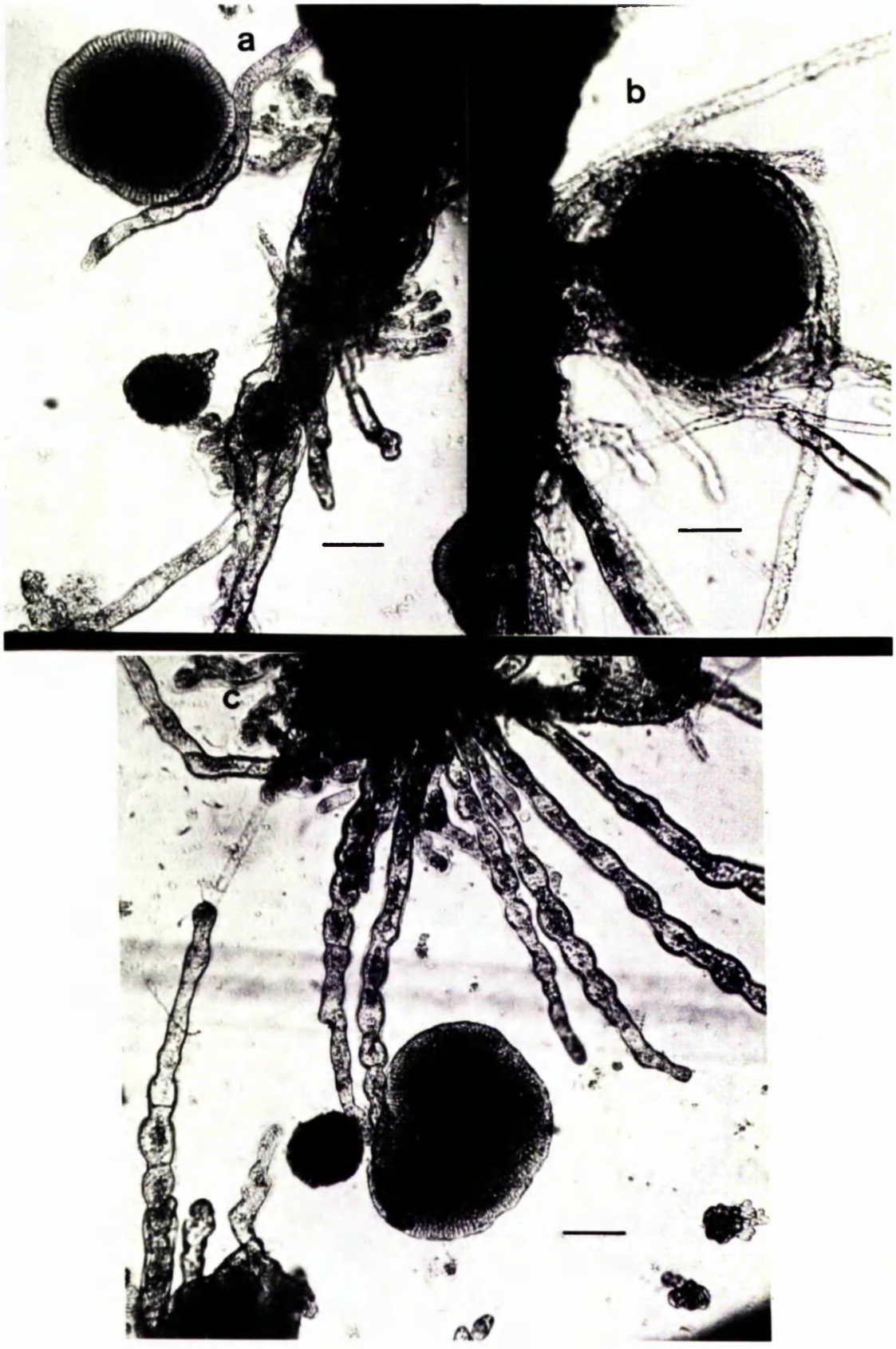
d, Long rhizoids of *Fucus* germlings which appear to be growing underneath the *Mastocarpus* sporelings. Scale bar= 33 μm.

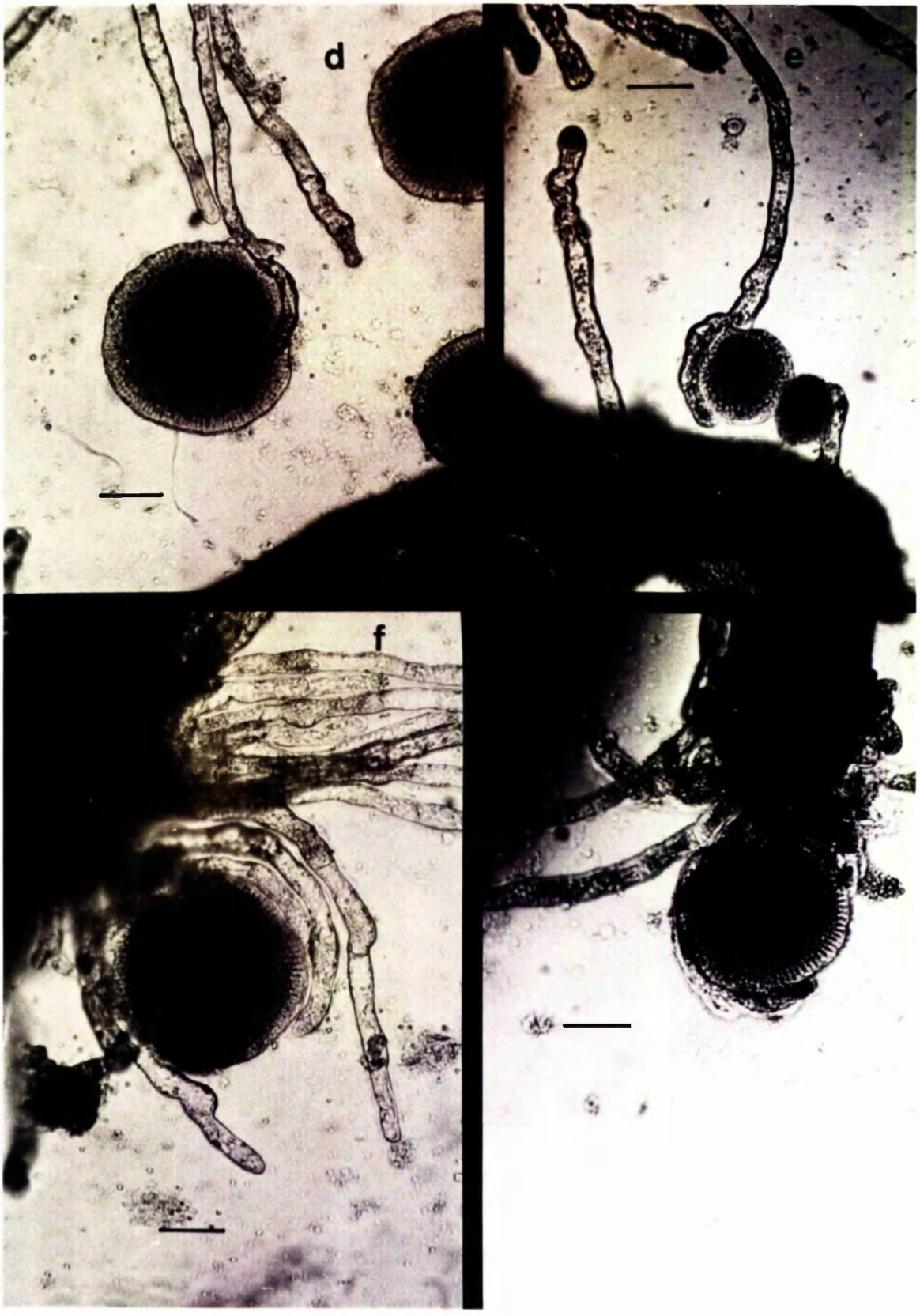
e, Long rhizoid of *Fucus* germlings growing around a *Mastocarpus* sporeling. Scale bar= 70 μm.

f, Several rhizoids of *Fucus* germlings growing around a *Mastocarpus* sporeling. Scale bar= 35 μm.

g, Showing many short rhizoids of *Fucus* germlings growing around a *Mastocarpus* sporeling. Scale bar= 56 μm.

h, Showing sporelings of *Mastocarpus* at a distance and in close proximity to *Fucus* germlings. Scale bar=38 μm.


a
b
c


d
e
f

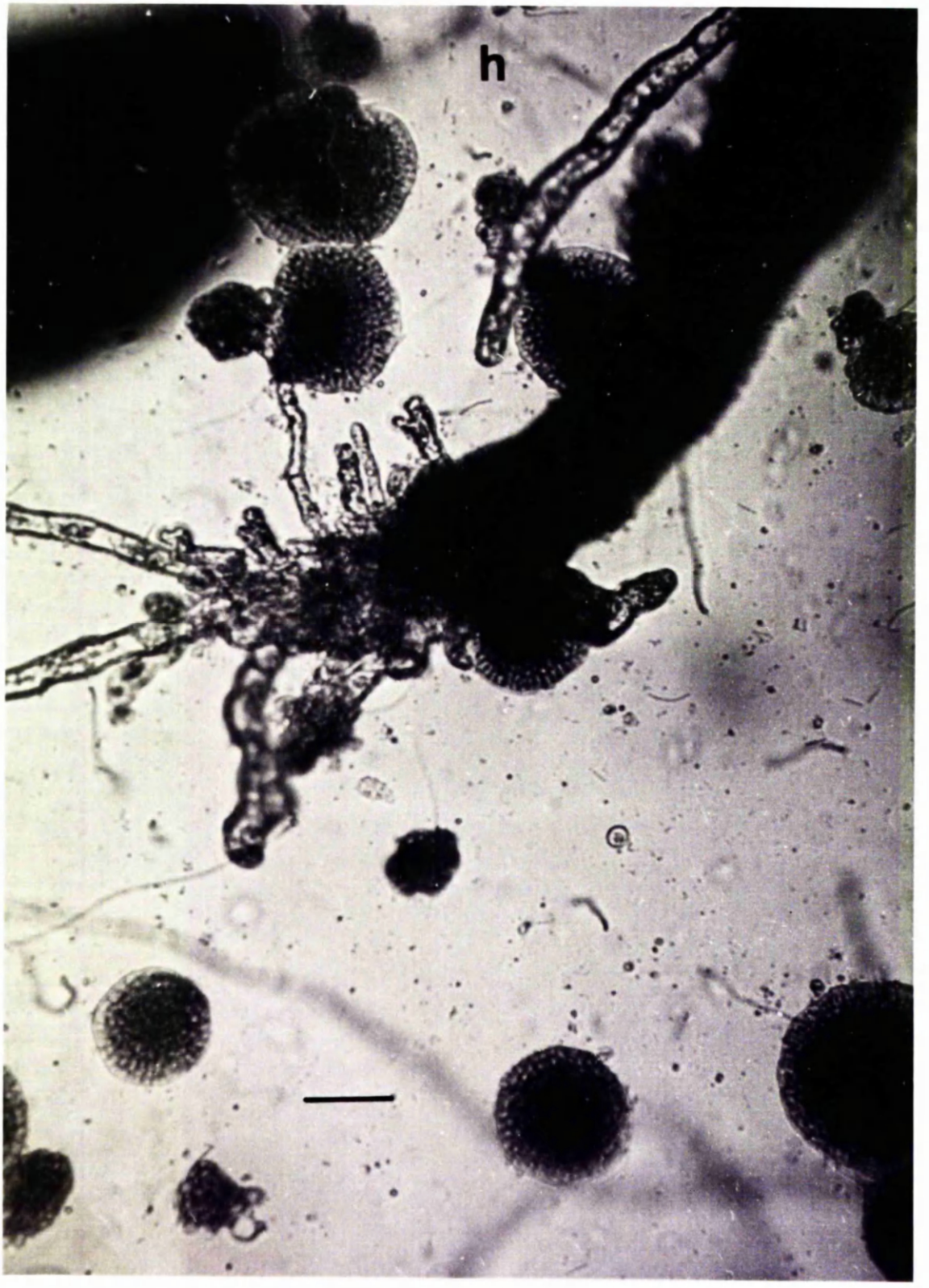

h

Table 4.3.3.2.1. Showing germination and growth of sporelings of *Mastocarpus* in close proximity to germlings of *Ulva lactuca* and at a distance. 25 sporelings measured, selected at random. Temperature 10 ^{o}C ± 2 ^{o}C, and light intensity 17.5 µmol m^{-2} s^{-1}. (Mean±S.D). D = dead.

Time/ weeks	Mean diamerer of control sporelings (µm)	Mean diameter of sporelings at a distance from *Ulva* (µm)	% growth inhibition	Mean diameter of sporelings in close proximity to *Ulva* (µm)	% growth inhibition
1	36.5	36.5	0	36.5	0
2	62.1±9.13	55.5±13.4	10.6	54.8± 10.5	11.8
3	73	63±19.13	13.7	60.9± 12.4	16.6
4	116.6± 8.36	85.4 ± 8.7	26.8	75.2± 11	35.5
5	153.5± 9.31	85.4±8.7	44.4	75.2±11	51
6	237.1 ± 17.9	D	D	D	D
7	270.2±20.3	D	D	D	D

Fig. 4.3.3.2.1. Growth of sporelings of Mastocarpus in the presence of *Ulva lactuca* sporelings.

■ Mean diameter of control sporelings

▨ Mean diameters of *Mastocarpus* sporelings at a distance from *Ulva*.

▩ Mean diameters of *Mastocarpus* sporelings in close proximity to *Ulva*.

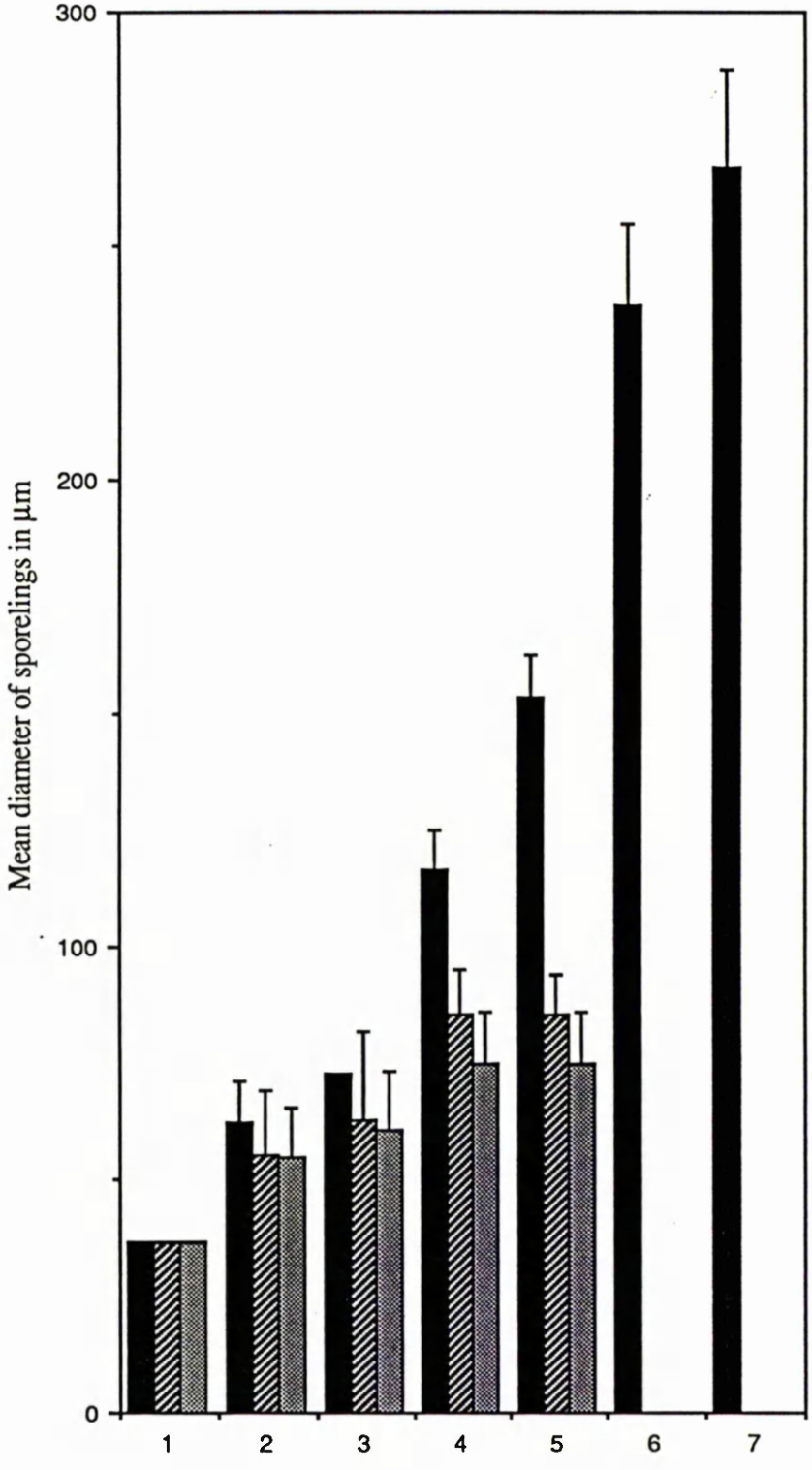

300
200
100
0
Mean diameter of sporelings in µm
1
2
3
4
5
6
7
Time in weeks

Fig. 4.3.3.2.2. Showing germination and growth of *Mastocarpus* sporelings with *Ulva* sporelings.

a, Low magnification showing growth of *Mastocarpus* sporelings at a distance and in close proximity to *Ulva* sporeling at an early stage. Scale bar = 285 μm

b, Higher magnification showing *Mastocarpus* sporelings in close proximity to *Ulva* sporelings. In centre *Ulva* rhizoids growing around *Mastocarpus* sporelings. Scale bar = 70 μm

c, Showing dead *Mastocarpus* sporeling in close proximity to *Ulva* sporelings. Scale bar = 109 μm

d, Showing dead *Mastocarpus* sporeling at a distance from *Ulva* sporelings. Scale bar = 85 μm

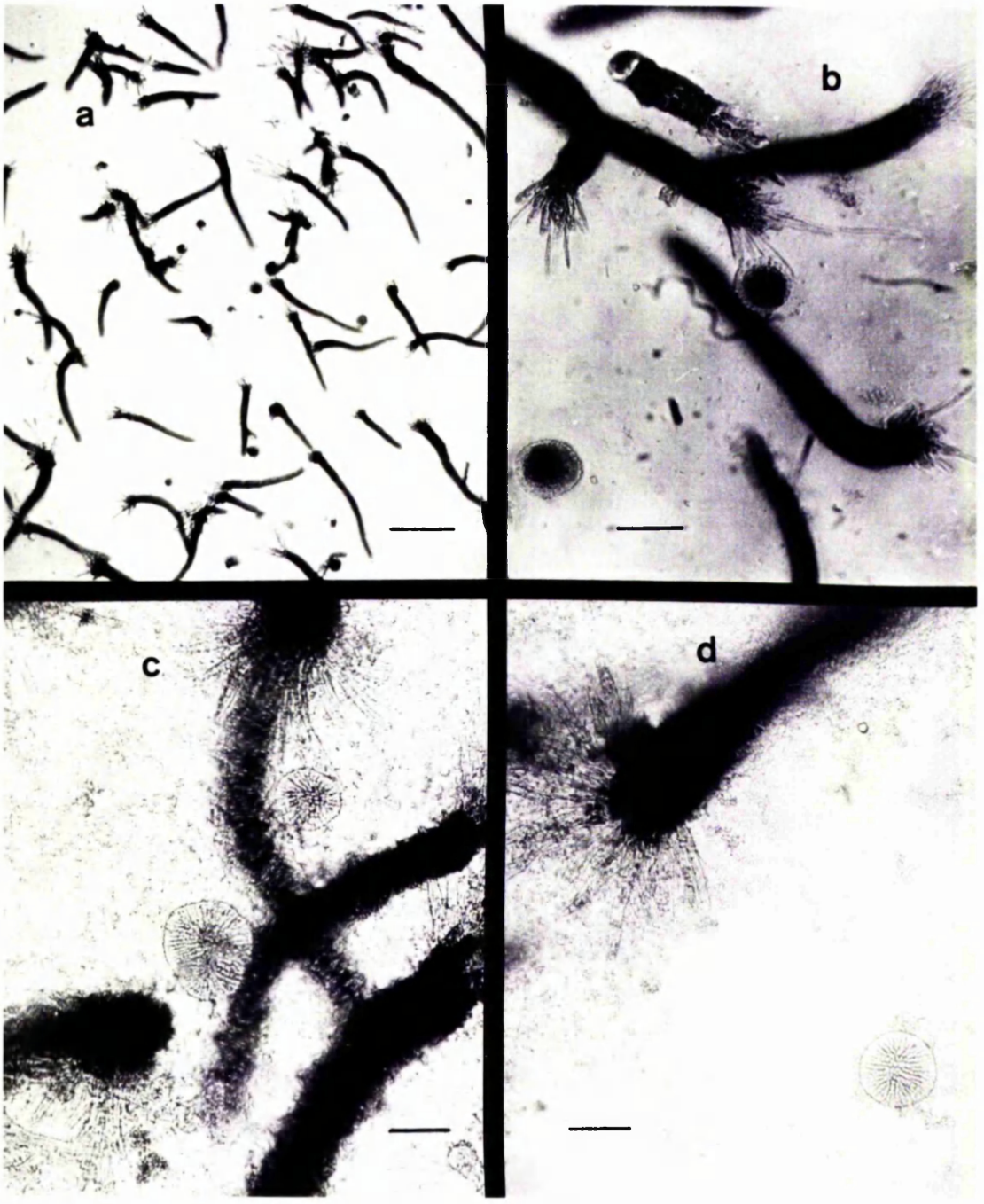

a
b
c
d

Table 4.3.3.2.2 Showing germination and growth of sporelings of *Mastocarpus* in close proximity to *Enteromorpha intestinalis* and at a distance. 25 sporelings measured, selected random. Temperature 10 ^{o}C ± 2 ^{o}C, and light intensity 17.5 µmol m^{-2} s^{-1}. (Mean±S.D). D = dead.

Time/ weeks	Mean diameter of control sporelings (µm)	Mean diameter sporelings at a distance from *Enteromorpha* (µm)	% growth inhibition	Mean diameter sporelings in close proximity to *Enteromorpha* (µm)	% growth inhibition
1	36.5	36.5	0	36.5	0
2	65.1±9.1	60	7.8	59.7±14.9	8.3
3	75	70.6±14.9	5.9	70	6.7
4	112.6±8.4	100.2±17.4	11	80.3±13.9	28.7
5	150.5±9.3	127±17.9	15.6	101.5±14	32.6
6	235.1±17.9	131.4±29.9	44.1	102.2±14.6	56.5
7	269.2±20.3	D	D	D	D

Fig. 4.3.3.2.3. Growth of sporelings of *Mastocarpus* with *Enteromorpha intestinalis* sporelings.

- Mean diameter of control sporelings.
- Mean diameters of sporelings at a distance from *Enteromorpha* sporelings.
- Mean diameters of sporelings in close proximity to *Enteromorpha* sporelings.

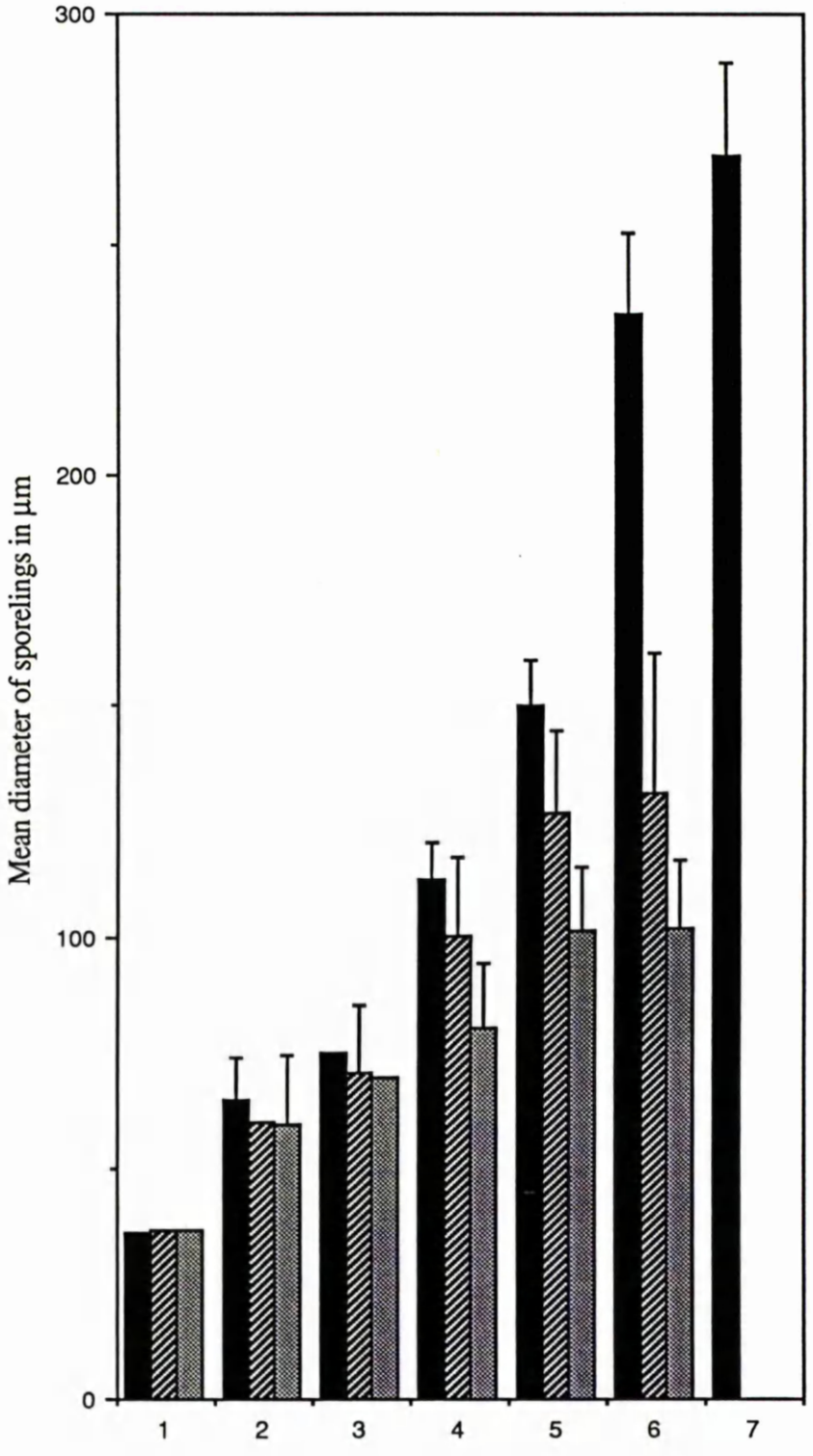

300
200
100
0
Mean diameter of sporelings in µm
1
2
3
4
5
6
7
Time in weeks

Fig. 4.3.3.2.4. Showing germination and growth of *Mastocarpus* sporelings with *Enteromorpha* sporelings.

a,b; Low magnification showing *Mastocarpus* sporelings with *Enteromorpha* sporelings at an early stage. Scale bar = 584 μm

c, Some *Enteromorpha* sporelings (arrowed) growing on *Mastocarpus* sporeling. Scale bar = 64 μm

d,e; Showing a rhizoid of *Enteromorpha* growing around and possibly underneath a sporeling of *Mastocarpus*. Scale bar = 43 μm

f, Showing dead *Mastocarpus* sporelings with *Enteromorpha* Scale bar = 102 μm

g, High magnification of *Mastocarpus* sporelings with *Enteromorpha*. Scale bar = 161 μm

h, High magnification showing dead *Mastocarpus* sporelings due to the presence of *Enteromorpha*. Scale bar = 161 μm


a
b
c
d

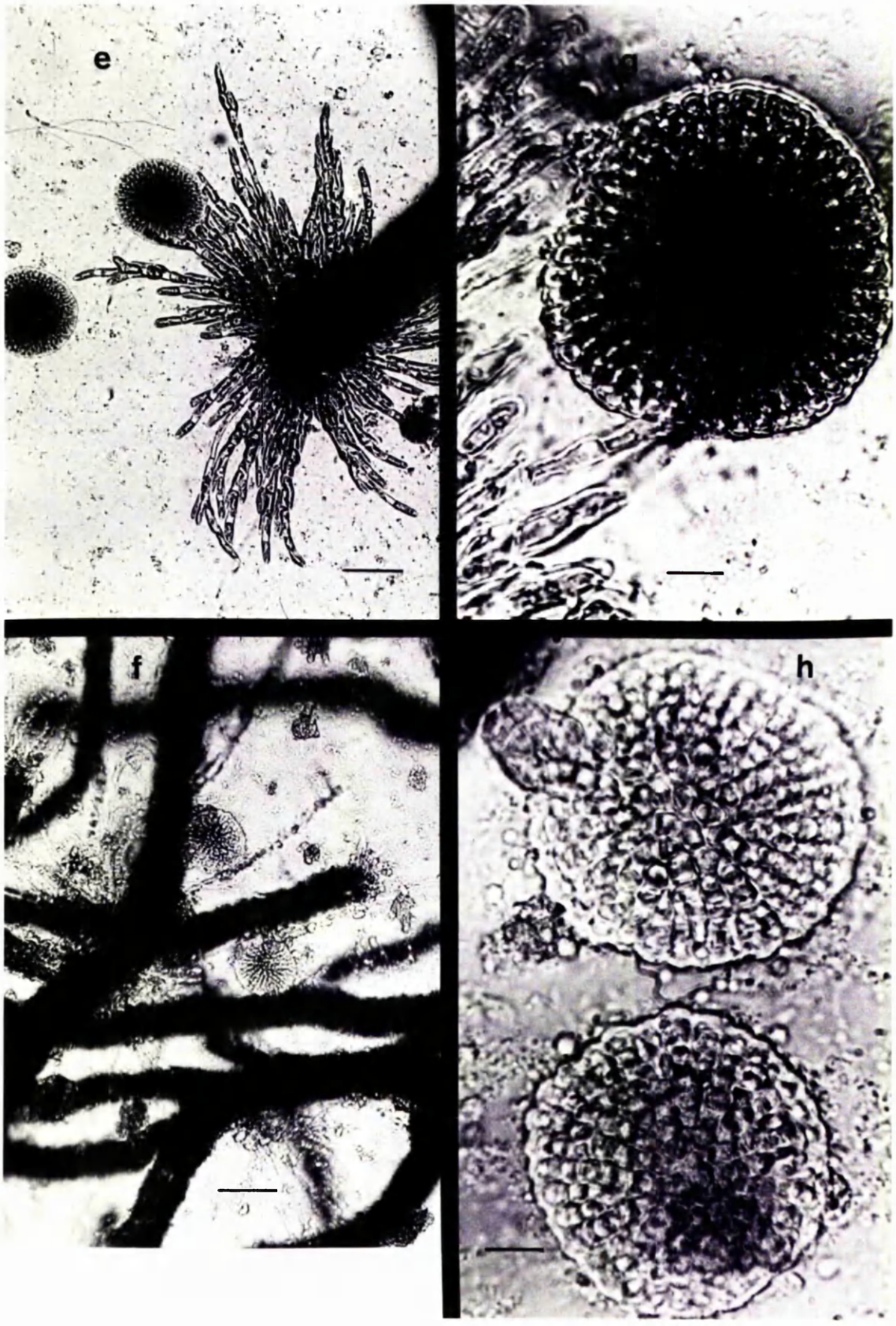

e
g
f
h

Fig. 4.3.3.2.5. SEM photomicrographs showing dead *Mastocarpus* sporelings with *Enteromorpha* sporelings .

a, b; Dead sporelings of *Mastocarpus* with some debris. Scale bar = 10 μm

c, Dead sporelings of *Mastocarpus* with *Enteromorpha*. Scale bar = 100 μm


a
b


C

4.3.3.3. *Mastocarpus* sporelings with *Porphyra* and *Porphyra* with *Mastocarpus* and free from it.

Table 4.3.3.3.1 and Fig. 4.3.3.3.1 summarizes the germination and growth of *Mastocarpus* sporelings at a distance and in close proximity to the *Porphyra*. Sporelings growing at a distance and in close proximity to the *Porphyra* did not show any significant growth inhibition over 1-3 weeks. Sporelings at a distance and in close proximity to the *Porphyra* showed growth inhibition of 20%-42% after 4-7 weeks. In general there was not much difference in growth inhibition between sporelings of *Mastocarpus* at a distance and in close to the *Porphyra* sporelings.

Table 4.3.3.3.2 and Fig. 4.3.3.3.2 shows the growth of *Porphyra* (measured as the surface area of the young fronds) in close proximity to *Mastocarpus* sporelings. The sporelings of *Porphyra* did not show any significant growth inhibition after 2-3 weeks, but after 4-7 weeks showed growth inhibitions of 22%-27%.

Fig. 4.3.3.3.3 shows the growth of sporelings of *Mastocarpus* with *Porphyra*, and with some inhibition of the growth and development.

4.4. Discussion

The attachment and develpment of *F. spiralis* germlings normally takes place on rocky habitats high in the eulittoral zone, so that *Mastocarpus* as a substratum was unusual since sporelings of *Mastocarpus* grow in the lower eulittoral area. The zygotes of *Fucus spiralis* on *Mastocarpus* sporelings very rapidly became attached by mucilage secretion. The property of the adhesive mucilage is to spread out over the surface of the substratum, and to infiltrate the uneven areas of the substratum, so that the surface area contact is increased and the attachment is strengthened (Hardy and Moss, 1979b). The production of mucilage by *Fucus spiralis* germlings on the surface of *Mastocarpus* sporelings followed very much this process.

Germlings of *Fucus serratus* on *Mastocarpus* sporelings showed significant inhibitions of growth and development, with the production of short rhizoids and short bodies. Germlings of *Fucus* growing well away from *Mastocarpus* on glass

Table 4.3.3.3.1. Showing growth of *Mastocarpus* sporelings in close proximity to *Porphyra umbilicalis*, and attached on glass surface. 25 sporelings measured, selected at random. Temperature 10 $^{o}C \pm 2\ ^{o}C$, and light intensity 17.5 µmol m^{-2} s^{-1}. (Mean ± S.D).

Time/ weeks	Mean diameter of sporelings control (µm)	Mean diameter of sporelings at a distance from *Porphyra* (µm)	% growth inhibition	Mean diameter of sporelings in close proximity to *Porphyra* (µm)	% growth inhibition
1	36.5	36.5	0	36.5	0
2	62.05±9.1	43.9±8.7	29.3	48.9±8.7	21.2
3	73	65.8±6.1	9.9	60.9±8	16.6
4	115.6±8.4	86.1±15.4	25	83.8±8	20.4
5	155.5±9.3	108±26.3	30.5	110.6±6.1	40.6
6	234±20.3	135.7±30.3	42	146.3±7.1	37.5
7	165.2±20.3	186.2±32.9	29.9	190.7±20.4	28.1

Fig. 4.3.3.3.1. Growth of *Mastocarpus* sporelings with *Porphyra umbilicalis* sporelings.

- ■ Mean diameter of control sporelings.
- ▨ Mean diameters of sporelings at a distance from *Porphyra* sporelings.
- ▩ Mean diameters of sporelings in close proximity to *Porphyra* sporelings.

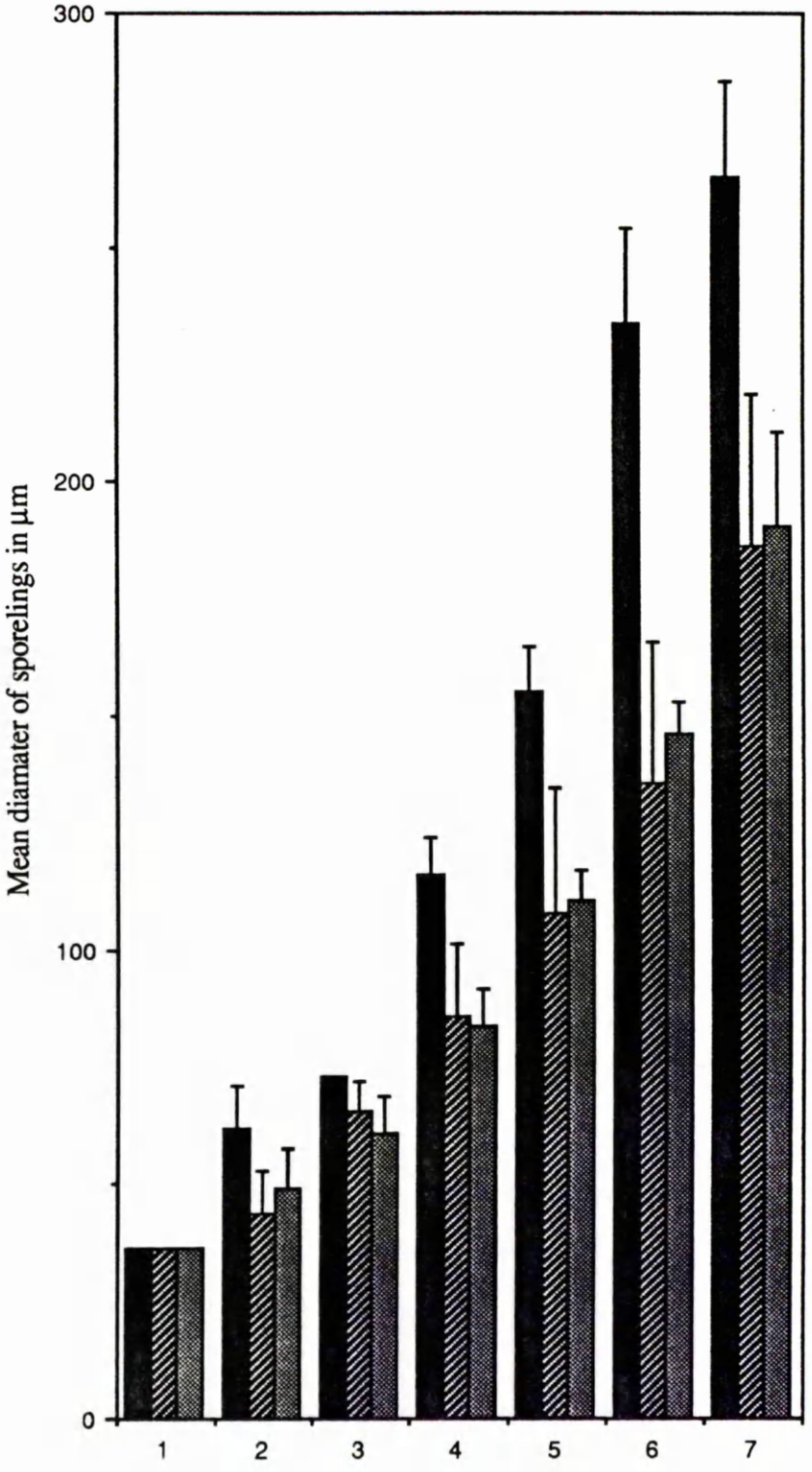

300
200
100
0
Mean diamater of sporelings in µm
1
2
3
4
5
6
7
Time in weeks

Table. 4.3.3.3.2. Showing growth of surface area of *Porphyra umbilicalis* in close proximity to *Mastocarpus* sporelings and attached on glass surface. 25 sporelings measured, selected at random. Temperature 10 $^{o}C \pm 2\ ^{o}C$, and light intensity 17.5 µmol $m^{-2}\ s^{-1}$. (Mean ± S.D). ---, not measured.

Time/ weeks	Mean surface area of *Porphyra* control (mm^2)	Mean surface area of *Porphyra* in close proximity to *Mastocarpus* (mm^2)	% growth inhibition
1	---	---	---
2	$0.58x10^{-6} \pm 1.2x10^{-7}$	$0.57x10^{-6} \pm 1.5x10^{-7}$	0.70
3	$2.34x10^{-6} \pm 6.6x10^{-7}$	$1.93x10^{-6} \pm 8x10^{-7}$	17.5
4	$6.02x10^{-6} \pm 2.3x10^{-6}$	$4.42x10^{-6} \pm 2.02 \pm 10^{-6}$	26.6
5	$9.66x10^{-6} \pm 1.8x10^{-6}$	$7.50x10^{-6} \pm 1.8x10^{-6}$	22.4
6	$11.7x10^{-6} \pm 4.7x10^{-6}$	$8.66x10^{-6} \pm 4.7x10^{-6}$	25.7
7	$14.5x10^{-6} \pm 3.2x10^{-6}$	$10.5x10^{-6} \pm 7.5x10^{-6}$	27.4

Fig. 4.3.3.3.2. Growth (as surface area of young plants) of *Porphyra umbilicalis* with *Mastocarpus* sporelings.

■ Surface area of control *Porphyra* sporelings.

▨ Surface area of *Porphyra* in close proximity to *Mastocarpus* sporelings.

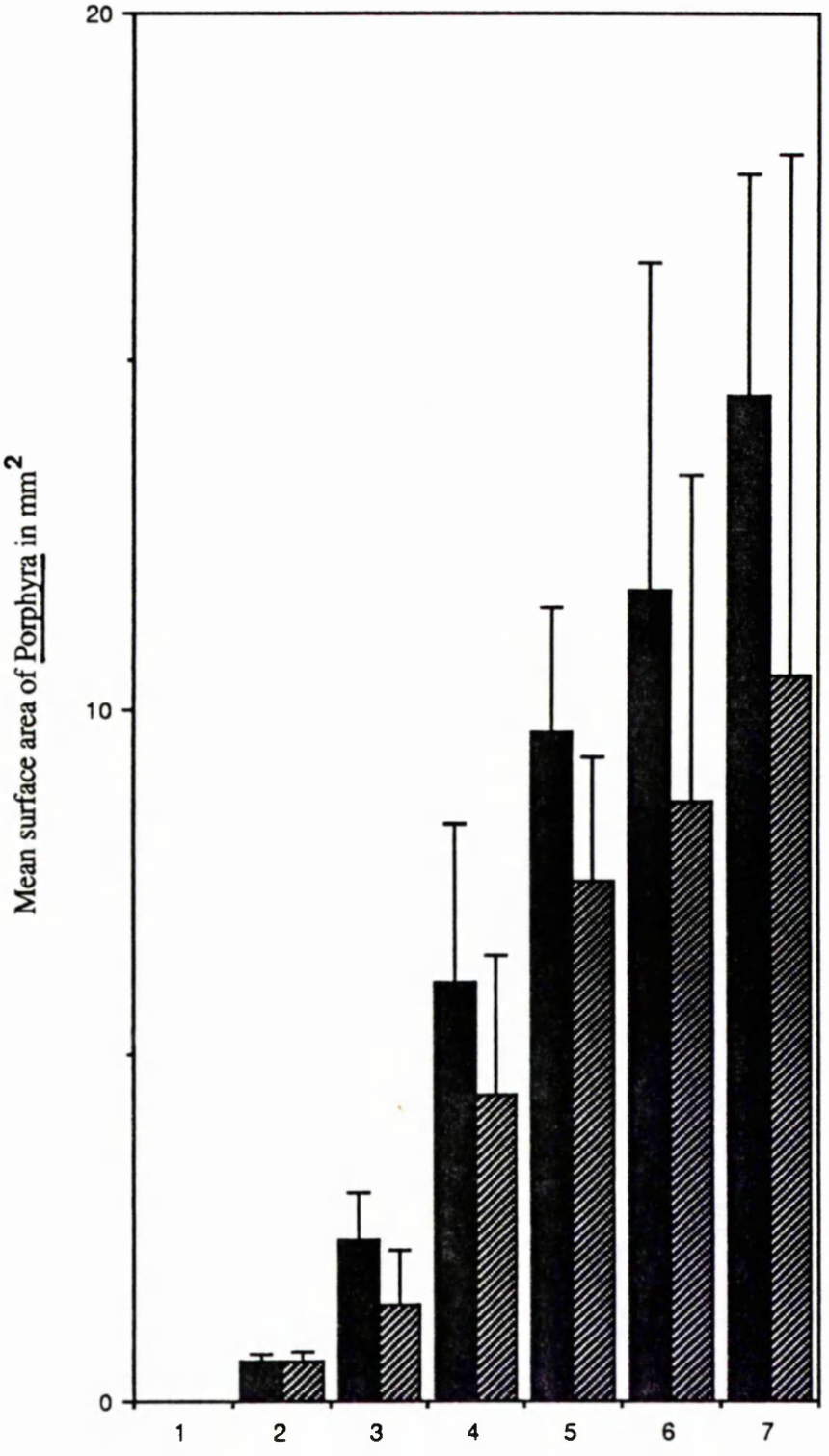
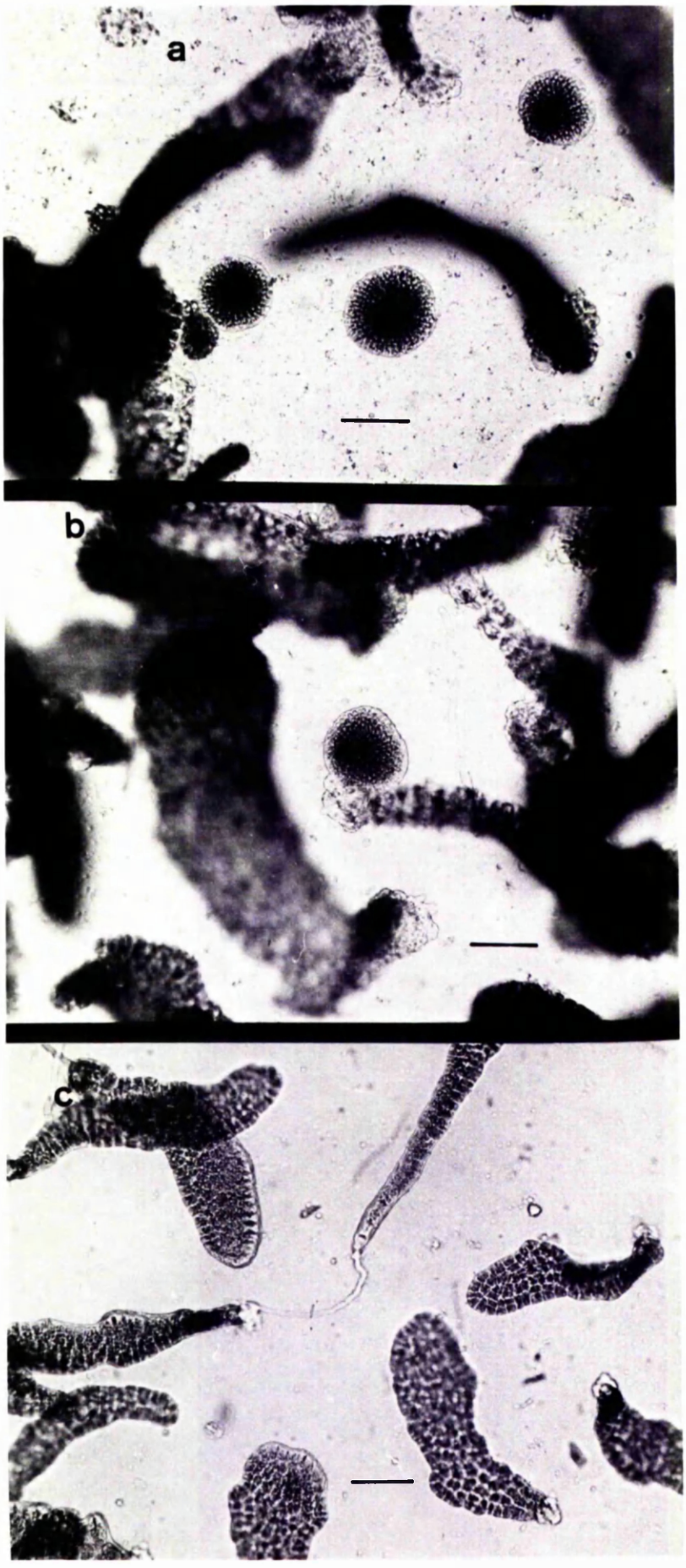

20
10
0
1
2
3
4
5
6
7
Mean surface area of Porphyra in mm2
Time in weeks

Fig. 4.3.3.3.3. Showing germination and growth of *Mastocarpus* sporelings with *Porphyra* sporelings, with the *Porphyra* sporelings out of focus.

a, b, unharmed *Mastocarpus* sporelings with *Porphyra* sporelings. Scale bar = 75 μm (applies only to *Mastocarpus*).

c, *Porphyra* sporelings of the same age as above in focus. Scale bar = 3 mm.


a
b
c

formed long rhizoids and long bodies. These results raise the question whether the *Fucus* growth inhibition on *Mastocarpus* was due to the production of chemical substances by the latter. However, when germlings of *Fucus* were grown in the vicinity of young *Mastocarpus* sporelings, the long young rhizoids seemed to be attracted to the sporelings, as shown by their growth patterns.

The attachment of *Fucus* to *Mastocarpus* sporelings indicated that the primary rhizoids of germlings of *Fucus serratus* are able to penetrate the 'cuticle' of sporelings and so produce very close "bonding", while with older germlings the secondary rhizoids also penetrated the 'cuticle' of sporelings without any damage to the *Mastocarpus* cells. Whilst the close "bonding" of the rhizoid tips with the *Mastocarpus* 'cuticle' involved some slight penetration there was no convincing evidence that the rhizoids actually entered the underlying *Mastocarpus* tissue.

Mastocarpus sporelings growing with *Fucus* germlings at a distance did not show any significant growth inhibition over 1-4 weeks, but between 5-7 weeks there was evidence of slight inhibition. Sporelings in close proximity to germlings showed significant growth inhibition after 4-7 weeks. It might be that sporelings of *Mastocarpus* are affected by extracellular substances produced by *Fucus*. McLachlan and Grigie (1964) showed that the yellow extra-cellular phenolic substances of *F. vesiculosus* were toxic to other algae. This effect is probably a quantitative one, since isolated *Fucus* germlings growing on *Mastocarpus* had no effect on sporeling growth. The numbers of *Fucus* germlings were not sufficient to cause any shading effect on the prostrate *Mastocarpus* sporelings.

Similarly *Fucus* germlings growing near *Mastocarpus* sporelings showed evidence of growth inhibition after 4-7 weeks, and rhizoid growth when in close proximity was noticeably reduced, and there appeared to be some growth curvatures of the rhizoids towards the sporelings. If the growth inhibitions of *Fucus* were due to a chemical interaction with *Mastocarpus*, it is puzzling that the rhizoids were seemingly attracted to the *Mastocarpus* sporelings. Light shading would not be important here, because the *Fucus* germlings do not shade the *Mastocarpus* sporelings. The question raised is whether reduced growth of primary and secondary rhizoids is

accompanied by a similar reduced ... growth of the 'body' of the *Fucus* germlings. Longer germling 'bodies' seem to accompany longer rhizoids.

Ulva sporelings caused a significant inhibition of *Mastocarpus* sporelings growth from a very early stage before light shading effects could be effective, and the *Mastocarpus* sporelings died after 6 weeks. There was less growth inhibition with *Enteromorpha*, but, ultimately the *Mastocarpus* sporelings again died. These results have important ecological implications. When any part of the shore is cleared of plants and animals (e.g. by abrasion, or as in the case of the "Torry Canyon" disaster by the wholesale use of toxic oil dispersants), the primary colonizes are first of all diatoms, then fast growing green algae, including *Ulva* and *Enteromorpha*. When Khfaji (1978) experimentally cleared a strip of shore in the Isle of Cumbrae passing through the *Mastocarpus* zone, the early colonizes were an green algae, followed by fucoids. No evidence of *Mastocarpus* recolonization was obtained after 2.5 years. Possibly a time factor was here involved. *Mastocarpus* sporelings would not have appeared until the green algae had been completely replaced, and then would have slowly appeared in competition with *Fucus*.

Porphyra is another alga of membranaceous form. In contrast with *Ulva* and *Enteromorpha*, the *Mastocarpus* sporelings were not killed, but did suffer some growth inhibition after 6-7 weeks. This inhibition was not solely a light shading effect since the *Mastocarpus* sporelings growing at a distance from the juvenile *Porphyra* plants did show evidence of growth inhibition.

5. Interactions of *Mastocarpus* sporelings with a mucilage-producing diatom, and with juvenile plants of other marine algae.

5.1. Introduction.

Spore attachment and development in algae are enhanced by excreted mucilaginous substances which bind them to the substratum (Moorjani and Jones, 1972; Quatrano and Cryton, 1973; Forbes and Hallam, 1979; Boney, 1975b, 1981). The nature of the substratum will also be important in bio-adhesion process (Baier, 1970; Corpe, 1970; Norton and Fetter, 1981). Studies on growth in culture show that insufficient contact of spores with the substratum result in a prostrate holdfast of regular or irregular form with both *Gigartina stellata* (*Mastocarpus stellatus*) and *Chondrus crispus* (Chen and McLachlan, 1972; Chen *et al*, 1974; Prince and Kingsburg, 1973). Hendey (1951, 1964) found that many diatom species in the littoral zone produce large amounts of mucilaginous substances. Scheer (1945) stated that in nature a bryozoan community provided favourable conditions for the settlement of the diatoms and the resulting mucilage films aggregated soil grains and organic debris and undoubtedly provides fouling macroorganisms with both a nutrient source and place for settlement and attachment. Schonbeck and Norton (1979) and Huang and Boney (1984, 1985) have shown that the growth of juvenile stages of marine algae can be effected by the presence of littoral diatoms. Huang and Boney (1983) have also shown that dried diatom mucilages can effect the growth and morphology of young plants. Little information is available about the chemical natures of diatom substances. Lewin (1958) and Lewin *et al* (1958) showed that the mucilaginous material in *Amphipleura rutilans* and capsular substances in *Phaeodactylum tricornutum* contained both protein and polysaccharides. Daniel *et al* (1987) examined the cytochemical reactions of the attachment mucilage of 12 diatom genera. In each case the mucilages were found exclusively to be of polysaccharide, except in *Berkeleya rutilans*, where a low molecular weight protein was discovered. All the polysaccharides contained anionic

constituents with carboxyl groups predominant in the pad forming species, and in the early of attachment of the stalk-forming species.

The mucilage producing littoral diatom *N. ramosissisma* (Ag.) Cleve was used in a study with *Mastocarpus* sporelings, *Fucus* germlings and *Porphyra* sporelings, in which the effects of diatom mucilage were studied on the development, growth and morphology of *Mastocarpus* sporelings and their interaction with *Fucus* germlings and *Porphyra* sporelings.

5.2. Materials and Methods

The diatom *Navicula ramosissisma*, originally isolated from intertidal habitats in the Firth of Clyde, was supplied by Miss E. McDonnell of the Botany Department in Glasgow University. When grown in unialgal culture, this diatom produces large amounts of mucilaginous substance, which gradually form a thick film on cover slips. The diatoms were cultured on cover slips in petri dishes which contained enriched seawater medium and grown in light intensity of 17.5 μmol m^{-2} s^{-1}, 10 $^{o}C \pm 2\ ^{o}C$ at 16 hours daylength. The diatoms were kept growing in culture for 4 -5 weeks without change of medium. The medium was then changed. After that fresh spores of *M. stellatus* , zygotes of *Fucus* and spores of *Porphyra umbilicalis* were cultured on living diatom films at the same time under the laboratory conditions already described in Chapter 2. Fertile plants of *Ulva lactuca* and *Enteromorpha intestinalis* were not available.

5.3. Results

The results showed that both the growth and development of the juvenile algae were affected in culture with and without diatoms. Table 5.3.1 shows the mean diameter of *Mastocarpus* sporelings with *Fucus* germlings on a clean surface, in which the growth of *Mastocarpus* was inhibited when compared with control sporelings. Sporelings with *Fucus* germlings on diatom mucilage also showed inhibition of growth when compared with sporelings on diatom mucilage grown as controls. *Mastocarpus* sporelings grown on diatom slime were larger than those on clean glass,

Table 5.3.1. Germination and growth of *Mastocarpus* sporelings with germlings of *Fucus spiralis* on clean surface and on diatom mucilage after 23 days. Duplicate samples of 25 sporelings measured. Temperature 10±2 ^{o}C, light intensity 17.5 µmol m^{-2} s^{-1}.

	Mean of size ± S.D			
	Mastocarpus sporelings control		*Mastocarpus* sporelings with *Fucus* germlings	
	Mean diameter (µm)	Mean surface area (µm^2)	Mean diameter (µm)	Mean surface area (µm^2)
Clean surface	80.7±3.6	5114.9	55.4±3.9	2410.5
Plus diatom mucilage	100.9±7.3	7996	75.8±9.3	4512.6

and whilst *Mastocarpus* sporelings grown with *Fucus* on clean glass showed a 45% growth inhibition, those on the diatom slime with *Fucus* showed 24% growth inhibition. Table 5.3.2 shows that *Fucus* germlings on diatom slime were shorter and thinner than those on clean glass, but with longer rhizoids. Whilst the *Fucus* germlings on clean glass were of a similar size in the presence of the *Mastocarpus* sporelings, those *Fucus* germlings grown on diatom slime with *Mastocarpus* were of similar length, but thinner and with much longer rhizoids. Terminal hairs were absent. Table 5.3.3 shows the mean diameter size of *Mastocarpus* sporelings with *Porphyra* sporelings on a clean surface, in which their was some inhibition when compared with control *Mastocarpus* sporelings. *Mastocarpus* sporelings grown on diatom slime were again larger in size than those on clean glass, and whilst sporelings of *Mastocarpus* grown with *Porphyra* on clean glass showed a 20% growth inhibition, those on diatom slime with *Porphyra* showed 23% growth inhibition. Table 5.3.4 shows that the surface areas of *Porphyra* were similar with all the treatments given.

Mastocarpus sporelings on diatom mucilage can produce sporelings of irregular shapes (Fig. 5.3.1B), whilst the sporelings on clean glass surface were normal and disc-like (Fig. 5.3.1A). Whilst growth of the rhizoids of *Fucus spiralis* was induced by the diatom mucilage, the apical hairs of *Fucus* in the presence of the diatoms did not grow both with germlings growing alone on diatom slime and germlings with *Mastocarpus* sporelings on diatom mucilage (Fig. 5.3.1D, F), whilst on the clean surface terminal hair production was observed (Fig. 5.3.1C). Sporelings with *Fucus* germlings on diatom mucilage were of irregular shapes , but normal on clean surfaces (Fig. 5.3.1E, F), and *Mastocarpus* sporelings with *Porphyra* on diatom slime were of irregular shapes , but on clean glass were normal (Fig. 5.3.1G, H). Both *Porphyra* with *Mastocarpus* sporelings and control *Porphyra* sporelings on diatom slime developed long rhizoids (Fig. 5.3.1H, J), but *Porphyra* with *Mastocarpus* sporelings and control *Porphyra* on clean glass developed short rhizoids (Fig. 5.3.1G,I).

5.4. Discussion

Table 5.3.2. Germination and growth of germlings of *Fucus spiralis* with *Mastocarpus* sporelings on clean surface and on diatom mucilage after 23 days. Duplicate samples of 25 germlings measured. Temperature 10±2 ^{o}C, light intensity 17.5 µmol m^{-2} s^{-1}. L, germling length; W, germling width; R, length of germling rhizoids; T, length of germling terminal hairs. NF, not found.

	Mean of size ± S.D							
	Mean measurements of germlings of *Fucus* (control) (µm)				Mean measurements of germlings of *Fucus* with *Mastocarpus* sporelings (µm)			
	L	W	R	T	L	W	R	T
Clean surface	466±4	201.6±25.2	651±147	222.6±96.6	415.8±163.8	172.2±4	445±25	352.8±17
Plus diatoms mucilage	336±50	113.4±29.4	840±84	NF	441±21	117±25	945±21	NF

Table 5.3.3. Germination and growth of sporelings of *Mastocarpus stellatus* with sporelings of *Porphyra umbilicalis* after 23 days on clean surface and on diatom mucilage. Duplicate samples of 25 sporelings measured. Temperature 10 ± 2 ^{o}C , light intensity 17.5 µmol m^{-2} s^{-1}.

	Mean of size ± S.D			
	Sporelings of *Mastocarpus* control		Sporelings of *Mastocarpus* with *Porphyra*	
	Diameter (µm)	Surface area (μm^2)	Diameter (µm)	surface area (μm^2)
Clean surface	78.7±17	4864.5	62.9±11.2	3107.4
Diatom mucilage	95.9±9.4	7223.2	73.9±12.9	4289.2

Table 5.3.4. Germination and growth of sporelings of *Porphyra umblicalis* with *Mastocarpus stellatus* after 23 days on clean surface and on diatoms mucilage. Duplicate samples of 25 sporelings measured. Temperature $10 \pm 2\ ^{o}C$, light intensity 17.5 $\mu mol\ m^{-2}\ s^{-1}$.

	Mean surface area of *Porphyra* control (mm^2)	Mean surface area of *Porphyra* with *Mastocarpus* (mm^2)
Clean surface	$1.8X10^{-6}\pm0.5X10^{-6}$	$1.3X10^{-6}\pm3.7X10^{-6}$
Plus diatoms mucilage	$1.6X10^{-6}\pm0.4X10^{-6}$	$1.3X10^{-6}\pm0.4X10^{-6}$

Fig. 5.3.1. Showing growth and development of sporelings and germlings with diatoms after 23 days.

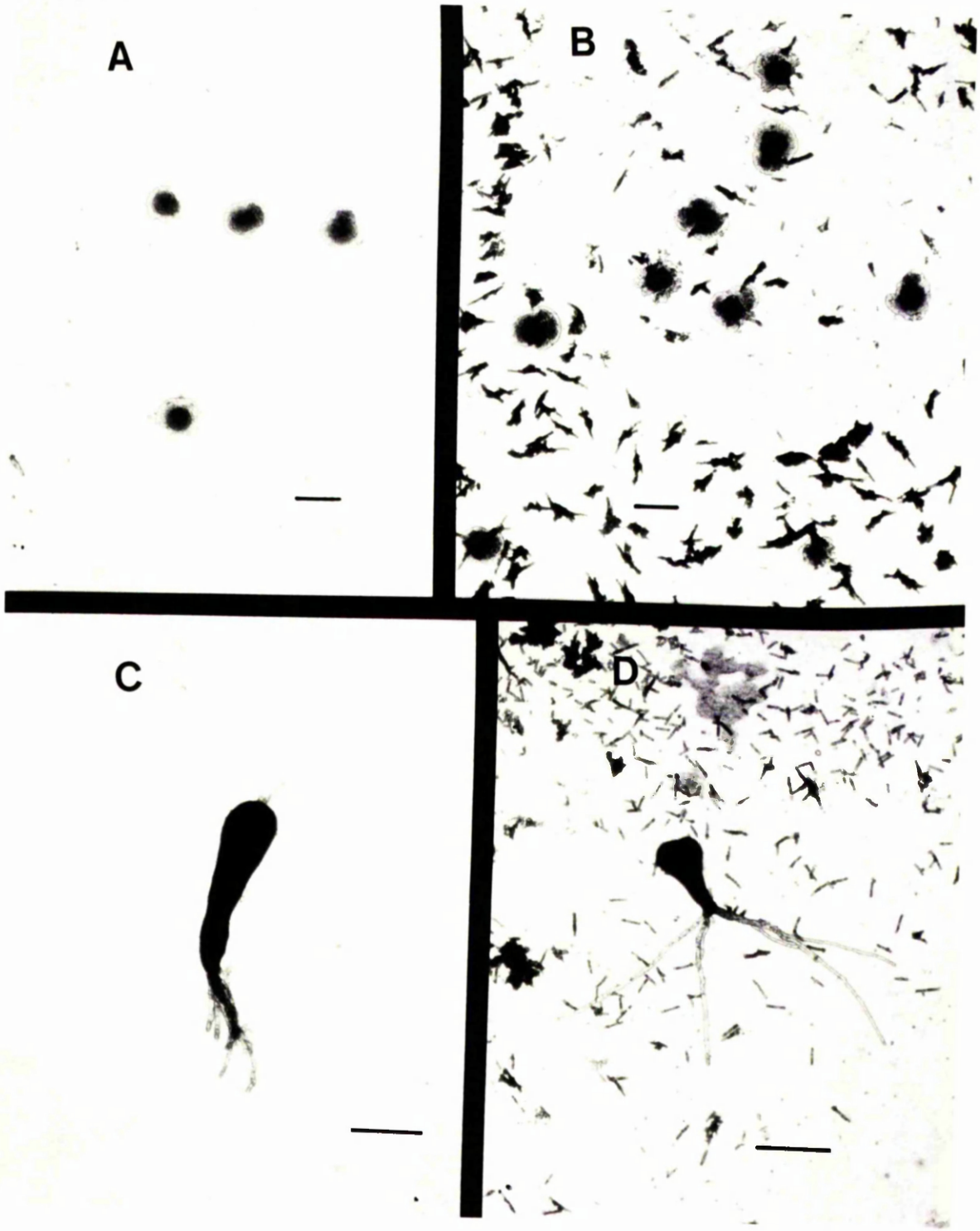
A, B, *Mastocarpus stellatus*; A, on clean surface; B, on diatom mucilage. Scale bar = 81 μm, and 101 μm.

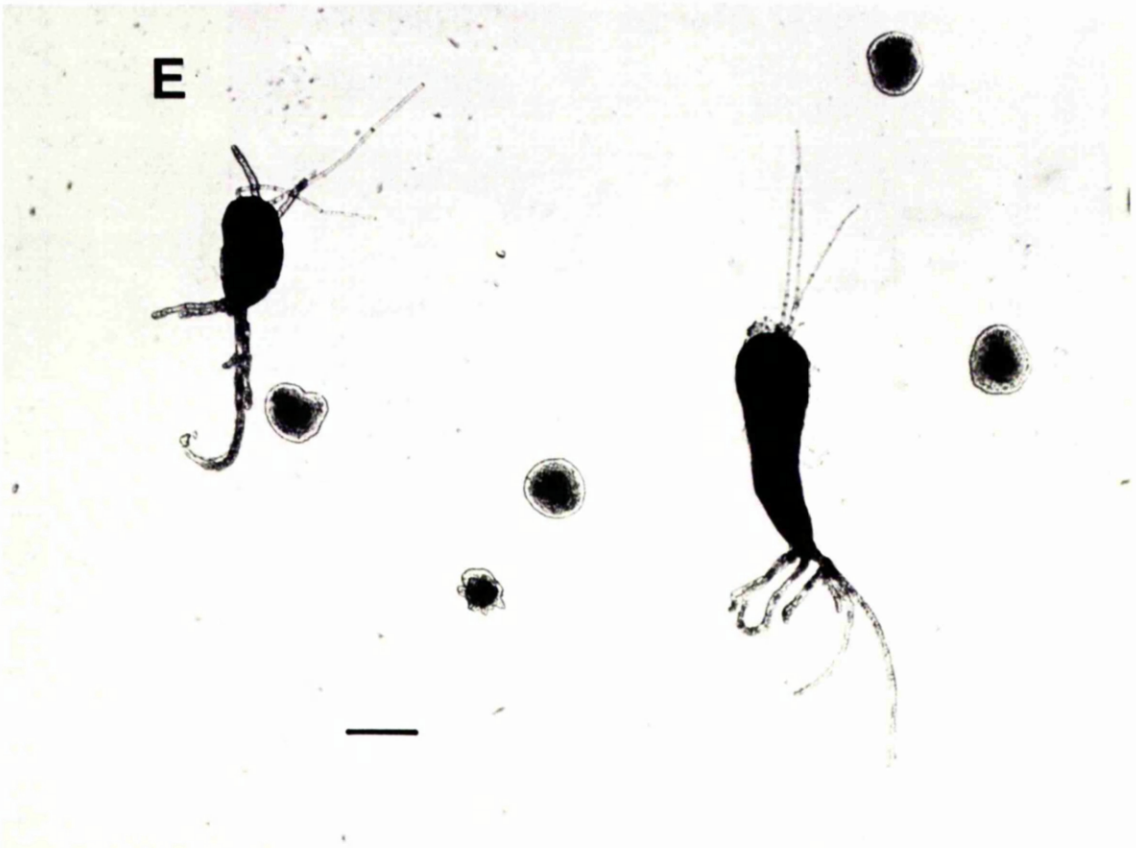
C, D, *Fucus spiralis* germlings; C, on clean surface; D, on diatom mucilage. Scale bar = 186 μm, and 280 μm.

E, F, *Mastocarpus* sporelings and *Fucus* germlings ; E, on clean surface; F, on diatom mucilage. Scale bar = 56 μm, and 76 μm.

G, H, *Mastocarpus* sporelings and *Porphyra umbilicalis*; G, on clean surface; H, on diatom mucilage. Scale bar = 63 μm, and 74 μm.

I, J, *Porphyra* sporelings ; I, on clean surface; J, on diatom mucilage. Scale bar = 3 mm.


A
B
C
D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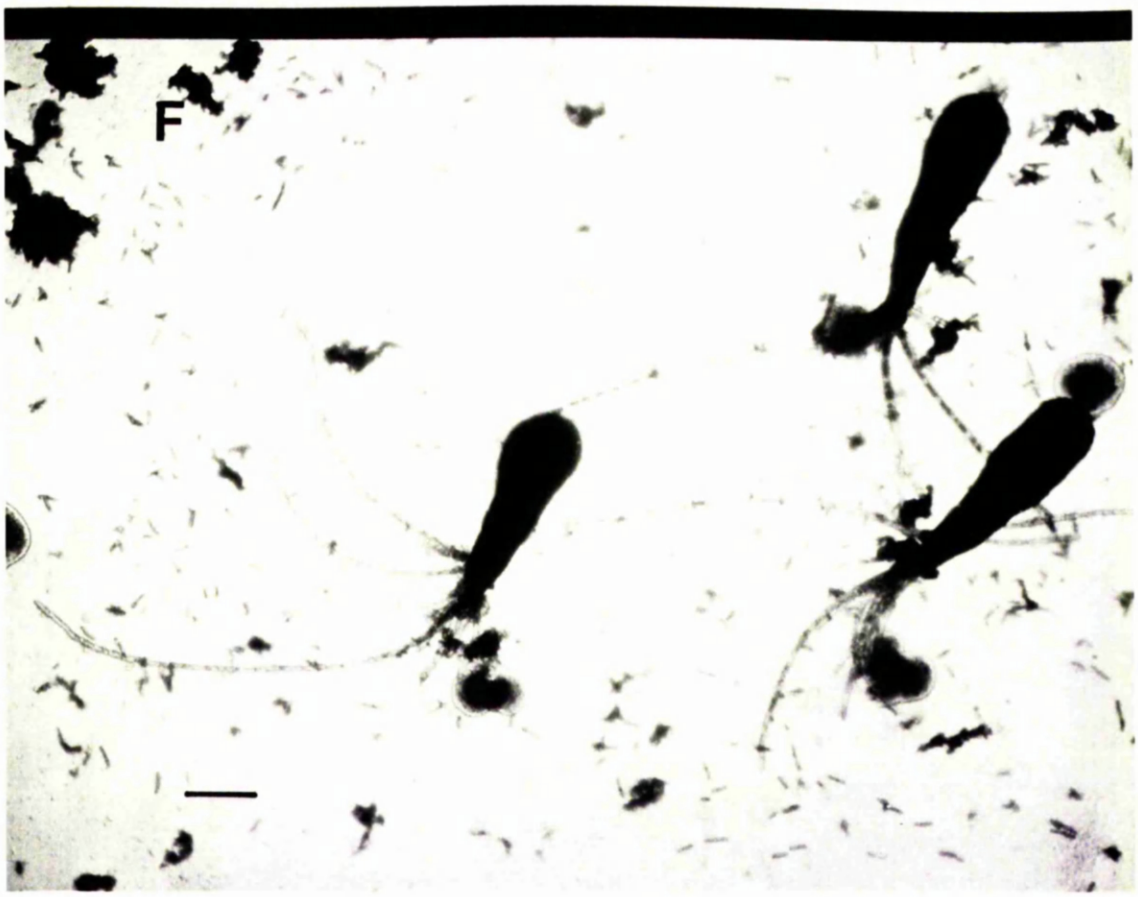
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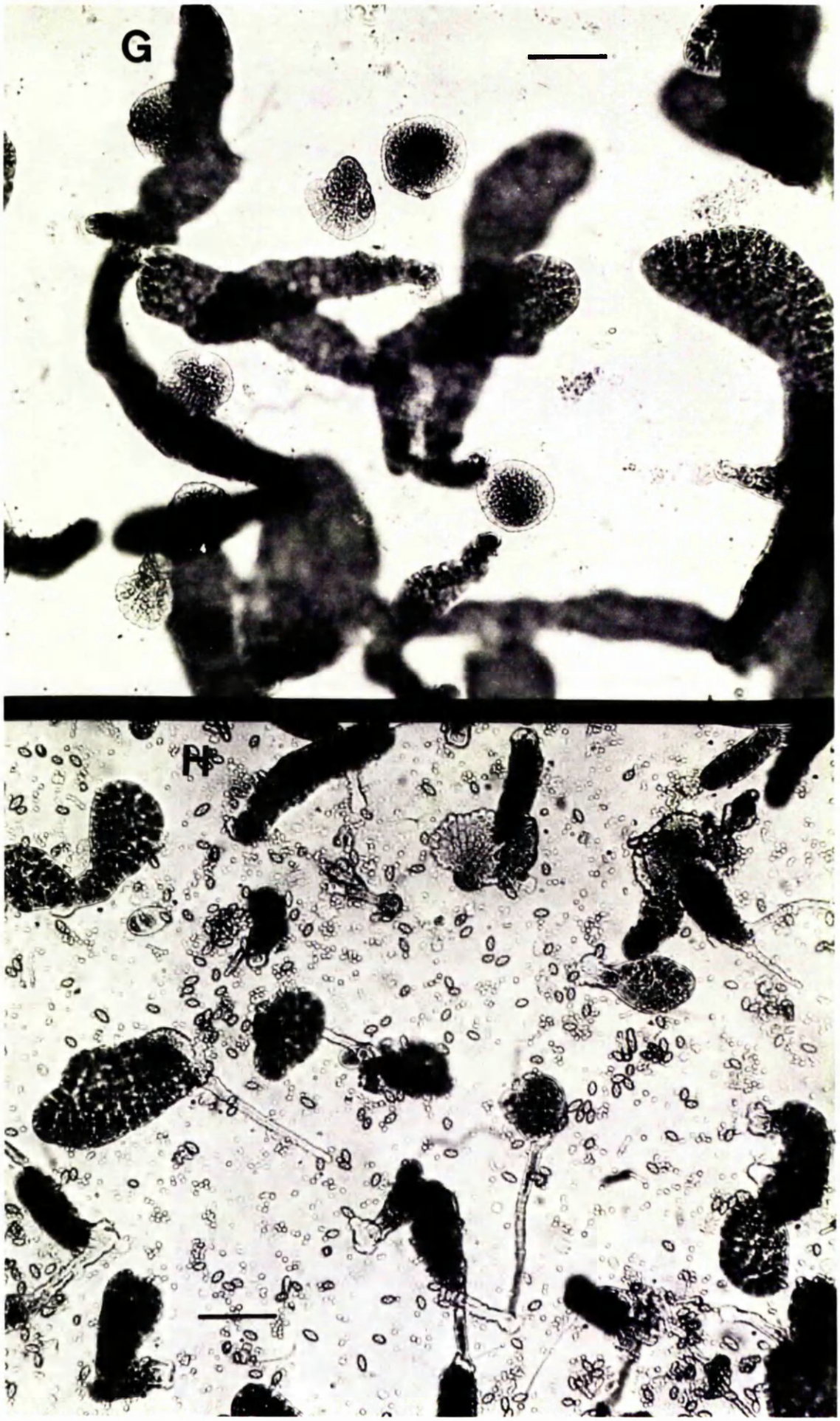
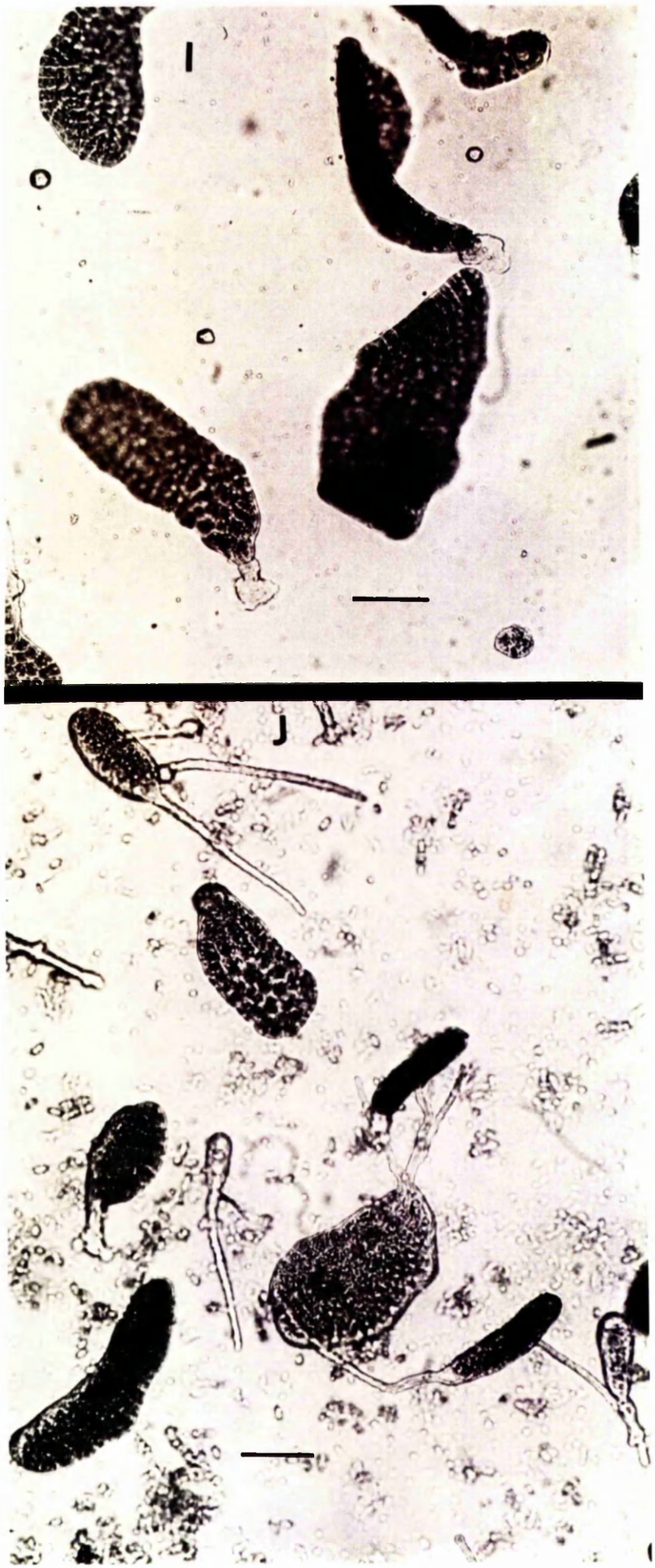

G
PH


I
J

The effect of diatom mucilage on growth and developmental morphology varied with the species observed. The mean size of *Mastocarpus* sporelings grown on diatom mucilage with and without *Fucus* were larger than those on a clean surface. The diatom mucilage affected both rhizoids and germling development with *Fucus*. Quatrano (1968) showed that the presence of ribonucleic acid and protein were important pre-requisites in rhizoid formation. Adhesion in brown algal germlings is dependent upon production of the sulphated fucoidan in the rhizoidal cells (Forbes and Hallam, 1979; Quatrano and Cryton, 1973). In this experiment increased rhizoid production and their growth in length was shown with germlings of *F. spiralis* on diatom slime. As shown by Huang and Boney (1983) increased rhizoids production was observed with germlings of *F. spiralis* when contact with dried diatom mucilage. The *Porphyra* sporelings also showed increased rhizoid production and growth in length on diatom slime. It would seem that the diatom mucilage interferes with the attachment process to the extent that it is only by increasing the surface area of the rhizoids in contact with the substratum that the young plants become fixed.

Mastocarpus sporelings grown on diatom mucilage tended to produce some irregular shapes. Huang and Boney (1983) also showed that dried diatom mucilage produced irregular disc-like sporelings with *Mastocarpus* (*Gigartina*) sporelings.

The diatoms affected the development and morphology of sporelings and germlings. These experiments showed that the mucilage containing living diatoms can induce growth changes. *Mastocarpus* sporelings are larger on the mucilage whether on their own or with *Fucus* germlings. The fact that they tend to be less regular in shape on the diatom slime may be a further example of the attachment and anchorage problem as seen with the *Fucus* rhizoids. *Fucus* germlings induce growth inhibitions with the *Mastocarpus* which are probably due to chemical interactions. The reduced growth of *Mastocarpus* with *Porphyra* sporelings is probably due to the shading effect of the membranaceous juvenile *Porphyra* plants. These results agree with Huang and Boney (1983) indicating that living and dried diatom mucilage affects the attachment and morphogensis of algal sporelings and germlings.

6. Observation on photosynthesis by the reproductive structures, spores and sporelings of *Mastocarpus stellatus*.

6.1. Introduction

The general equation for photosynthesis in plants can be written as:

$$CO_2 + 2H_2O \xrightarrow[\text{chlorophyll}]{\text{light}} (CH_2O) + H_2O + O_2,\ \Delta G = 469\ \text{KJ mole}^{-1}.$$

(CH_2O) represents stored carbohydrate. The reaction requires 469 KJ $mole^{-1}$ for each hexose molecule produced, when CO_2 is reduced to CH_2O and water is oxidized to O_2 by dehydrogenation. The oxygen derived in photosynthesis thus comes from the water rather than from CO_2. The chloroplast pigments are involved in the light reactions of photosynthesis, especially the capture of photosynthetically active radiation (**PAR**). Seaweeds are obligate photolithotrophs wholly dependent upon ambient light energy for synthetic metabolism. In nature seaweeds can be found in different photic zones. Thus the light reaching these plants is variable in quantity, quality, and duration. The ability of these plants to capture **PAR** is the means to growth, reproduction, physical adaptation and biological interaction. The process of photosynthesis is more complicated than it appears from the general equation above. The release of O_2 cannot be directly related to CO_2 fixed in the process.

Algal material has been used in pioneering photosynthetic studies, *Spirogyra* sp. (Chlorophyta) was used by Engelmann in 1883 to demonstrate that the chloroplast is the seat of photosynthesis and by the use of aerotactic bacteria demonstrated the accompanying oxygen release from the cells, a simple demonstration of an action spectrum (Hipkins 1985). Whilst chlorophyll a is common to all red algae the principal accessory pigments are carotenoids and the phycobilins, phycoerythrins (red) and phycocyanins (blue). Comparisons of the absorption spectra (light absorbed by thallus) with the action spectra (those wavelengths which promote photosynthesis, the photosynthetically active radiation **PAR**), and with the absorption spectra of the

extracted pigments, have shown that for red algae the action spectrum follows closely the absorption spectrum of the phycobilin pigments (Haxo and Blinks, 1950; Luning in Dring, 1982).

Red algae are known to inhabit shaded habitats more than other seaweeds. This is due to the role of accessory pigments as wavelength antennae present in their photosynthetic apparatus, and their ability to adjust the quantities of these pigments under different light intensities.

The concept of chromatic adaptation, according to Engelmann's (1883) proposals, is based on the view that the pigmentation of the major groups of algae is an adaptation (complementary) to the depths at which they are found in the oceans. Thus the red algae in the deeper water would be illuminated by the deeper penetrating green light, and the green algae would be commonly found in shallower waters. For some time this viewpoint held sway (e.g. Tilden, 1935). However, Larkum *et al* (1967) showed that for a vertical submarine face in the sea off Malta, the upper 15 m of the subtidal zone was dominated by brown algae and that the green algae were found down to 75 m depth. The red algae were numerous in shaded subtidal habitats. Levring (1968) showed that the photosynthesis of green marine algae was considerable at depths below 30 m in a Scandinafian Fjord. Schneider (1976) found that 25% of the green algae were to be found below 50 m in the Carolinas, and Doty *et al* (1974) found in Hawaiian coastal waters that the green algae progressively increased in numbers with depth, being the most numerous at 90 m. More recent viewpoints are that chromatic adaptation is not supported by the evidence from either physiological activity or distributional studies (Ramus, 1981; Dring, 1981). A green alga *Johnson-sea-linkia profunder*, at 157 m depth is the deepest growing fleshy alga (Littler *et al*, 1985), whilst encrusting red algae and green algae have been found at greater depths. Any adaptations which may occur are now thought to be due to decreased irradiances more than spectral distribution changes (Ramus *et al* 1976). Kirk (1986) has stated that algal depth distributions are controlled by other factors (temperature, grazing resistance, and substrata) as well as light. Yu *et al* (1981) stated that phycoerythrin in red algae showed virtually identical light absorption spectra. Comparison of the typical

phycoerythrin of red algae showed the presence of different bilins. These differences in the relative amounts of the bilin prosthetic groups account in large measure for the differences between the absorption spectra of the native proteins. The ratio of phycourobilin (**PUB**) and phycerythrobilin (**PEB**) can be modulated by varying the light intensity during growth.

Photosynthesis in the red alga *Gigartina harveyana* was studied by Emerson and Green (1934) who used a piece of *Gigartina* weighing less than 150 mg, suspended in 8 ml of normal sea water. Photosynthesis rose to a maximum in about 15 minutes, and soon afterwards entered an almost linear decline. This decline in the rate of photosynthesis was not due to irreversible injury from experimental conditions, but to a steady fall in the carbon dioxide concentration because of its removal in photosynthesis. Mathieson and Burns (1971) measured the photosynthesis and respiration of *Mastocarpus stellatus* (as *Gigartina stellata*) using discs 6 mm in diameter removed from the thin flattened portions of the thalli. The rates of oxygen exchange of the sample were recorded in a Gilson Differential Respirometer. Results of this study showed that the light saturation intensity at (approx. 420 $\mu E\ m^{-2}\ s^{-1}$). of *Mastocarpus stellatus* was higher than that of eulittoral plants. Maximum rates of photosynthesis and respiration were recorded at 20 ^{o}C and 40 ‰. Khfaji (1978) measured oxygen liberation rates of plants of *Mastocarpus stellatus* (as *Gigartina stellata*) from 3 different localities in Firth of Clyde. He found that rates of photosynthesis of plants from the sheltered Loch Long were lower than for plants from Cumbrae Island and Portencross, both more exposed localities. Plant dried for 12 hours showed that the rates of photosynthesis of plants on re-immersion from Portencross were much higher from the other localities. Rates of photosynthesis at different salinities were also studied. Rates of oxygen release increased gradually from 4‰ to 32‰.

In the present work some observations were made on the photosynthesis of spores, sporelings and reproductive structures of *Mastocarpus* using the oxygen electrode (p. 29), and of fertile and non-fertile fronds using Winkler's method of measuring O_2 yield (p.20).

6.2. Results

6.2.1. Spore suspensions

Measurements of oxygen release for spore suspensions proved difficult in that large quantities of released spores had to be used to obtain a detectable measurement using the oxygen electrode. These quantities prevented satisfactory replication of measurements. It was also found that in all cases the spore suspension failed to liberate oxygen, and that the oxygen content of the sea water steadily decreased. Measurements were therefore made of the absorption of oxygen by the spore suspension. Fig.6.2.1.1a-c compares the rates of oxygen uptake by (a) spores utilised immediately after their release from fertile papillae; (b) released spores left undisturbed for 15 h at 15 ^{o}C in light; (c) released spores continuously shaken by means of a laboratory shaker for 24 h at 10 ^{o}C in the light. This last treatment might be considered more typical of conditions in nature. If the uptake of oxygen is to be regarded as an expression of spore metabolism, then the results summarized on Fig. 6.2.1.1 would suggest that the metabolic rates of suspended spores increase with time, and were much higher in suspensions kept circulating in the water prior to the experiment. In a further experiment with a spore suspension taken immediately after release the rates of oxygen uptake were examined in the light, and with an intervening dark period (Fig. 6.2.1.2). The rate of oxygen uptake increased significantly with the onset of the dark period, and tended to continue at a similar rate with the return of lighted conditions.

6.2.2. Experiments with sporelings

The previous experiments with suspended spores indicated that their metabolism involved more uptake of oxygen than release by photosynthesis. Spores on settlement take 1-2 days to attach to substrata. It was impossible to obtain measurements of the photosynthetic activity of newly attached spores and the early sporelings stages. Hence sporelings of 3-4 months of age were mainly used, since these are large enough to be detached from the glass surface. By trial experiments it was found that 30-40 sporelings of this age gave oxygen yields measurable with the oxygen electrode.

Fig. 6.2.1.1. Oxygen uptake by a *Mastocarpus* spore suspensions at 20 ^{o}C and quantum irradiance of 11.6 μmol m^{-2} s^{-1}.

a- ⊡ spores measured immediately after release.

b- ● spores left 15 h in the light before measurement at 15 ^{o}C.

c- ⊡ spores continuously shaken for 24 h at 10 ^{o}C in the light before measurement in the oxygen electrode.

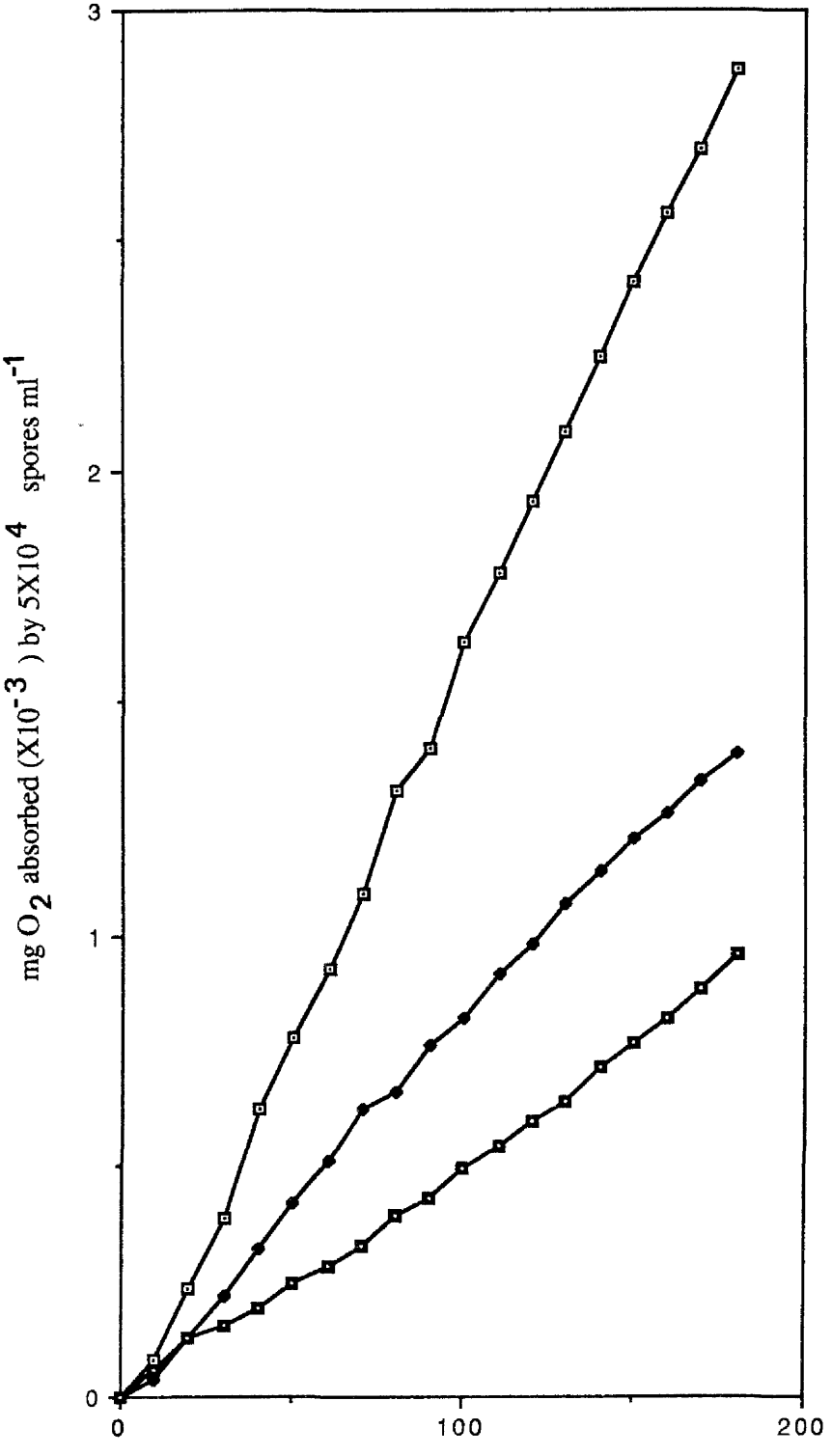
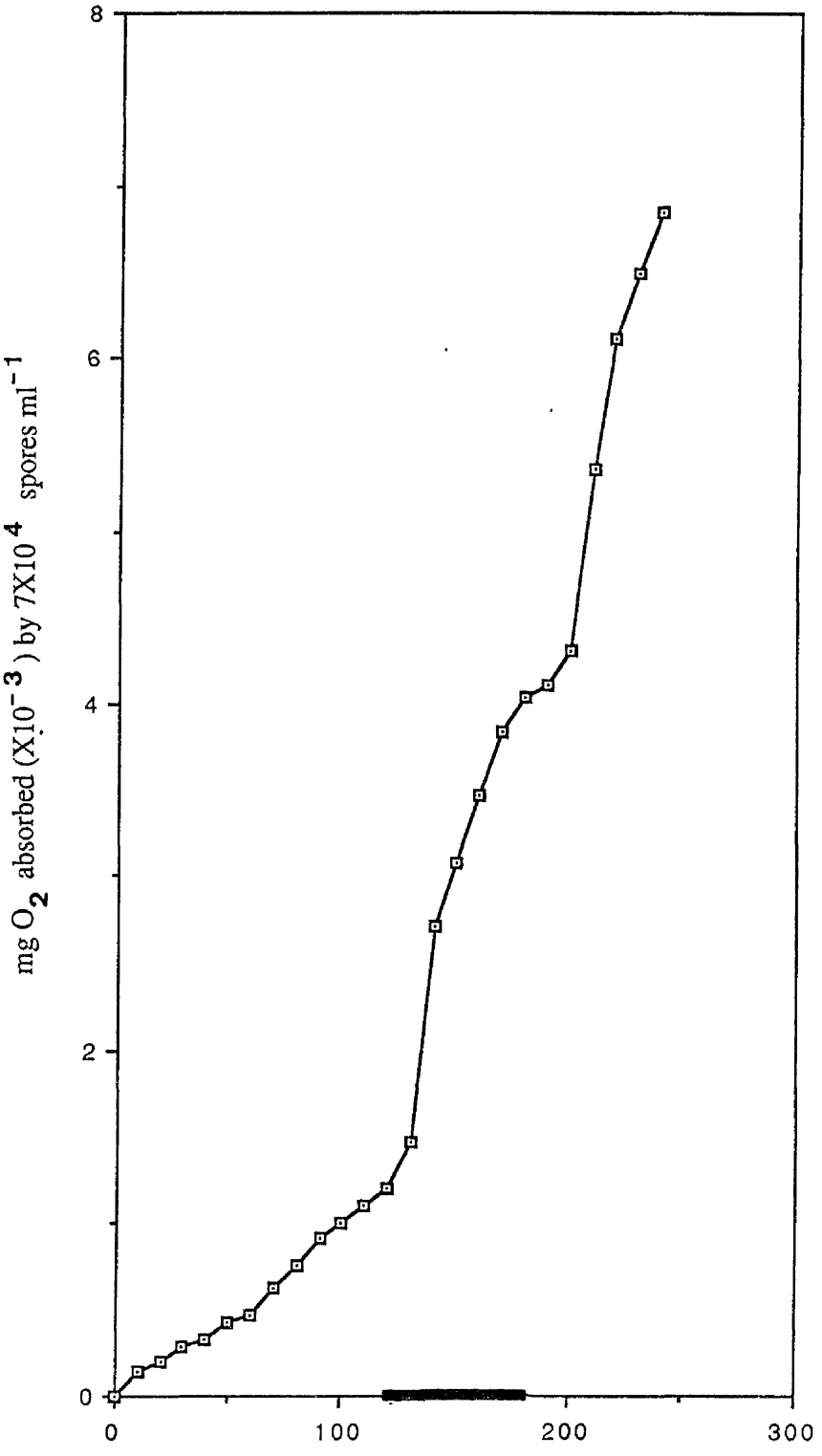

3
2
1
0
0
100
200
mg O_2 absorbed ($X10^{-3}$) by $5X10^4$ spores ml^{-1}
Time in minutes

Fig. 6.2.1.2. Oxygen uptake by a *Mastocarpus* spore suspension take immediately after release, measured in the light with an intervening dark period (temperature 20 ^{o}C and quantum irradiance of 11.6 μmol m^{-2} s^{-1}).


8
6
4
2
0
0
100
200
300
mg O_2 absorbed ($X10^{-3}$) by $7X10^4$ spores ml^{-1}
Time in minutes

Sporelings were detached by means of glass needles. Only undamaged sporelings were used, i.e those sporelings which showed evidence of tearing of their structure were discarded. For this reason it was not always possible to replicate experiments. The methods of measuring sporeling surface areas have been described (p. 15).

In all of the experiments the sporelings were suspended in circulating sea water in the electrode cell. Both upper and lower surfaces were thus in contact with the surrounding medium, whilst in nature the upper surface of the attached sporelings is the principal means of light and nutrient absorption. Whilst chloroplasts are to be found in the basal attaching cells of the sporelings, the chloroplasts are much larger in the uppermost cells (Fig. 6.2.2.1). Whilst the total sporeling surface areas have to be taken into account in all measurements, the greater quantity of photosynthetic activity is probably from the uppermost regions of the sporelings. In nature the sporelings are stationary on rock substrata, with water flowing over them. In the experiments with the oxygen electrode the sporelings circulated in suspension , but this water movement would more closely resemble the situation in nature. The need for water circulation to maintain measurable photosynthesis by the sporelings was demonstrated when the circulation control (the magnetic 'flea') was switched off for 30 min. during the course of an experiment (Fig. 6.2.2.2). There was an immediate fall in the rate of the oxygen release by the now settled sporelings. When circulation was restarted the rate of oxygen release returned to the previous level, and continued to increase at a rate similar to that obtained before the circulation of the water was stopped. In such 'still water' situations the photosynthetic activity of the sporelings was reduced to the levels which could not be measured with the oxygen electrode.

Fig. 6.2.2.3 shows the rates of oxygen release of sporelings in light and dark periods. Sporelings in light after 50 min. had produced $1.3X10^{-4}$ mg O_2 mm^{-2} surface area, and at 90 min. $2.3X10^{-4}$ mg O_2 mm^{-2}. The sporelings were then kept in dark the for 30 min., when the oxygen level fell to $1.85X10^{-4}$ mg O_2 mm^{-2}, probably due to respiration. With sporelings again in the light they immediately responded and the rate of oxygen release after 50 min. was $2.95X10^{-4}$ mg O_2 mm^{-2}, and at the end of measurements the sporelings showed a rate of oxygen release of $3.84X10^{-4}$ mg O_2

Fig. 6.2.2.1. Section of sporelings showing chloroplasts in basal cells. Scale bar = 16 µm

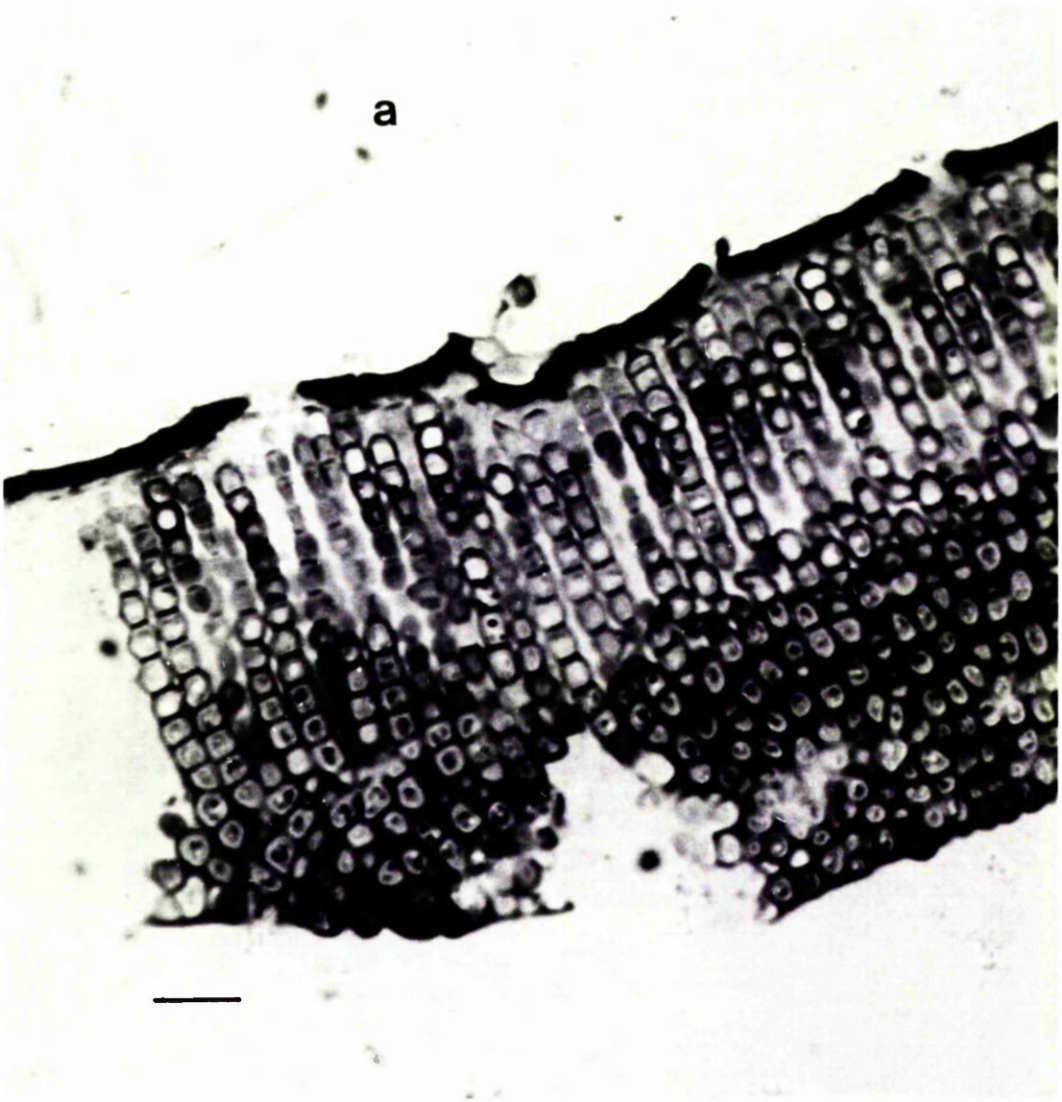

a

Fig. 6.2.2.2. Oxygen release by *Mastocarpus* sporelings when circulating in the electrode cell and when settled. Sample too small to allow replication. 1= magnetic 'flea' stopped; 2= magnetic 'flea' started again.

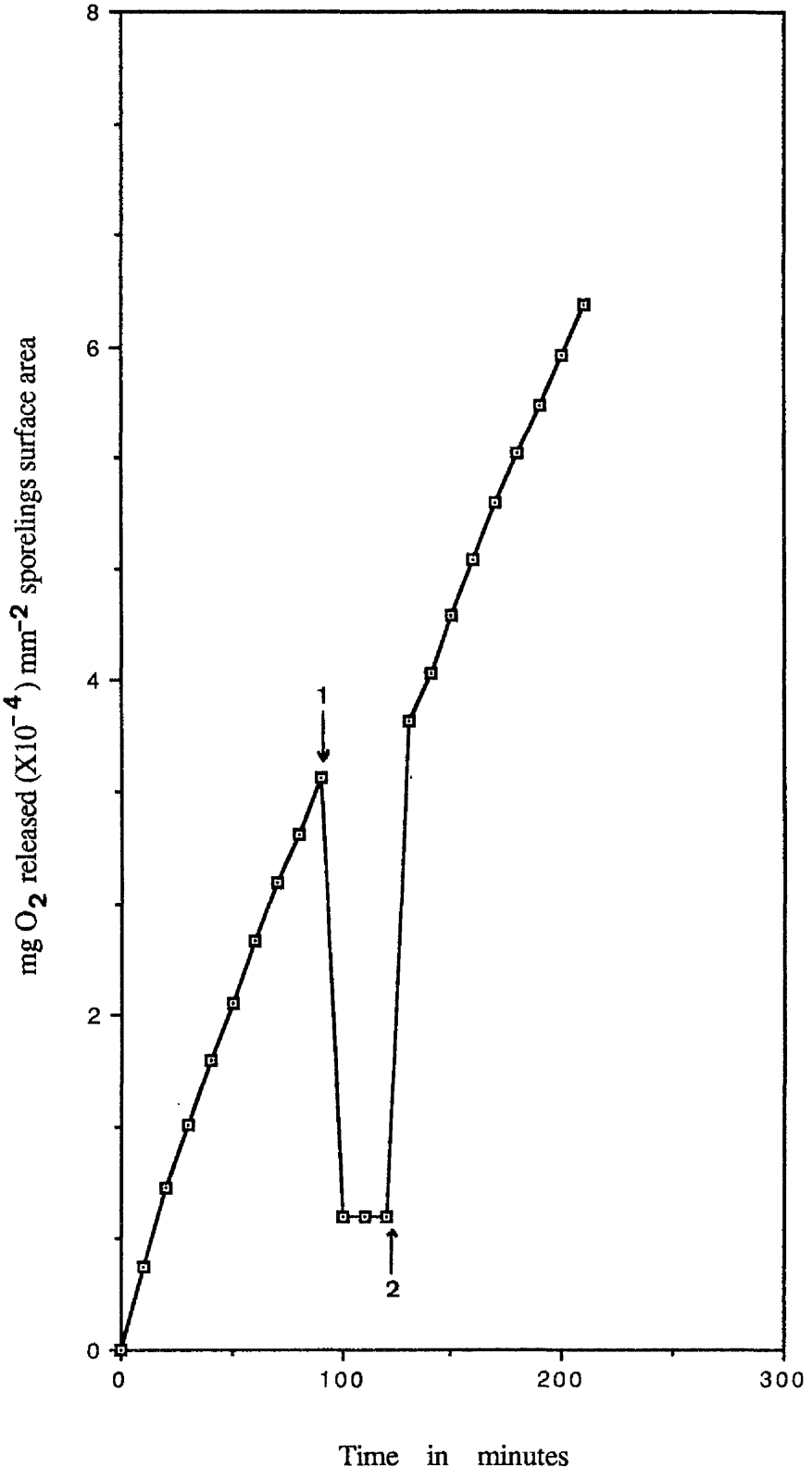
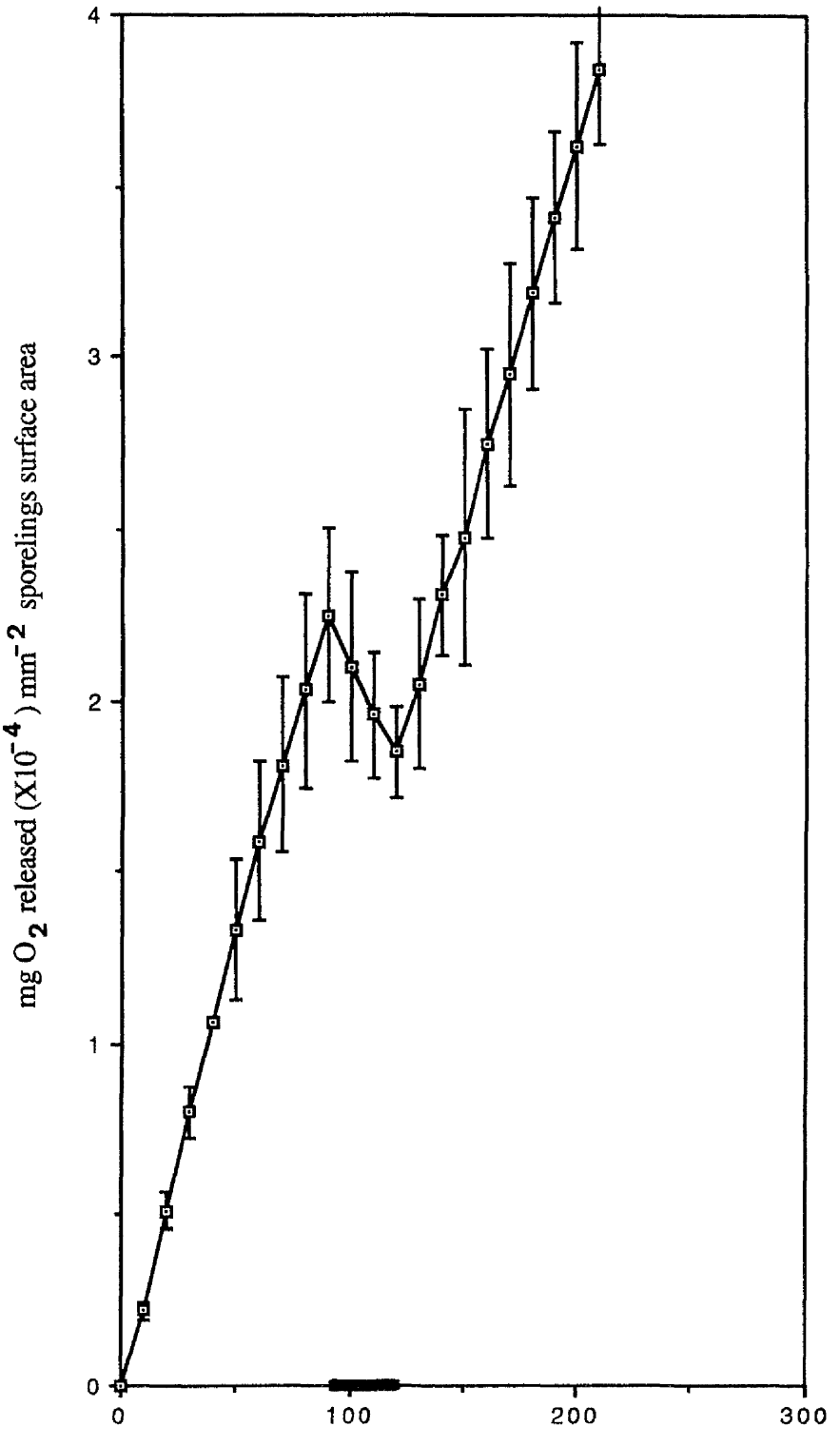

8
6
4
2
0
0
100
200
300
1
2
mg O_2 released ($X10^{-4}$) mm^{-2} sporelings surface area
Time in minutes

Fig. 6.2.2.3. Oxygen release by *Mastocarpus* sporelings in light and dark periods (temprature 20 ^{o}C and quantum irradiance of 11.6 μmol m^{-2} s^{-1}). Two samples measured.


4
3
2
1
0
0
100
200
300
mg O_2 released ($X10^{-4}$) mm^{-2} sporelings surface area
Time in minutes

mm^{-2}. The results suggest that the sporelings can immediately assume a similar photosynthetic rate after the of intervention a dark period.

Fig. 6.2.2.4 shows the rates of oxygen release at different quantum irradiances. In these experiments layers of muslin were used as neutral filters. After the initial 'surge' of photosynthetic activity in the first 20 minutes at 11.6 μmol m^{-2} s^{-1} irradiance (the quantum irradiance used in all other experiments), over the remaining 90 min. of the experiment the rate of oxygen production proceeded at a steady rate (mean production of $0.22\pm0.03X10^{-4}$ mg O_2 mm^{-2} sporeling surface area in each 10 minutes). With sporelings at an irradiance of 5.3 μmol m^{-2} s^{-1} in the first 50 min. the oxygen output was at a steady rate ($0.32\text{-}0.38X10^{-4}$ mg O_2 mm^{-2} sporelings surface area at 10 min. intervals), proceeding at a lower but steady rate ($0.15\pm0.06X10^{-4}$ mg O_2 mm^{-2}) for the remainder of the experiment. Sporelings at an irradiance of 2.5 μmol m^{-2} s^{-1} showed an even rate of O_2 production over the first 80 min. ($0.16\pm0.03X10^{-4}$ mg O_2 mm^{-2} per 10 min. interval), falling to 0.05- 0.09 mg O_2 mm^{-2} per 10 min. in the remaining 40 minutes of the experiment. Sporelings at irradiance of 1.3 μmol m^{-2} s^{-1} failed to exhibit any measurable photosynthetic activity in the first ten minutes, and over the next 30 min the oxygen output was low, but increased to a steady rate ($0.11X10^{-4}$ mg O_2 mm^{-2} at each 10 min. interval) for the remainder of the experiment. At 0.78 μmol m^{-2} s^{-1} there was no measurable photosynthetic activity over the period of experiment.

Fig.6.2.2.5 shows the results of experiments a which sporelings were subjected to irradiances of 11.6 μmol m^2 s^{-1}, and 242 μmol m^{-2} s^{-1}, a twentyonefold increase. Whilst the sporelings as expected, showed increased rates of photosynthesis at the higher irradiance the amount of oxygen released was just over double that at the lower light level. This suggests that there was light saturation at the higher irradiance.

The experiment with an intervening dark period showed that the sporelings immediately recovered when re-illuminated. The effects of longer dark periods at a lower temperature were examined (an attempt to simulate the more extreme conditions possible with sporelings in shade habitats in the depths of winter). Sporelings were kept in the dark for periods of 1-5 days at 5 ^{o}C and their photosynthetic activities

Fig. 6.2.2.4. Oxygen release by a *Mastocarpus* sporelings at different quantum irradiances. Two samples measured at each irradiance level.

- sporelings (as control) at irradiance of 11.6 μmol m^{-2} s^{-1}.
- sporelings at irradiance of 5.3 μmol m^{-2} s^{-1}.
- sporelings at irradiance of 2.5 μmol m^{-2} s^{-1}.
- sporelings at irradiance of 1.3 μmol m^{-2} s^{-1}.
- sporelings at irradiance of 0.78 μmol m^{-2} s^{-1}.

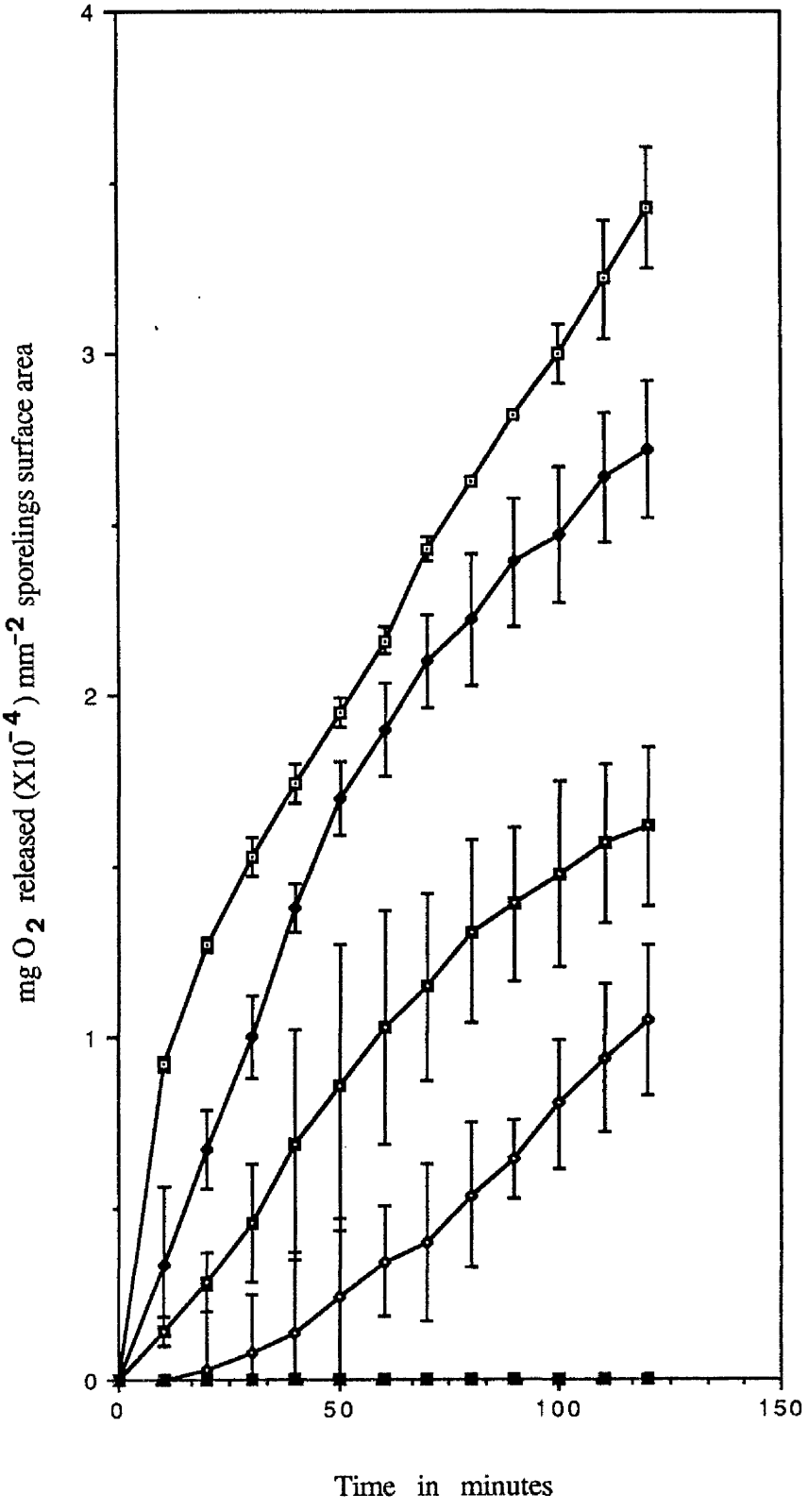
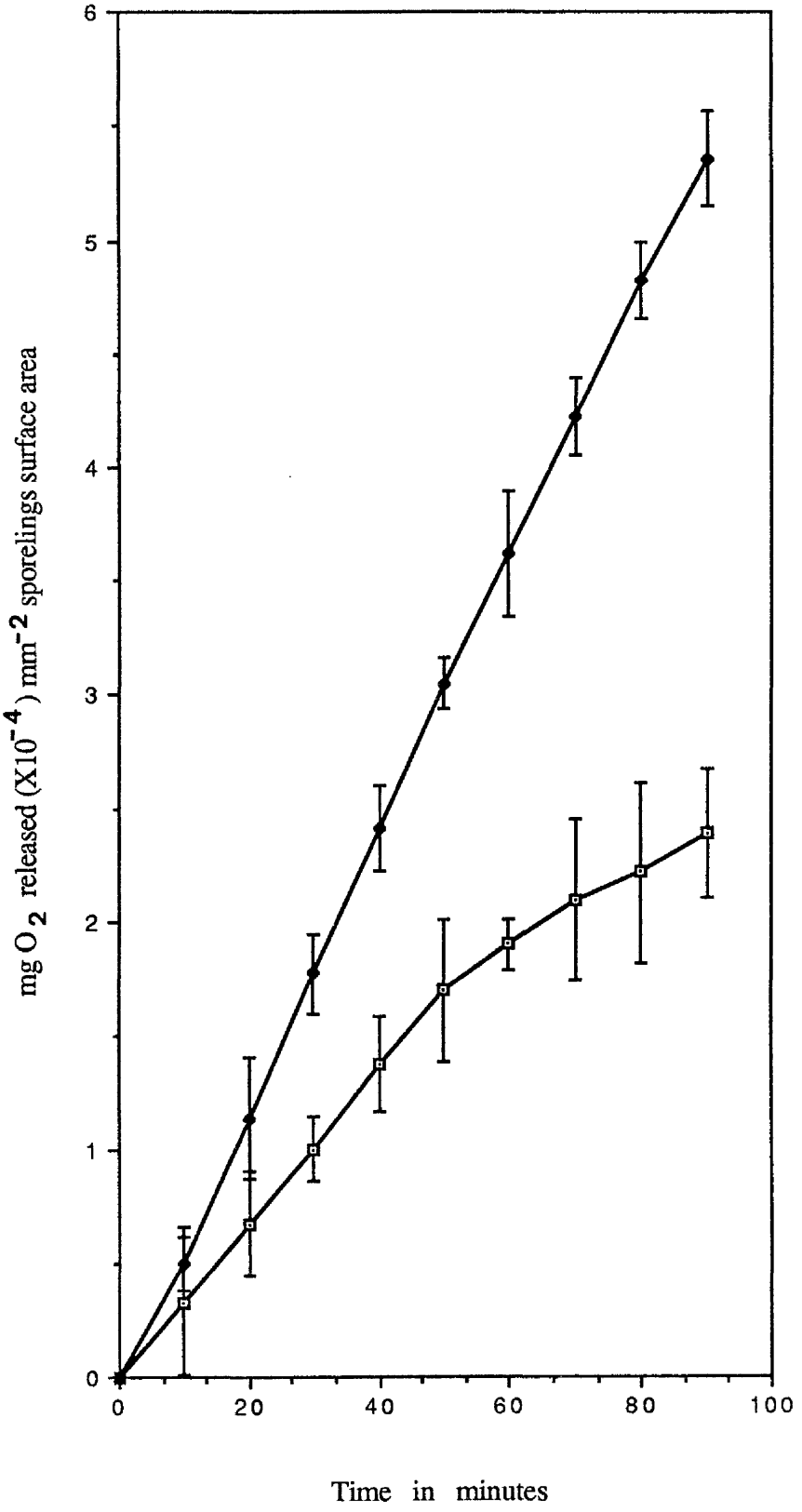

4
3
2
1
0
0
50
100
150
mg O_2 released ($X10^{-4}$) mm^{-2} sporelings surface area
Time in minutes

Fig. 6.2.2.5. Oxygen release by a *Mastocarpus* sporelings with low and strong light. Two samples measured at each irradiance level.

- at irradiance 11.6 μmol m^{-2} s^{-1}.
- at irradiance 242 μmol m^{-2} s^{-1}.


6
5
4
3
2
1
0
0
20
40
60
80
100
mg O_2 released ($X10^{-4}$) mm^{-2} sporelings surface area
Time in minutes

immediately measured at 20 °C and 11.6 μmol m^{-2} s^{-1}. The results are summarised in Fig. 6.2.2.6. These show that sporelings kept for 1-2 days in the dark exhibited enhanced photosynthetic rates compared with the controls (sporelings kept under normal culture conditions see p. 10) when placed in the warmer and lighted conditions of the oxygen electrode vessel. Sporelings kept for 3 and 4 days in the dark showed lower photosynthetic rates than the control sporelings, and those kept for 5 days in the dark failed to show any measurable photosynthetic recovery.

In time the attaching discs of the sporelings produce cylindrical erect branches. Experiments were carried out with 6 month old sporelings, each bearing one erect branch with lengths varying between 1.3-3.4 mm, and the photosynthetic activity compared for the attaching systems and the separated erect branches. Results are summarised in Fig. 6.2.2.7 and Table 6.2.2.1 These shown that the photosynthetic activity of the erect branches was significantly greater than that of the attaching systems, and that these differences in the rates remained much the same over the period of experiment.

Sea temperature fluctuations are probable environmental factors. In the Clyde Sea the winter minimum lies at about 6-7 °C, and the early autumn maxima about 15 °C. Localized increases of a higher order can take place during summer in rock pools left by the receding tide. The effects of temperature change on sporeling photosynthesis were investigated.

Fig. 6.2.2.8 shows the photosynthetic rates of sporelings measured directly at different temperatures (5, 20, 30, and 35 °C). With sporelings at 5 °C the higher photosynthetic activity was in the first 10 min., but thereafter proceeded at a lower but steady rate. As with all previous experiments, at 20 °C there was an even rate of oxygen production over the 90 minutes ($0.27\pm0.04X10^{-4}$ mg O_2 mm^{-2} sporelings surface area in each 10 min.). Sporelings at 30 °C showed increased photosynthetic activity in the first 10 min. ($0.39X10^{-4}$ mg O_2 mm^{-2}), but after this the oxygen production rate varied between $0.07\text{-}0.14X10^{-4}$ mg O_2 mm^{-2}. At 35 °C there was no measurable photosynthetic activity in all the period of experiment.

Fig. 6.2.2.9 shows rates of oxygen release of sporelings which were kept 6 h in

Fig. 6.2.2.6. Oxygen release by *Mastocarpus* sporelings kept under dark conditions for various periods (1-5 days) at 5 ^{o}C. (temperature 20 ^{o}C and quantum irradiances of 11.6 µmol m^{-2} s^{-1}). Two samples measured with each regime.

- sporelings kept in 'normal' light regime.(temprature 20 ^{o}C and quantum irradiances of 11.6 µmol m^{-2} s^{-1} and day length 16 h).
- sporelings kept 1 day in dark.
- sporelings kept 2 days in dark.
- sporelings kept 3 days in dark.
- sporelings kept 4 days in dark.
- sporelings kept 5 days in dark.

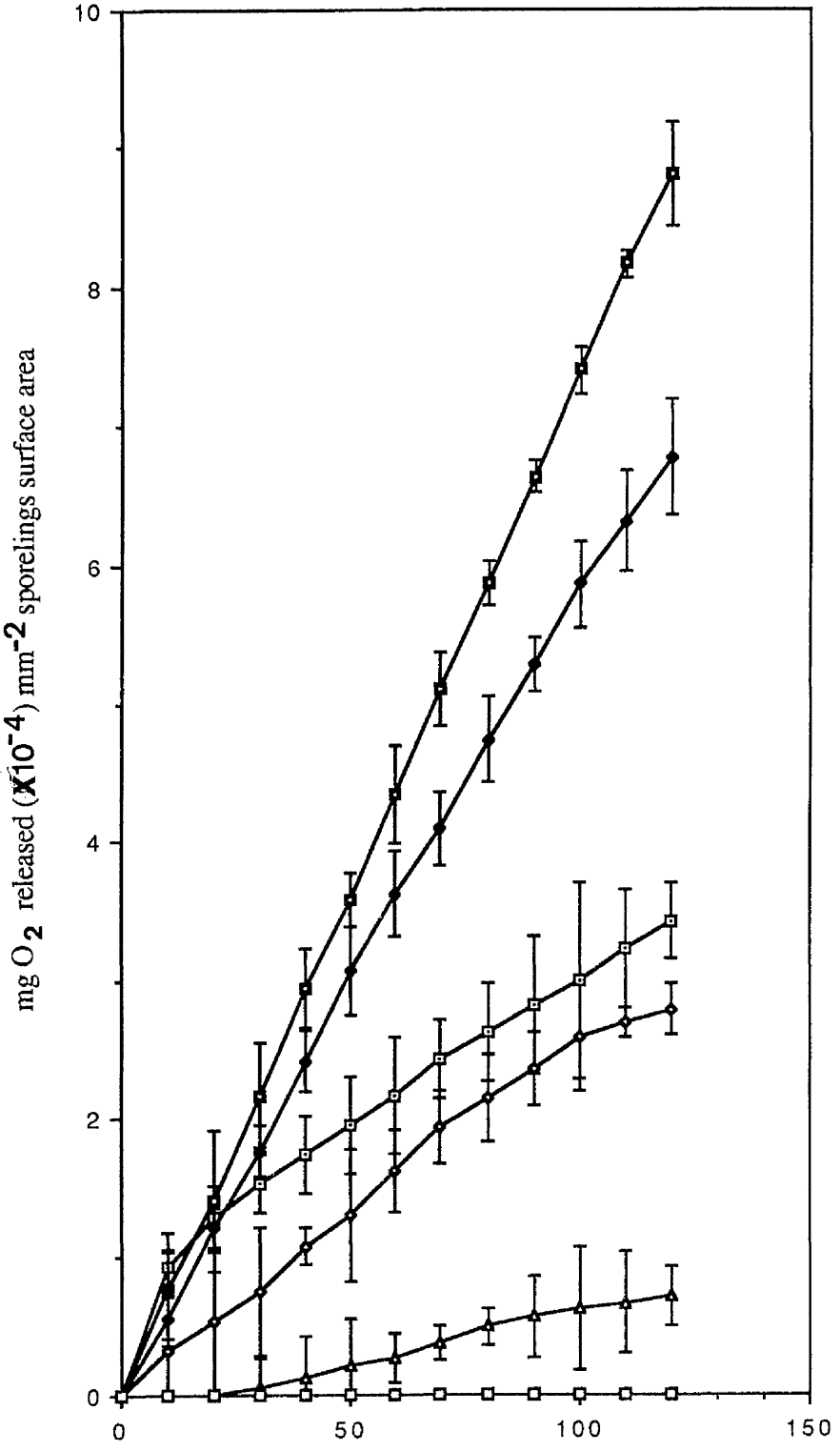

10
8
6
4
2
0
0
50
100
150
mg O_2 released ($\times 10^{-4}$) mm^{-2} sporelings surface area
Time in minutes

Fig. 6.2.2.7. Oxygen release by attaching discs of the sporelings and by the erect branches. (20 ^{o}C and quantum irradiance of 11.6 μmol m^{-2} s^{-1}). Two samples measured in each case.

-□- Attaching discs

-◆- erect branches

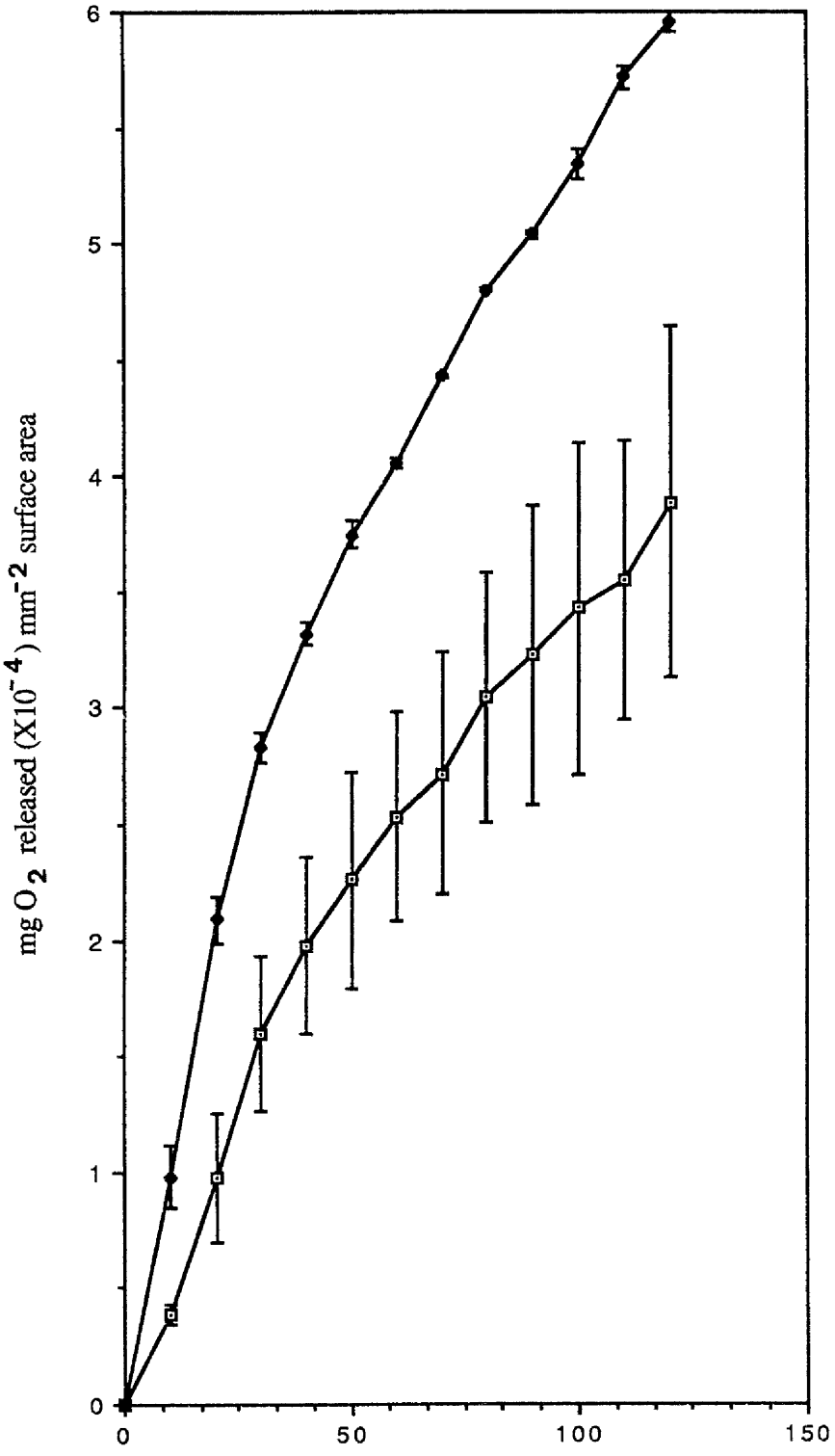

6
5
4
3
2
1
0
0
50
100
150
mg O_2 released ($X10^{-4}$) mm^{-2} surface area
Time in minutes

Table 6.2.2.1. Relative contributions of attaching discs and erect branches to overall photosynthesis based on total O_2 production mm^{-2} of surface.

	%	
Time (minutes)	Discs	Erect branches
10	29	71
20	32	68
30	36	64
40	37	63
50	38	62
60	38	62
70	38	62
80	39	61
90	39	61
100	39	61
110	38	62
120	39	61

Fig. 6.2.2.8. Photosynthetic rates of sporelings measured directly different temperatures (quantum irradiance of 11.6 µmol m^{-2} s^{-1}). Two samples measured at each temperature.

- 5 °C
- 20 °C
- 30 °C
- 35 °C

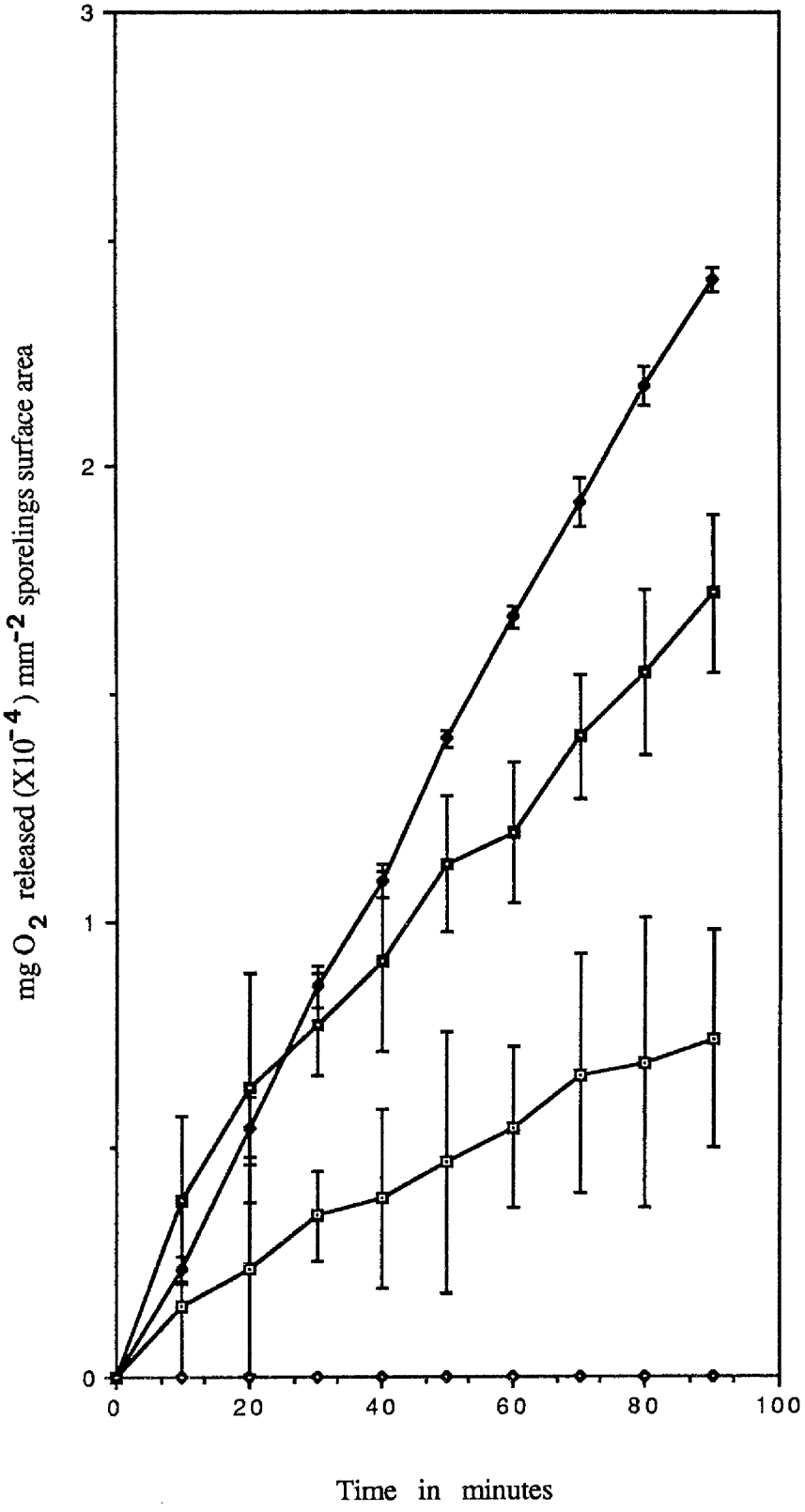
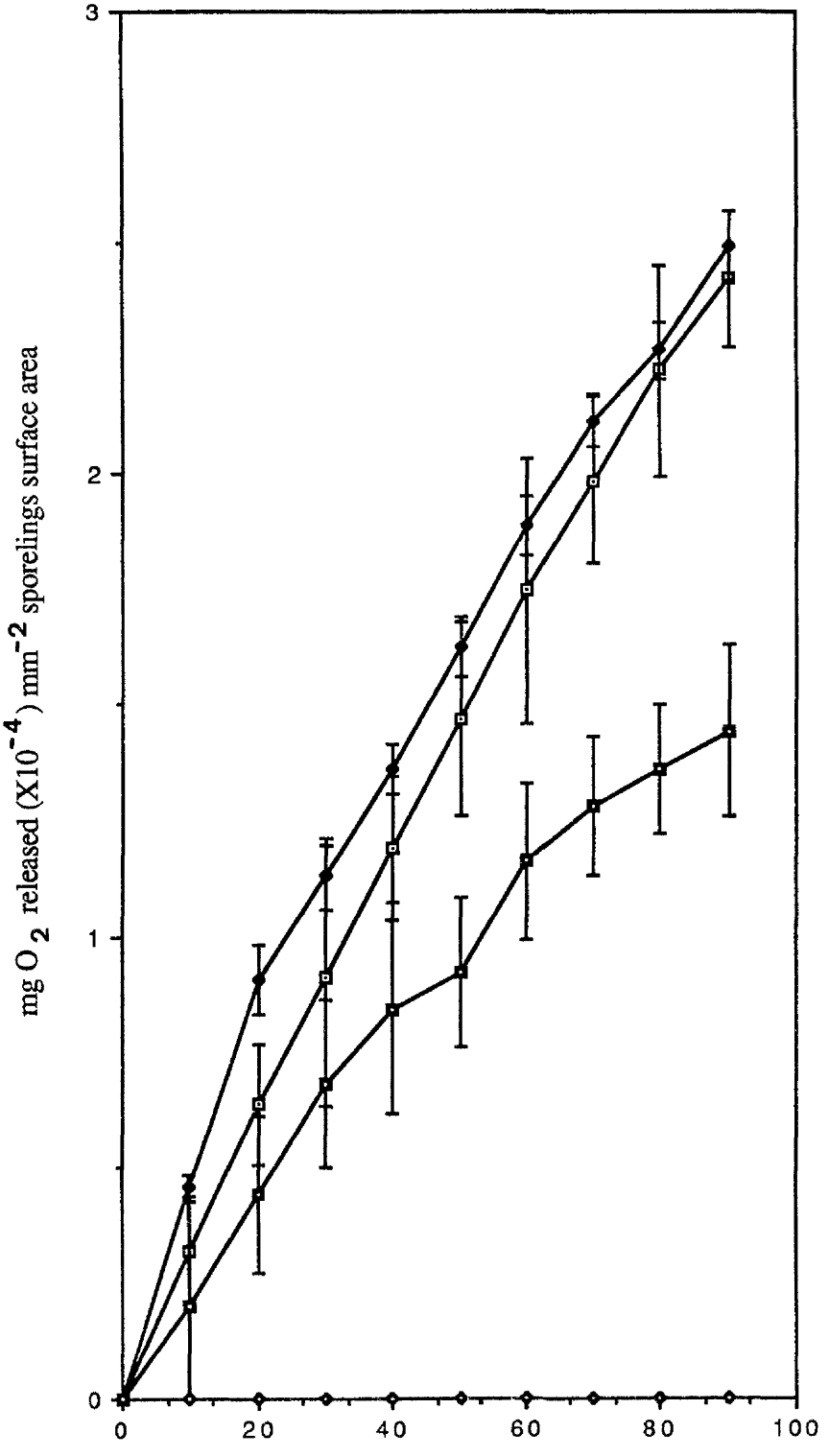

3
2
1
0
0
20
40
60
80
100
mg O_2 released ($X10^{-4}$) mm^{-2} sporelings surface area
Time in minutes

Fig. 6.2.2.9. Photosynthetic rates of sporelings at which were kept 6 h in different temperatures, and then measured at 20 ^{o}C and quantum irradiance of 11.6 µmol m^{-2} s^{-1}. Two samples measured at each temperature.

- 5 ^{o}C
- 20 ^{o}C
- 30 ^{o}C
- 35 ^{o}C


3
2
1
0
0
20
40
60
80
100
mg O_2 released ($X10^{-4}$) mm^{-2} sporelings surface area
Time in minutes

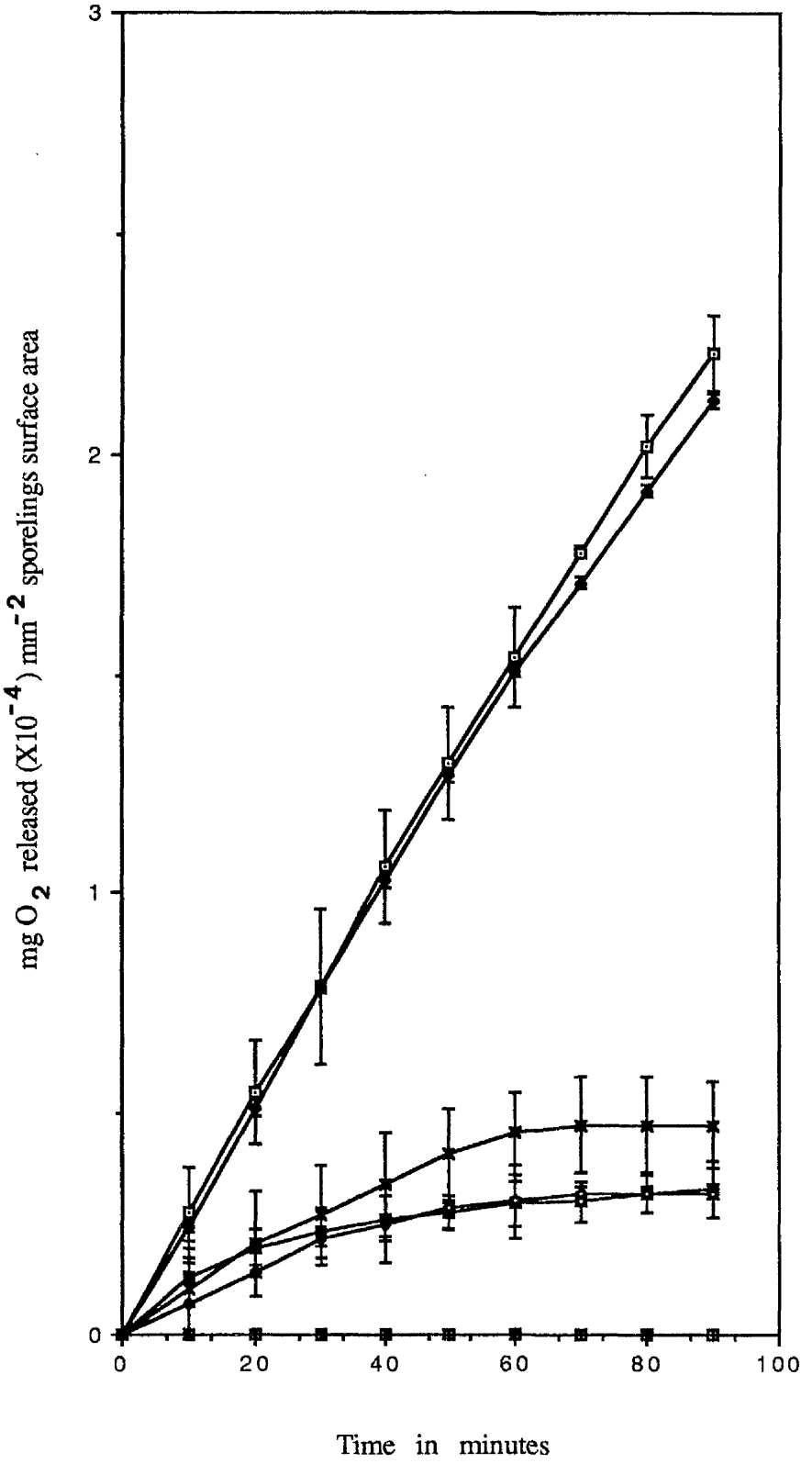
different temperatures (5, 20, 30, and 35 ^{o}C), and then measured at 20 ^{o}C. In this experiment the photosynthetic activity of the sporelings kept at 5 ^{o}C was at a steady rate in the first 60 min., (0.28-0.32X10^{-4} mg O_2 mm^{-2} in each 10 min. interval), followed by a lower but steady rate (0.19-0.24X10^{-4} mg O_2 mm^{-2}). Sporelings at 20 ^{o}C showed in the first 20 min. an oxygen release of 0.45-0.46X10^{-4} mg O_2 mm^{-2}, and over the remaining 80 min. of the measurements the rate of oxygen release was lower but at a steady rate (0.15-0.27X10^{-4} mg O_2 mm^{-2} per 10 min.). Sporelings kept at 30 ^{o}C showed an even rate of oxygen release in the first 30 min. (0.23X10^{-4} mg O_2 mm^{-2} per 10 min. interval), proceeding at a lower but unsteady rate (0.13±0.06X10^{-4} mg O_2 mm^{-2}) for the remainder of the experiment. With sporelings kept at 35 ^{o}C no photosynthesis was recorded.

Variations in local salinity levels are a possible ecological factor in intertidal habitats. These may arise through the effect of rainfall when plants are exposed to the air when emersed by tidal movements, or local variations in rock pools similarly isolated. Fig. 6.2.2.10 shows the rates of oxygen release at different salinities ('normal' sea water or 33‰, 16.5, 10, 7.5, 5, and 45‰) at 20 ^{o}C. when sporelings were directly transfered to the test salinities. Sporelings in normal sea water showed a steady rate of oxygen release in all 10 min. intervals (mean production was 0.25±0.02X10^{-4} mg O_2 mm^{-2}). Sporelings in a salinity of 16.5‰ showed almost identical rates of O_2 release with those in 'normal' sea water. Sporelings at 10‰ showed the highest photosynthetic activity in the first 10 min., but thereafter proceeded at lower and steady rates (0.01-0.07X10^{-4} mg O_2 mm^{-2} at each 10 min. interval). With sporelings at 7.5‰ there were steady rates of oxygen production in the first 70 min. (0.02-0.07X10^{-4} mg O_2 mm^{-2} per 10 min. interval), but after this the sporelings failed to produce any increase in the quantity of oxygen released. Sporelings at 5‰ failed to show any measurable photosynthetic activity in all the period of experiment. With sporelings at 45‰ the oxygen output in the first 70 min. was at a steady rate (0.05-0.10X10^{-4} mg O_2 mm^{-2} at each 10 min. interval), and at the remaining period of experiment there was no increase in oxygen production.

The previous experiments with different salinities might be regarded in the nature of

Fig. 6.2.2.10. Rates of oxygen release of sporelings at different salinities at 20 ^{o}C and quantum irradiance of 11.6 µmol m^{-2} s^{-1}. Two samples measured at each salinity level.

- 'normal' sea water (33‰)
- 16.5‰
- 10‰
- 7.5‰
- 5‰
- 45‰


3
2
1
0
0
20
40
60
80
100
mg O_2 released ($X10^{-4}$) mm^{-2} sporelings surface area
Time in minutes

'shock' effects. Further experiments were carried out to examine the effects of longer term immersions.

Fig. 6.2.2.11 shows the measurements of oxygen release of sporelings were kept for 24 h at each salinity ('normal' sea water, 16.5, 10, 7.5, 5, and 45‰), and then the rates of oxygen release measured in 'normal' sea water. Sporelings in normal sea water produced a steady rate of oxygen release as in the previous experiment, with a mean production of $0.24\pm0.03X10^{-4}$ mg O_2 mm^{-2} at each 10 min. interval. Sporelings kept for 24 h in media of salinity 16.5‰ showed a reduced but steady rate of oxygen release when placed in normal sea water (mean rate $0.16\pm0.02X10^{-4}$ mg O_2 mm^{-2} surface area at each 10 min. interval). The photosynthetic rates were reduced after the 24 h immersion at the lower salinity when compared with the previous experiment. Sporelings kept at 10‰ showed similar rates of oxygen output to those directly transferred, and similar results were obtained with sporelings kept in media of 7.5‰. After 24 h in media of 5‰ there was some measurable photosynthetic activity which soon tailed off. Sporelings kept in media of 45‰ for 24 h showed slightly enhanced rates of oxygen release when transferred to 'normal sea water compared with sporelings placed directly in the higher salinity, but this rate of oxygen release slowed and stabilized within the period of experiment.

On the shores of the Firth of Clyde the dense growths of *Mastocarpus* are found on the lower shore, coinciding with the zoning of the brown seaweed *Fucus serratus*. *Mastocarpus* is abundant on rocky outcrops not fully colonised by *F. serratus*. Earlier experiments have shown some competitive effects of interactions between *Mastocarpus* sporelings and *Fucus serratus* germlings, and it was thought worthwhile to determine whether these interactions could be observed in the photosynthetic activities of *Mastocarpus* sporelings after varying periods in the presence of developing germlings of *F. serratus*. *F. spiralis* is found on the upper shore, but similar experiments were carried out with *F. spiralis* germlings.

Figs. 6.2.2.12-13 show the rates of oxygen release of sporelings with *F. spiralis* and *F. serratus* germlings. *Mastocarpus* sporelings of age 45 days were cultured with newly formed zygotes of *Fucus* spp. The rates of oxygen release of the sporelings

Fig. 6.2.2.11. Rates oxygen release of sporelings were kept for 24 h at different salinities and then measured in 'normal' sea water at 20 °C and quantum irradiance of 11.6 μmol m^{-2} s^{-1}.

- 'normal' sea water (33‰)
- 16.5‰
- 10‰
- 7.5‰
- 5‰
- 45‰

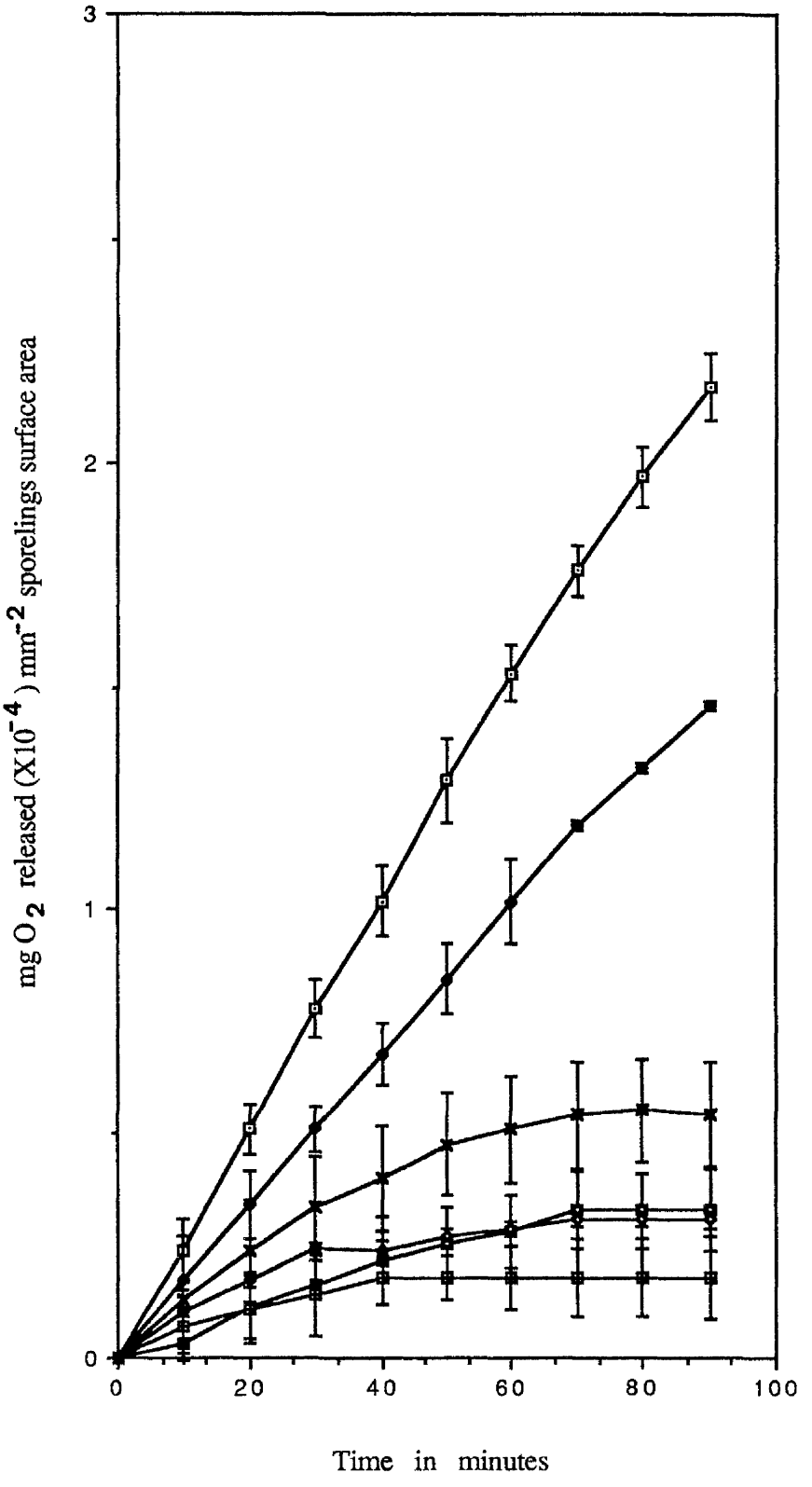
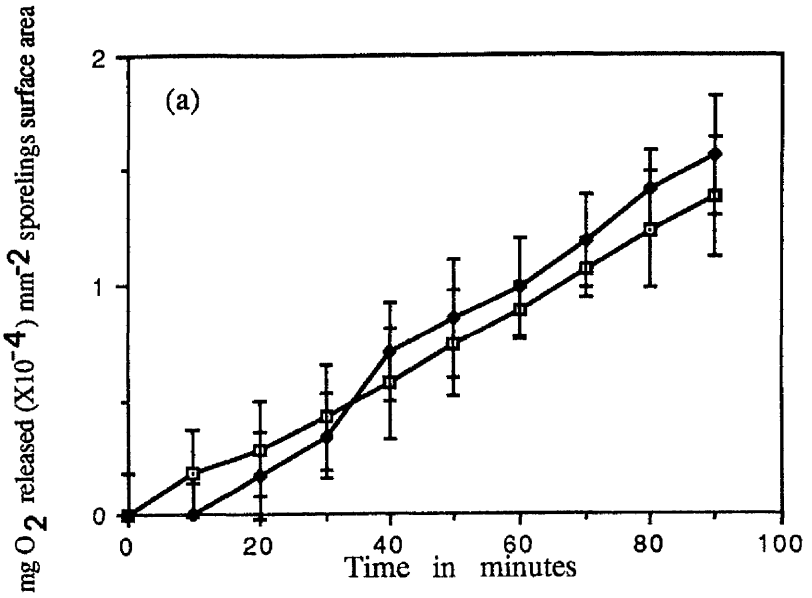
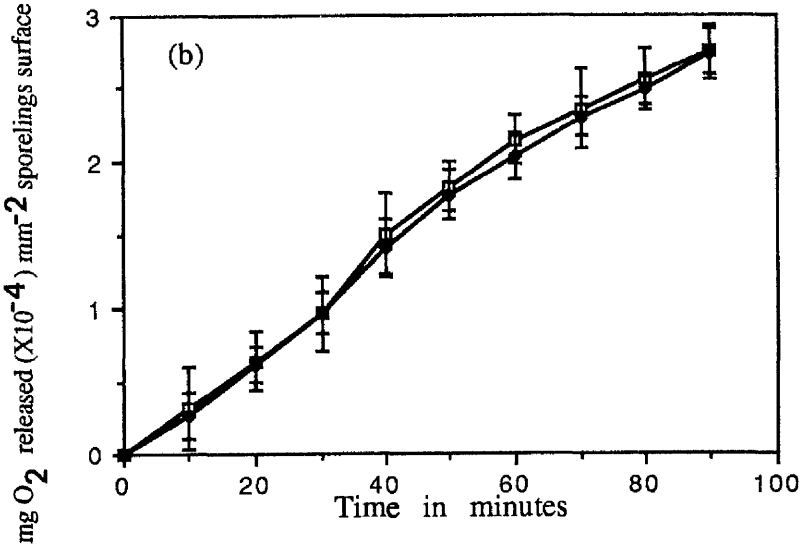

3
2
1
0
0
20
40
60
80
100
mg O_2 released ($X10^{-4}$) mm^{-2} sporelings surface area
Time in minutes

Fig. 6.2.2.12a-c. Rates of oxygen release of sporelings with *Fucus serratus* germlings at intervals of 1, 4, 8 weeks. Measured at 20 ^{o}C and quantum irradiance of 11.6 µmol m^{-2} s^{-1}. Two samples measured in each case.

- 1, 4, and 8 weeks for control sporelings
- 1, 4, and 8 weeks for sporelings with germlings.


(a)
mg O2 released (X10-4) mm-2 sporelings surface area
0
1
2
0
20
40
60
80
100
Time in minutes


(b)
mg O2 released (X10-4) mm-2 sporelings surface area
0
1
2
3
0
20
40
60
80
100
Time in minutes

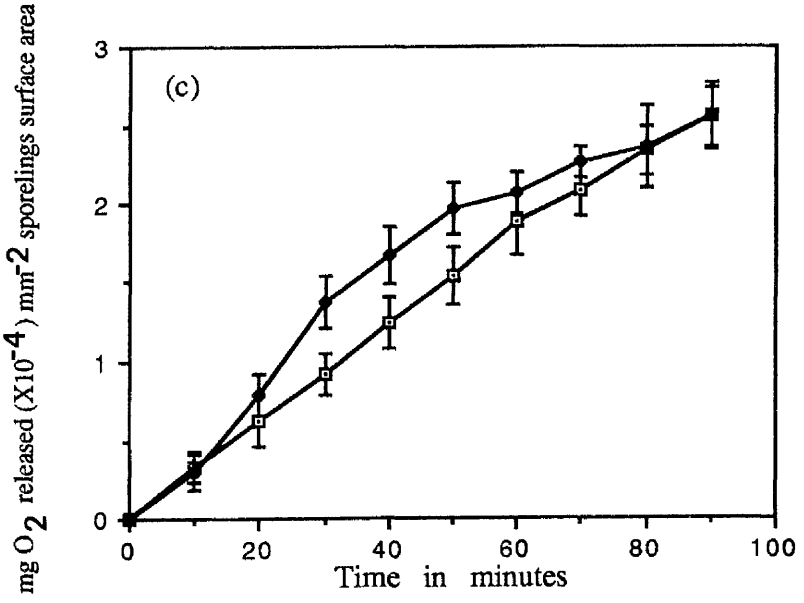
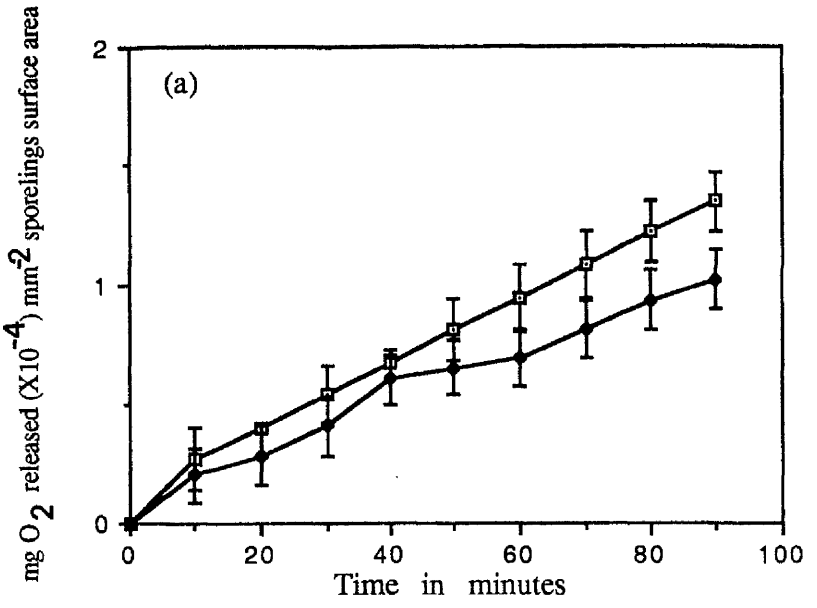
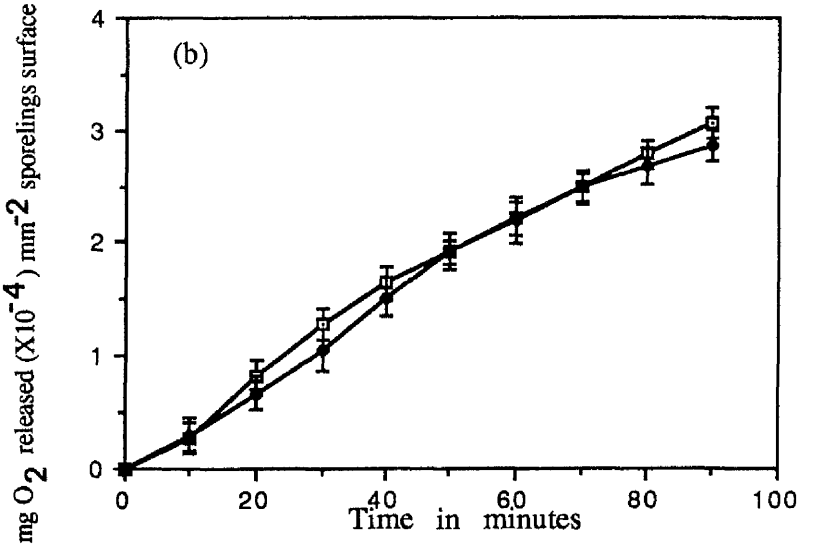

(c)
mg O2 released (X10-4) mm-2 sporelings surface area
0
1
2
3
0
20
40
60
80
100
Time in minutes

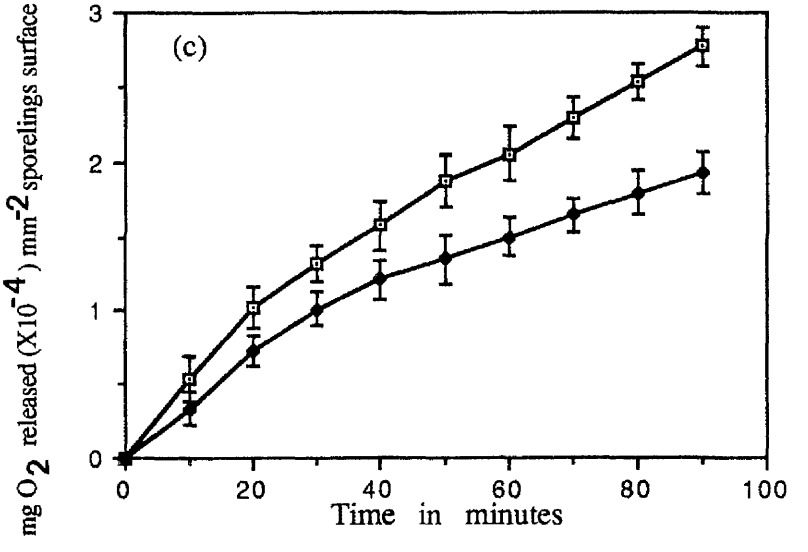
Fig. 6.2.2.13a-c. Rates of oxygen release of sporelings with *Fucus spiralis* germlings at intervals of 1, 4, 8 weeks. Measured at 20 ^{o}C and quantum irradiance of 11.6 $\mu mol\ m^{-2}\ s^{-1}$. Two samples measured in each case.

-□- 1, 4, and 8 weeks for control sporelings

-◆- 1, 4, and 8 weeks for sporelings with germlings.


(a)
mg O_2 released ($X10^{-4}$) mm^{-2} sporelings surface area
Time in minutes
0
1
2
0
20
40
60
80
100


(b)
mg O_2 released ($X10^{-4}$) mm^{-2} sporelings surface area
Time in minutes
0
1
2
3
4
0
20
40
60
80
100


(c)
mg O_2 released ($X10^{-4}$) mm^{-2} sporelings surface area
Time in minutes
0
1
2
3
0
20
40
60
80
100

were measured at intervals of 1, 4, and 8 weeks from the time of settlement of the *Fucus* zygotes. In general the control sporelings and sporelings with *F. serratus* showed similar rates of oxygen release in all the periods time of experiment at 1 and 4 weeks (mean production was 0.15 and $0.17X10^{-4}$ mg O_2 mm^{-2} at each 10 min. interval). Sporelings with *F. serratus* after 8 weeks showed a similar production of oxygen in the first 10 min when compared with control sporelings, and although the oxygen production of the sporelings with germlings was slightly increased thereafter after 70 min. the production of oxygen was at the same rate with control sporelings and those with germlings. Sporelings after 1 week with *F. spiralis* showed a slight effect on sporeling photosynthesis when compared with the controls ($0.11\pm0.06X10^{-4}$ mg O_2 mm^{-2} and $0.15\pm 0.05X10^{-4}$ mg O_2 mm^{-2} at each 10 min. interval), but sporelings after 4 weeks with *F. spiralis* showed similar rates of oxygen release with germlings when compared with control sporelings (mean production of $0.32\pm0.09X10^{-4}$ mg O_2 mm^{-2} in each 10 min.). Sporelings after 8 weeks with *F. spiralis* showed a more significant effect on rates of oxygen release (mean production $0.20\pm.10X10^{-4}$ mg O_2 mm^{-2}) when compared with the control sporelings ($0.31\pm0.12X10^{-4}$ mg O_2 mm^{-2}).

6.2.3. Experiments with reproductive structures

The papillate outgrowths on the frond surface of *Mastocarpus* are a well known and characteristic feature (see Frontispiece). The fertile spore bearing papillae assume various shapes, similar to those that are non-fertile. In nature the fronds will move to and fro when covered by the sea, so that the papillae will be constantly bathed in sea water containing dissolved CO_2, O_2 and nutrients. In all the following experiments detached fertile and non-fertile papillae of similar size and shapes were used, and it was found in preliminary experiments that 13-15 papillae (and separated spore masses) were sufficient to being about a measurable oxygen release. Whilst detached papillae are not in their natural state, the circulation of the papillae in the sea water of the electrode cell could be regarded as to some extent simulating the water movements in nature. The irradiance used in all the experiments was 11.6 µmol m^{-2} s^{-1}, at 20 °C. The need for

continuous circulation was shown when the papillae (fertile and non-fertile) were allowed to settle in the electrode cell by switching off the magnetic 'flea' (Fig. 6.2.3.1). The immediate fall in the rate of oxygen release was followed by a return to a similar photosynthetic rate when the circulation was restarted.

Fig. 6.2.3.2 shows the rates of oxygen release at different salinities ('normal' sea water, 16.5, 10, 7.5, 5, and 45‰) at 20 ^{o}C when reproductive structures were directly transferred to the test salinity. With the reproductive structures (fertile, non-fertile and spore masses) in 'normal' sea water the fertile and non-fertile papillae showed similar rates of oxygen release over the 90 min. of the experiment (mean production 0.002 mg O_2 released by 0.01 g fresh weight at each 10 min. interval), whilst the spore masses showed a much lower oxygen production steadily over the 90 min. At 16.5‰ the non-fertile papillae gave a slightly higher oxygen release compared with fertile papillae and spore masses, but all produced steady rates over the 90 min. of the experiment. At 10‰ the oxygen output by the fertile papillae was slightly reduced compared with the other salinities tested, and at the remaining salinities (7.5‰ and 5‰), and at 45‰ there was little sign of any photosynthetic activity. With both the non-fertile papillae and the spore masses there was little measurable photosynthetic activity in the 5-10‰ range, and at 45‰.

With the experimental material kept at the various salinities for 24 h, and then the rates of oxygen measured in 'normal' sea water, whilst similar rates of oxygen were obtained with both fertile and non-fertile papillae and the spore masses at all salinities as in the previous experiment (see Fig. 6.2.3.3).

Plants in intertidal habitats can be subjected to drying when exposed to air by tidal movements of the sea. Whilst the overlapping nature of *Mastocarpus* fronds on the shore may prevent this, there is the possibility that the papillae may be exposed to drying. This factor was examined by subjecting fertile and non-fertile papillae to dry air (in a desiccator at room temperature 20-22 ^{o}C). In preliminary experiments the papillae were exposed to dry air for periods up to 1 h. It was found that no photosynthetic activity was measurable with papillae kept for more than 20 min. under these conditions. Fig. 6.2.3.4 shows the effect of dry air on the oxygen release of

Fig. 6.2.3.1. Rates of oxygen release by fertile and non-fertile papillae whilst circulating in the electrode cell and when settled. Measured at 20 ^{o}C and quantum irradiance of 11.6 $\mu mol\ m^{-2}\ s^{-1}$. Two samples measured in each case.

-⊡- fertilepapillae

-●- non-fertile papillae.

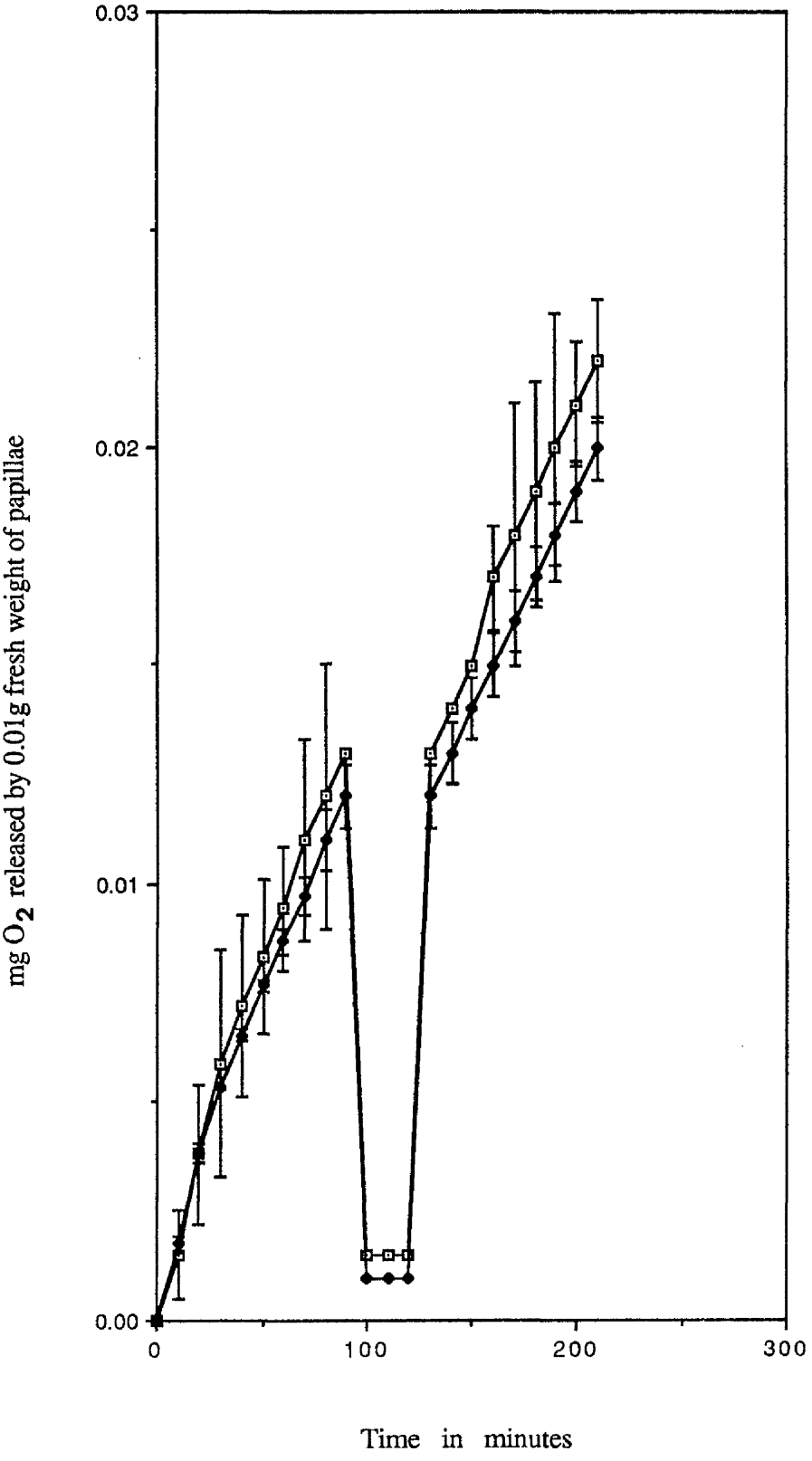

0.03
0.02
0.01
0.00
0
100
200
300
mg O_2 released by 0.01g fresh weight of papillae
Time in minutes

Fig. 6.2.3.2. Rates of oxygen release of fertile and non-fertile papillae and spore masses when measured directly at different salinities at 20 ^{o}C and 11.6 µmol m^{-2} s^{-1}. Small samples available; replication not possible.

a: fertile papillae:

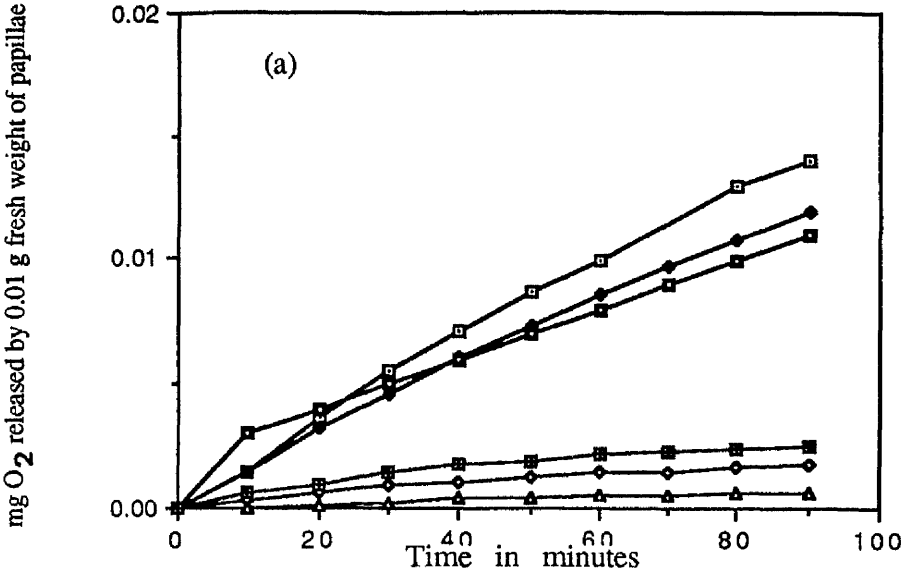
- 'normal' sea water (33‰)
- 16.5‰
- 10‰
- 7.5‰
- 5‰
- 45‰

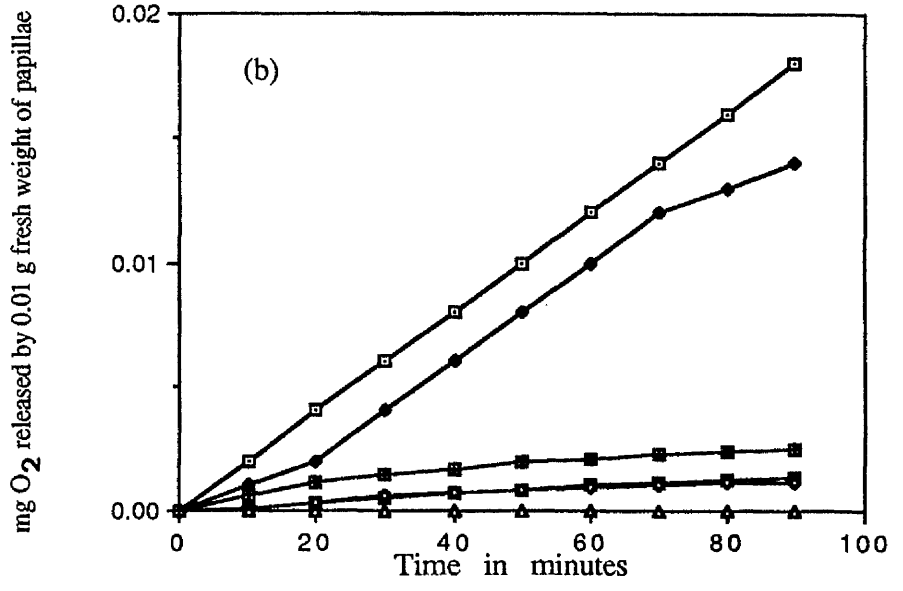
b: non-fertile papillae

- 'normal' sea water (33‰)
- 16.5‰
- 10‰
- 7.5‰
- 5‰
- 45‰

c: spore masses

- 'normal' sea water (33‰)
- 16.5‰
- 10‰
- 7.5‰
- 5‰
- 45‰


(a)
0.02
0.01
0.00
0
20
40
60
80
100
Time in minutes
mg O2 released by 0.01 g fresh weight of papillae


(b)
0.02
0.01
0.00
0
20
40
60
80
100
Time in minutes
mg O2 released by 0.01 g fresh weight of papillae

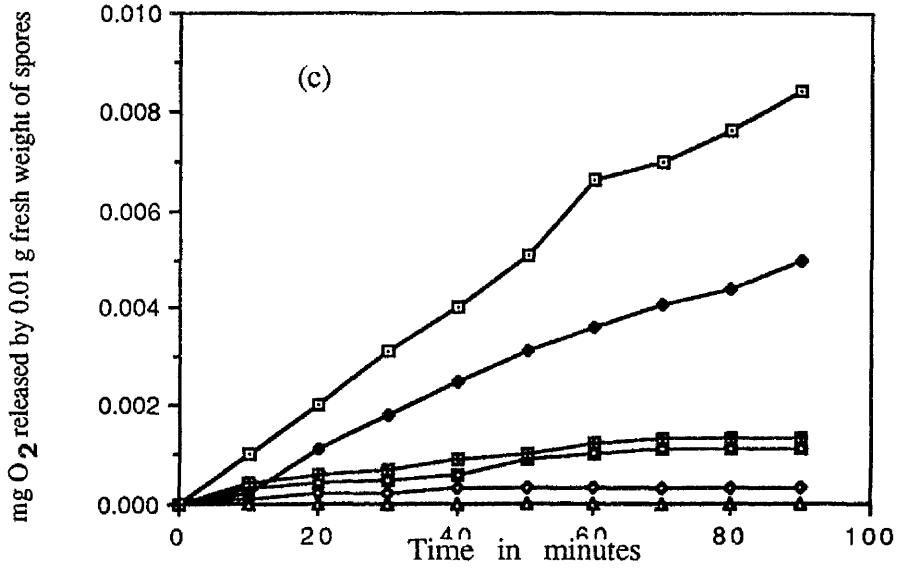

(c)
0.010
0.008
0.006
0.004
0.002
0.000
0
20
40
60
80
100
Time in minutes
mg O2 released by 0.01 g fresh weight of spores

Fig. 6.2.3.3. Rates of oxygen release of fertile and non-fertile papillae and spore masses kept for 24 h at each salinity ('normal' sea water, 16.5, 10, 7.5, 5, and 45 ‰), and then measured in 'normal' sea water at 20 °C and light intensity of 11.6 µmol m^{-2} s^{-1}. Small samples available; replication not possible.

a: fertile papillae.

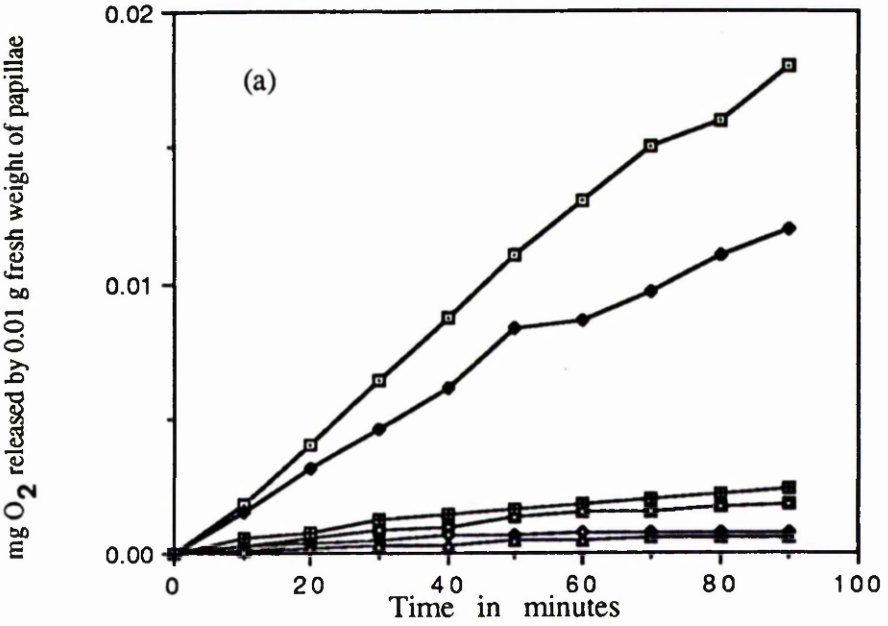
- 'normal' sea water (33‰)
- 16.5‰
- 10‰
- 7.5‰
- 5‰
- 45‰

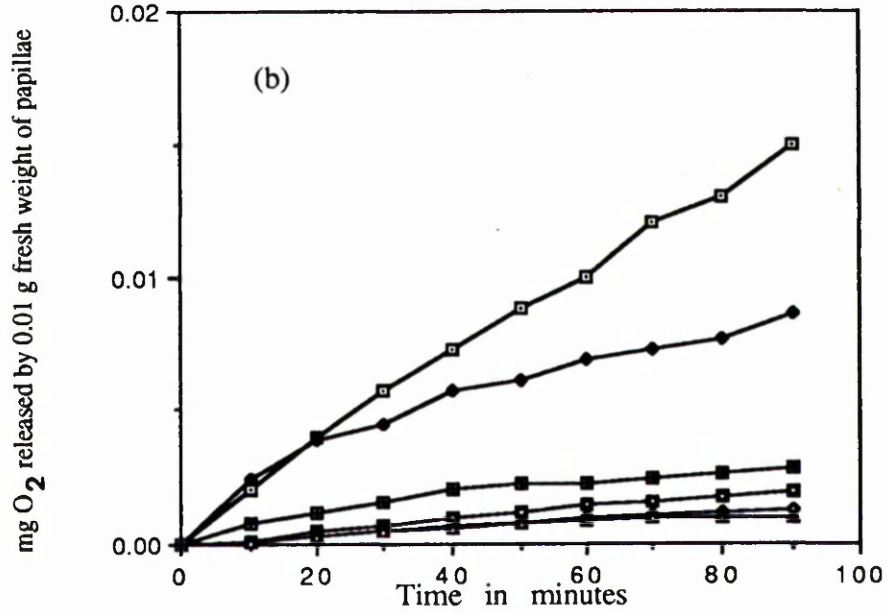
b: non-fertile papillae

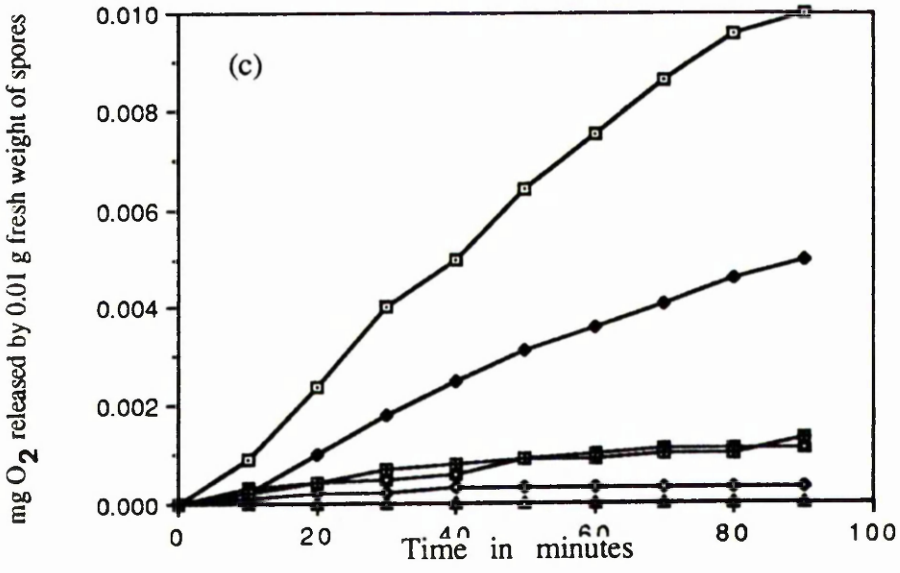
- 'normal' sea water (33‰)
- 16.5‰
- 10‰
- 7.5‰
- 5‰
- 45‰

c: spore masses

- 'normal' sea water (33‰)
- 16.5‰
- 10‰
- 7.5‰
- 5‰
- 45‰


(a)
0.02
0.01
0.00
0
20
40
60
80
100
Time in minutes
mg O_2 released by 0.01 g fresh weight of papillae


(b)
0.02
0.01
0.00
0
20
40
60
80
100
Time in minutes
mg O_2 released by 0.01 g fresh weight of papillae


(c)
0.010
0.008
0.006
0.004
0.002
0.000
0
20
40
60
80
100
Time in minutes
mg O_2 released by 0.01 g fresh weight of spores

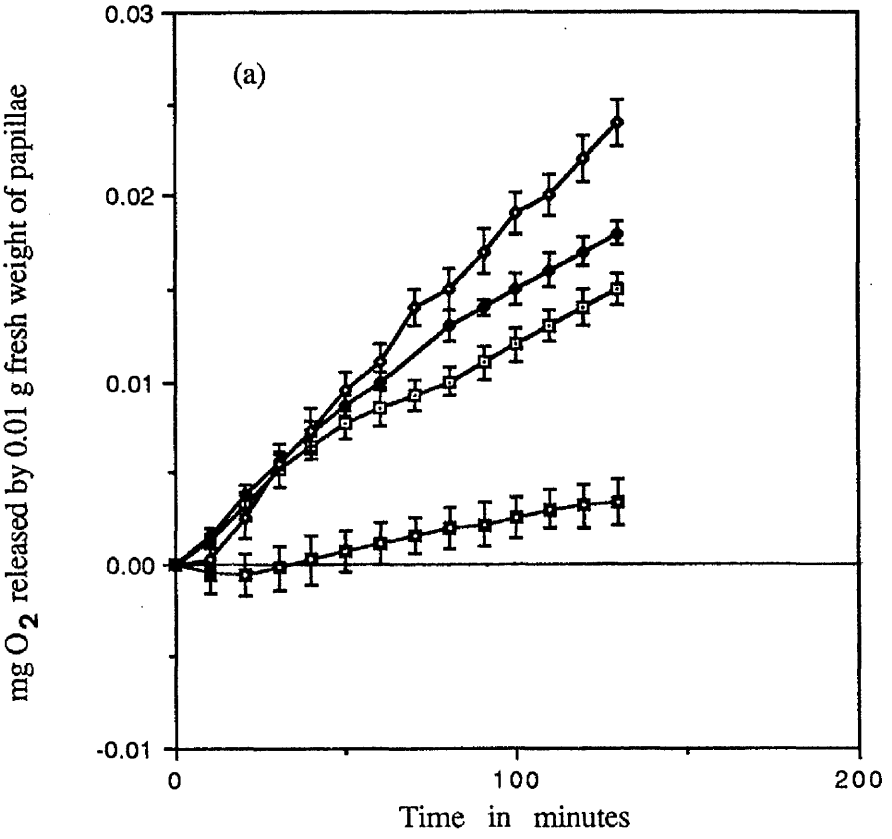
Figs. 6.2.3.4. Effect of dry air at room temperature on photosynthesis of fertile and non-fertile papillae. Oxygen release measured at 20 ^{o}C and 11.6 μmol m^{-2} s^{-1}. Two samples measured with each treatment used.

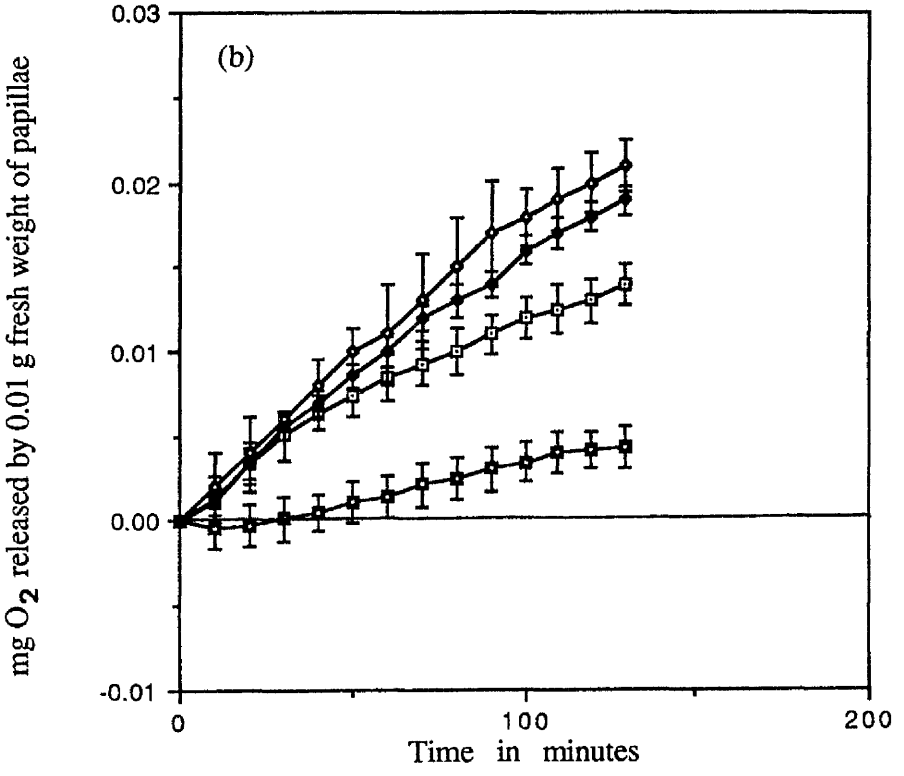
a: fertile papillae

- control (continuously immersed in sea water)
- 5 minutes in dry air
- 10 minutes in dry air
- 15 minutes in dry air

b: non-fertile papillae

- control (continuously immersed in sea water)
- 5 minutes in dry air
- 10 minutes in dry air
- 15 minutes in dry air


(a)
0.03
0.02
0.01
0.00
-0.01
0
100
200
Time in minutes
mg O_2 released by 0.01 g fresh weight of papillae


(b)
0.03
0.02
0.01
0.00
-0.01
0
100
200
Time in minutes
mg O_2 released by 0.01 g fresh weight of papillae

fertile and non-fertile papillae of *Mastocarpus* plants when treated for different times (5, 10, and 15 minutes), and then transferred direct to the electrode cell, compared with control papillae which were kept immersed in sea water. Both fertile and non-fertile papillae at 5, 10 min. and control showed steady rates of oxygen production over the 130 min. of the experiment, although at slightly lower rates following exposure to air. Both fertile and non-fertile papillae after 15 min. exposure to air showed little indication of any photosynthetic activity over the period of experiment. Fig. 6.2.3.5 shows the effects of dry air on fertile and non-fertile papillae when dried in a desiccator for 5, 10, and 15 min. at room temperature, and then kept for 24 h in 'normal' sea water before measuring photosynthetic rates. For the fertile papillae there was no effect up to 5 min., but significant reduction in photosynthesis occurred after 10 and 15 min. exposure. For the non-fertile papillae there were similar rates of oxygen release after exposure to dry air for up to 10 min., with a marked reduction after 15 min.

6.2.4. Oxygen release by *Mastocarpus* fronds (with and without papillae).

The use of detached papillae, as in the previous experiments gave some indications of the relative rates of photosynthetic activity. Some further experiments were carried out using whole fronds, with and without papillae, with incubations carried out on Winkler bottles at 20-22 ^{o}C and 42 μmol m^{-2} s^{-1} irradiance, with the quantities of oxygen measured by the Winkler method (p. 20). Results on seasonal basis are summarized in Table 6.2.4.1.

Rates of dissolved oxygen were generally higher during summer and autumn than in winter. The annual value observed ranged between 0.03-0.25 mg O_2 g^{-1} dry weight h^{-1} of fronds with papillae and 0.10-0.59 mg O_2 g^{-1} dry weight h^{-1} of fronds without papillae. During the summer (June) oxygen production by the fronds with papillae was at the highest value, whilst during the autumn (October) the fronds without papillae showed the largest yield of oxygen when compared with other seasons. The fronds with papillae in the winter of 1985 gave low yields of oxygen, but in spring time the photosynthetic rate increased to 0.21 mg O_2 g^{-1} dry weight h^{-1}, and during

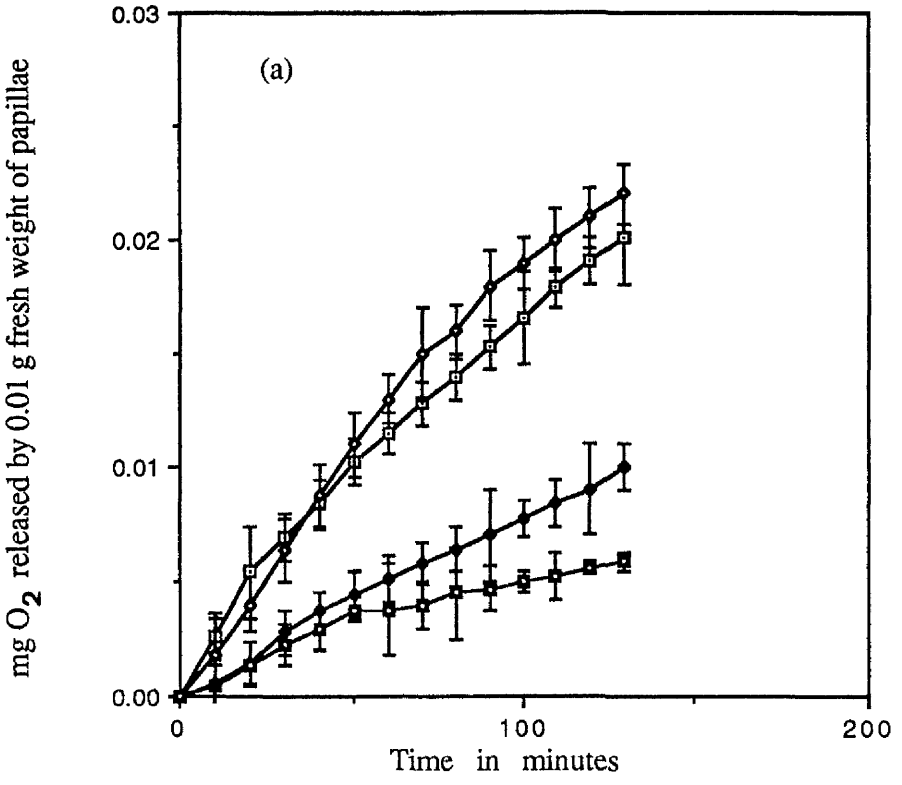
Figs. 6.2.3.5. Effects of dry air at room temperature followed by 24 h in 'normal' sea water on fertile and non-fertile papillae. Oxygen release measured at 20 ^{o}C and 11.6 μmol m^{-2} s^{-1}. Two samples measured with each treatment used.

a: fertile papillae

- control (continuously immersed in sea water)
- 5 minutes in dry air
- 10 minutes in dry air
- 15 minutes in dry air

b: non-fertile papillae

- control (continuously immersed in sea water)
- 5 minutes in dry air
- 10 minutes in dry air
- 15 minutes in dry air


(a)
0.03
0.02
0.01
0.00
0
100
200
Time in minutes
mg O_2 released by 0.01 g fresh weight of papillae

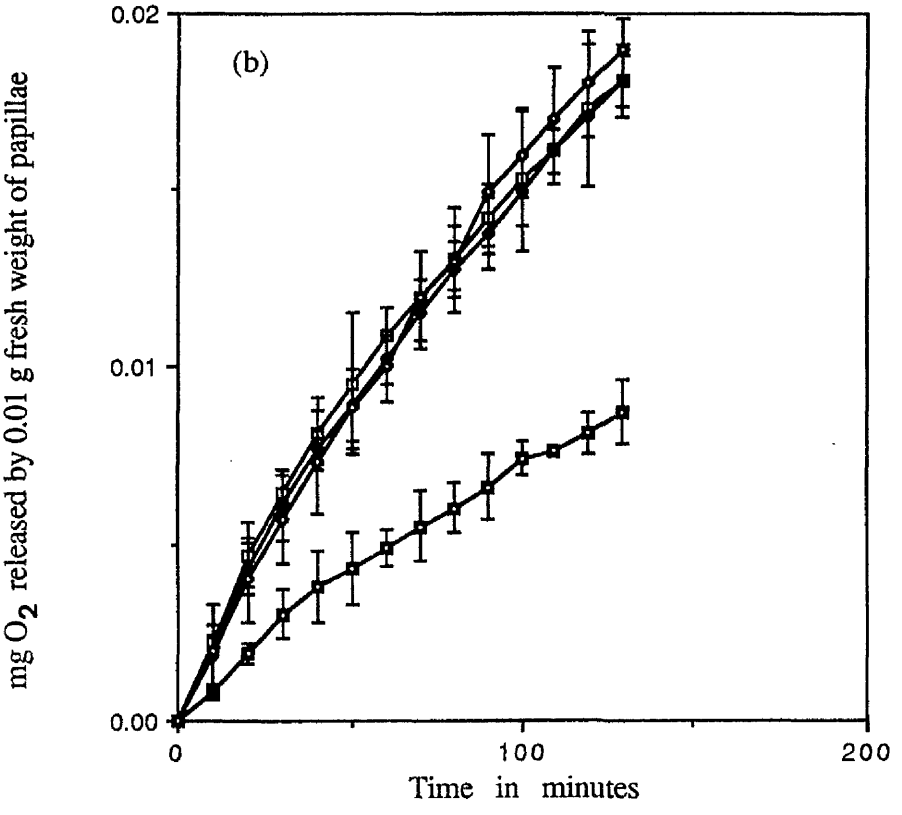

(b)
0.02
0.01
0.00
0
100
200
Time in minutes
mg O_2 released by 0.01 g fresh weight of papillae

Table 6.2.4.1. showing dissolved oxygen in mg O_2 g^{-1} dry weight h^{-1} of different regions of *Mastocarpus stellatus* from Firth of Clyde, Island of Cambrae during the period December 1985-January 1987.

Date collected	Fronds with papillae mg O_2 g^{-1} dry wt. h^{-1}	Fronds without papillae mg O_2 g^{-1} dry wt. h^{-1}
15 December 1985	0.03	0.10
15 March 1986	0.21	0.23
30 June 1986	0.25	0.25
8 October 1986	0.22	0.59
12 January 1987	0.14	0.46

summer the maximum rate of oxygen was 0.25 mg O_2 g^{-1} dry weight h^{-1}, and similar results were obtained in the autumn. The dissolved oxygen yield in winter 1987 was small (0.14 mg O_2 g^{-1} dry weight h^{-1}). The fronds without papillae in winter 1985 gave a photosynthesis rate equal to 0.10 mg O_2 g^{-1} dry weight h^{-1}. In the following spring the rate of oxygen increased to 0.23 mg O_2 g^{-1} dry weight h^{-1}, and during the summer reached 0.25 mg O_2 g^{-1} dry weight h^{-1}. Maximum rates of photosynthesis were obtained in autumn and early winter (0.59, 0.46 mg O_2 g^{-1} dry weight h^{-1} respectively).

6.3. Discussion

The spores failed to show any measurable photosynthetic activity but gave some evidence of oxygen uptake. This may be a measure of their metabolism after release in which they drew on reserves for the synthesis of the precursors of the mucilaginous materials which are immediately released on settlement and attachment. Spores of red algae are known to be well endowed with reserves of Floridean starch (Boney 1975c), and this may be the energy source for their metabolism. It was noticeable that the spore masses dissected from fertile papillae did produce some measurable photosynthetic activity although small. Such activity was sensitive to the more extreme salinity changes. It might be concluded that whilst within the papillae, the immature spores are photosynthetically active, this activity is suppressed on release. However, the spore masses are intimately associated with papillae tissue, and this cannot be entirely removed. Hence the small rates of oxygen release recorded may be more a measure of the activity of the associated tissue than of the spores themselves.

Earlier on it was shown that no matter the degree of injury, the sporelings of *Mastocarpus* will always tend to return to the discoid condition or near to it. The results of experiments with detached sporelings clearly show that photosynthetic activity is associated with the amount of water movement over the surface of sporelings. The constant removal and replenishment of the contact layer is important.Despite the fact that they are closely adherent to their substratum, the turbulent nature of the natural habitat with ensure this exchange. That the

photosynthetic activity of the sporelings immediately responds to removal of the light source is to be expected, as well as the immediate onset of oxygen release on re-illumination. Protracted dark storage (which might be a factor due to the continuing dull short days in the depths of winter) does produce evidence of a stress which may take some time to overcome. Noticeably, sporelings kept for 1-2 days under dark conditions showed higher rates of photosynthesis than the control sporelings, as if a return to lighted conditions induces a surge of activity to compensate for the reduced metabolism in the dark and at a lower temperature. Measurable photosynthetic activity was recorded at very low irradiances. In nature the sporelings would be appreciably shaded by neighbouring plants. The temperature sensitivity of the sporelings is perhaps to be expected. Thier normal habitat would rarely reach the temperature which inhibited photosynthetic activity in the experiments. A temperature of 35 ^{o}C might be attained in parts of a rock pool during periods of low tide, but would be less likely on the shore. Unprotected sporelings covered by water films would suffer severely under intense insolation. However, some aclimatization to temperature changes seems possible. Exposure to variations in salinity indicated that sporeling photosynthesis appeared sensitive to lowered salinities and to raised levels-conditions which might well arise in an intertidal habitat. Perhaps here again the factor of protection from neighbouring plant material may mitigate aganist this apparent sensitivity to extreme salinity variations.

The photosynthetic activity of *Mastocarpus sporelings* seems to be unaffected by the presence of growing *Fucus serratus* germlings in close proximity, although some evidence of interactions was obtained in earlier growth experiments. Some inhibitory effects an oxygen release were observed with 8 week-old *Fucus spiralis* germlings, but these grow in a higher shore zone than *Mastocarpus*.

It is perhaps not surprising that the on the growing sporelings the erect branches are the more active photosynthetically. They are the centres of rapid seasonal growth, and proportionally their contribution to the photosynthetic 'budget' is the greater. Nevertheless, the prostrate discs continue to grow. An outgrowth of several erect branches must be balanced by compensatory growth of the attaching system.

As with the sporelings the photosynthetic activity of fertile and non-fertile papillae requires water circulation. Both types of papillae show similar photosynthetic activity at 'normal' sea water and at 16.5‰ and on less active at lower salinities. They would seem to be sensitive to any increases in local salinity.

Exposure to dry air is clearly a critical factor for both types of papillae. When measured immediately after the exposure periods there were measurable suppressions of photosynthetic activities which increased with the longer the periods of exposure to dry air. the 24 h 'recovery' period of re-immersion in sea water would appear to have allowed some return to near normality with the non-fertile papillae in terms of photosynthetic activity, except with those left for 15 min. in dry air. As already mentioned, irreversible changes accompanied exposure for 20 min. The fertile papillae appeared the sensitive to dry of 10 min. and over possibly due to the sensitivity of their spore contents.

The rates of oxygen release of *Mastocarpus* fronds with and without papillae showed that fronds without papillae during autumn and winter periods were more active photosynthetically than fronds with papillae. These variations were probably due to the fronds without papillae being of more fleshy form.

7. Calorific values of spore masses and spore bearing structures of *Mastocarpus stellatus*.

7.1. Introduction

Calorific values are measures of the potential energy content of plant tissues. The oxidizable materials are converted into heat and expressed in energy units (calories or kcalories). Measurements of the calorific values of marine algae have been mainly studied in terms of their food values to marine grazers. Paine and Vadas (1969) studied 70 macroalgae from intertidal and subtidal habitats. Calorific values varied according to phyletic affinity, water purity, depth of immersion, and factors such as growth form, generation time, and relative susceptibility to herbivory. McQuaid (1985) studied the ash and calorific values of 9 intertidal algae. He found ash values were lowest in winter and highest in spring or summer. Calorific values were at a minimum in winter or early spring with summer and autumn maxima in seven species. Different types of bombs had been developed for measurements of calorific values, adiabatic and non-adiabatic, macro and micro-oxygen bombs. The type of calorimeter to be used is determined by the amount of material available. The Phillipson's Micro-Bomb Calorimeter was found to be suitable for *M. stellatus*, in that it combusted samples less than 100 mg dry weight (see Materials and Methods).

The aim of the present investigation was to examine whether there were noticeable differences in calorific values of the spore bearing structures and the spore masses which might reflect differences in chemical natures (e.g the large quantities of mucilage and of Floridean starch in the spore masses). To obtain measurable quantities of spore material proved difficult, and required dissection of the spore mass from the fruiting papillae. Reproductive material samples were measured in the months of October to March, at a time of high fertility.

7.2. Results

Figs. 7.1-9 show the firing curves of the *Mastocarpus stellatus* tissues used,

Fig. 7.2.1. Firing curve obtained from combustion of benzoic acid sample used for calibration of the micro bomb and determination of calorific values of *Mastocarpus stellatus* (refer to labeling already described in Materials and Methods Fig. 2.6).

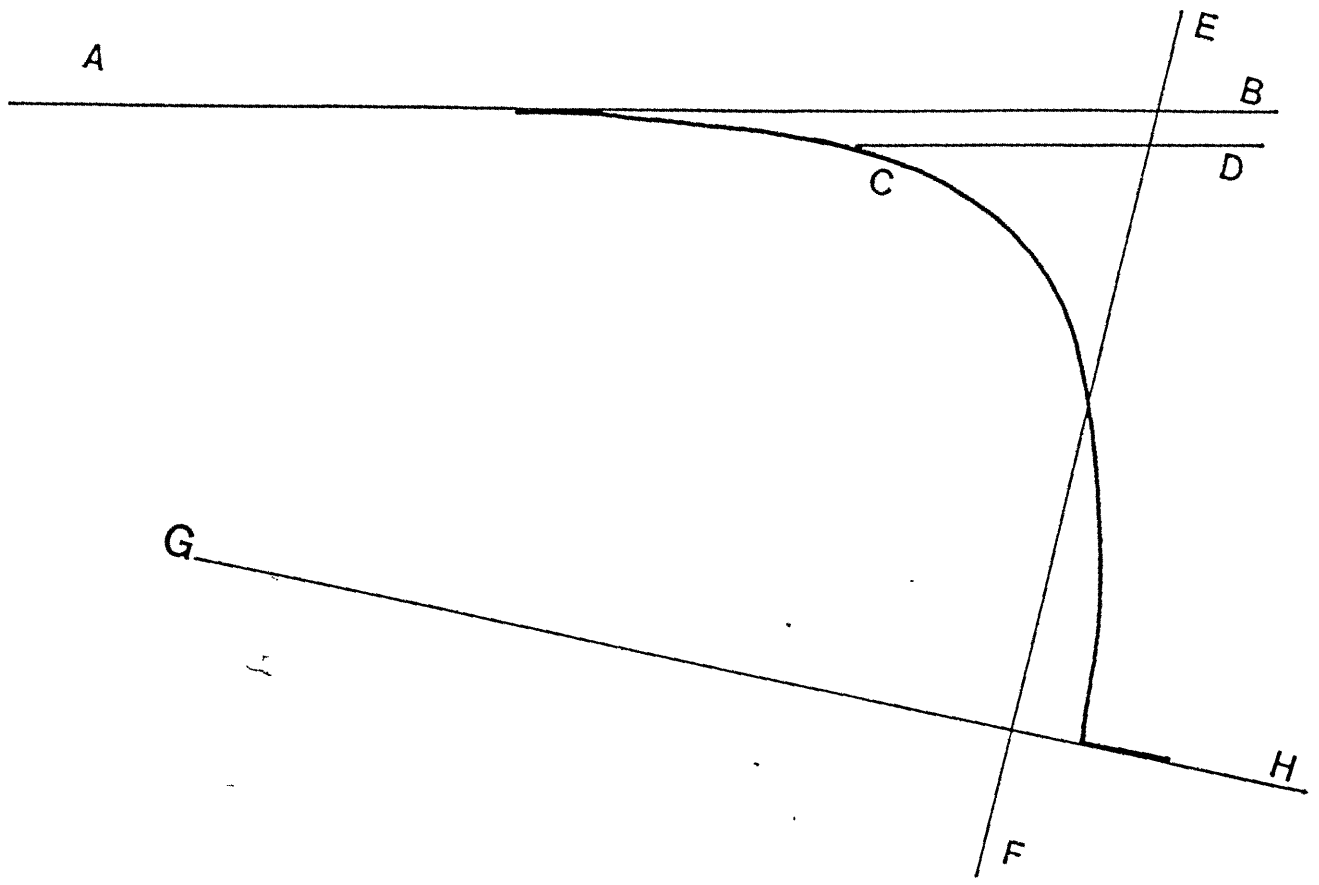

A
B
C
D
E
F
G
H

Fig. 7.2.2. Firing curve obtained from combustion of spore masses of *M. stellatus* sample (dry weight varied between 7.85-14 mg).

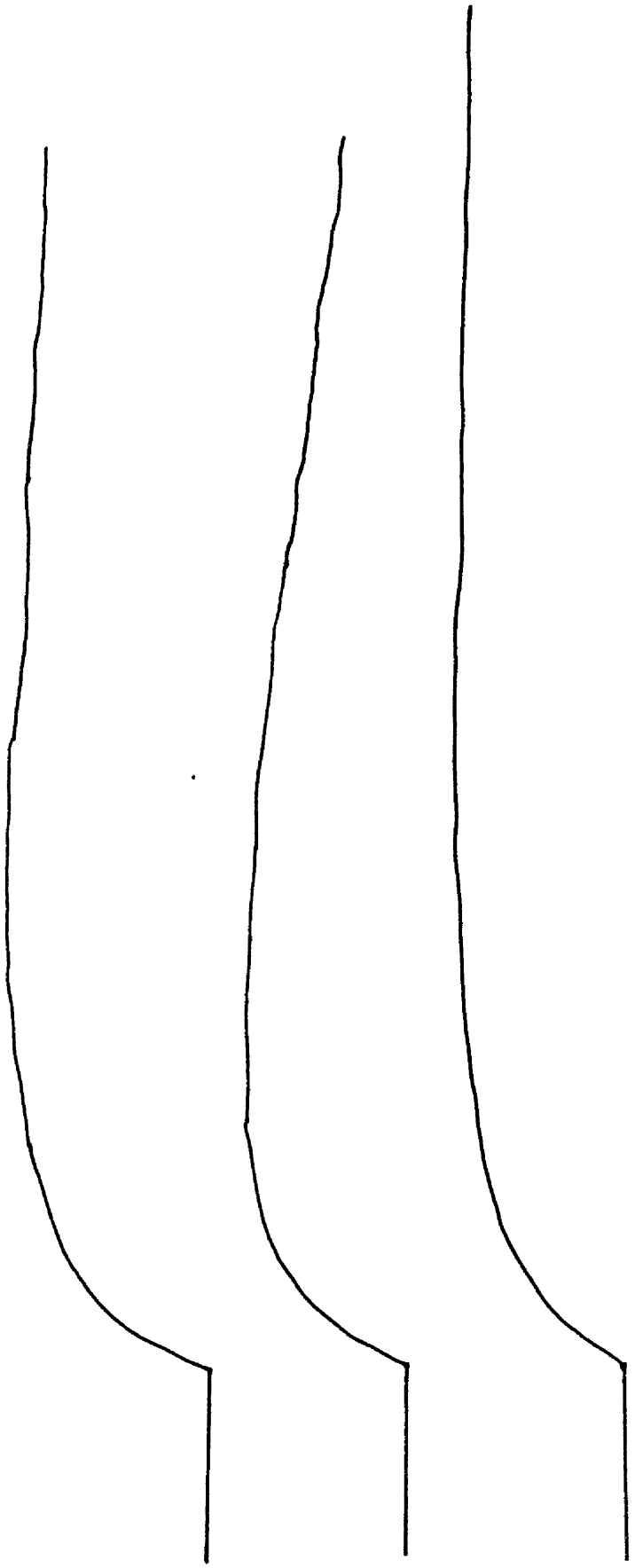


Fig. 7.2.3. Firing curve obtained from combustion of fertile papillae of *M. stellatus* sample (dry weight varied between 12.10-19.37 mg).

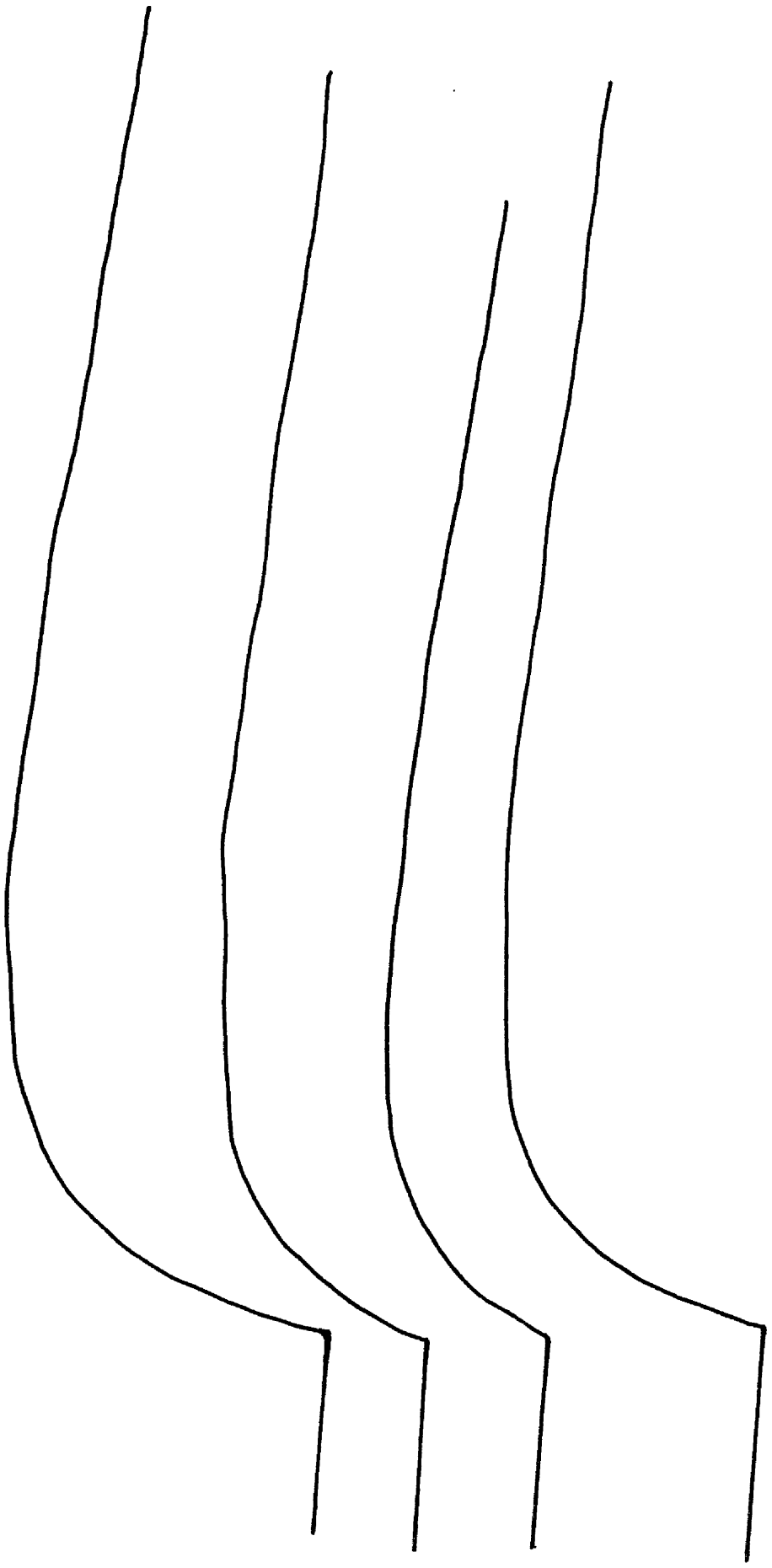


Fig. 7.2.4. Firing curve obtained from combustion of non-fertile papillae of *M. stellatus* sample (dry weight varied between 7.80-19.37 mg).

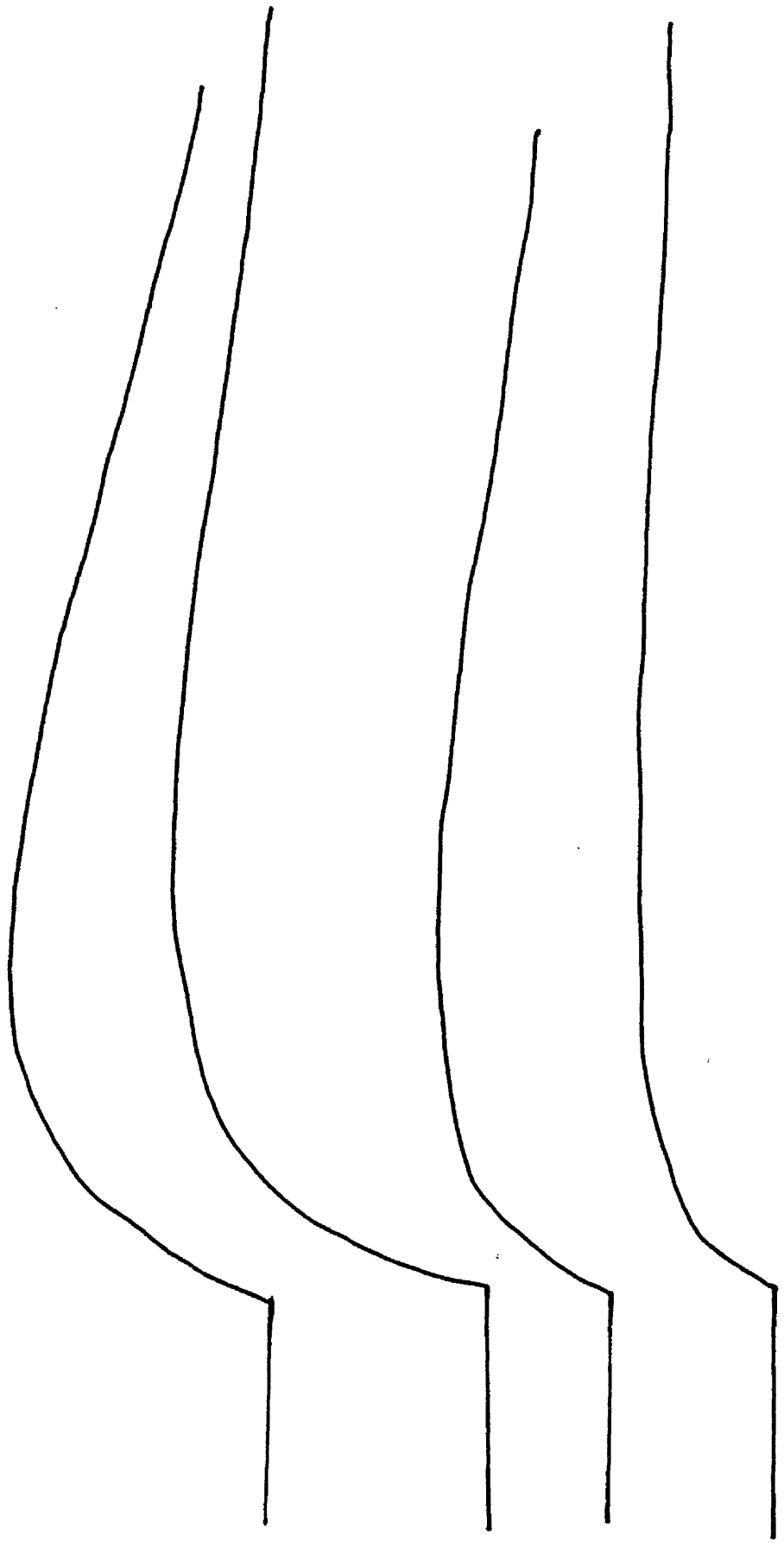


Fig. 7.2.5. Firing curve obtained from combustion of papillae tissue of *M. stellatus* sample (dry weight varied between 7.15-14.80 mg).

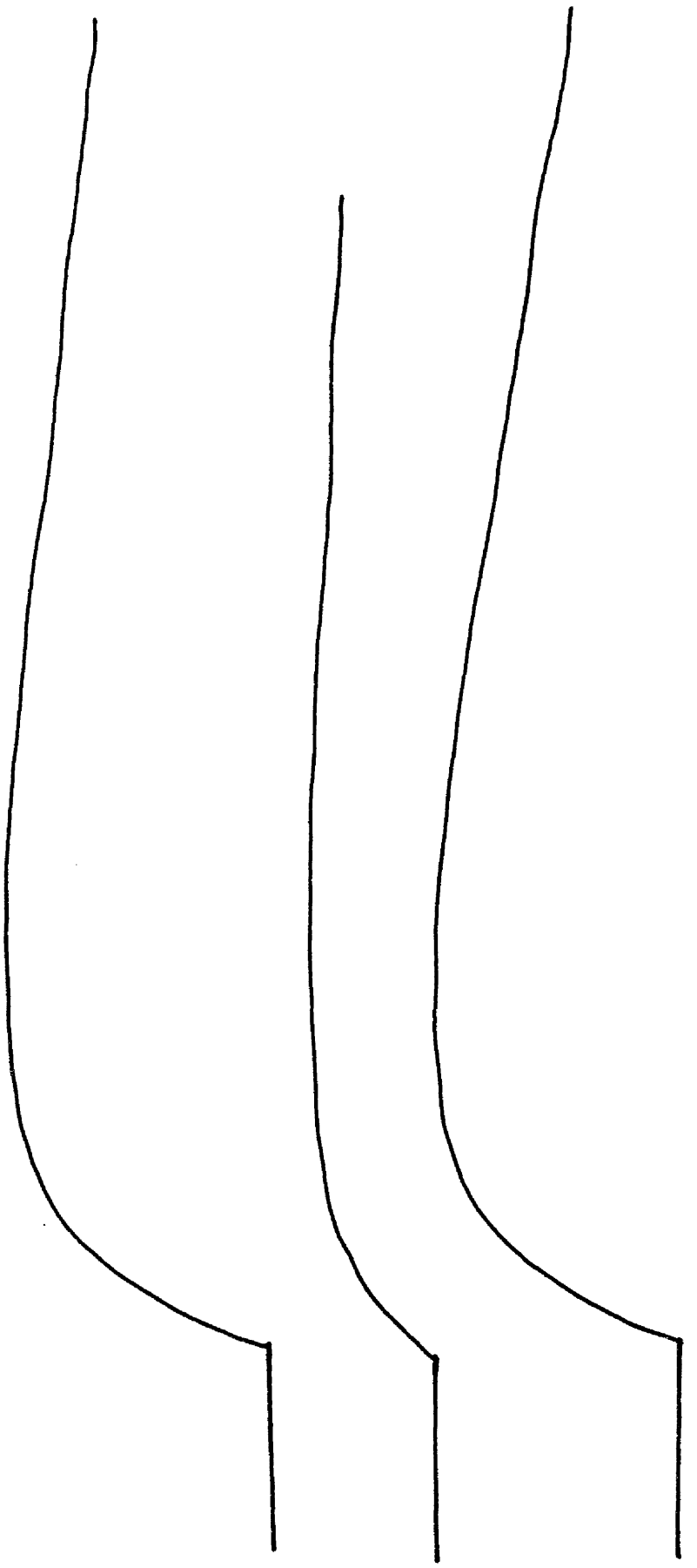


Fig. 7.2.6. Firing curve obtained from combustion of frond without papillae of *M. stellatus* sample (dry weight varied between 10-15.57 mg).

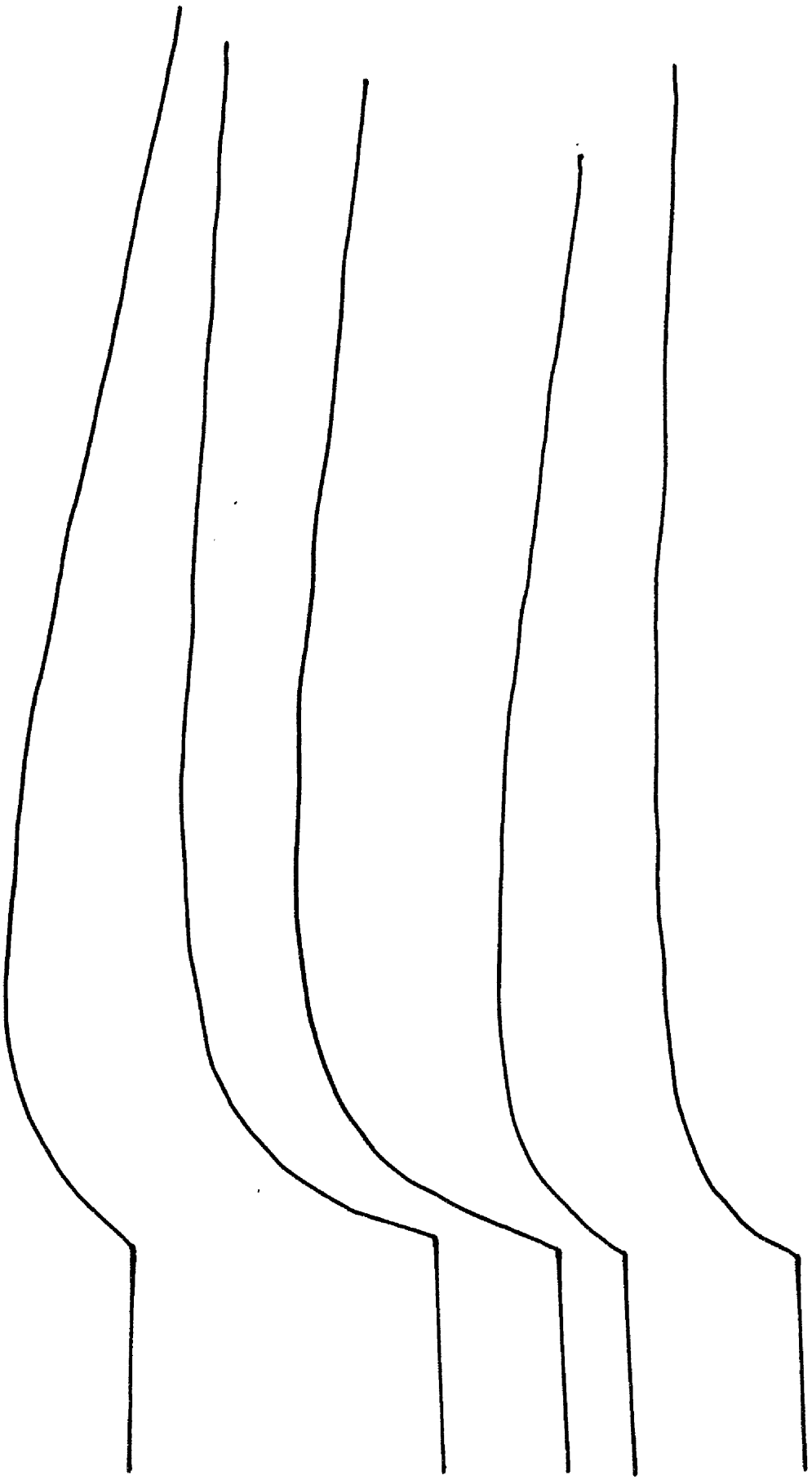


Fig. 7.2.7. Firing curve obtained from combustion basal of frond of *M. stellatus* sample (dry weight varied between 8.25-19.27 mg).

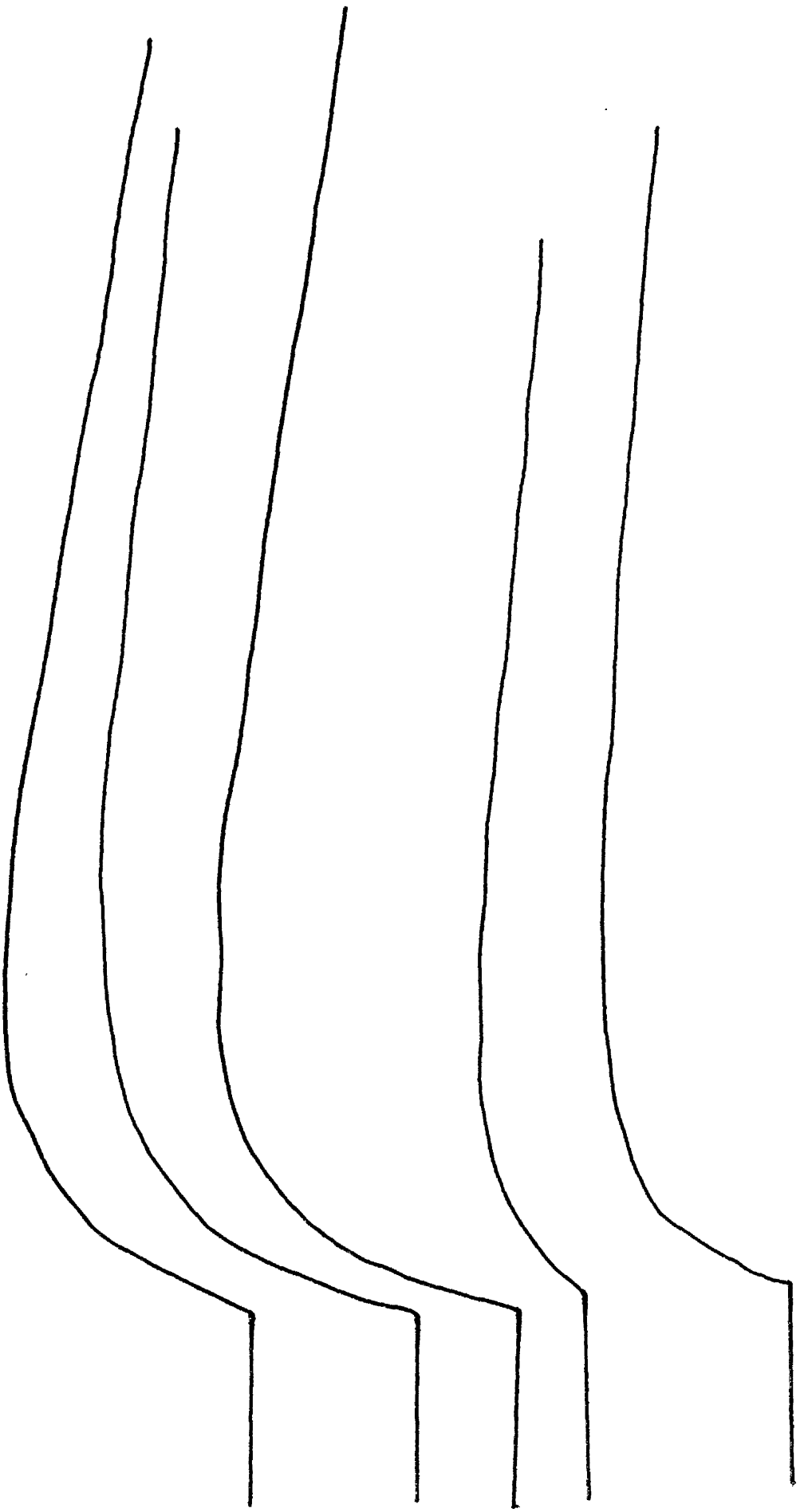


Fig. 7.2.8. Firing curve obtained from combustion of stalk of *M. stellatus* sample (dry weight varied between 12.50-19.60 mg).

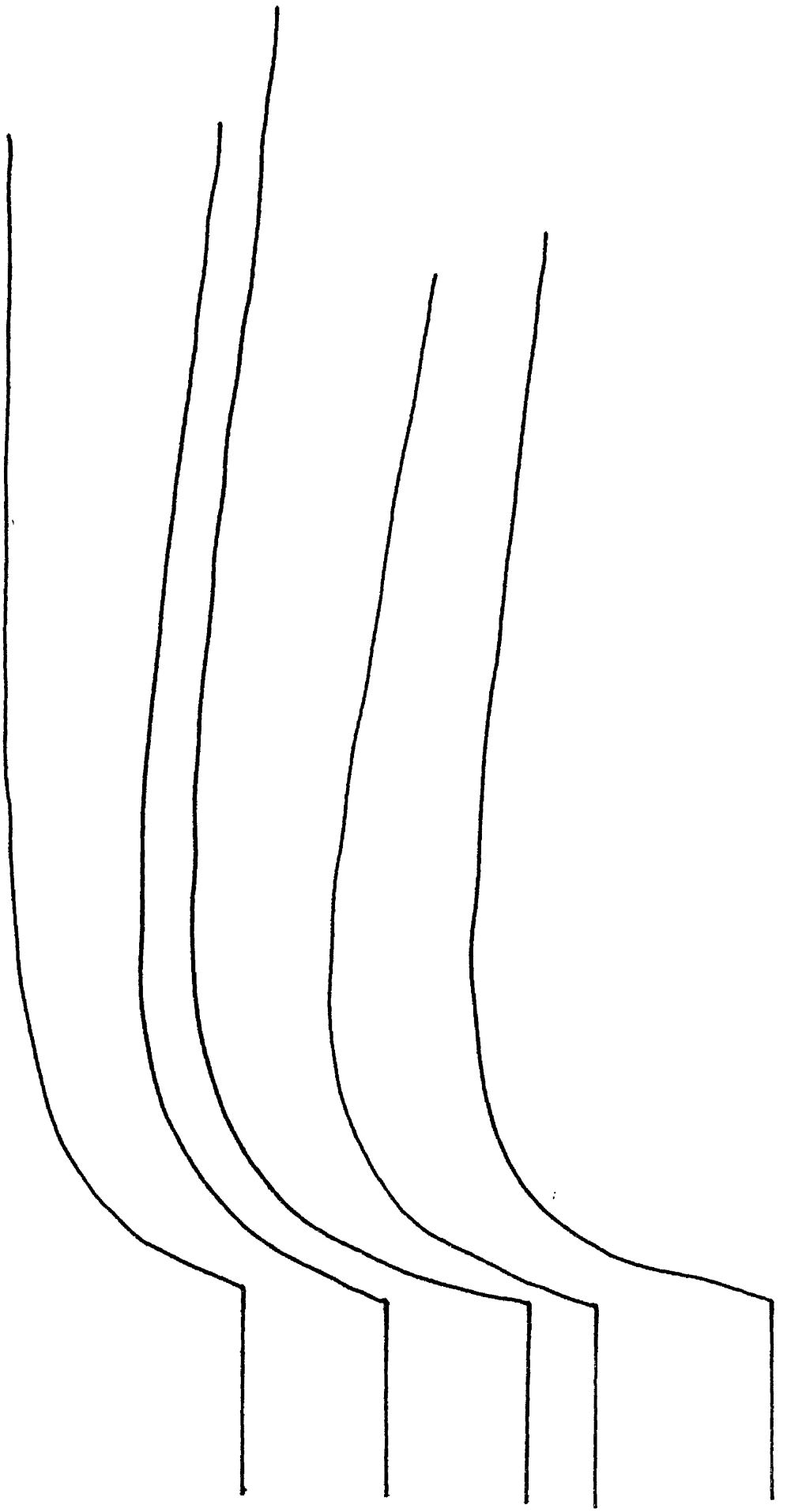
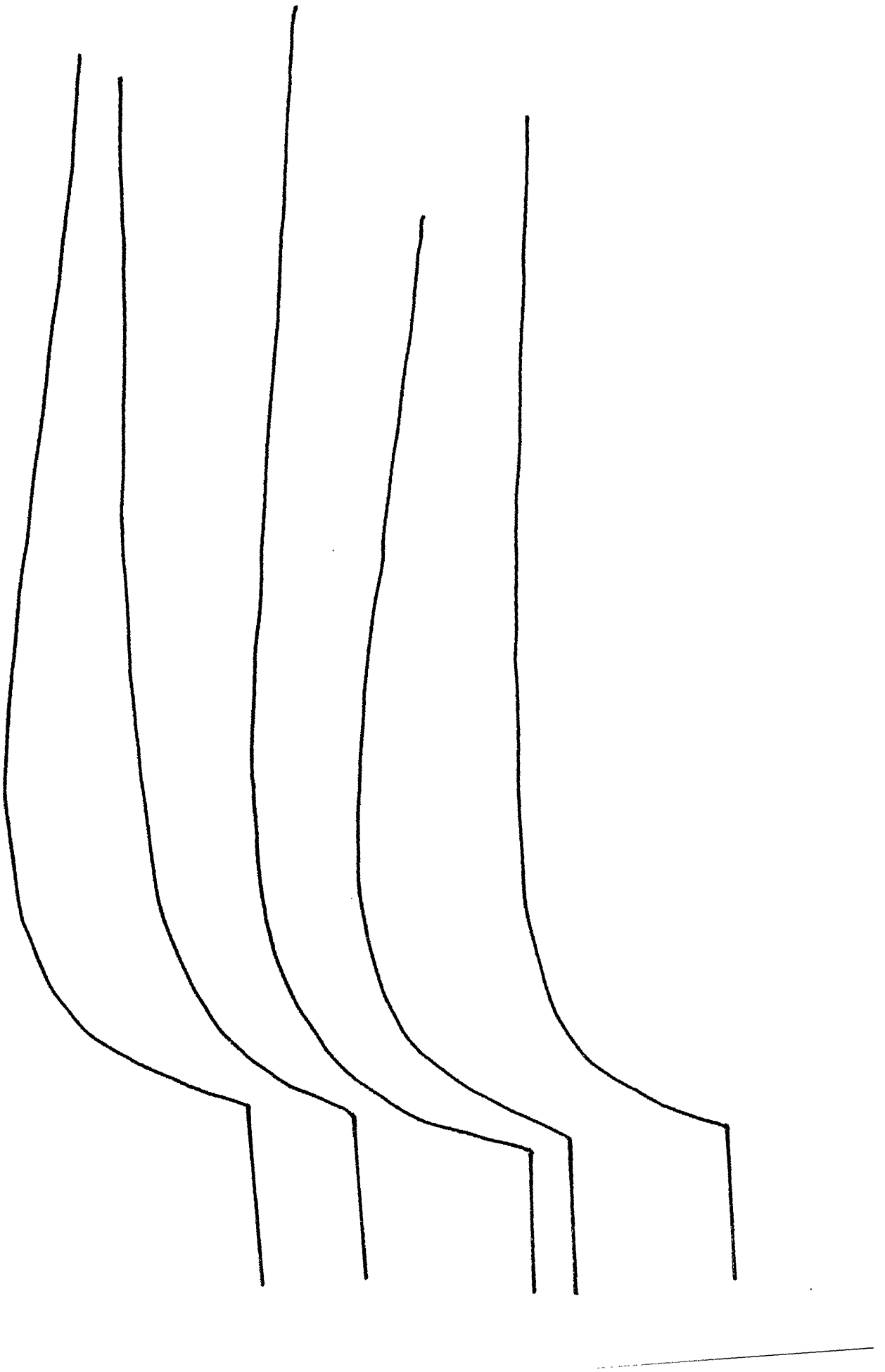


Fig. 7.2.9. Firing curve obtained from combustion of holdfast of *M. stellatus* sample (dry weight varied between 15.18-19.18 mg).



and Tables 7.2.1-2 show the calorific values obtained. The dry weight of the material used varied between 7.15-19.6 mg.

Table 7.2.1 shows the calorific values of spore masses, the fertile and non-fertile papillae, and papillae tissue. Spore masses of *Mastocarpus* sporelings were of mean dry weight 10.79 mg, and produced mean calorific values of 3.65 calories per mg ash free dry weight, and this dry weight gave 1.49 mg ash (15%). Fertile papillae had a mean dry weight of 15.19 mg, and produced similar calorific values to those of the spore masses (3.68 calories per mg ash free dry weight), with 1.85 mg ash dry weight (12%). Non-fertile papillae had a larger mean dry weight than the spore masses (14.27 mg), but had calorific values less than spore masses and fertile papillae (3.45 calories per mg ash free dry weight), with mean ash weight similar to the spore masses (1.5 mg) equal to 11%. Papillae tissue had a similar mean dry weight to the spore masses 11.28 mg, with calorific values (3.86 calories per mg ash free dry weight), with higher ash weight of 1.79 mg (16%). In general the papillae tissue produced the highest calorific values, whilst the measurements for the spore masses and fertile papillae were similar. Noticeably the values for entire non-fertile papillae were lower than those for segments of papillae tissue.

Table 7.2.2 shows the calorific values of upper frond tissue (free from papillae frond tissue), stalk, and holdfast regions of *Mastocarpus*. The upper frond tissue had a mean dry weight of 12.76 mg, with a calorific value of 3.35 calories per mg ash free dry weight, (ash weight 1.54 mg or 12%). The basal region of fronds (mean dry weight higher 15.53 mg), had a similar calorific value of 3.34 calories per mg ash free dry weight, with ash weight 1.82 mg (12%). The stalks had a mean dry weight of 16.91 mg, and showed also a similar calorific value to those of the frond tissue and basal region of fronds (3.42 calories per mg ash free dry weight), with ash weight 1.57 mg (9%). The holdfast region had a larger mean dry weight than the fronds, basal region and stalk (17.92 mg), but had a calorific value of 3.65 calories per mg ash free dry weight with a higher ash weight of 3.84 mg (22%).

Table 7.2.3 shows calorific values and ash free contents of species of marine red

Table 7.2.1. Showing a comparison of the calorific values of spore mass, fertile papillae, non-fertile papillae,and papillae tissue. S.M, Spore mass; F.P, fertile papillae; N.F.P, non-fertile papillae; P.T, papillae tissue.

Part	Dry weight before combust-ion (mg)	Ash weight after combust-ion (mg)	%	Cal. mg^{-1} dry weight	Cal. mg^{-1} ash free dry weight
S.M	7.85	1.65	21	3.32	4.19
	11.10	1.40	13	2.94	3.36
	14.0	1.50	11	3.05	3.40
Mean	10.97	1.49	15	3.10	3.65
S.D	3.10	0.12		0.2	0.47
F.P	19.37	1.71	8	3.07	3.37
	12.70	1.30	10	3.34	3.72
	12.10	1.80	15	3.05	3.58
	16.60	2.60	16	3.24	3.84
Mean	15.19	1.85	12	3.18	3.68
S.D	3.43	0.54		0.14	0.20
N.F.P	19.37	1.73	9	2.86	3.16
	18.37	1.80	10	3.14	3.49
	12.60	1.95	15	2.94	3.47
	7.80	0.9	11	3.25	3.67
Mean	14.27	1.50	11	3.05	3.45
S.D	5.10	0.47		0.18	0.12
P.T	14.80	2.54	18	3.21	3.87
	7.15	1.15	16	3.28	3.90
	11.95	1.86	15	3.25	3.80
Mean	11.28	1.79	16	3.25	3.86
S.D	3.87	0.7		0.04	0.05

Table 7.2.2. Showing a comparison of the calorific values of fronds without papillae, basal of fronds, stalk, and holdfast of *M. stellatus*. F.W.P, frond without papillae; B.F, basal of fronds; S, stalk; H.F, holdfast.

Part	Dry weight before combas-ion (mg)	Ash weight after combast-ion (mg)	%	Cal. mg^{-1} dry weight	Cal. mg^{-1} ash free dry weight
F.W.P	10.04	1.4	14	2.77	3.22
	15.10	1.72	11	2.52	2.84
	15.57	1.85	11	3.17	3.60
	10.00	1.4	14	3.04	3.53
	13.10	1.65	12	3.09	3.54
Mean	12.76	1.54	12	2.92	3.35
S.D	2.76	0.23		0.27	0.32
B.F	18.37	2.77	15	2.67	3.15
	18.90	2.17	12	3.25	3.67
	19.27	1.8	9	3.12	3.44
	8.25	1.0	12	2.76	3.14
	12.85	1.35	11	2.96	3.30
Mean	15.53	1.82	12	2.95	3.34
S.D	4.48	0.69		0.24	0.22
S	12.50	1.13	9	2.81	3.10
	15.09	1.14	8	3.13	3.38
	18.53	2.37	13	3.42	3.92
	19.20	1.5	8	3.03	3.29
	19.60	1.7	8	3.13	3.81
Mean	16.91	1.57	9	3.10	3.42
S.D	3.20	0.15		0.23	0.30
H.F	19.05	3.4	18	2.56	3.12
	15.18	4.83	34	2.63	3.85
	19.18	2.75	14	3.63	4.24
	18.60	2.9	16	2.72	3.23
	17.60	5.3	30	2.66	3.81
Mean	17.92	3.84	22	2.84	3.65
S.D	1.65	1.16		0.45	0.47

Table 7.2.3. Comparison of calorific values of members of the Gigartinales and different regions of *Mastocarpus* plants.

Species	Ash-free content %	Cal./mg dry weight	Cal/mg ash-free dry weight
*Schizymenia pacifica**	66.0	2.95	4.47
*Opuntiella californica**	72.0	3.34	4.64
*Gigartina corymbifera**	70.0	3.05	4.36
*Gigartina papillata**	78.0	3.38	4.33
Iridaea sp.*	75.0	2.92	3.89
Mastocarpus stellatus (spore masses)	85.0	3.10	3.65
Mastocarpus stellatus (fertile papillae)	88.0	3.18	3.68
Mastocarpus stellatus (non-fertile papillae)	89.0	3.05	3.45
Mastocarpus stellatus (papillae tissue)	84.0	3.25	3.86
Mastocarpus stellatus (fertile without papillae)	88.0	2.95	3.35
Mastocarpus stellatus (basal of fronds)	88.0	2.95	3.34
Mastocarpus stellatus (stalk)	91.0	3.10	3.42
Mastocarpus stellatus (holdfast)	78.0	2.84	3.65

* Paine and Vadas (1969)

algae of the Order Gigartinales as given in Paine and Vadas (1969), and different parts of *Mastocarpus stellatus* as measured in the present work. The ash free content was as low as 66% for *Schizymenia pacifica* (4.47 calories per mg ash free dry weight), and as high as 78% for *Gigartina papillata* (*Mastocarpus papillatus*) (4.33 calories per mg ash free dry weight). These red algae had generally higher calorific values (3.89-4.64 calories per mg ash free dry weight) than the different parts of *Mastocarpus*. The red alga *Iridaea* sp. had a calorific value similar to the papillae tissue of *Mastocarpus* (3.89 and 3.86 calories per mg ash free dry weight respectively), with different ash contents (75% and 84%).

7.3. Discussion

The amount of heat from combustion of the spore mass and spore bearing structures of *Mastocarpus stellatus* when converted into calories showed that there is little difference between them. Any differences in body composition are likely to be due to age and biochemical contents. Pain and Vadas (1969) attributed the differences they obtained to additional growth factors and immersion. McQuaid (1985) found that calorific values of 9 intertidal algae reach a peak between mid summer and autumn. Since the energy content of spore masses, fertile papillae, and papillae tissue were of similar calorific value probably the close packing of the spores would seem to be equivalent to the walled cells of the tissues in organic nature and content. Frond tissue without papillae, the base of fronds, and stalks had similar calorific values and presumably similar biochemical (oxidizable) material. The holdfast tissue produced the highest calorific value, probably because of the close packing of smaller cells and thicker walls. The spore masses and holdfast tissue have similar calorific values (3.65 calories per mg ash free dry weight) with different ash weights of 1.49 and 3.48 mg or (15% and 22%). They have similar quantities of oxidizable organic content. The fertile papillae have a similar calorific value (3.68 calories per mg ash free dry weight) with ash weight of 1.85 mg (12%). Frond tissue without papillae and base of tissue from the fronds have a similar calorific value (3.35 calories per mg ash free dry weight) with ash weight of 1.54 and 1.82 mg (12%), they probably have a similar biochemical material. Non-fertile papillae and stalk tissues have a similar calorific value (3.45

calories per mg ash free dry weight). *Gigartina papillata* (*Mastocarpus papillatus*) and holdfast tissue of *M. stellatus* gave ash free values of 78%, but produced difference calorific values (4.33 and 3.65 calories per mg ash free dry weight respectively). The *Iridaea* sp. and papillae tissue of *Mastocarpus stellatus* had a similar calorific values (3.89 and 3.86 calories per mg ash free dry weight), but with different ash percentages (75% and 84%). These diferences are probably due to ecological factors and the biochemical contents in each species.

8. Observation on the organic carbon contents of reproductive structures of *Mastocarpus stellatus*.

8.1. Introduction

The method involved the wet oxidation of carbon by acid dichromate and was based on the procedure described on p. 22. The determination of low chemical oxygen demands of surface waters by dichromate oxidation was studied by (Moor and Walker, 1956). Maciolek (1962) stated that the procedure was adapted to samples of different sizes by adjusting the volume or normality of the dichromate. Dichromate ions ($Cr_2O_7^{-2}$) give orange coloured solution which is reduced to green or bluish-violet chromic (Cr^{+3}) derivatives. The reaction is given by the ionic equation:

$$Cr_2O_7^{-2} + 14\,H^+ + 6e \longrightarrow 2\,Cr^{+3} + 7\,H_2O$$

Preliminary experiment examined the oxidizable carbon in different regions of *Mastocarpus stellatus* at different seasons by wet oxidation.

8.2. Results

Table 8.2.1. shows the amount of dichromate consumed and the quantities of oxidizable carbon with different regions of *M. stellatus* at different seasons. Each is the mean of 3 samples determinations. There would seem to be some evidence of seasonal variations in the quantities of oxidizable carbon. Allowing for individual discrepancies, which may well be due to difficulties associated with the methodology, the data suggest that for the most part the quantities are of a similar order in the parts of the plant examined. One exception seems to be with the fronds lacking papillae, which tended always to show lower values.

8.3. Discussion

When the method is acknowledged as being open to possible discrepancies, it is difficult to formulate clear conclusions from such results. There would seem to be seasonal differences, and the presence of papillae, fertile and non-fertile would seem to

result in increased levels of oxidizable carbon.

Table 8.2.1. Showing oxidizable carbon of different regions of *Mastocarpus stellatus* at different period time (December 1985- december 1986). FP, fertile papillae; non-fertile papillae; FWP, fronds without papillae, BF, basal of fronds; s, stalk; HF, holdfast. NM, not measured.

Date collected	Region	Mean number of ml titrant of sample with $Na_2S_2O_3$	% oxidizable
31 December 1985	FP	9.0	52
	NFP	9.8	57
	FWP	5.7	33
	BF	9.0	52
	S	9.5	55
	HF	9.0	52
15 March 1986	FP	10.7	62
	NFP	5.3	31
	FWP	7.6	44
	BF	9.5	55
	S	8.7	50
	HF	10.4	60
30 June 1986	FP	NM	NM
	NFP	NM	NM
	FWP	5.8	34
	BF	7.0	41
	S	7.8	45
	HF	6.9	4O
23 December 1986	FP	13.3	7 3
	NFP	14.8	82
	FWP	12.0	6 6
	BF	12.7	7 0
	S	12.1	67
	HF	13.5	74

9. General discussion

Sporelings and juvenile plants of *Mastocarpus* assume an attached and discoid form, increasing in size by centrifugal growth from marginal meristematic regions. They are thus closely adherent to the substratum, and will tend in nature to be shaded by a canopy layer of larger marine algae, including fully grown *Mastocarpus* plants. There are two possible sources of physical damage; abrasion due to small stones and the browsing action of herbivorous molluscs. Watson (1983) stated that with juvenile *Mastocarpus* both the erect fronds and the basal discs were generally destroyed by molluscs such as *Littorina* spp., although occasionally the discs were left undamaged while the fronds were broken off. The evidence from the present work in which attached sporelings have been subjected to varying degrees of physical damage by artificial means suggests that, irrespective of the amount of tissue removed, recovery growth always attempts to effect a return to the discoid condition. Not all the energy is channelled solely into recovery growth; growth also continues at the same time from undamaged marginal meristem regions. However, even when up to 75% of the original tissue is removed, complete recovery seems possible. It is also apparent that following severe physical damage and loss of the original meristematic tissue, new meristematic areas can soon arise on the damaged surfaces, and these are the sources of the recovery growth.

The closely adherent discoid form of the sporeling presents a larger surface area for absorption of radiant energy nutrients and gaseous exchange. It will also ensure a constant change of the contact layer of sea water. Growth control mechanisms seem to be linked to the attachment process. When detached and free floating sporelings were subjected to similar physical damage their recovery growth produced irregular forms lacking any resemblance to the attached sporelings. Ageing of the sporelings affected the degree of recovery after damage. Thus sporelings of age 39 days showed much more rapid recovery than those 129 days old. In time the attaching discs produce erect branches, which are at first cylindrical in form.

The discoid adherent form of the sporelings renders them susceptible to exposure to

air and insolation if there is loss of the protection canopy layer. In experiments with seemingly killed sporelings it was observed that isolated 'islands' of living cells within the dead tissue could increase in number and in time would grow between the dead cells and spread onto the surface of the dead tissue and grown in size. Whether such a recovery is possible in nature would depend on the degree of protection available and presumably on the number of viable cells within the dead tissue. In the experiments this new living tissue eventually died, probably due to invasion from bacteria and fungi from the associated decaying tissue.

Rhizoids of *Fucus* germlings on *Mastocarpus* sporelings produced at first thin strands of attaching mucilage. After several days there was more abundant mucilage production. *Fucus serratus* germlings on *Mastocarpus* developed short rhizoids and 'bodies'. Rhizoids of *F. serratus* on *Mastocarpus* effect a very close bonding to the 'cuticle' of *Mastocarpus* and there is evidence of some penetration of the *Mastocarpus* cuticle by the *Fucus* rhizoids. In magnified surface view the *Mastocarpus* sporelings show an irregular appearance, so that *Fucus* rhizoid attachment is quickly achieved.

Mastocarpus sporelings and *F. serratus* grow in same zone on the shore. Experiments showed that attached *F. serratus* germlings had some effect on the growth and development of *Mastocarpus*, possibly due to some local production of extracellular substances by *Fucus*, *Mastocarpus* sporelings grown in close proximity with *F. serratus* germlings also showed some inhibition of growth over the 3 weeks of the experimental periods. These inhibitions may again be due to the germlings secreting some chemical substances.

Ulva and *Enteromorpha* are common in intertidal habitats and in rock pools. The results of experiments indicated that *Mastocarpus* sporelings in the presence of sporelings of *Ulva* and *Enteromorpha* showed some growth inhibition, and died after 6 weeks. Sporelings of *Enteromorpha* are 'opportunist colonisers' of shore habitats. Dense settlements of *Enteromorpha* zoospores close to *Mastocarpus* sporelings might thus have severe side effects. *Mastocarpus* sporelings with *Porphyra* sporelings showed some retardation of growth due to a shading effect, whilst *Porphyra* with *Mastocarpus* showed little growth inhibition of the surface area of fronds.

The diatom *Navicula* releases large amounts of mucilaginous substance. The *Navicula* mucilage had marked effects on both the growth and morphogenesis of *Mastocarpus* sporelings and *Fucus spiralis* when present both combined and singly. The sizes of *Mastocarpus* sporelings diatoms mucilage with and without *Fucus* were larger than on clean surfaces. *Fucus spiralis* germlings on the diatoms mucilage showed both rhizoids and development of germlings were effected. Some irregular shapes of *Mastocarpus* sporelings were observed on diatom mucilage with and without germlings, with less effective attachment to the substratum . The *Fucus* germlings showed increases in the size of rhizoids in the presence of diatom mucilage. As indicated by Huang (1982), the presence of diatom mucilage on a substratum can have effects on the growth of sporelings or germlings that settle on it.

Measurements of photosynthetic activity with the spore suspensions produced unexpected results, in that no measurable photosynthetic activity could be detected, but there was some respiratory action. The question is whether these spores when free floating metabolise their reserve material. It is known that the spores are surrounded by a mucilage sheath which helps flotation and primary attachment, but since attachment requires immediate and abundant mucilage secretion at the point where a spore touches the substratum, perhaps some synthesis of attaching mucilage precursors proceeds in the pelagic phase, and it is this activity which is responsible for the uptake of oxygen.

Water movements are important for the photosynthesis of *Mastocarpus* sporelings. Water flowing over the surface of attached sporelings is necessary for photosynthetic activity. The turbulence of water in the habitat with ensure this exchange. In nature the turbulent nature of the sea water flowing over the rock face will ensure the constant change of the contact layer, and it would seem that for continued photosynthetic activity the *Mastocarpus* sporelings require this constant change. The discoid and flattened habit of the sporelings is accompanied by a compacted arrangement of the constituent cells. Gaseous exchange and nutrient absorption by the assimulatory cells, which tend to be concentrated towards the upper surface, may well require a more active water movement over the upper surface of the sporeling.

Sporelings of *Mastocarpus* could be sensitive to extreme light intensities. In nature the incident irradiance would be reduced through shading by other algae after attenuation by the overlying sea water. Measurable photosynthetic activity was observed at low irradiances. Photosynthetic rates increased with an increase in light intensity, although evidence of light saturation was obtained.

In the northern seas it is likely that the winter months will ensure long periods of very low irradiances, due to short days and cloud cover. The results of experiments in which the *Mastocarpus* sporelings were subjected to the extreme conditions of dark storage at low temperature indicated that whilst the length of time could influence the subsequent rate of photosynthetic activity, even under the more extreme conditions there was some measurable restoration of photosynthetic activity when higher levels of irradiance became available. The results would suggest that whilst the shortened days and very low levels of irradiance may lead to marked reductions in photosynthetic rates, to be extent that little assimulatory activity takes place (although with an accompanying reduction in respiratory activity), given some localised increases in light levels (short periods of winter sunshine), the sporelings may be able to take advantage of this limited irradiation budget in sufficient amount to maintain a reduced metabolism until warmer and sunnier periods return. There on some indications that after the shorter dark periods the sporelings may show enhanced rates of photosynthetic when re-illuminated.

As expected, the photosynthetic activity of the sporelings showed sensitivity to temperature changes. The shock effect of sudden temperature changes are such that at 5 ^{o}C and 30 ^{o}C there was reduced photosynthesis compared with that obtained at 20 ^{o}C. This temperature is a few degrees above the annual late summer maximum for the sea in the Firth of Clyde. At 35 ^{o}C photosynthetic was inhibited. When some acclimatisation to the increased and reduced temperatures was allowed, photosynthetic rates were higher at 5 ^{o}C than at 30 ^{o}C, which would suggest that for this northern *Mastocarpus* 'strain' there is some temperature sensitivity in the higher ranges. Whilst salinity variations are a likely environmental factor in an intertidal habitat, the *Mastocarpus* sporelings (as indicated by their photosynthetic activity) appear to be sensitive to lowered and increased salinities. Perhaps such extreme conditions are less

likely in nature on the lower shore, where the sporelings will have the additional protection of the overlying canopy layer of algae.

It should be remembered that the discoid sporeling phase needs to be well established before the growth of the erect branches. These then became the principal sites of assimilation and growth, and formation of reproductive structures. The most noticeable feature is that the first juvenile erect branches tend to be evenly spaced on the attaching disc, which may be in part an explanation of the tendency of damaged very young sporeling to attempt to regain the discoid form in its recovery growth. Again, when relative rates of photosynthetic activity are compared, that of the juvenile erect branches is appreciably greater than the attaching disc from which it arises. This would seem to be associated with their function as active growth sites, projecting as they do above the substratum, and with the swirling water around them and a greater degree of illumination than that obtained with the attaching discs.

The papillate outgrowths, both fertile and non-fertile are characteristic features of the channelled fronds of *Mastocarpus*. It is not suprising that the spore masses from within the papillae appear highly sensitive to features arising from exposure to air desiccation and increased salinity. The papillae themselves are very sensitive to extreme desiccation, when assessed in terms of their photosynthetic activity. In nature the channelled nature of the fronds in which the papillae lay may allow some protection from the extremes of drying when the fronds are exposed to air. The overlapping nature of the crowded fronds may also be an important factor. When covered by the sea the movements of the fronds will ensure water circulation around the papillae, since experimental evidence suggest that this constant change of the contact layer is necessary for their effective photosynthesis.

The biochemical natures at different regions of *Mastocarpus* plant were indicated by the bomb calorimeter. There was some difference in energy content, these probably being due to the chemical contents and ecological factor.

One of the more interesting developments in this study of *Mastocarpus* spores and sporelings is that the photosynthetic rates of quite juvenile stages can be measured using the oxygen electrode. The preliminary results obtained show that some

environmental stress effects are quickly registered in the subsequent assimilatory activity of the sporelings. Methods may therefore be developed for an examination of the more fundamental aspects of sporelings photosynthesis, and the more subtle effects of environmental stresses, both natural and man made.

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