EXPERIMENTAL AND ECOLOGICAL STUDIES ON THE RED ALGA GIGARTINA STELLATA (STACKH. IN WITH.) BATT.

Abdul Karim Khfaji, B.Sc., M.Sc.

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SUMMARY:

1. Plants of Gigartina stellata from three different localities in the Firth of Clyde were examined and showed some clear cut variation in their morphological characters.

2. The field transplant experiments in which plants of Gigartina from the three localities were transplanted in reciprocal fashion indicated that the morphology of Gigartina is principally controlled by environmental conditions with possible genetic variability.

3. Plants of Gigartina stellata required more than three years to establish themselves from spores on the denuded and sterilized areas of the shore.

4. Plants of Gigartina regenerate only when parts of the flattened fronds were left intact on the basal crusts. Regeneration is a slow process and some plants formed numerous small proliferations from the margin of the fronds.

5. The period of maximum spore discharge in Gigartina from all three localities was recorded in September through December, whilst the period of minimum spore discharge was recorded in April through July.

6. Culture of fronds of Gigartina from the different localities under the same conditions showed similar growth rates in all plants except plants from Loch Long, which showed slightly slower growth rates which may suggest a lack of ability to adapt to the experimental conditions.

7. Culture of fronds of Gigartina from one locality (Cumbrae Island) under a variety of laboratory induced environmental conditions emphasised the important role of environmental conditions and confirmed the results of field transplant experiments.

8. Culture of carpospores of Gigartina under laboratory conditions showed that the disc-like sporelings grew healthily for 5 months in which time the average diameter of sporelings reached 0.5 mm, then they detached and lost into the surrounding medium with no sign of forming the erect branches. The carpospores of the related species Chondrus crispus grew much faster and reached 0.8 mm in 3.5 months in which time they formed their first erect branches. The sporelings of Chondrus crispus were found to produce an antibiotic substance but not those of Gigartina.

9. Carrageenan showed some obvious variation in its quantity and quality in plants from the three localities. Portencross plants contained higher quantities of carrageenan but with weaker gel strength, and plants from Loch Long contained lower carrageenan content with stronger gel, while plants from Cumbrae Island showed an intermediate value in both carrageenan contents and gel strengths.

10. Carrageenan showed seasonal variations in its quantity and quality. Maximum carrageenan content was recorded in March through May, whilst the minimum carrageenan content was recorded in September through December. Strongest gels were recorded in December through March whilst the weakest gel was recorded in April through August.

11. The transplants experiments indicated that both carrageenan contents and gel strengths are controlled by environmental conditions.

12. Plants of Gigartina collected from one locality (Cumbrae Island) and kept two weeks under varieties of laboratory induced environmental conditions showed the important role of all conditions studied in controlling both carrageenan contents and gel strengths.

13. The most fertile plants of Gigartina in all the three localities contained lower carrageenan contents with stronger gel, whilst the sterile plants (with no papillae) had higher carrageenan contents, but with weaker gel strength.

14. Plants from Portencross were better able to postpone the tissue dehydration than plants from Loch Long.

15. Plants from Portencross were also better able to control the rate of chloride ions loss than plants from Loch Long.

16. Plants of Gigartina from the different localities have similar rates of photosynthesis. Plants from Portencross were better able to recover after drying for 12 hours under laboratory conditions and have considerably high photosynthetic rates. The various salinities were found to affect the photosynthesis in similar ways as the effects observed on the growth rates of fronds of Gigartina.

CHAPTER 1.

INTRODUCTION

The marine red alga Gigartina stellata (Stackh. in With.) Batt. is a member of the family Gigartinaceae, of the order Gigartinales and the class Florideophyceae. The name Gigartina is derived from the Greek word for grape stone and refers to its emergent cystocarps. Gigartina together with the related Chondrus crispus Stackh. is also called carrageen (Marshall, Newton & Orr 1949). In the Firth of Clyde, Gigartina stellata is abundant, dominating the lower littoral and upper sublittoral zones of the rocky shores. Thus it is a very good area for studies on the biology of this carrageenan seaweed . Although genera of the order Gigartinales form the basis of a large seaweed industry, little seems to be known about the basic feature of their biology (Burns 1971).

1.1 Taxonomy and morphology:

The definitive type specimen (lectotype) is Stackhouse's description in Withering "An arrangement of British Plants" published in 1796 and described as Fucus stellatus (Dixon and Irvine 1977). Goodenough and Woodward (1797) called the plant Fucus mamillosus. The genus Gigartina seems to have been first proposed by Stackhouse (1809) but few copies of this paper now exist (Marshall et al 1949). Lamouroux (1813) unknowingly also proposed the genus Gigartina but the species attributed to the genus by Lamouroux were numerous and apparently unrelated, few of which are today considered to belong to Gigartina as currently accepted. Gigartina was also described by Greville (1830), Kutzing (1843) and J.A. Agardh (1899), who in his

final revision brought the number of species to 69. Setchell and Gardner (1933) pointed out that species attributed to Gigartina have passed successively and temporarily through various genera and they brought the number of species to 88. These species were to be found on the coast of New Zealand (one quarter of those described) on the coast of Western North America (one third of the species) and the remainder are to be found on the Mediterranean and Atlantic coasts of Europe, Northeast of North America and South Africa. Thus Gigartina with its numerous species has a wide global distribution.

The variable form of the plant has been recognised from earlier descriptions, such as Turner (1808-1819, Vol.4) who described several varieties. Goodenough and Woodward (1797) described four varieties for the type species Fucus mamillosus. These varieties were:

1 - var. β. linearis: frond narrow, linear apices bluntish and emarginate.

2 - var. γ. prolifer: frond proliferous at both the disc and margin with very numerous clustered oblong tuberculate processes.

3 - var. δ. echinatus: the branches all dilated upwards nearly naked on one side.

4 - var. ε. incurvatus: frond nearly linear, apices rounded, peduncles of the tubercules incurved.

Holmes and Batters (1890-1891), who described the plant as Gigartina mamillosa, have listed the same four varieties described by Goodenough and Woodward (1797). Batters (1902) called the plant

Gigartina stellata and listed the four varieties as: var. linearis, var. echinata, var. genuina and var. acuta and described f. prolifera Turn. and f. incurvata Turn. as distinct forms. Newton (1931) followed Batters's classification but listed only three varieties; var. prolifera Turn., var. incurvata Turn. and var. acuta Good. & Woodw. and included f. genuina in the type species.

Marshall et al (1949) have examined large numbers of fresh specimens of Gigartina stellata from very varied localities on the west coast of Britain and they stated that only one variety, var. acuta Good. & Woodw. can be distinguished. However, they added that there is a wide range of polymorphism in the species and under certain conditions extreme forms are produced but these have not been regarded as true varieties.

Gigartina stellata is characterised by the broad segments of the frond and the regular dichotomy of branching. It varies from 4-17 cm. in length and 3-5 cm. in width, the branching is sparse and the channeling of the frond, which is one of the distinguishing characteristics of the species, is very well marked. The apices of the frond are rounded or wedge-shaped. The surface of the upper parts of the thallus, except in very young specimens, is usually covered with papillae in which carpogonia and then carposporangia develop. These latter are not usually marginal. The colour of the frond is reddish-brown to black, except when the plant becomes greenish under conditions of strong illumination, or when new vegetative growth is taking place in Spring. The newly formed branches of the thallus are deep pink in colour, sometimes turning olive green at a later stage. The plants are attached to

rocks, often in very exposed situations where the coast is surf washed, or grow in rock pools.

The var. acuta Good. & Woodw. as described by Marshall et al (1949) is characterised by the almost linear form of the thallus and the irregular and profuse branching; the branches are often twisted; the length of the thallus is from 4-10 cm. and the width from 0.2 - 0.5 cm. In Cardigan Bay the whole plant, except the apices of the branches, is often covered by an epiphytic polyzoan Membranipora pilosa (L.). The apices of the thallus are often acute. The plant is generally very dark in colour, although greenish specimens have been found under conditions of strong illumination. There are few papillae on the surface of the thallus, but in addition to the carpogonia borne in them, others are sometimes developed in small outgrowths from the edge of the thallus, this form is usually found growing on sand covered rocks.

Also two growth forms have been described by Marshall et al (1949). The first growth form is a very distinctive, parsley-like growth. It is almost black in colour, but greenish if exposed to strong illumination. Papillae bearing carpospores partly cover the thallus surface and amongst them are numerous small proliferations. This form always grows in water of low salinity and is often found in localities of sandy shingle beach. Possibly the mechanical irritation by angular fragments causes the excessive development of papillae and proliferations. This form was found almost completely buried in the sand in some localities on the Isle of Skye.

The other growth form is the dwarf-form. The plant on the whole is similar to the parsley-type but is distinguished from it by

the sparseness of proliferations and branches, together with its very small size. The thallus is rarely more than 3 cm long. The papillae which bear the cystocarps are usually born in tufts at the apices of the thallus. The extremities of the branches are rounded and the thallus is reddish-brown in colour. This form is found growing on rocks beneath constantly dripping fresh water, at the base of the promenade wall at Aberystwyth. It has never been found elsewhere.

The most recent comprehensive review of the genus is that by Kim (1975) who stated that the most confusing feature of members of the family Gigartinaceae is the extreme variability of the external morphology. Earlier investigators were aware of this extreme variability. They used characters of external morphology such as colour, size and shape of the frond, the pattern of branching, the form and distribution of cystocarps and tetrasporangial sori to distinguish species. In a few studies, internal structures such as the size of mature cystocarps, the thickness of the frond and the diameter of medullary filaments, were considered. Unfortunately, most of these characteristics are subject to so much variation under varied environmental conditions that they must be used with great caution in distinguishing species. Kim (1975) proposed a new classification system in which he suggested that the Gigartinaceae has only two genera, Gigartina and Chondrus. He subdivided genus Gigartina into two subgenera, Gigartina and Iridaea. In distinguishing the different species Kim used only internal characteristics such as:

1. The presence or absence of enveloping tissue around the gonimoblast.
2. The amount and compactness of enveloping tissue.
3. The occurrence of enlarged cells of female filaments around the gonimoblast filaments.
4. The position of gonimoblast filaments between female filaments (interlaced or not interlaced).
5. The shape of cells in young gonimoblast filaments (elongate or round).
6. The abundance of absorbing filaments.
7. The depth of tetrasporangial sori in the thallus.
8. The method of formation of tetrasporangial mother cells (intercalary, accessory or both).

1.2 Life history studies on Gigartina:

Members of the order Gigartinales are characterised by carpogonia which arise from apical cells of undifferentiated filaments of the thallus; development of the carposporophyte follows transfer of the zygote nucleus to an auxiliary cell which is always an unspecialised vegetative cell of the thallus, and may be a supporting cell of the carpogonial branches or a cell at distance from this. Carposporangia liberate either one carpospore or four carpotetraspores; gametangial and tetrasporangial plants are of similar or totally dissimilar organisation.

Kylin (1932) in defining the order, stated that the auxiliary

cell is formed before fertilization of the carpogonium. Schmitz and Hauptfleisch (in Engler and Prantl's "Die naturlichen pflanznfamilien" published in 1896) described the Gigartinales as one of four orders of the Florideae. 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 Gigartina stellata, the gametophytic and cystocarpic phases only were recognised with an apparent absence of a tetrasporophyte. Taylor (1957) found that some members of the order Gigartinales have a triphasic cycle in which the gametophyte is morphologically similar to tetrasporophyte (i.e. a Polysiphonia type of life history). Knaggs (1969) and Dixon (1970) pointed out that the occurrence of sexual, cystocarpic and tetrasporangial plants of any given species cannot be in itself proof that the species has a Polysiphonia type life history. It must be appreciated that there may be several expressions of life history for any species (Kim 1975).

Lindauer (1939) reported a tetrasporophyte of Gigartina alveata (Turn.) J. Ag. in New Zealand and stated that the tetrasporophytes of G. alveata resembled the cystocarpic plants very closely with the branching being strictly dichotomous. The tetrasporangia first make their appearance in one or more small oval patches and become large and well raised above the surface of the frond. However, Marshall et al (1949) who failed to observe the tetrasporic phase of Gigartina stellata in nature, stated that G. alveata is very different in habit from any of the northern forms. Isaac and Simons (1954) reported plants of Gigartina pistillata (Gmelin) Stackhouse from Port Alfred, South Africa, with a record of plants bearing both tetrasporangia and

carposporangia on the same frond.

Damman (1930) grew the carpospores of Halarachnion ligulatum (Woodw.) Kutzing and obtained crustose plants from the carpospores. These crustose plants were later shown by Boillot (1965) to be the tetrasporophyte of H. ligulatum and to resemble Cruoria rosea (Crouan frat.) Crouan frat. Marshall et al (1949) obtained only basal discs from carpospores of Gigartina stellata. They stated that they were aware of the results of Damman (1930), but they were unsuccessful in associating a crustose stage with G. stellata. They therefore speculated that the gametophyte was formed directly from carpospores. A similar conclusion was drawn by Hinchman (1964) in his study of Gigartina papillata complex of the North Eastern Pacific. According to Marshall et al (1949) and Hinchman (1964) the carpospores might develop apomictically or minute tetrasporophytes were yet to be found in nature.

Edelstein, Chen and McLachlan (1974) found that the carposporophytes of Gigartina stellata develop without any evidence of fertilization which suggests an apogamous development in the species. They stated that unlike some species of Gigartina with a diplobiontic life cycle, only sexual plants of G. stellata have been found. Moreover, most of the records of the male plants for the species are doubtful, and because the tetrasporic phase is apparently lacking, reproduction was assumed to be only by means of carpospores. Chen, Edelstein and McLachlan (1974) completed the life history of Gigartina stellata in culture started from carpospores produced by plants in nature. The alga was taken through two generations within a 3-year period; in all cases carpospores

gave rise directly to gametophytic plants without intervention of tetrasporophyte. Male plants with spermatia were absent in culture and were not noted in the field.

Boillot (1965, 1972) and Codomier (1972) have shown that in some species of Gigartinales, the foliose gametophyte alternates with a small crustose tetrasporophyte.

West (1972) reported on the culture of tetraspores of Petrocelis fransiscana Setch. et Gardn. from California. Sporelings gave rise to fertile gametophyte which resembled Gigartina agardhii Setch. et Gardn. and although the carpospores were viable, their fate was not followed beyond germination. This result was confirmed by West and Polanshek (1972) who reported briefly on culture started from carpospores of Gigartina papillata (c.Ag.) J. Ag. also from California, some spores gave rise directly to gametophytic phase whereas others formed crustose plants and while not becoming fertile, resembled Petrocelis anatomically. More recently, Polanshek and West (1975) confirmed again their previous results by culturing the tetraspores of Petrocelis from Alaska and California. They found that the tetraspores gave rise to foliose, dioecious gametophyte, and the fertilized female plants produced cystocarps and carpospores gave rise to crustose plants anatomically similar to field-collected Petrocelis sporophytes.

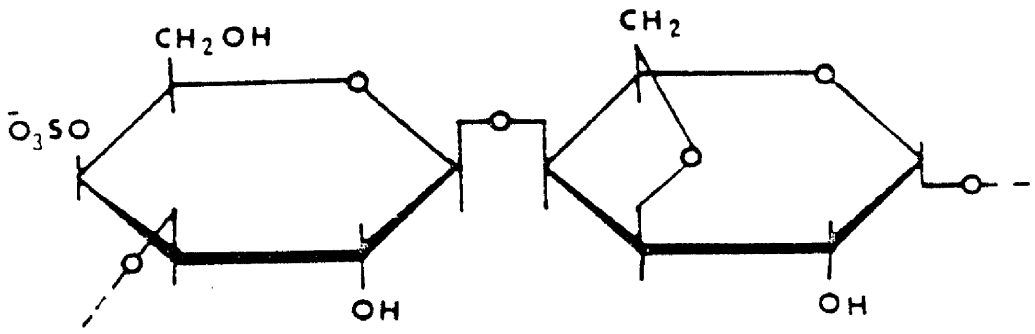
West, Polanshek and Guiry (1977) cultured the tetraspores of Petrocelis cruenta J. Ag. from County Waterford, Ireland. Again they found that the tetraspores gave rise to foliose, dioecious gametophytes,

and carpospores from this foliose phase gave rise to Petrocelis-like crustose phase that has not reproduced in culture. They also stated that Gigartina stellata is postulated to represent the naturally occurring gametophyte of Petrocelis cruenta in Ireland and possibly elsewhere. Rueness (1978) found that carpospores of Gigartina stellata from the Norwegian west coast gave rise directly to carposporic plants throughout two successive generations in culture. The male plants and tetrasporophytes were absent in culture. A remarkable result was found by Rueness (1978) in that the carpospores germinated as crustose discs at 12°C and 17°C, 200-300 lux and 1500 lux and under photoperiod of L/D 16:8. At the higher levels of temperature and light intensity, erect axes were formed and grew to reproductive maturity in about 12 months with only slight increase in the discs diameter after establishment of erect axes. Under conditions of low temperature and light intensity the basal discs increased in diameter through marginal growth, attaining diameter of about 25 mm after 4 years of incubation with apparent suppression of erect axes.

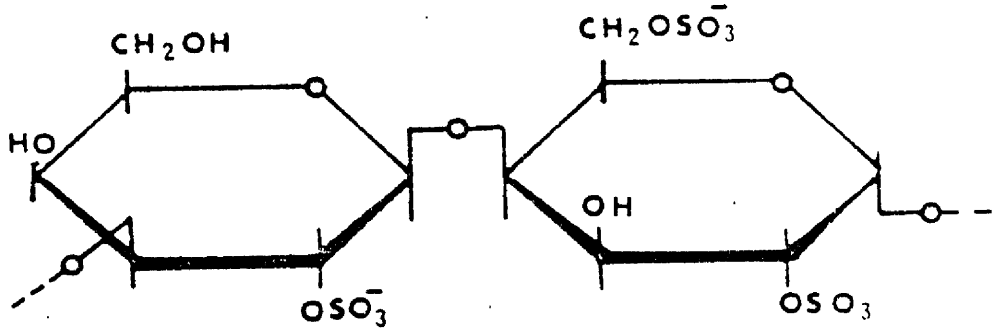
1.3 Studies on Carrageenan:

Gigartina stellata in common with several other members of the Gigartinales contains the phycocolloid carrageenan. This substance is a sulphated polysaccharide which exists in 3 main forms κ, λ and μ carrageenan as shown in Figure (1). The name Carrageenin to designate the extract from Chondrus crispus was first used by Stanford in 1862 (De Rosa 1972), but a polysaccharide with similar properties had been isolated and described by Schmidt (1844). The term Carrageenan is more

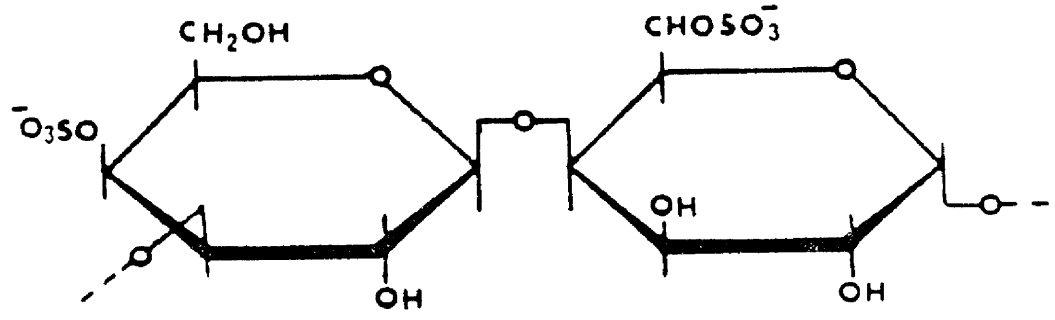
Figure 1: Showing the structure of the three main types of carrageenan.



K– CARRAGEENAN



λ– CARRAGEENAN



μ– CARRAGEENAN

recent and has been used by several authors after 1950. The chemical and physical properties of Carrageenan make it useful as a stabilizer as well as a gelating, stiffening and thickening agent. Food, pharmaceutical, cosmetic and other industries incorporate Carrageenan in products as diverse as tooth paste, chocolate milk, ice cream, insect sprays and paint (Hoppe and Schmid 1962).

Sebor in 1900 (as reported by Haas (1921a)) extracted the mucilage of Chondrus crispus and described the Carrageen mucilage as a complex carbohydrate. Haas (1921a) and Haas and Hill (1921) reported that the hot water extract of Chondrus crispus is not a single substance but is composed of at least two constituents, which can be separated by making use of their different solubility in cold water, and they called the two constituents the cold extract and hot extract. The cold extract produces thick viscous solutions, while the hot extract produces solutions which tend to gelatinise on cooling. A few years later Butler (1934) studied the extract of Chondrus crispus and stated that the extract is a mixture of sulphates together with other substances such as phosphates. Carrageenan of both Gigartina stellata and Chondrus crispus was found to be similar (Dewar and Percival 1947) and Marshall et al 1949) who also found that both species yield about 50% of dry weight as an extract. They used the term "Agar of G. stellata" without mentioning the possibility of fractionation.

Smith and Cook (1953), Smith, Cook and Neal (1954) and Goring and Young (1955) studied the fractionation of carrageenan and showed that it contains two or more polysaccharides, and that the κ-carrageenan is precipitated by addition of potassium chloride, while λ-carrageenan

remains in solution. Black, Blakemore, Colquhoun and Dewar (1965) who studied carrageenan from several red marine algae including Gigartina stellata, indicated that carrageenan from G. stellata gave poor separation into κ and λ fractions. This result was confirmed by Waaland (1975) who studied Carrageenan from several species of Gigartinacea (Iridaea cordata, I. heterocarpa, I. lineare, I. cornucopiae, Gigartina exasporata and G. papillata) and found that the total yield from the species studied ranged from 52-66% of dry weight, and in all species, the carrageenan in gametophyte was 93% κ-carrageenan while in tetrasporophyte it was 95% λ-carrageenan. A similar result was found by Mathieson and Tveter (1975) who reported that the λ -carrageenan "the non-gelling fraction" occurs in tetrasporophytes of Chondrus crispus. McCandless, Craigie and Walter (1973) found a similar result when they analysed carrageenan from Chondrus crispus. They stated that the sporophyte of Chondrus crispus contains only λ- carrageenan, whereas the gametophyte contains a mixture of κ- carrageenan, μ- carrageenan and x- carrageenan.

Chen, McLachlan, Neish and Shacklock (1973) stated that the ratio of the concentration of κ- to λ- carrageenan is low in tetrasporic plants of Chondrus crispus and relatively high in the gametophytes. The κ/λ ratio in Gigartina stellata was high in gametophyte as in C. crispus. Therefore, they suggested that the commercial quality of carrageenan can be correlated with the biological status of the harvested plants. McCandless and Craigie (1974) analysed carrageenan produced by carposporic plants of Chondrus crispus from Nova Scotia and found that the plants have 75% κ-carrageenan. The soluble carrageenan is not λ-carrageenan, but a mixture of κ-precursor, μ-carrageenan and a

new sulphated galactan. McCandless, Craigie and Hansen (1975) found similar results to Chondrus crispus when they analysed the carrageenan from Iridaea cordata and they also found that male plants of I. cordata yield proportionately more κ- carrageenan than did the female. Pickmere, Parsons and Bailey (1975) studied the carrageenan composition in three New Zealand species of Gigartina (G. decipiens, G. atropurpurea and G. angulata) and found similar results to the previous authors, i.e. the tetrasporophytes contain λ- carrageenan and little or no κ-carrageenan. They also found that juvenile gametophytes contain more μ-carrageenan than the fertile ones.

The chemical structure of carrageenan has been studied by many authors. For example, Tollen (1914) as reported by Haas (1921a) placed carrageenan among the fructosans, without assigning a definite formula to the substance. Also Haas (1921a) quoted the work of Sebor (1900) who stated that the complex carpohydrate of Chondrus crispus is produced by the union of galactose, dextrose and leavulose residues together with small quantities of pentosan or methyl pentosan as impurity. Smith, O'Neill and Perlin (1955), Morgan and O'Neill (1959), Dolan and Rees (1965) and Black (1966) studied the chemical structure of carrageenan in detail and showed that the κ- and λ- fractions were structurally related, but as stated by Smith et al (1955), that κ- arrageenan indicates general chemical homogeneity but physical heterogeneity and it is chemically more homogeneous than the λ-carrageenan.

Anderson, Dolan, Penman, Rees, Mueller, Stancioff and Stanley (1968) studied the variation in carrageenan structure and characterisation of sulphate ester by infrared spectroscopy, and showed that the separation

of carrageenan into series of subfractions suggests that it might contain a mixture of related molecules. Carrageenans as stated by Black, Blakemore, Colquhoun and Dewar (1965) are characterised by having a higher sulphate content (20-50% cal. as SO_3Na) and are readily differentiated grom the agars extracted from Ahnfeltia, Gelidium, Gracilaria, Phyllophora and Pterocladia species which have much lower sulphate contents.

Rees (1963) suggested that λ- carrageenan may be the biological precursor of κ- carrageenan and that the seaweed may contain several enzymes capable of metabolising sulphate esters including one that effects the 6-sulphate. Farber (1959) made a comparative study of κ- and λ- carrageenan of Chondrus crispus in solution. He studied the behaviour of the two fractions as polyelectrolytes in order to be able to differentiate them into fractions. He found κ- carrageenan is of lower molecular weight than the λ- carrageenan. He considered both fractions as stiffer than vinyl-type polymers. Studies on the molecular structures of carrageenan have been reviewed by many workers, e.g. Smith and Montgomery (1959), Anderson, W. (1967) and De Rosa (1972). A reference list was compiled by Harvey and McLachlan (1973).

Little is known of the role of environmental conditions in determining the concentration and properties of carrageenan in plants. Haas (1921b) and Haas and Hill (1921) found that the cold and hot extracts of Chondrus crispus have different physical and chemical properties. Butler (1934, 1936) studied the seasonal variation of carrageenan in Chondrus crispus and found that the maximum yield of

carrageenan was obtained in summer months.

The physical properties of extracts of Gigartina stellata and Chondrus crispus was studied by Marshall et al (1949). They found that both the method of extraction and the time of extraction affects the viscosity, specific gravity and gel strength of extract from the two species. Goring (1956a) and Young and Goring (1958) studied some physical properties of carrageenan from Chondrus crispus and found that the gel strength and viscosity of carrageenan was affected by storage of the dried seaweed. After two years they noticed marked losses in the value of gel strength and viscosity at all temperatures of storage. Yaphe (1959) tried to use the carrageenan content as a factor in classification of the Rhodophyceae. He analysed the carrageenan content of 30 species of Rhodophyceae including Gigartina stellata and Chondrus crispus and found that the species studied could be subdivided into 3 groups on the basis of the quantity of carrageenan and regarded both G. stellata and C. crispus as member of group 2 which contains medium amounts. Fuller (1971) in his Ph.D. dissertation, made an extensive ecological study of carrageenan in Chondrus crispus from 5 different locations in New Hampshire, U.S.A. in order to find the interrelation between carrageenan content, gel strength, viscosity and ratio of κ/λ and season of harvest. No correlation was found between gel strength and viscosity, but gel; strength and the ratio of fractions showed a direct relationship. Plant age, reproduction, vertical position and exposure to wave action had no effect on the quantity or properties of carrageenan. The quantity of carrageenan, gel strength and the size of kappa fraction were greater in plants of coastal than estuarine locations. The

viscosity showed an opposite trend. Carrageenan concentration, gel strength and viscosity were highest in winter months, while the lowest value were recorded during the spring-early summer. Fuller (1971) stated that a complex intraction of many factors (including temperature, salinity and nutrients) is probably responsible for the seasonal trends. Pickmere et al (1975) found that the total yield of carrageenan from 3 species of Gigartina reached about 70% in summer and autumn. Mathieson and Tveter (1975) correlated the carrageenan content, viscosity and gel strength with the time of harvest. They found that these properties exhibited maximum value in autumn and early winter. They also suggested possible correlation between variation in fractions of carrageenan of Chondrus crispus and exposure to wave action.

1.4 Ecological Studies on Gigartina stellata:

The ecology of Gigartina stellata has been studied by Marshall et al (1949) from the point of view of general distribution of harvestable areas in Britain, and from the more detailed view point of its occurrence in relation to other shore algae. They have shown that the most productive areas are the coast of Skye and its associated Islands, the west coast of Mull, the coast of the Firth of Clyde and adjacent islands, part of Ayrshire and Wigtownshire, Anglesey, the south of Lleyn peninsula and the south of Cardigan Bay, St. David's Head, the Gower Peninsula, the coast of Devon and Cornwall and part of the coast of the southeast Scotland and Northeast England and Northern Ireland.

On the European coasts, Gigartina has been recorded from the northern coast of Norway to as far south as the Atlantic coast of Morocco. Details of the distribution of Gigartina stellata in Iceland was given by Munda (1972, 1976).

Marshall et al (1949) related the distribution of Gigartina stellata in Britain to many factors such as exposure to and shelter from wave action, salinity of seawater and topography of the shore and substrata. They also found that the plants grow healthily in water that is well aerated and free of mud. They stated that there is some evidence of the tolerance of Gigartina for sewage pollution with high bacterial account and high proportion of dissolved nitrogen.

Munda (1972, 1976) studied the distribution of Gigartina stellata and Chondrus crispus in the Icelandic waters and found that G. stellata has a wider range of distribution than C. crispus. The distribution of G. stellata appears to be controlled by temperature in that the species is not present when there is a high sea temperature. C. crispus is controlled by the lowered winter temperature and is found only in the south and southeast of Iceland. The ecology of Gigartina stellata has not been investigated extensively. However, Burns (1971) in his Ph.D. dissertation made some contribution to the ecology of the species. He found that the salinity is the dominant factor influencing the distribution of G. stellata, the populations exhibiting maximum biomass and frond size on the open coast, whilst on estuaries the populations showed reduced biomass and stunted plants. Maximum photosynthesis/respiration ratios were recorded in 40‰ at 20°C. Spores exhibited maximum germination in 30‰ at 11°C, and maximum growth of sporelings was recorded in 25‰, 30‰, and

35‰ at 19°C. In studying the seasonal growth and reproduction, Burns found that the maximum growth was recorded in spring and summer and the maximum carospores release was recorded between October and March. The annual growth was initiated between February and May and the growth coincided with temperature, with populations reaching maximum biomass in August and September.

Mathieson and Burns (1971) measured the photosynthesis and respiration of both Chondrus crispus and Gigartina stellata under a variety of light intensities, temperature, salinities and degrees of desiccation. They found that the apparent photosynthesis of C. crispus is light saturated at about 1000 ft-C, while that of G. stellata at about 2100 ft-C. The optimal temperature for photosynthesis in both species is about 20°C, but they have a high photosynthesis capacity over a wide range of temperature.

C. crispus showed its maximum photosynthesis and minimum respiration at salinity of 24‰ while G. stellata exhibited maximum photosynthesis and reduced respiration at 40‰. The apparent photosynthesis and respiration of G. stellata is more flexible than that of C. crispus and adversely affected by a high degree of dehydration. They also stated that the local abundance and distribution of both species are correlated with their photosynthetic responses. Burns and Mathieson (1972a) grew the carpospores of Gigartina stellata and Chondrus crispus under variety of light intensities, temperature and salinities and found that the carpospores of C. crispus exhibited a rapid increase in growth concurrent with increasing intensities up to 440 ft-C, and tended to level off above this intensity. G. stellata

exhibited a less rapid but more constant increase in cell production coincident with increasing light intensity through 770 ft-C. The growth of both species was accelerated with increasing temperature to 19°C. Spores of C. crispus germinated and grew rapidly over a broad range of salinities (15‰ - 45‰ at 19°C) G. stellata exhibited a more restricted tolerance to reduced salinity (20‰ at 19°C). Burns and Mathieson (1972b) studied the growth in situ of populations of Gigartina stellata at three locations in New Hampshire. The annual growth of the species began between February and May, population reaching maximum biomass and size in August and September, and general decrease in biomass and size occurred during the reproductive period from October to February. Fastest growth coincided with increasing summer temperatures, while maximum carpospore release occurred during the period of coldest temperature. The largest plants and maximum biomass were found on the open coast (at a semi-exposed site), while smaller plants and reduced biomass were evident within the estuary system. Salinity is a dominant factor influencing both local distribution and growth of plants. G. stellata is primarily restricted to the littoral zone and its maximum length and biomass occur between + 0.45m and $\bar{+}$ 1.0 m above M.L.W. Summer harvesting allowed control levels of biomass and population structure to be reached after one year, while winter harvest attained controlled levels after 19 months. Denuded and sterilized quadrats required more than three years to establish themselves.

Prince (1971) in his Ph.D. dissertation studied the ecology of Chondrus crispus in Plymouth, Massachusetts, and found that the growth of carpospores and tetraspores of this species was controlled

primarily by temperature, with maximum growth at 21°C and no growth at 4.4°C. Growth rate increased during spring and summer months. Maximum growth of 0.37 mm/day (erect shoots) occurred during August and so did the increase in weight of plants. Light intensity of 0.35m Watt/cm^2 was near optimal at 21°C. The reproductive level of Chondrus population in situ exhibiting a small peak in May and large peak in October with minima in August and December through March. Colonization by Chondrus spores commenced within a few days after exposure of the surface. A period of 10-13 months was required, however, for the weathering of a cement surface before it was suitable for colonization by Chondrus spores.

Pybus (1977) studied the ecology of Gigartina stellata and Chondrus crispus in the neighbourhood of Galway Bay, Ireland, Pybus made collections of both species at regular intervals from three localities and found that the growth of both species was continuous throughout the year with some seasonal variation in growth rate. The standing crop could be correlated with changes in plant density. While both species were epiphytized by a variety of organisms, plants of Gigartina from the most exposed shore were the cleanest. The greatest biomass estimates were made on a population of Gigartina on the exposed coast. The best growth of both species occurs in summer months with slight reduction in growth in the winter.

The purpose of the present work is therefore to study some aspects of the biology of Gigartina stellata using laboratory methods as well as field observations. G. stellata was chosen because:

1. It is economically important, and yield about half of its dry weight as carrageenan.
2. It grows abundantly in the Firth of Clyde and especially in the Isle of Cumbrae where detailed studies were made.
3. It is found to grow in different habitat conditions and shows some variations in morphological characteristics and adaptations to reduced salinity.
4. Whilst studies on its biology have been made in recent years in other parts of the world, a combined experimental and ecological study on this common seaweed of the coast of Great Britain appears to be lacking.

CHAPTER 2

MATERIAL & METHODS

A. Gigartina stellata, collection, transportation and storage:

Most of the algal materials used in culture experiments and other laboratory work were collected from Davy's Rock on the Isle of Cumbrae (Figure 2). Plants were also collected from Arrochar in Loch Long and from Portencross as required (Figure 3). The plants were kept damp but not submerged in polythene bags and brought to the laboratory within a period of 2 hours at temperature not exceeding 15°C and stored under refrigeration (0-5°C) until used.

It was found that vegetative materials could be stored for several days without any damage. For obtaining viable spores, the fertile plants with papillae were stored for not more than 2 days under these conditions.

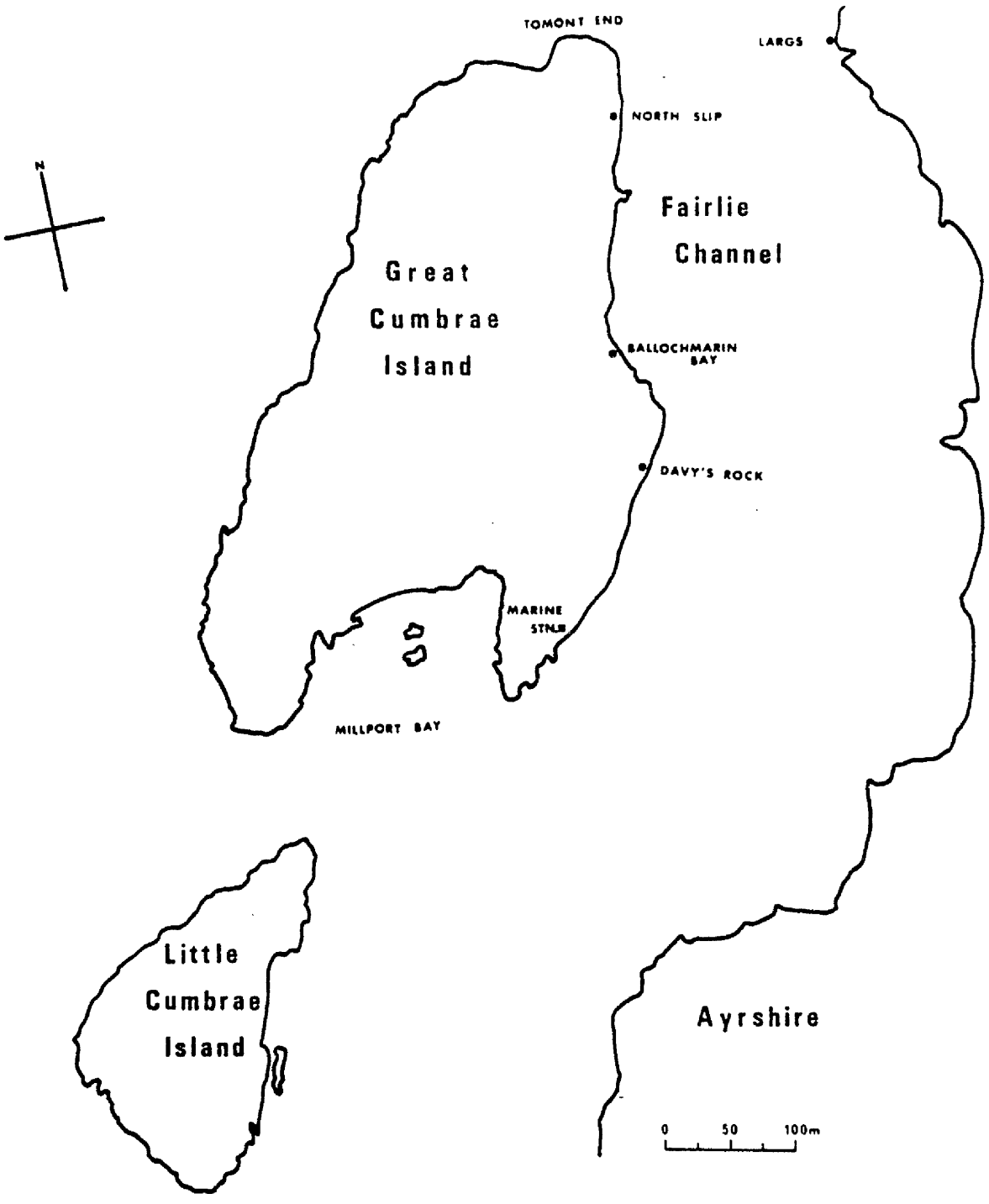
B.1 Determination of tidal levels:

The tidal levels of the experimental sites in Cumbrae Island were determined as follows:

On a calm day, the time was noted when the site was just being submerged or exposed by the tide. The tidal levels at each of these times were then obtained from continuous records maintained by the tidal gauge at the University's Marine Biological Station at Millport.

The tidal levels at two sites on Cumbrae Island, Davy's Rock and Tomont End (Figure 2) were determined on one occasion at Davy's Rock (6.4.78) and at Tomont End (4.7.78).

Figure (4) shows the vertical distribution of Gigartina stellata at the two sites on Cumbrae Island.


TOMONT END
LARGS
NORTH SLIP
N
Fairlie
Channel
Great
Cumbrae
Island
BALLOCHMARIN
BAY
DAVY'S ROCK
MARINE
STNS
MILLPORT BAY
Little
Cumbrae
Island
Ayrshire
0
50
100m

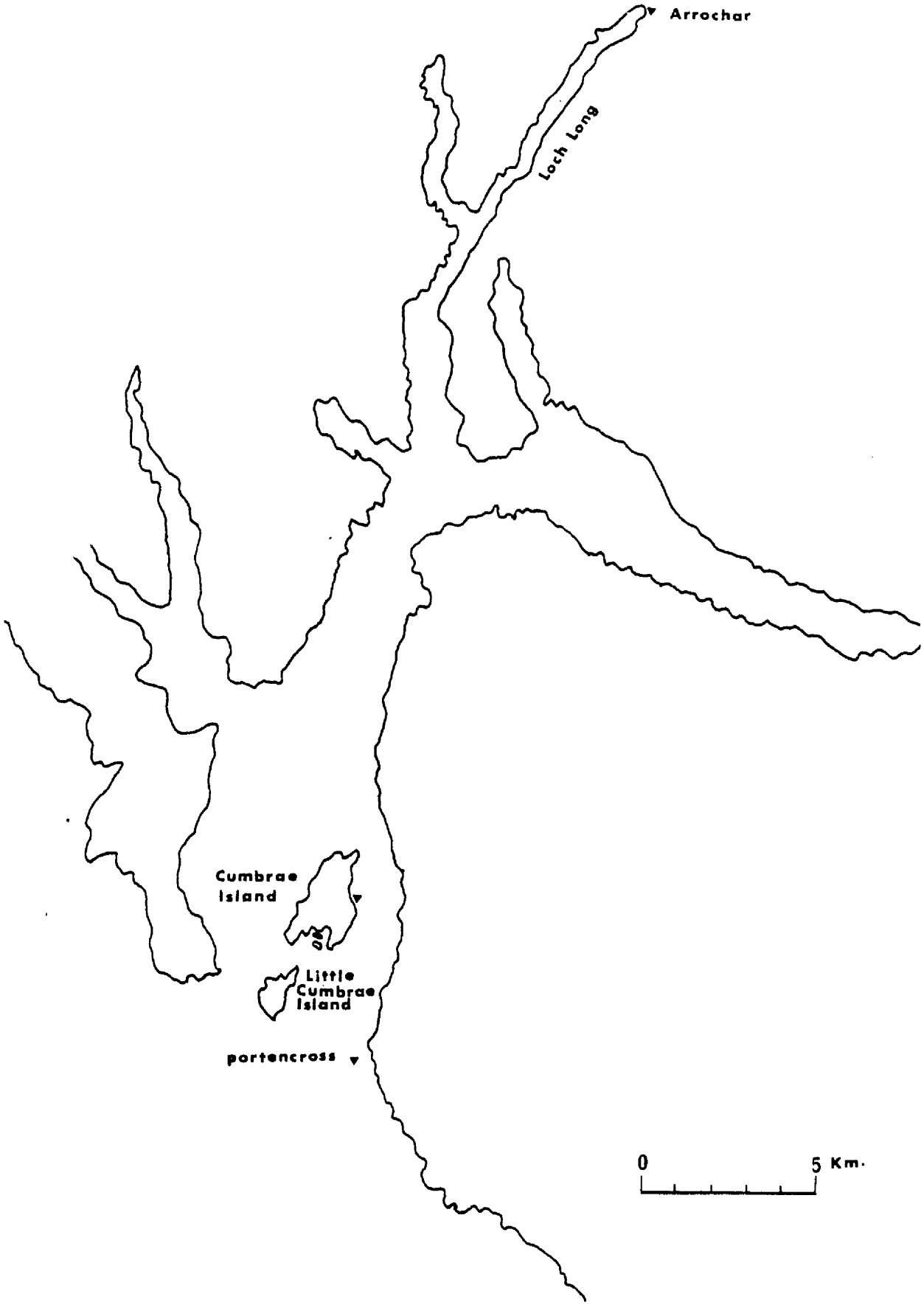

Arrochar
Loch Long
Cumbrae Island
Little Cumbrae Island
portencross
0
5 Km.

Figure 4: Showing the vertical distribution of G. Stellata in relation to tidal level at two sites in Cumbrae Island.

Elevation relative to zero tide datum point (m)

0·0 1·0 2·0 3·0

Tomont End

= 50 %

Davy's Rock

B.2. Method of sampling shore populations:

Sampling of Gigartina stellata for analysis of morphological characters and other laboratory work was made by collecting any plants of Gigartina present within a 25 cm square quadrat randomly placed in the middle region of the vertical range of Gigartina zone. Plants from 3 quadrats (about 75 plants) were collected from each locality.

B.3. Method of clearing shore of Gigartina and other algae:

A strip was cleared, 25 cm wide and up to 4 m in length, traversing the Gigartina zone from before Ascophyllum nodosum down to the Laminaria zone. The strip was first prepared by cutting away the larger frond. The area was then scraped and the remaining plants were removed by a wire brush. The strip was then burned using a blow torch and finally formalin solution was poured on the strip to make sure that nothing viable remained in the interstices of the rock.

Successive strips were cleared at different times of the year in order to follow any seasonal variability in recolonization.

B.4. Regeneration studies:

Gigartina fronds were removed in situ by cutting at two levels. In the first, fronds were cut about 2 cm from the basal crust leaving only part of the stalks to regenerate. Other plants were cut at a higher position, so leaving parts of the fronds to regenerate.

Plants in two 25 cm square quadrats were cut at Davy's Rock in Cumbrae Island.

B.5. Transplant experiments:

Stones and boulders with attached plants of Gigartina were transplanted in the following way:

1. Plants from Cumbrae Island were transplanted to Loch Long and Portencross.
2. Plants from Loch Long were transplanted to Cumbrae Island and Portencross.
3. Plants from Portencross were transplanted to Cumbrae Island and Loch Long.

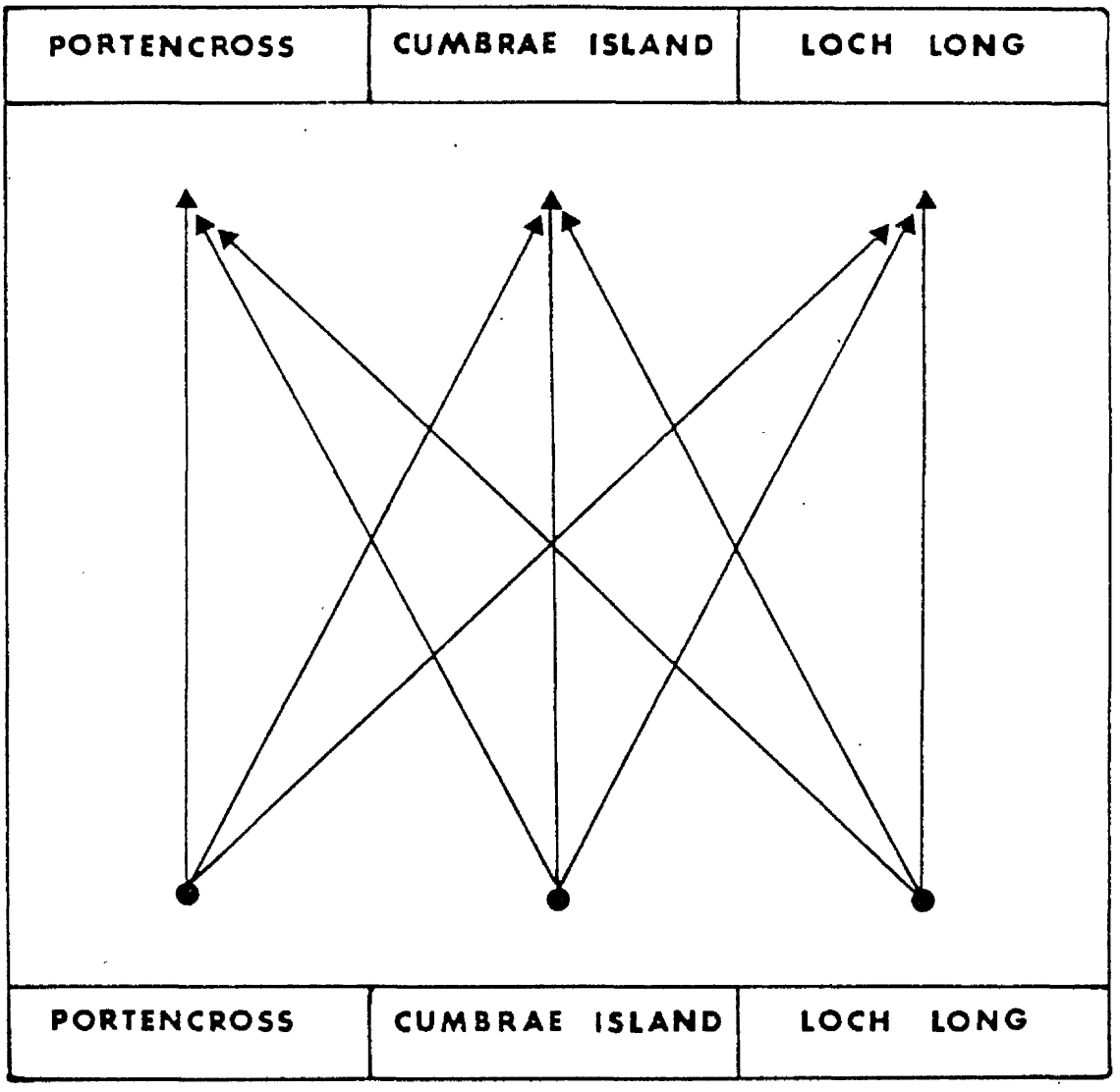
Figure (5) shows the pattern of transplant throughout the three localities. At the same time control experiments were carried out by transplanting stones with plants of Gigartina in its own zone at each locality.

In all cases, the stones were cemented to the rock using quick drying cement mixed with sand (the ratio of cement to sand was 1:2) plate (1). Although cementing stones does not seem to have any effect on the growth of plants, large stones could be placed in a permanent new position without need of cementing.

C. Analysis of morphological characters:

The morphological characters of natural populations of Gigartina stellata sampled by the method described before were measured and the data expressed by means of bar diagrams. The characters measured (Figure 6) were:

LOCALITIES RECEIVING TRANSPLANT



SOURCE OF TRANSPLANT

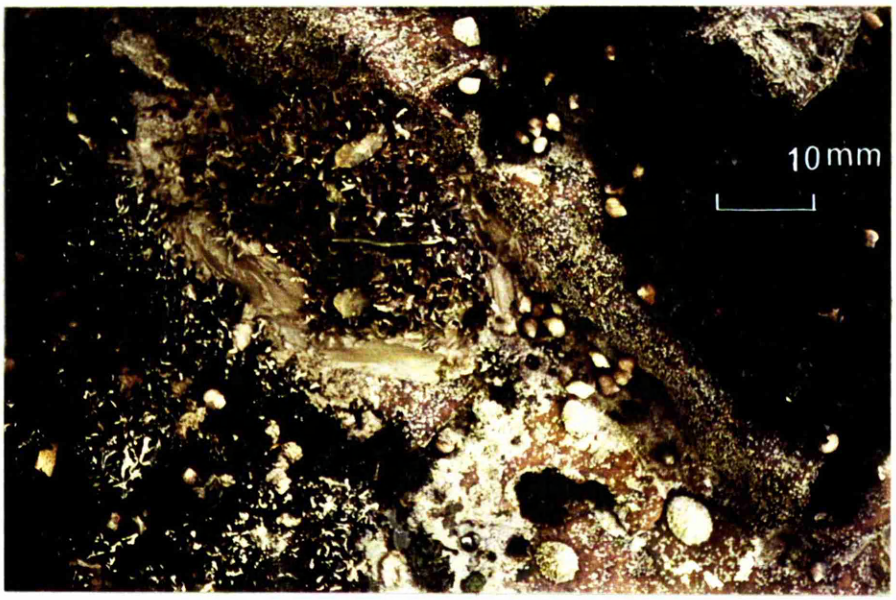
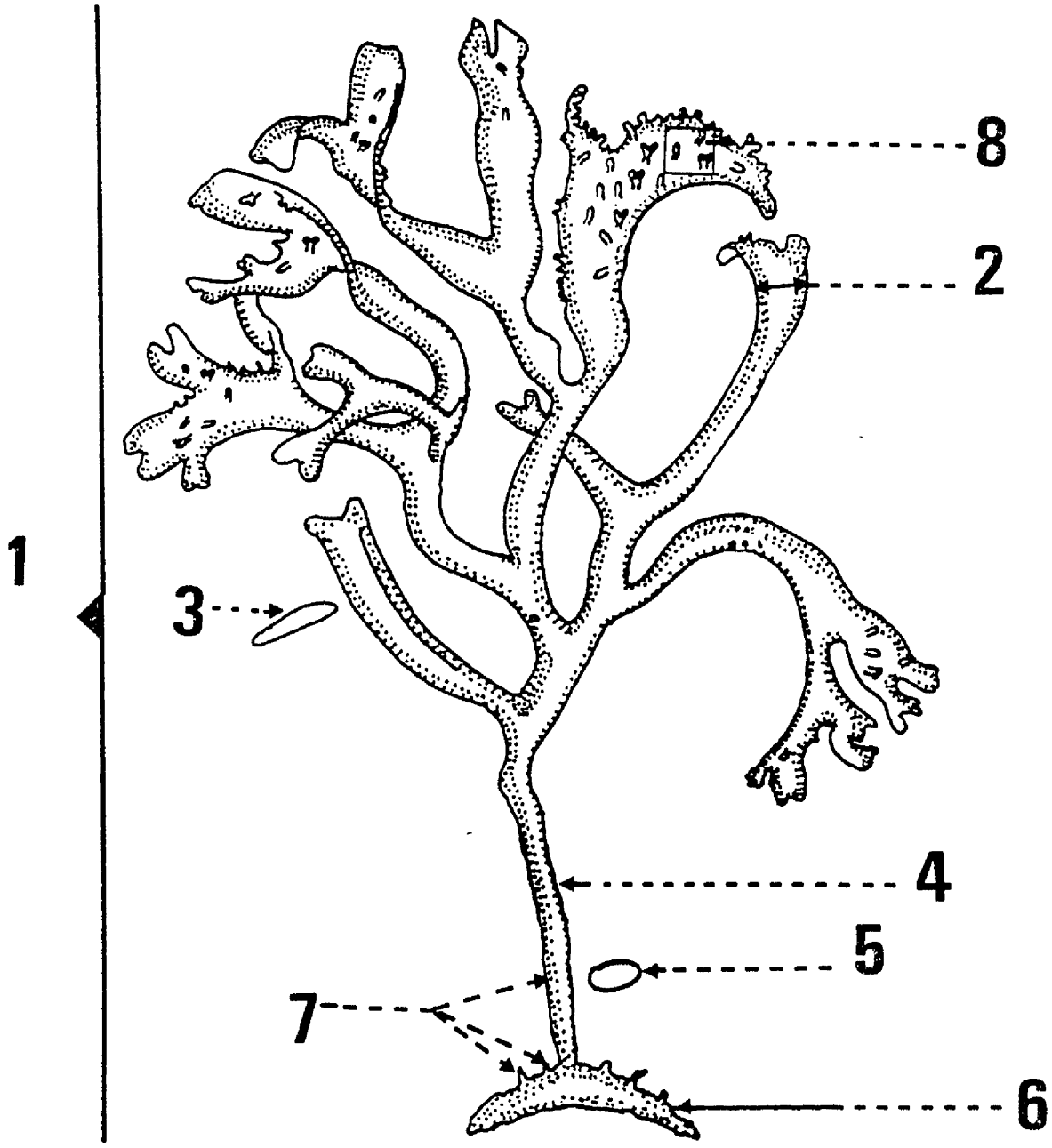


Plate 1: Showing the method of cementing stones.

Figure 6: Showing plant of Gigartina stellata with the morphological characters analysed:
1 = Length of frond, 2 = width of frond,
3 = thickness of frond, 4 = length of stalk,
5 = diameter of stalk, 6 = basal crust,
7 = number of erect fronds and 8 = number of papillae per unit of surface area.


1
2
3
4
5
6
7
8

1. The maximum length of frond in mm.
2. The maximum width of frond in mm.
3. The maximum thickness of frond in mm.
4. The maximum length of stalk in mm.
5. The maximum diameter of stalk in mm.
6. The average area of basal crust in sq. mm.
7. The average number of erect frond per basal crust.
8. The average number of papillae per sq. mm.

D. Measurement of fertility of different populations of Gigartina:

Five different stages of maturity of plants of Gigartina can be distinguished as follows:

1. Stage 1. Fronds without any papillae.
2. Stage 2. Fronds with initiation of papillae.
3. Stage 3. Fronds with many immature papillae.
4. Stage 4. Fronds with some mature papillae.
5. Stage 5. Fronds with all papillae fertile.

The maximum fertility was determined by counting the number of fronds with all papillae fertile (stage 5) and expressing the value as a percentage of the total number of fronds.

The fertility of papillae was tested by their ability to release viable spores and the spores viability was tested by allowing the spores to germinate and grow for 48 hours.

The maximum fertility of plants from the three different populations was determined from monthly collections of plants from the 3 localities. This experiment was initiated in June 1976 and terminated in November 1977.

E.1. Media and culture vessels:

All algae were cultured in an enriched seawater medium recommended by Professor A. D. Boney. Seawater collected from the Firth of Clyde was aged for at least two weeks, filtered through a double layer filter paper No.1 and then pasteurized by heating it twice to 75°C at room temperature. The formula of the medium is as follows:

To 1ℓ of filtered and pasteurized seawater were added:

60 ml of solution A, which contained:

50 ml of 4% $NaNO_3$ in distilled water

2 ml of each of the following:

1.47 g/ℓ. $MnSO_4$. 4 H2O.

0.0023 g/ℓ. $CuSO_4$. 5 H2O.

0.064 g/ℓ. $CoCl_2$ 6 H2O.

0.23 g/ℓ. Na_2MoO_4. 2 H2O.

0.005 g/ℓ. LiCl H2O.

15 ml of solution B, which contained:

2.6 g/ℓ. tetrasodium salt EDTA.

0.12 g/ℓ. Fe SO_4. 7 H2O.

1.5 ml of solution C, which contained:

1.5 g/ℓ. Na_2 HPO_4. 12 H2O.

2 ml of solution D, which contained:

4.98 g/ℓ. Zn SO_4. 7 H2O.

Each of the above solutions was autoclaved separately and stored in a refrigerator until used. The addition of these nutrient solutions was found to reduce the salinity of seawater so the final salinity of the medium was ~ 32‰.

In most culture experiments, whole fronds as well as sporeling attached to glass slides were cultured in rectangular glass tanks which measured 180 x 250 x 170 mm and had a capacity of about 5 litres. The tanks were covered with glass plates.

E.2. Control of contamination:

In the cultures of vegetative fronds of Gigartina contamination is not so serious a problem as with cultures of spores and germlings. However, contamination was successfully controlled by:

1. Using the carefully washed papillae instead of using parts of the fertile fronds.
2. Changing the medium twice a week, especially in the early stages of growth.
3. Using an antibiotic mixture consisting of 200 µg/ℓ Streptomycin and 800 µg/ℓ penicillin (1625 units/mg).
4. Using Germanium dioxide at a concentration of 5 mg/ℓ. to control diatom growth.

E.3 Illumination:

The cultures were illuminated from above with two six feet Atlas Daylight 85 Watt fluorescent tubes at a distance of 230 mm. The glass shelves upon which the culture vessels were placed were covered with opaque construction paper to eliminate illumination from the tubes over the shelf below. Two tubes give an illumination of 2400 lux. The illumination was measured with an EEL portable photo-electric photometer serial No.1132. The tubes changed whenever necessary to keep the illumination at 2400 lux. The daylength was 12 hours.

E.4 Aeration and temperature:

In most culture experiments, the culture vessels were aerated by bubbling compressed air continuously through the culture medium. The air was cleaned and humified by passing the tubes through a flask containing tap water.

All culture experiments were run at $10^{\circ}C \mp 2^{\circ}C$ in a thermostatically controlled culture cabinet.

F.1 Culture of vegetative fronds:

About 2 cm long fronds of Gigartina were cleaned by removing the epiphytes and epizoa and washed several times in cold seawater.

10 healthy fronds were placed in a culture tank containing 5ℓ. of seawater medium. The medium was changed every 10 days. The tank was aerated and evaporation was kept to a minimum by covering with a glass plate.

F.2 Measurement of growth of vegetative fronds:

The growth of vegetative fronds was measured by two methods:

a - Increase in surface area:

The vegetative fronds were removed from the culture tank and quickly arranged on a glass sheet. The plants were then gently covered with a sheet of clean glass plate and photocopied. The plants

were then returned immediately to their culture tank. The area of the plants was then measured using a planimeter, each measurement being made three times and the area calculated as the mean value.

b - Increase in fresh weight:

The fresh weight of each vegetative frond was taken at the beginning of the experiment and then measured weekly. The increase in fresh weight was expressed on a relative basis as follows:

$$\frac{\text{latest weight} - \text{initial weight}}{\text{initial weight}} \times 100$$

F.3 Culture of spores and sporelings:

Spores of Gigartina were obtained from fertile papillae cut from mature plants freshly collected from the shore. Papillae were washed several times in sterile seawater and then laid on glass slides ground at one side and incubated at 2400 lux at 10°C for 24 hours in small square petri-dishes containing sterile seawater.

Spores from washed papillae rather than from the whole plants or part of the fertile fronds, effectively reduced the contamination. The cultures were thinned by brushing the sporelings gently with a fine brush in order to avoid overcrowding. The sporelings attached to the glass slides were then placed in larger culture tanks containing 5ℓ of seawater medium. The medium was changed at first twice a week and later every week.

F.4 Measurement of growth of sporelings:

Growth of sporelings was measured by the increase in diameter of disc-like sporelings. The mean values of maximum and minimum diameter were obtained. The mean diameter of 50 sporelings selected at random were measured from 5 different microscopic field of each slide.

G. Tidal simulator:

The apparatus employed in this study to simulate a tidal cycle is shown in Figure (7) and is based on the system described by Townsend and Lawson (1972),(Hruby 1977). A rack which could be lowered and raised on a 12 hour sinusoidal cycle within a glass tank containing 5 litres of seawater. One driving motor, a Crouzet type 82-344 (1 Rev./12 hrs) was used to power two racks, each one acting as the counterweight to the other. Sporelings of Gigartina were cultured on a glass plate 39 x 157 mm and ground on one side. The vegetative fronds of Gigartina were mounted on 160 x 230 mm plates of 6.5 mm thick clear perspex in which a 5 x 5 array of holes about 4 mm in diameter were drilled.

The time during which each level was submerged per cycle was calculated using the following algorithm:

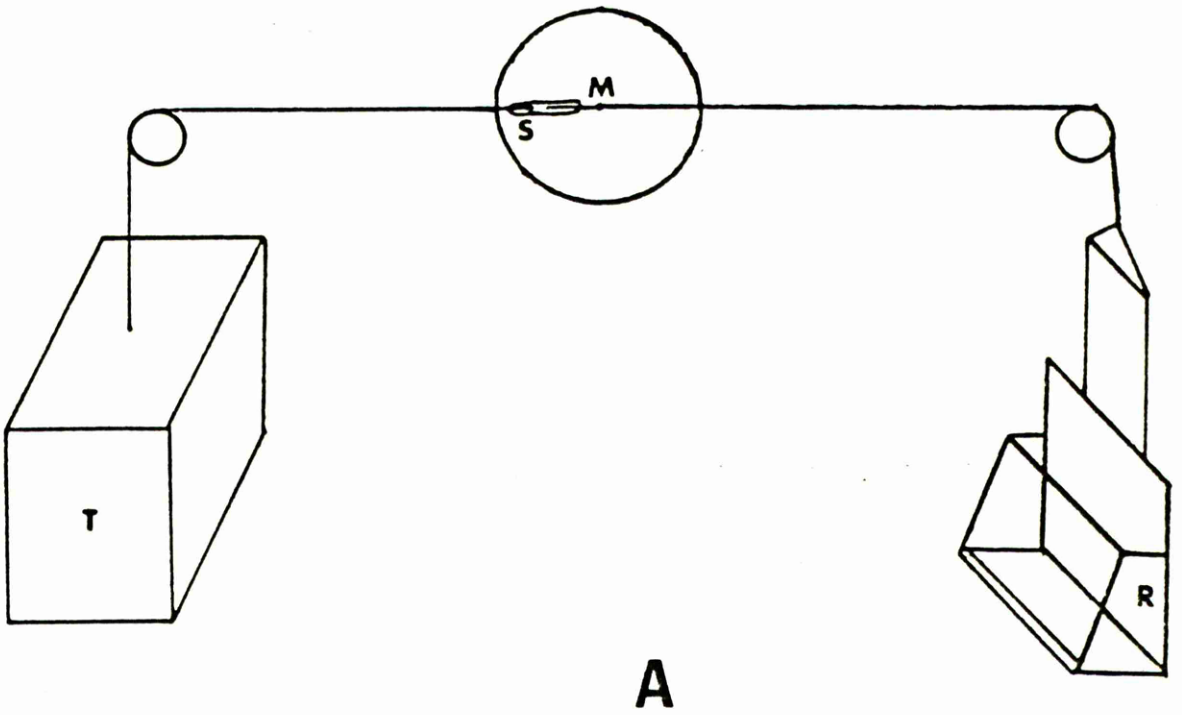
$$T = 12 \times \frac{\cos^{-1}(2(D/M)-1}{\pi}$$

where T is the time submerged, M is the maximum distance on the slides or the plates between the lowest level which was submerged and D is the distance between the lowest level of emersion and the level on which the plants were measured.

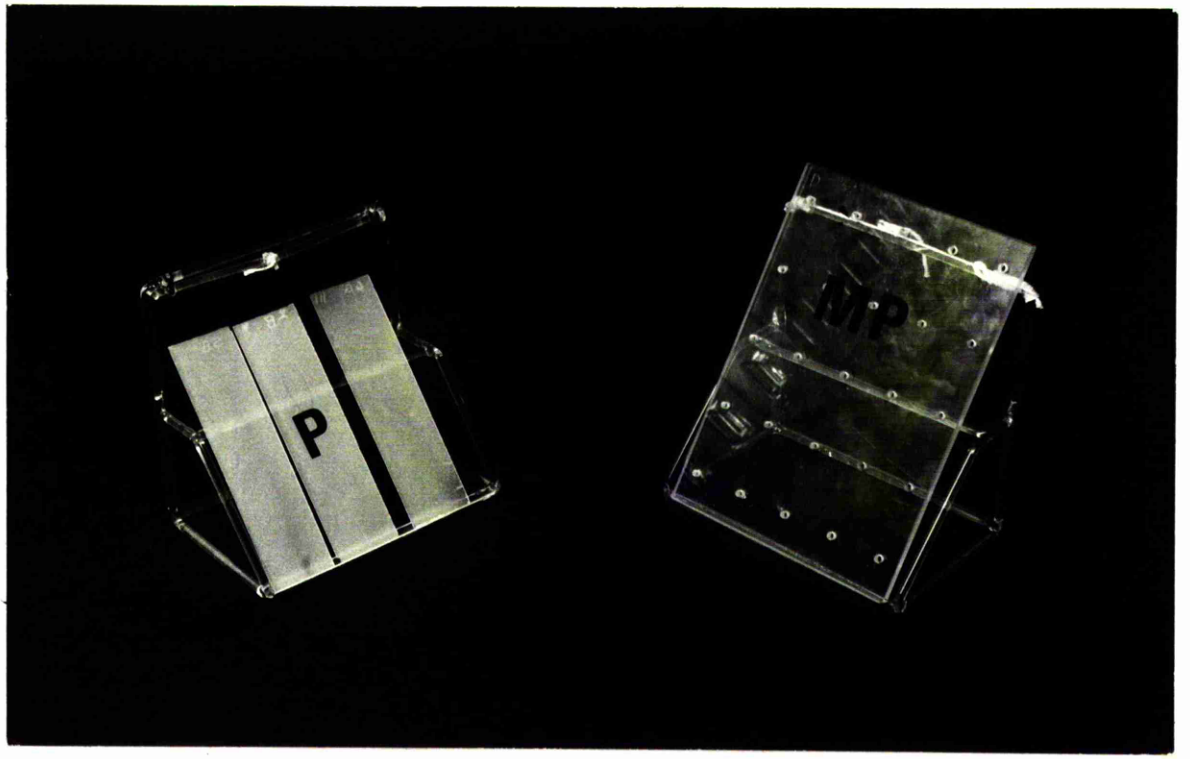
Figure 7:

A. diagram of tidal simulator showing placement of culture tank (T), supporting racks (R) and motor (M), a crouzet type 82-344 (1 rev./12 hours). The distance between the maximum and minimum elevation on a cycle was adjusted by a movable bolt held in slots (s).

B. photograph showing the supporting rack holding 3 (39 x 157 mm) glass plates (P) or one (160 x 230 mm and 6.5 mm thick) clear perspex mounting plate (MP).



A



B

H.1 Extraction of Carrageenan and measurement of quantity:

The freshly collected Gigartina was cleaned by removing the epiphytes and epizoa. The plants were immersed very quickly in distilled water to remove excess of salt. The washed plants were dried in an oven at 80°C - 90°C until constant weight was obtained. The method employed in extracting the carrageenan is based on the method described by Black, Blakemore, Colquhoun and Dewar (1965) with some modifications.

5g of the dried milled seaweed was stirred with 0.2% sodium chloride solution (200 ml) at pH 7.0 for 15 minutes at room temperature, centrifuged and the supernatant discarded. The residue was then extracted with 0.2% sodium chloride solution (500 ml) at 90°C for 5 hours with rapid stirring, then centrifuged and the extract was dialysed against 0.1 m sodium chloride to remove ions other than sodium ions. The pH was adjusted to 7.0 with sodium hydroxide solution and solid potassium chloride added slowly with stirring to a concentration of 0.25 m. The bulky thin gel was then centrifuged, dissolved by dialysis against 0.1 m sodium chloride and reprecipitated by the addition of potassium chloride to a concentration of 0.25 m. The resulting thick gel was again separated by centrifugation, diluted to 300-400 ml and the suspension heated to 90°C and solid sodium chloride added to a concentration of 0.5 m. The solution was dialysed against 0.5 m sodium chloride with rapid stirring to remove potassium ions and then against water. The viscous solution was centrifuged and added with stirring to three volumes ethanol. The precipitated carrageenan was washed with 80% ethanol, absolute ethanol and ether and air dried at 50°C.

The carrageenan content was determined as dried material and expressed as a percentage of dry weight of seaweed used.

H.2. Measurement of gel strength:

The apparatus employed in this study is shown in Figure (8), and is based on the apparatus described by Goring (1956b). The apparatus consists of two parts; a 400 g balance and an electric motor with speeds of 0.36 mm/sec, 0.88 ,,/sec., 1.45 mm/sec., and 2.21 mm/sec.

As shown in the figure, the motor driven plunger (0.8mm diameter) depresses the gel which rests on the pan of the balance. As the pointer of the balance moves, it moves an indicator needle mounted on a special scale. At the point of rupture, the plunger penetrates the gel and the pointer moves to its maximum. The motor was stopped and the reading was taken as gel strength in grams.

I. Measurement of photosynthesis:

The photosynthesis rate of plants of Gigartina was measured by determining the rate of oxygen evolution.

About 12 hours before the determination a stoppered 10ℓ capacity glass bottle equipped with a tap near the bottom was filled with seawater and partially deoxygenated by bubbling nitrogen through the seawater for 30 seconds. The bottle was then placed in the 12°C culture cabinet and allowed to equilibrate to this temperature. Then a series

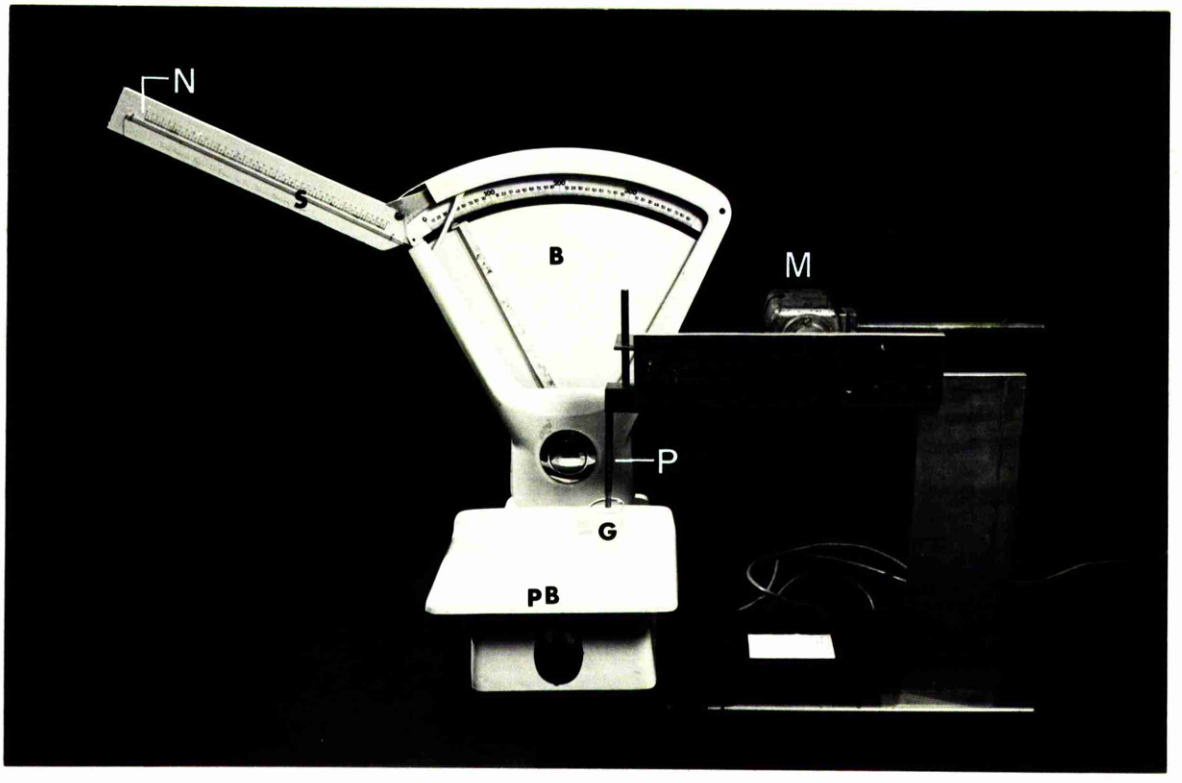


Figure 8: Showing the gelometer, which consists of a balance (B) and electric motor (M). The plunger (P) depresses the gel (G) which rests on the pan of the balance (PB), and moves an indicator needle (N) mounted on calibrated scale (S).

of 275 ml glass bottles equipped with grounded glass stoppers were carefully filled from the large bottle via a short length of plastic tubing connected to the tap. Care was taken to avoid turbulence and an excess of seawater was allowed to overflow from each bottle to ensure that the seawater inside was homogenous in oxygen concentration. The first and last bottles filled were used as controls to determine the initial oxygen concentration of the seawater.

One plant sample was introduced into each of the remaining bottles. Each plant was fastened to a thread passing through the ground glass connection between the bottle neck and the stopper, this was found not to cause any leakage or allow intake of air into the bottle during incubation. The bottles were then incubated in the light for two hours. They were illuminated from above by 2 six-foot Atlas Daylight 85 Watt fluorescent tubes. Immediately after incubation, the algal samples were withdrawn and the oxygen concentration of the seawater in each bottle was determined by Winkler's method as described by Strickland 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 in μ moles O_2/g fresh weight/hour was calculated as follows:

$$\text{Rate} = \frac{(\text{net change in } O_2 \text{ concentration in } \mu\text{moles/litre})(\text{bottle volume in litre})}{(\text{plant weight in gram})(\text{length of incubation period in hour})}$$

J. Water loss from Gigartina plants:

After the wet weight was determined, the plants were dried simultaneously and reweighed after intervals of times.

The plants were suspended by a thread or paper clip during drying so that all surfaces of the plant were equally exposed to the air. Also plants were dried in a desiccator containing silica gel for comparison.

All weights were taken to the nearest 0.1 mg on a Mettler type H6 single pan balance.

K. Rate of loss of halide ions:

The method employed in this study was described by Gessner and Hammer (1968). In this method, the rate of loss of chloride ions was determined by means of titration against 0.01 N $AgNO_3$ using potassium chromate (K_2CrO_4) at concentration of 3.5 g/ℓ as indicator.

Only complete and healthy fronds of Gigartina were used to test the rate of loss of chloride ions after various time intervals. Each complete test usually lasted only a few minutes in order to avoid secondary damage by the distilled water.

CHAPTER 3.

LOCALITIES AND HABITAT CONDITIONS

The degree of exposure to wave action is a factor of prime importance in the intertidal habitats. As reported by Ballantine (1961) the degree of exposure is controlled by many factors such as speed of wind, its duration and the distance of open water over which it can blow. Waves are often refracted and their form altered as they approach a shore, and the distribution of their energy on the shore itself is governed largely by the slope and detailed configuration of the rock. These factors have to be known if the dimensions of the waves are to be calculated.

According to Ballantine (1961) continuously recording instruments placed below water can measure the height of the waves passing over them. Such instruments are expensive however, difficult to instal and to maintain and must be run for long periods to provide useful information.

Jones and Demetropoulos (1968) described an instrument which measures the dynamic pressure produced by waves. They stated that this dynamometer not only gives a simple method of comparing the intensity of wave action on different shores, but it is also easily and cheaply constructed. The results obtained did show that relatively small changes in wave force resulted in marked changes in species distributions.

Ballantine (1961) and Lewis (1964) suggested biological measurements of the status of exposure to and shelter from wave action. They stated that some species occurring on rocky shores

Plate 2 : Shows the habitat condition at Cumbrae Island.

have positive correlation with exposure and abundant only in wave beaten places. Others common in sheltered shores or have negative correlation with exposure. For example: Alaria esculenta (L.) Grev., Porphyra umbilicalis (L.) J. Ag. and Littorina neritoides (L.) Jeffreys are common in exposed shores and Ascophyllum nodosum (L.) Le Jol., Laminaria saccharina (L.) Lamour. and Littorina Littorea (L.) Jeffreys are common in sheltered shores.

Populations of Gigartina stellata were analysed from three different localities in the Clyde sea area [Fig.3 page (24)], Cumbrae Island, Loch Long and Portencross.

1. Cumbrae Island:

As shown in Fig.(2) page (22), Cumbrae is a rocky island of about 16 km circumference. According to Bassett (1958), Cumbrae is composed of Upper Old Red Sandstone conglomerates and sandstones overlain by mixed sediments which are probably of Calciferous Sandstone age. The rocks are folded into a shallow pitching syncline occupying the area west of Great Cumbrae Fault and a pitching anticlyne occupying the narrow tract to the east of the fracture. The sediments are cut by an exceptionally large number of dykes which are in general easily accessible and well exposed around the coast of the island.

Since the prevailing wind on the west coast of Scotland is south-west, the island is not frequently exposed to heavy seas (Gibb 1939). This feature and the fairly gentle slope tends to reduce the splash zone above high water mark. However, the degree of exposure to and shelter from wave action varies from place to place on the island. For example, the north side of the island at Tomont End is more exposed to wave action than the southern side i.e. Keppel Point. On the

Plate 3: Shows the habitat conditions at Loch Long.

western side of the island at Davy's Rock the shore is semi-exposed to wave action.

Except for a few sandy bays, the shore is mainly rocky and is colonised richly by Gigartina stellata which form a wide band above the Laminaria zone and extends up to Ascophyllum nodosum (plate 2.).

2. Loch Long:

Loch Long lies on the northern side of the Clyde sea area. The shore is mostly steep and fairly sheltered. It shows a typical fucoid domination, also Littorina littorea is abundant. Plants of Gigartina stellata are found growing on small scattered igneous stones (see plate 3) below the Ascophyllum band. Near the head of Loch Long, especially on the west side at Arrochar, Gigartina is much richer but appears as a dwarf form, already described by Marshall et al. (1949) where fresh water enters to the loch. Even if the freshwater is only a trickle over the stones the Ascophyllum dies out and Gigartina becomes dominant. At low tide plants of Gigartina at the water edge may be covered with almost freshwater (2‰). At high tide the salinity becomes 19.0‰ and may reach the maximum salinity of 29.9‰.

3. Portencross:

Portencross lies south west of Cumbrae island on the Ayrshire coast. The substratum here is of Old Red Sandstone similar to the Isle of Cumbrae.

The shore at Portencross is exposed to more intensive wave action

Plate 4: Shows the habitat conditions at Portencross.

than the other localities and on its most exposed area is dominated by barnacles with dense bands of small mussels (Mytilus edulis) L. on the lower shore.

Gigartina showsa patchy distribution on rocks on the more gently sloping shore below the rocky outcrops, but in the path of breaking waves (plate 4).

CHAPTER 4.

ANALYSIS OF MORPHOLOGICAL CHARACTERS

Plants of Gigartina stellata consist of a basal crust that grows radially on the substrate and fronds extending upward from the basal crust. [Fig.(6) page (25)]. The basal crust may persist for several years and it has been considered as pseudo-perennial (Marshall et al. 1949). The upright fronds show some obvious morphological variability which has already been discussed in Chapter 1.

The various populations of Gigartina were sampled in the summer of 1977 using the method discussed in Chapter 2, in which the whole plants of Gigartina (about 25 basal crusts) from a square 25 cm quadrat were collected from the middle region of the vertical range of Gigartina zone at each locality. Plants of three quadrats from each population were analysed. The characters analysed as shown in Fig.(6) are:

1 - Length of frond in mm.
2 - Width of frond in mm.
3 - Thickness of frond in mm.
4 - Length of stalk in mm.
5 - Diameter of stalk in mm.
6 - Area of basal crust in square mm.
7 - Number of erect fronds per basal crust.
8 - Number of papillae per square cm of fronds surface.

Measurements of morphological characters are shown in Appendices 1,2 and 3. Photographic silhouettes of various age groups of plants from different populations are shown in Figs. 9 - 11. These figures not only show variation in the morphological characters of different

Figure 9: Showing the various age groups of plants of *Gigartina* collected from Cumbrae Island.

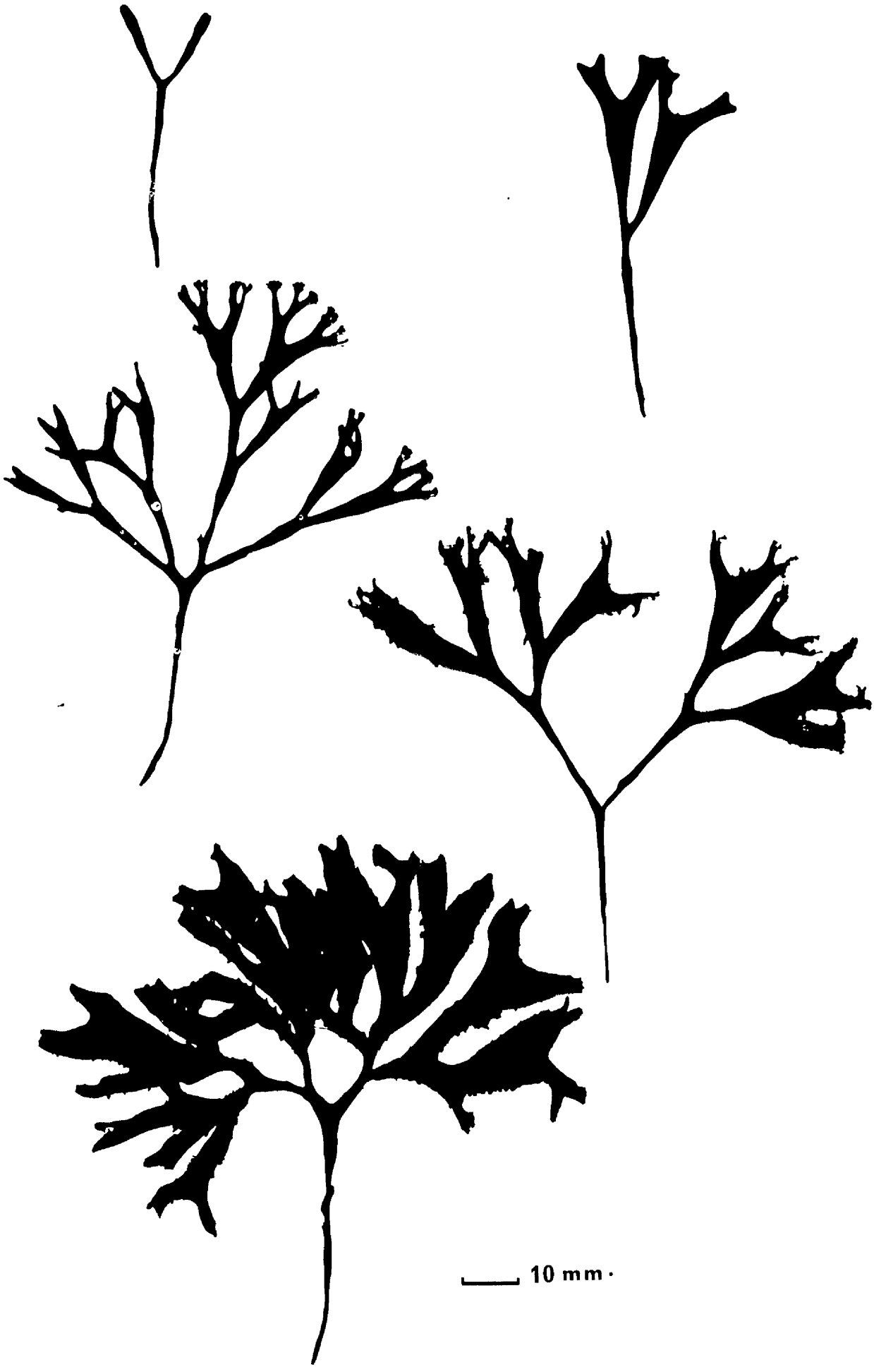

10 mm

Figure 10: Showing the various age groups of plants of Gigartina collected from Loch Long.

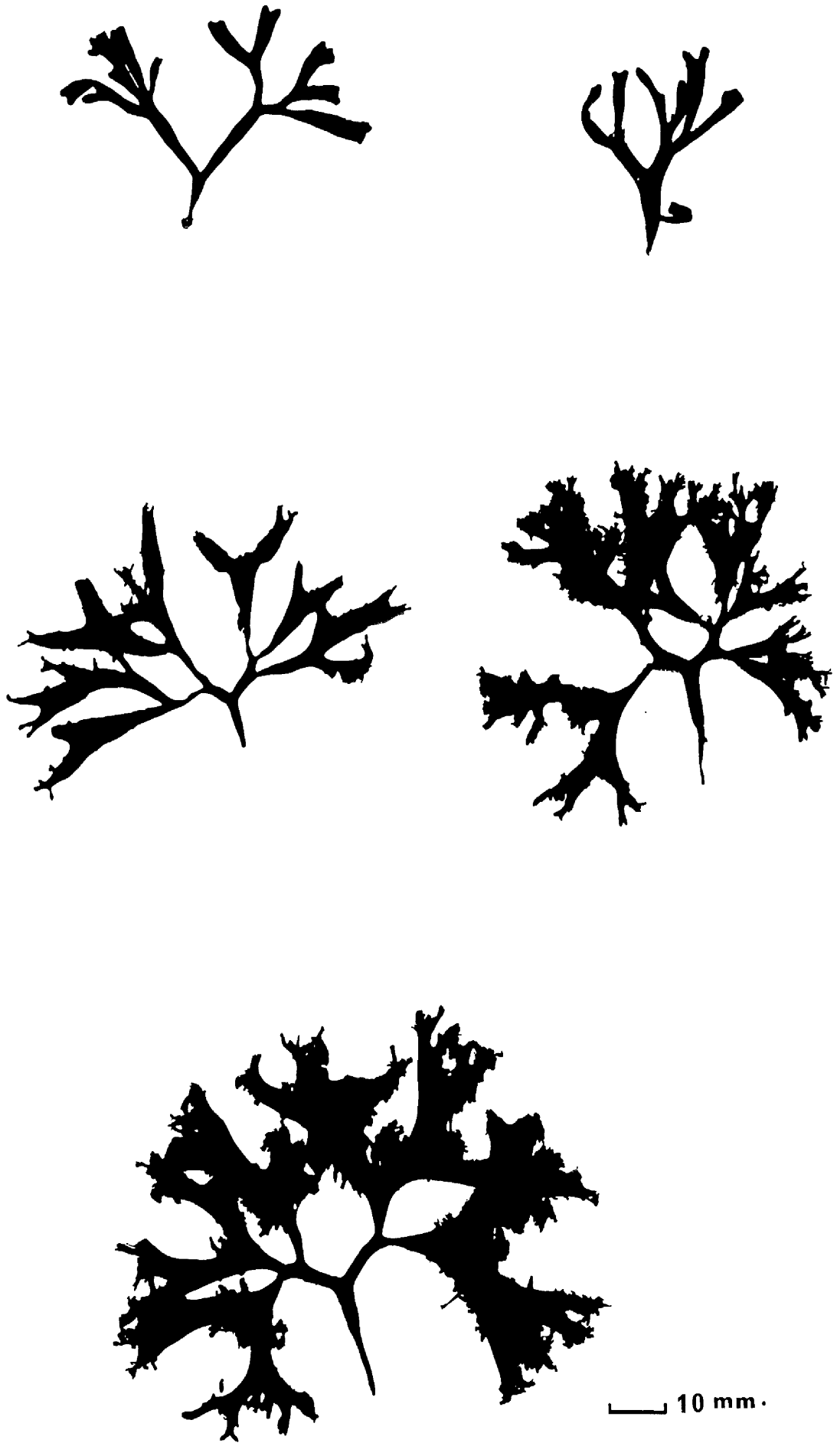

10 mm

Figure 11: Showing the various age groups of plants of *Gigartina* collected from Portencross.


10 mm.

populations of Gigartina but also they show that the variations in morphological characters occurred in all age groups of plants.

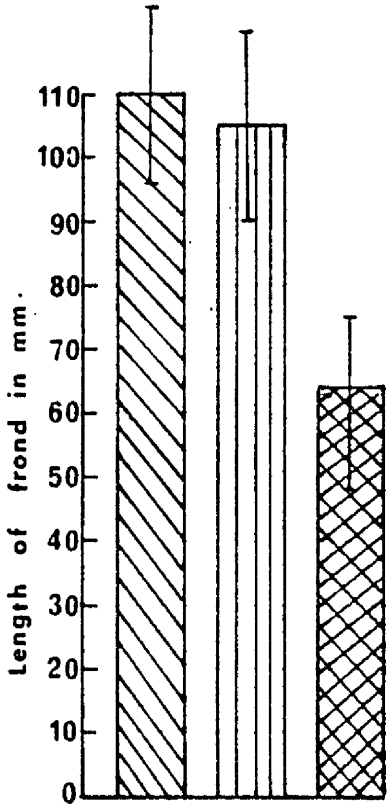
The results of measurements of morphological characters are presented in the form of bar diagrams. (Fig.12, (a-h)). It is clear from these data that in all characters, plants from Loch Long exhibited the smallest value. On the other hand, plants from Portencross exhibited the highest value of all characters except for the number of erect fronds per basal crust which was highest in plants from Cumbrae Island. Plants from Cumbrae Island exhibited intermediate values for all characters. Therefore, plants from Portencross are characterised by large, wide and thick fronds, long and thick stalk and large area of basal crust. Plants from Loch Long are characterised by short, narrow and thin fronds, short and thin stalk, a small number of erect fronds per basal crust, a small area of basal crust and a small number of papillae per square mm of frond surface. In some characters e.g. area of basal crust and the number of papillae per square cm, it was not possible to represent the standard deviation on the bar, because in all cases it was very large, but the mean values still showed no significant differences, also some characters e.g. length of frond showed no significant differences in plants from Portencross and Cumbrae Island.

In sampling the different populations of Gigartina for analysis of morphological characters, the plants from the uppermost region of the Gigartina zone was discarded because in all cases they are usually juvenile.

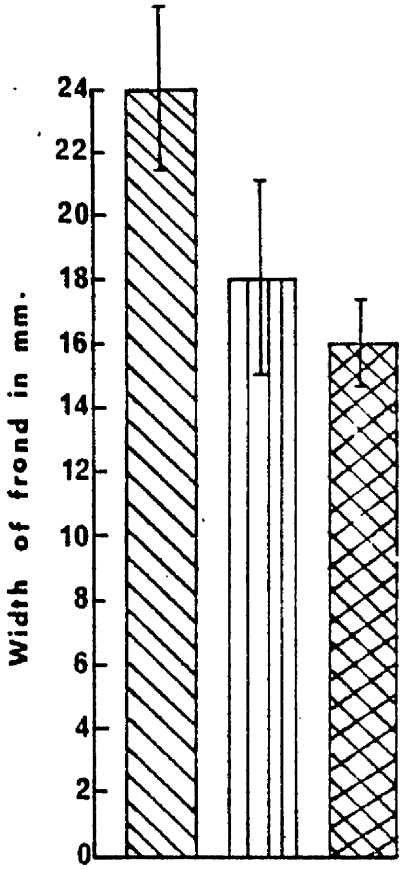
As already described in Chapter 3, Portencross, is an open coast and exposed locality while Loch Long is a very sheltered locality

Figure 12: Showing analysis of morphological characteristics of Gigartina stellata from the different populations:

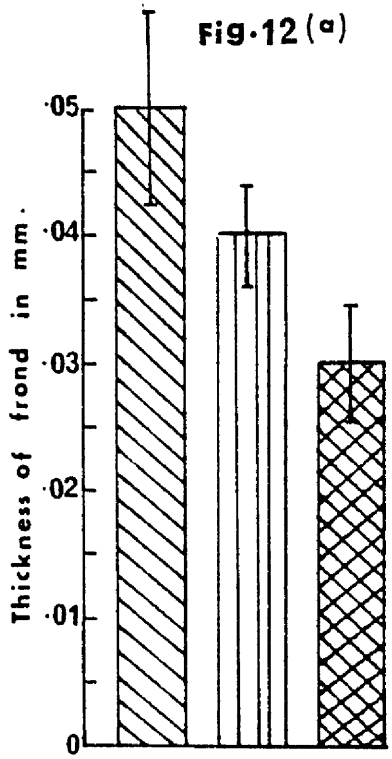
(a) The length of frond.
(b) The width of frond.
(c) The thickness of frond.
(d) The length of stalk.
(e) Diameter of stalk.
(f) Area of basal crust.
(g) Number of erect fronds/basal crust.
(h) Number of papillae/square cm.



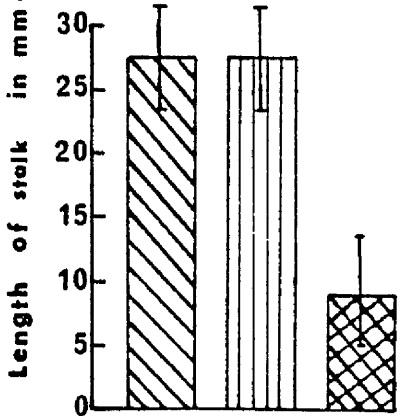
Fig·12 (a)



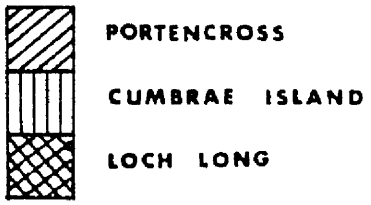
Fig·12 (b)

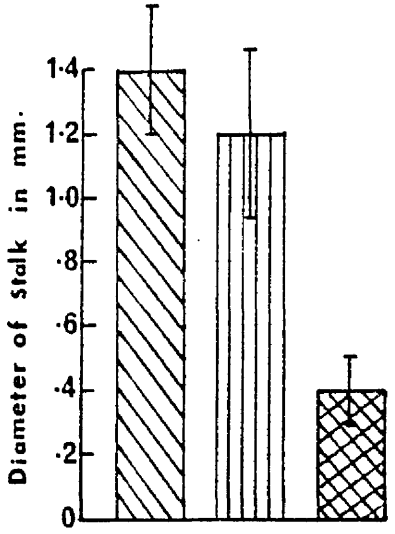


Fig·12 (c)

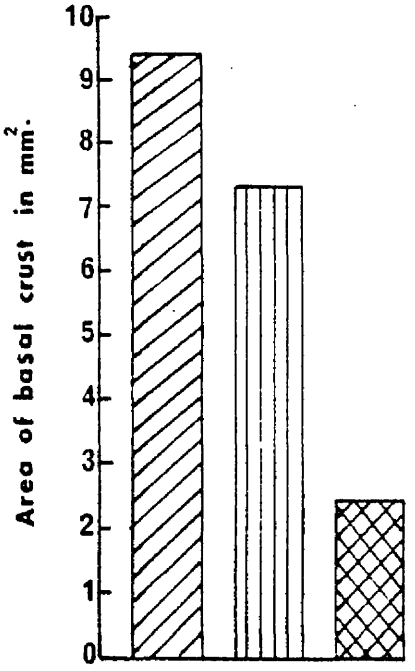


Fig·12 (d)

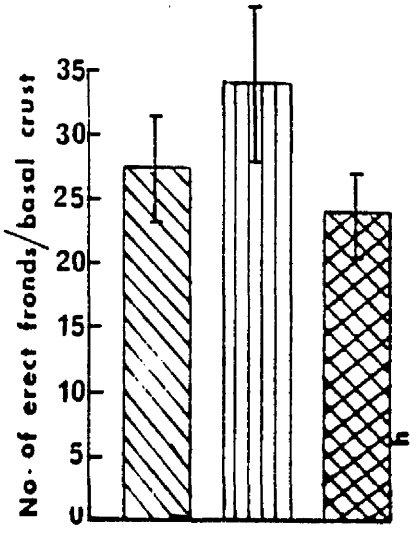




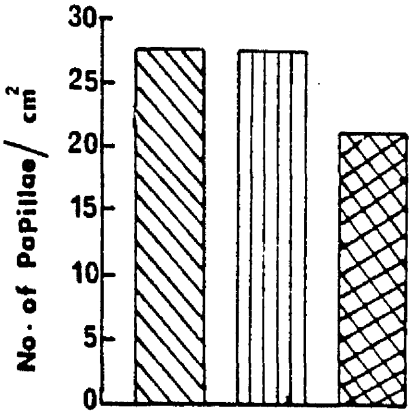
Fig·12(e)



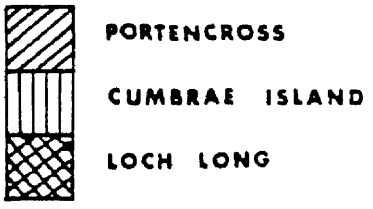
Fig·12(f)



Fig·12(g)



Fig·12(h)



with low salinity of seawater and Cumbrae Island is a moderately exposed locality. These variations in the habitat conditions probably explain the range of the morphological characters. To test the extent to which these morphological variation could be associated with the habitat conditions, some transplant experiments were planned with plants from these localities.

CHAPTER 5

FIELD STUDIES

5.1 Transplants:

The interrelation of genetic and environmental factors in the biology of higher plant species was investigated first by Turesson (1922) using field transplant methods in which material coming from different locations was grown in a standard environment to eliminate environmentally produced effects. He found some considerable genetically determined variation within certain species e.g. Lysimachia vulgaris L., Aster tripolium L., Leontodon autumnalis L. and Atriplex littoralis L. In spite of this success, the transplantation method has not been widely used with marine algae in general and Gigartina in particular, in spite of the opportunities it offers to understanding the development of forms in a species.

Burrows and Lodge (1951) described a transplant technique in which they settled the fertilised eggs of Fucus species on sterile bricks placed in running seawater in the laboratory. After 1-2 months the sporelings could be easily seen by the naked eye and the bricks plus the sporelings were cemented to chosen positions on the shore. The purpose of this experiment was to study the hybridization amongst Fucus plants. Good growth of sporelings was obtained in this way but problems arose through loss of plant material in heavy seas. This problem was overcome by Sundene (1961) working on Alaria esculenta by using large concrete blocks (5 x 15 x 60 cm) bound with nylon cord to which the haptera of the plants could be attached, the aim of this experiment was to study the factors controlling the distribution of

this species, and although this species does not occur on the south-east coast of Norway, the transplanted material grew well in the new habitat. Munda (1964) transplanted plants of Fucus vesiculosus L. var. vadorum Aresch. (with large number of vesicles) to a site beyond its natural limit of occurrence. She found that the plants survived and adapted themselves to the new habitats. The plants, after 7 months of transplantation, formed new parts which were different from the original plants in lacking vesicles and having extensive branching. Pollock (1969) has successfully transplanted two distinct forms of Fucus distichus L. using naturally occurring plants growing on stones and boulders. Interzone transplants of this plant showed that the upper shore plants failed to grow at lower levels, but that lower shore plants could grow successfully when transplanted to higher levels. Also Pollock (1969) established sporelings of Fucus distichus on corrugated plastic strip in the laboratory and then placed them at different levels on the shore. The results of this experiment indicated that the high or low shore conditions exerted no differential mortality on the different samples and the variation in morphology between the different forms of Fucus distichus arose sometimes after the first dichotomy. Norton (1969) found that the wave action produces three different forms of Saccorhiza polyschides (Lightf.) Batt. and the transplanted forms adopted the morphology of the population in the habitat to which they were moved. Floc'H (1969) transplanted two different forms of Chondrus crispus on the coast of Finistére in France in order to check on the possible connection between the morphology and the ecology of this alga. He found that after 7 months of the transplant, the environmental factors had no apparent influence upon the young shoots that were obtained from the two different

forms. Floc'H added that these results make clear that the shape of the thallus is not transient and that the possible effect of the environmental factors upon the morphology of Chondrus crispus can be but very slow. Waaland (1973) described a simple and successful method for experimental transplantation in which plants of Iridaea cordata (Turner) Bory and Gigartina exasperata Harvey and Bailey were attached to 6 mm diameter polythylene line. The two algae were transplanted to habitat in which they do not occur naturally. The purpose of this field transplant experiment was to find out the optimum depth for maximum growth of the two algae which was found to be about 3-5 m below M.L.L.W.

Thus the previous work has shown very clearly that the field transplant experiments offer a useful method of determining the cause of morphological variation and adaptation in a species.

The object of the present field transplant experiment was to try to correlate the morphological variability of the different populations of Gigartina stellata with habitat conditions. Plants of Gigartina stellata were transplanted throughout three different localities in the Clyde sea area in reciprocal fashion as shown in Figure (5).

The transplant experiment was initiated in June 1976 using the method described in page (24) and visits at regular monthly intervals were made.

The difficulties obtained with this fixed transplant experiment can be summarised as follows:

(a) Wave action removes the transplants either by dislodging the whole stones after cementing or by removing the plants from

fixed stones. [See plates (5) and (6)]. The loss of plants and stones was most obvious with plants transplanted from Loch Long to Portencross even for short periods. This can be explained by the fact that Loch Long plants have the smallest area of basal crust as shown in Chapter 4, and are least able to contend with severe wave action.

(b) In some cases, e.g. at Portencross, it was difficult to find the typical plants growing on the right size of stones.

(c) In a few cases the cemented stones proved attractive to visitors to the shore and some disturbances have been seen. Also, sometimes the local people objected to the removal of stones from the shore.

Results of the field transplant experiments show that all control experiments (in which stones bearing plants were cemented at the same site in their normal habitat) indicated that the physical effects of handling and cementing the stones is negligible. Control plants on the cemented stones grow as healthily as the undisturbed plants growing at the same shore sites. Details of the transplant experiment results are as follows:

1 - On Cumbrae Island:

(a) <u>After 3 months</u>:

Plants transplanted from Loch Long grew healthily in this locality. There were slight changes in the colour of plants which became darker. Very few plants were lost from the cemented stones and none of the cemented stones were removed from the rocks.

Plants transplanted from Portencross to this locality grew healthily as well as the undisturbed Cumbrae Island plants alongside. No changes in their morphological condition could be seen.

Plate (5): Showing stones bearing Gigartina plants from Loch Long transplanted to Portencross.
Taken 10 weeks after the transplant.
Note clear patches arising through loss of plant material.
Scale line = 10 cm.

Plate (6): Showing plants from Loch Long transplanted to Cumbrae Island. Taken 18 months after the transplant. The cemented stone (a) cleared of any Gigartina.
Scale line = 10 cm.

(b) After 6 months:

In spite of some losses with plants transplanted from Loch Long both these and plants transplanted from Portencross, appeared normal and still resembled the form in their original localities. The three types of plant forms could be easily recognised at Cumbrae Island site, i.e. the transplants from Loch Long and Portencross and the undisturbed plants of Cumbrae Island.

(c) After 12 months:

Noticeable losses in plants transplanted from Loch Long had occurred. Stones transplanted from Loch Long and Portencross both bore fronds of Gigartina which resembled now the form of the habitat in Cumbrae Island. This can be explained by the fact that new growths of Gigartina produced by the basal crust appeared on all transplanted stones and these new Gigartina fronds resembled these of the undisturbed plants on Cumbrae Island. In many cases it was difficult to distinguish between the 'native plants' of Cumbrae Island and plants transplanted from other localities.

2. At Loch Long:

(a) After 3 months:

Plants transplanted from both Cumbrae Island and Portencross were growing healthily with slight changes in the colour of the transplanted material which became paler and fronds of these plants became slightly twisted. Three groups of plants could be easily distinguished at the site, i.e. the undisturbed plants of Loch Long, plants transplanted from Portencross and plants transplanted from Cumbrae Island.

(b) After 6 months:

Although plants transplanted from both Cumbrae Island and Portencross were growing healthily, some changes in both plants were noticed. New fronds on the transplants now tended to resemble those of the 'native' plants at Loch Long.

(c) After 12 months:

Stones transplanted from both Cumbrae Island and Portencross were covered by plants of Gigartina with growth forms resembling that of the undisturbed plants of Loch Long. Later it became impossible to distinguish between the 'native plants' of Loch Long and the transplanted from other localities.

3. At Portencross:

(a) After 3 months:

A very high rate of loss of plants transplanted from Loch Long was noticed, even after the first few months. The remaining plants from Loch Long became darker in colour and tough in their texture. There were no noticeable changes in plants transplanted from Cumbrae Island. These plants were growing healthily and resemble the form shown in their original locality.

(b) After 6 months:

There was no record of any plants transplanted from Loch Long, since stones from this locality were bare or had disappeared. Plants transplanted from Cumbrae Island grew healthily with very few plants

lost despite the heavy seas. The fronds became darker, tougher in texture, twisted and showed some resemblance to the undisturbed plants of Portencross.

(c) After 12 months:

Plants transplanted from Cumbrae Island grew healthily and as well as the 'native plants' of Portencross. Both old fronds and the new ones produced from the basal crust resembled the native plants and at a later time it was very difficult to distinguish between the undisturbed plants and the transplanted ones.

Generally, plants of Gigartina were able to resist any changes in their morphology for sometime after transplantation to new habitats. After 3 months slight changes in colour, texture and spirality of fronds had occurred. At a later time (after 6 months) these changes became more obvious and plants adapted themselves to their new habitat condition at Loch Long and Portencross. Plants transplanted to Cumbrae Island (the moderately exposed locality) seemed able to resist any changes in their morphology for a longer period (up to 12 months). During this period the new fronds were produced from the basal crusts resembled the 'native plants' in all cases.

Thus, these results are in agreement with those obtained by Munda (1964), Pollock (1969) and Norton (1969). To some extent the results underline the work of Floc'H (1969) on Chondrus crispus in demonstrating that any morphological changes are very slow processes.

5.2 Recolonisation:

Studies on recolonisation on artificially denuded areas have shown that much valuable information can be gained and the technique should undoubtedly be extended. Such studies will throw some light on the factors determining the characteristics of zonation that can be observed (Chapman 1946, 1957). According to Marshall et al (1949) the recolonisation studies would help to elucidate the life history of the plants and indicate whether cultivation on artificial substrata, placed in the sea, would be commercially practicable. Bokenham (1938) was among the first to study colonisation in the intertidal zone in South Africa. He stated that the result of such study would make it possible to compare the growth rate of the same organism in warmer and colder water.

Rees (1940) found that the recolonisation is influenced by the season of the year, the nature of the substratum (e.g. whether wood, iron or concrete), tide level, angle of slope and competition between plants and animals.

Kitching (1937) stated that the sequence of recolonisation in area studied in all European waters appears to be much the same with diatoms generally returning first, followed by Enteromorpha spp and then by Fucus spp.

Umamaheswararae and Sreeramulu (1967) described the sequence of recolonisation in denuded areas in India and reported that the ability of algae to tolerate tidal exposure is important in determining the recolonisation.

Recolonisation on denuded rocky surfaces in the intertidal region undertaken by different workers has been reviewed by Chapman 1946, 1957

and Doty (1957).

This study was commenced in February 1976, by clearing a strip as described in page (23) at Davy's Rock, Cumbrae Island. Visits were made at regular monthly intervals and macroscopic recolisers were recorded.

For a period of 3 months, the strip was bare, and after this period, the strip recovered from the chemical effect of formalin solution. The first colonisers which appeared after 3 months were Cladophora rupestris (L.) Kutz. on the upper part of the strip and Enteromorpha spp. on lower part. After 6 months from the date of clearing the strip, the upper part was colonised by sporelings of Fucus, while the remaining part of the strip was covered by Enteromorpha spp., Cladophora spp. and a few scattered plants of Porphyra spp. After 8 months Fucus sporelings in the upper part of the strip reached about 5-8 cm in length, and more sporelings of Fucus were growing from underneath Enteromorpha and Cladophora in the lower part of the strip. After 10 months, Fucus plants in the upper part of the strip reached 10-15 cm in length with some plants forming receptacles. These were found to be Fucus spiralis. The lower part of the strip was still covered by Enteromorpha, Cladophora, Porphyra and small scattered plants of Ulva. However, under these species a carpet of Fucus sporelings were still growing. After 12 months from the date of clearing the strip, the whole strip was covered by Fucus plants, and while the lower part of the strip was covered by small plants of Fucus, the upper part was covered by fertile F. spiralis.

In July 1977, after about 18 months from the date of clearing the strip, the latter was covered by three species of Fucus, F. spiralis L. in the upper part, F. vesiculosus L. in the middle part and F. serratus L.

in the lower part of the strip. All Fucus plants were fertile.

At no time did Gigartina sporelings or any red crust appear on any part of the strip. After $2\frac{1}{2}$ years from the date of clearing the strip there was still no indication of any reappearance of Gigartina and the strip remained covered only by Fucus plants [see plate (7)].

To follow any seasonal variability in recolonisation, successive strips in the same area were cleared at different seasons of the year as follows:

*) Strip No.2. was cleared in June 1976.

*) Strip No.3. was cleared in September 1976.

*) Strip No.4. was cleared in April 1977.

The sequence of recolonisation was similar to the above described and in all cases Gigartina and red crust were absent, and all strips were covered by Fucus plants as shown in plates (8), (9) and (10).

Marshall et al (1949) reported that Gigartina fronds 0.5 - 1.0 cm in length reappeared 6 months after the date of clearing the area. The experiment of Marshall et al (1949) was made in July and they sterilised the area using a solution of mercuric chloride after scraping the surface of the rocks. Hruby (1977) scraped, brushed and burned small areas at Toment End on the northern side of Cumbrae Island, he found similar results to Marshall et al (1949).

Two successive strips were cleared at Tomont End in July 1977. On one strip the surface was cleared of plants by scraping and brushing, whilst the other was cleared by scraping, brushing, burning and formalin solution was poured on it.

After only 4 months from the date of clearing the strips, the first

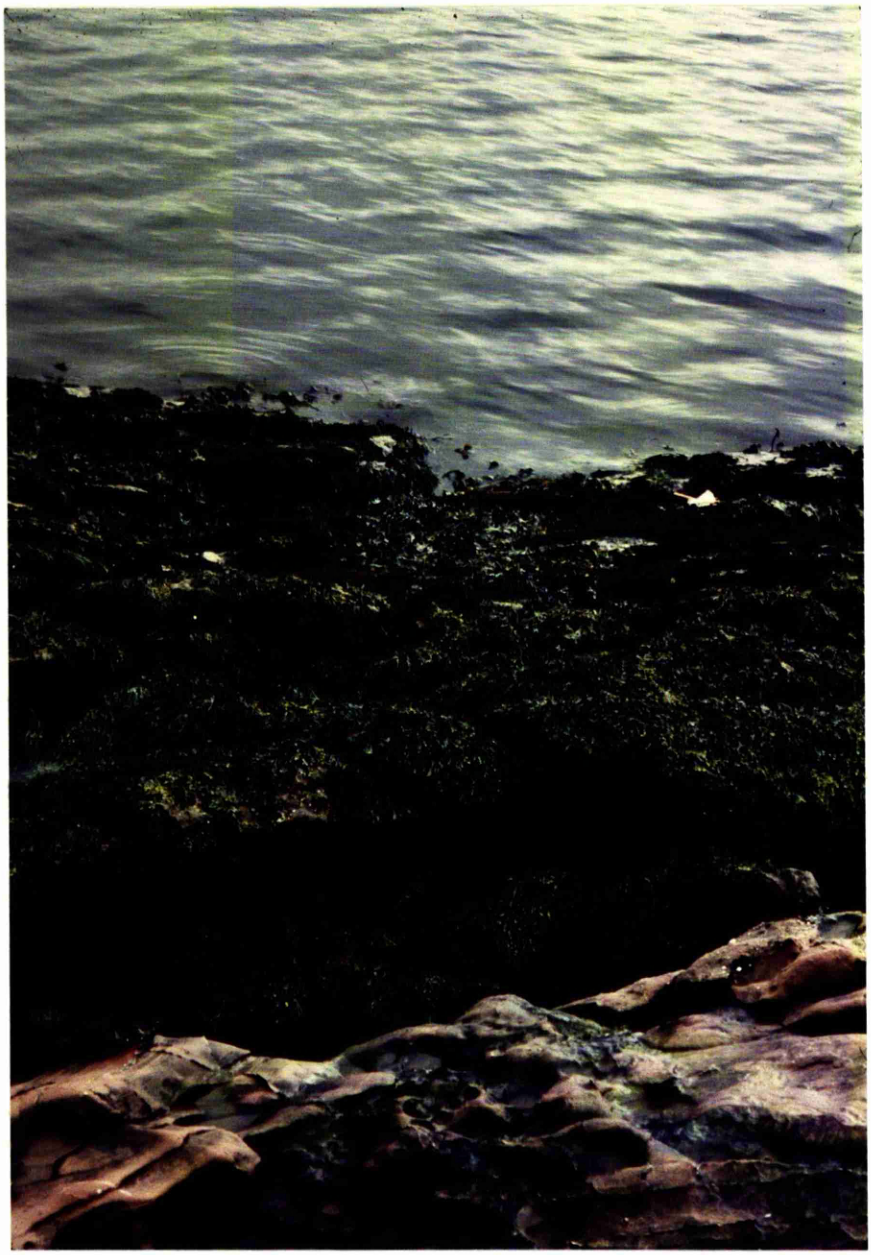


Plate 7: Showing strip No.1, cleared at Davy's Rock in February 1976.

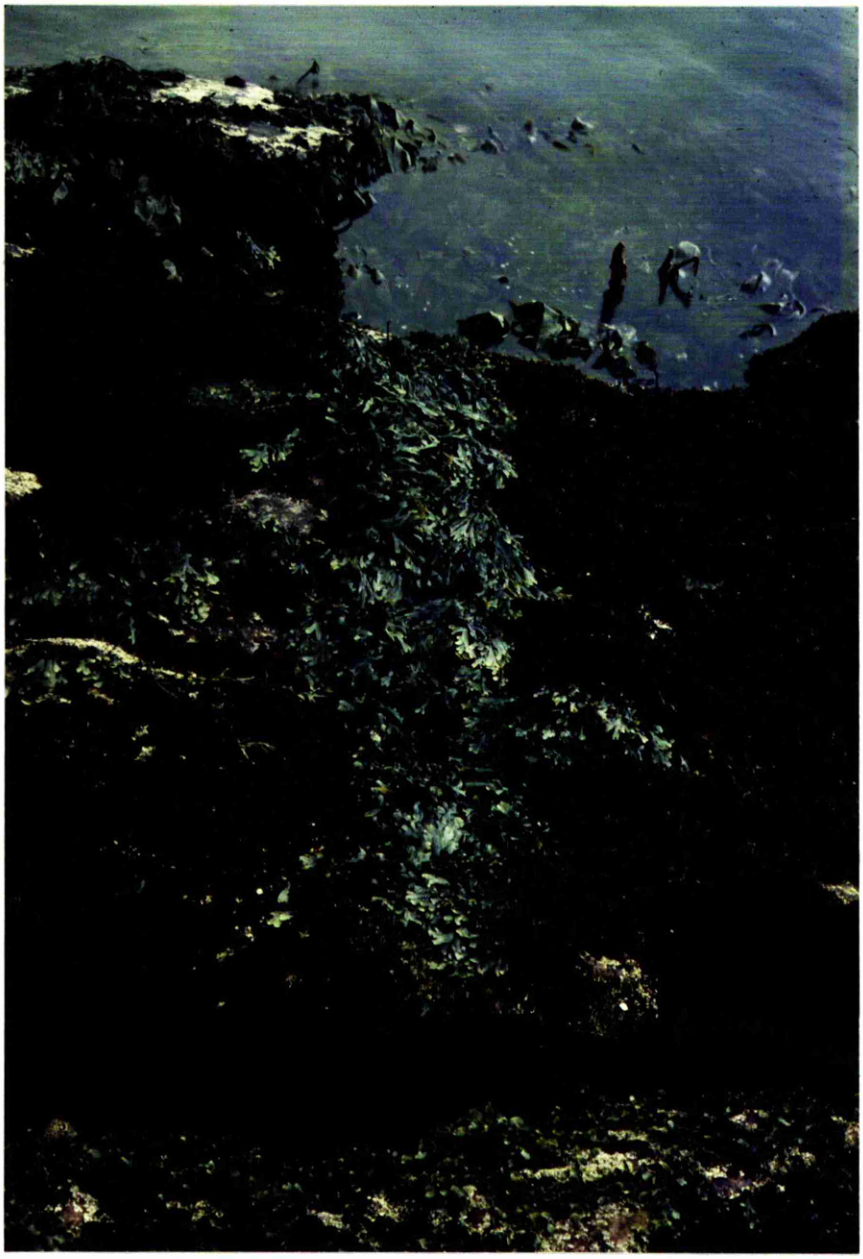


Plate 8: Showing strip No.2, cleared at Davy's Rock in June 1976.

Plate 9: Showing strip No.3, cleared at Davy's Rock in September 1976.

Plate 10: Showing strip No.4, cleared at Davy's Rock in April 1977.

strip which was not burned and had no formalin solution poured on it, was covered by a carpet of germlings of Gigartina [see plate (11)] . The result agreed with the results of Marshall et al (1949) and Hruby (1977). The other strip which was burned and had formalin solution poured on it was still bare after 4 months [see plate (12)]. This result agreed with the previous results obtained at Davy's Rock and also agreed with the result obtained by Burns (1971) who made similar experiments in New Hampshire in U.S.A. and found that plants of Gigartina need more than 3 years to recolonise themselves.

5.3 Regeneration:

Regenerative growth of thallus fragments of red algae is a well-known phenomenon. The ability of minute fragments, which have over-wintered or survived through some other adverse period, often buried under a mass of sand or other debris, to produce new thalli as soon as conditions improve is one of the most significant reasons for the persistence of red algae both in freshwater and the sea (Dixon 1973). Boney (1974) reported that when detached from actively growing plants the fragments demonstrate a clear-cut polarity and growth proceeds either from newly established or from existing apical cells, the former arising on the end facing the apex of the originally intact plant, and rhizoids will arise from any part of the plant fragment surface in contact with the substratum. Very small fragments (even individual cells as in Griffithsia corallinoides (L.) Batt.) can serve as sources of new plant material (Konard-Hawkins 1964a,b, 1968). As observed by Boney (1974) the excised pseudo-laterals of Heterosiphonia plumosa (Ellis) Batt. showed rhizoid formation either from the region

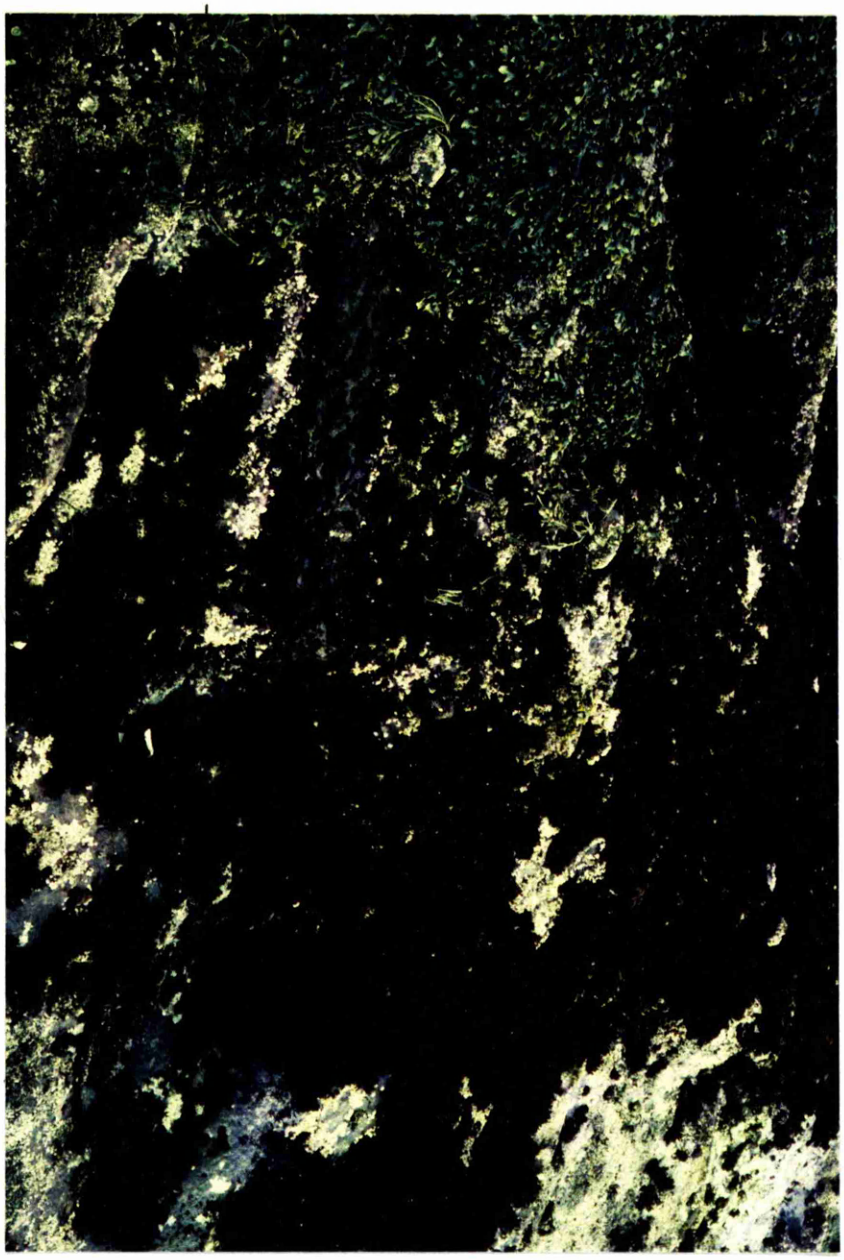


Plate **11**: Showing the strip cleared in July 1977 at Tomont End by scraping and brushing the area.

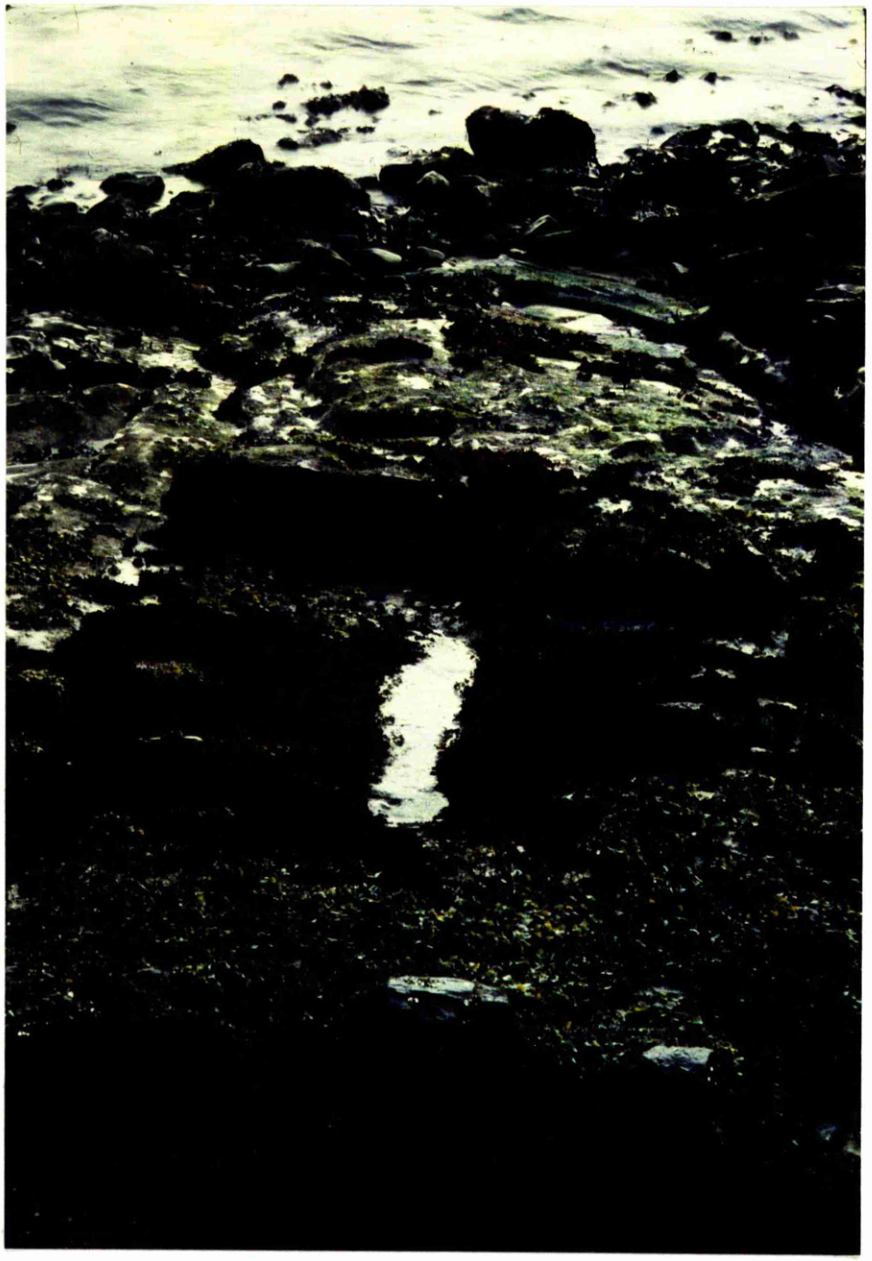


Plate **12**: Showing strip cleared in July 1977 at Tomont End by scraping, brushing, burning and formalin poured on the strip.

of the rhizoid in close proximity to a basal cell or directly from the basal cell.

Perrone and Felicini (1972) found that when cut into segments the fronds of Petroglossum nicaeense (Lamour. ex Duby) Schotter give riae to adventitious buds which are produced distally by proliferating activity of erect fronds while the creeping axes are formed from the regenerated buds. These facts clearly indicate a polarisation in the development of the algae and suggest that correlation 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 margin of the leafy fronds and removing the distal portion of erect frond of this alga induces outgrowth of adventitious branches. They also found that cessation of apical growth in older fronds is characterised by outgrowth of marginal proliferations. Both regeneration and proliferation are affected by Nitrogen starvation and by addition of IAA.

Regeneration studies on Gigartina have not been investigated extensively, However, Marshall et al (1949) reported that the damaged plants of Gigartina stellata have been found to be capable of forming new branches and regeneration normally takes place from the wounded surfaces if parts of the thallus are removed by injury or by harvesting. They also found if the apex of Gigartina thallus with papillae is wounded, new growth develops from the meristems that would normally produce reproductive papillae immediately behind the growing point.

Perrone and Felicini (1976) cultured segments of Gigartina acicularis (Roth) Lamouroux and found that the segments produced two types of buds: (1) proliferation and (2) regeneration. Either one

type or both may be found on cut surfaces. Suitable trophic conditions increase the relative frequency of regeneration in comparison with proliferations and segments from the distal part of the frond show a greater tendency to proliferate, while segments of basal part show more regenerations.

The present study was initiated on November 1976, by cutting plants of two 0.25 square quadrats at Davy's Rock on Cumbrae Island. Plants were cut at the middle of the stalk about 2 cm from the basal cut, leaving only parts of the stalk to regenerate. Visits at regular monthly intervals were made.

After 3 months, the cut fronds of Gigartina had disintegrated and the quadrats were dominated by Enteromorpha spp. and Cladophora spp. New growths from the basal crust of Gigartina had appeared under the carpet of the green algae, and these new growths were growing healthily and of normal appearance. This suggests that the growth of green algae was not the reason for the death of the Gigartina fronds.

This experiment was repeated in July 1977, by cutting plants of two 0.25 cm square quadrats in the same region of the shore at Davy's Rock on Cumbrae Island. In this experiment the top 2 cm were cut from Gigartina fronds leaving part of the flattened fronds as well as the stalk.

Results of this experiment showed that plants of Gigartina regenerate very slowly in nature and the new growth from the cut surface reached about 2 cm in 7 months from the date of cutting the fronds (see Figure (13)).

For some reason, certain fronds (only about 10-15% of the total fronds in the quadrat) did not regenerate from the cut surface but formed numerous proliferations from the margin of the fronds (see Figure (14)).

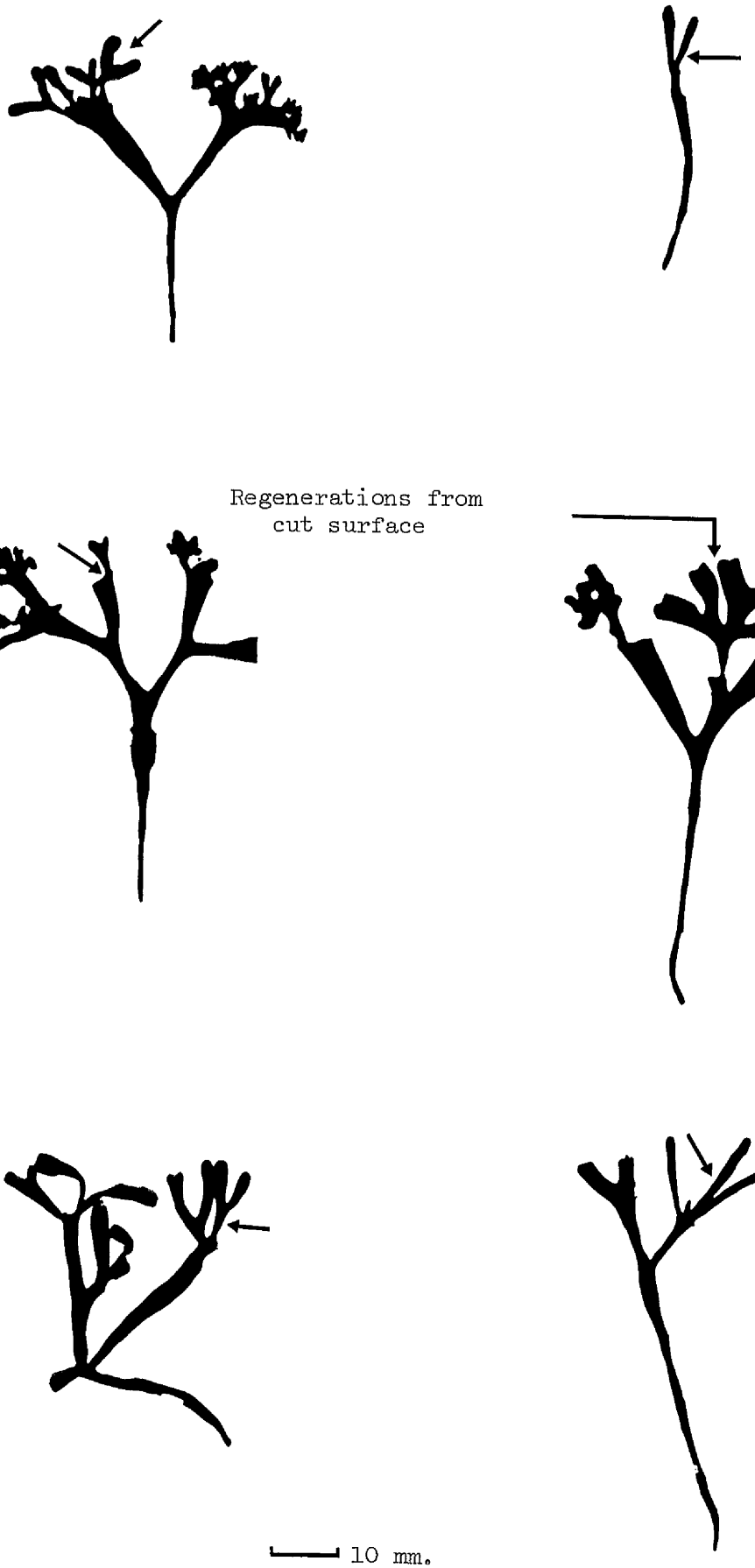

Regenerations from
cut surface
10 mm.

Figure 14: Shows proliferation from the margin of fronds. Taken after 7 months.


Proliferations from the
margin of frond.
10 mm.

5.4 Determination of reproductive period:

The aim of this study was to measure the period of maximum spore discharge of plants of Gigartina stellata.

This study was commenced in June 1976 and the degree of fertility was measured from monthly collection as described in Chapter 2. The experiment was terminated in November 1977, after two peaks of maximum spore discharge in two successive years were obtained.

The results are summarised in Figure (15). As shown from this figure:

1. In the different populations of Gigartina stellata the maximum spore discharge occurred from September through December. Also the minimum period of spore discharge occurred from April through July in all populations.
2. Maximum and minimum spore discharge periods occurred in the same months over two successive years.
3. Plants from Portencross were the most fertile, while plants of Loch Long were the least fertile and plants from Cumbrae Island show an intermediate degree of fertility.
4. In all populations a small number of plants bearing a few fertile papillae were found throughout the year.

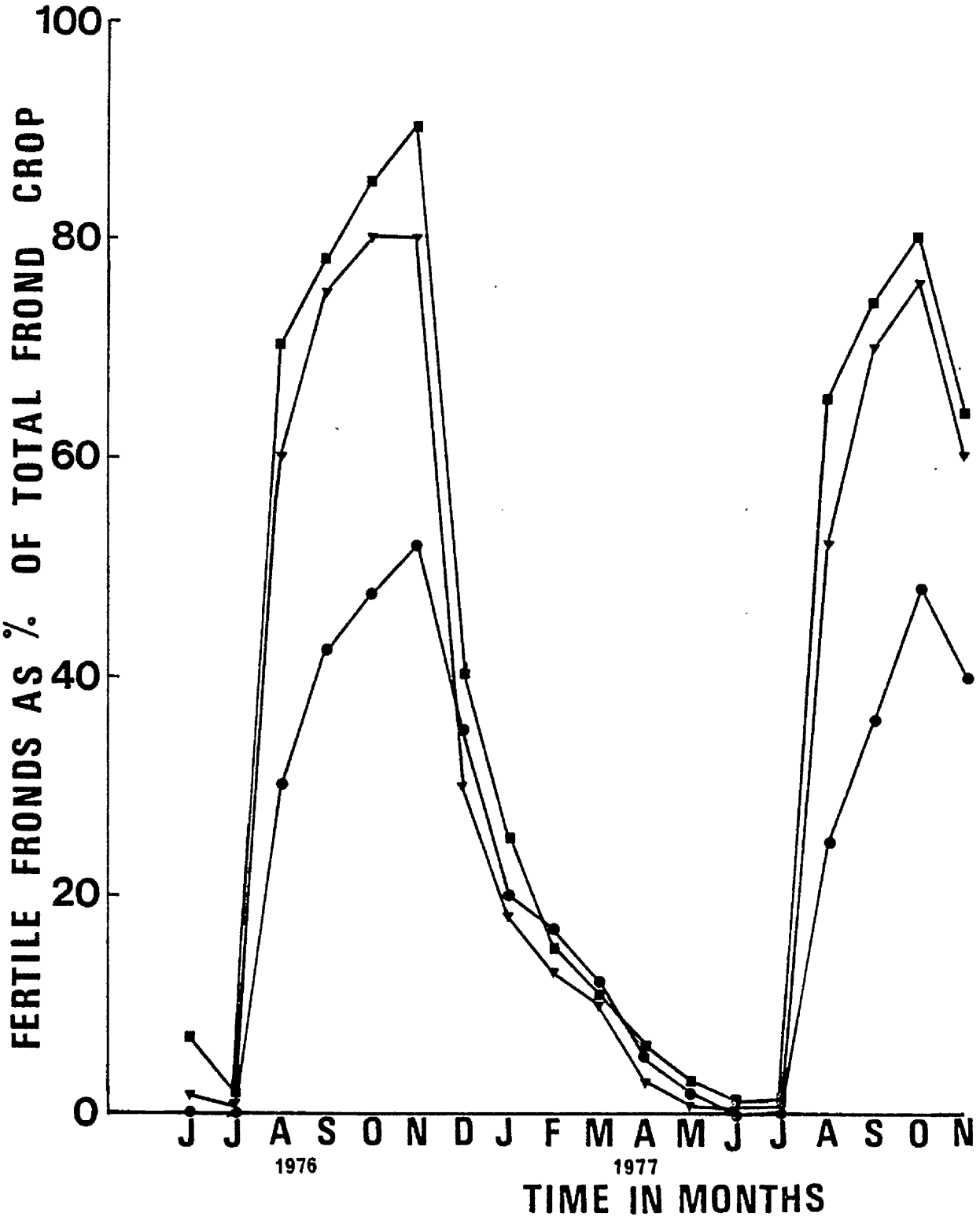
Results of this study agreed with the results obtained by Marshall et al (1949) who found that the maximum period of Gigartina spore discharge appears to be from September to December. Burns (1971) reported that the period of maximum spore discharge of Gigartina stellata in New Hampshire (U.S.A.) was from October through December. Burns

Figure 15: Shows the maximum and minimum spore discharge of plants of *Gigartina* from the different localities in the Firth of Clyde.

● plants from Loch Long

▼ plants from Cumbrae Island

■ plants from Portencross


100
80
60
40
20
0
FERTILE FRONDS AS % OF TOTAL FROND CROP
J J A S O N D J F M A M J J A S O N
1976
1977
TIME IN MONTHS

and Mathieson (1972) studied the seasonal periodicity of reproduction in plants of Gigartina from New Hampshire, and found that the cystocarpic papillae developed on fronds during late June and July and the maximum number of papillae per plant was recorded during September through December. They also added that few papillae-bearing thalli were found throughout most of the year.

It has also been reported by McConnel (1977) that reproductive papillae of Gigartina stellata from Anglesey, North Wales were observed mainly between June and September.

CHAPTER 6

CULTURE STUDIES

6.1 Culture of fronds of Gigartina stellata

The morphological variability in Gigartina discussed so far can be related to the effect of environmental conditions as well as to inherited characters. To investigate some aspects of the variability observed, culture experiments were carried out in which experimental conditions simulating some environmental factors were examined.

Culture studies on Gigartina fronds do not seem to have been investigated extensively. Burns (1971) and Burns and Mathieson (1972) cultured the vegetatine discs removed by a cork borer from the thin flattened portion of the thalli of Gigartina stellata and the related species Chondrus crispus in order to study the effect of environmental factors on the photosynthesis and respiration rates of the vegetative fronds of the two species. They found that for Gigartina stellata the maximum photosynthesis/respiration ratios were recorded at 20°C and at 40‰ and light saturation intensity for photosynthesis of Gigartina is higher than that for many sublittoral plants. They also found that Gigartina exhibited a broad tolerance to dehydration. Neish and Shacklock (1971) and Neish and Fox (1971) grew detached plants of Chondrus crispus lacking holdfasts in large culture tanks in a greenhouse. Their investigation included the effect of flushing with freshly pumped seawater and turbulence, and the effect of adding fertilizers in several combinations. They not only found that the detached whole plants of Chondrus crispus continued to grow in culture, but also that pieces of Chondrus thallus would also continue to grow. Brinhuis and Jones (1974) studied the photosynthesis

in whole plants of Chondrus crispus by ^{14}C uptake. They cultured plants of Chondrus 5-10 cm long in 50 ℓ culture tanks supplied with running seawater. Results of this experiment showed a direct relationship between light intensity and temperature in terms of rate of photosynthesis in this alga.

In the present experiments 10 healthy cleaned fronds of about 2 cm long were placed in a culture tank containing 5 ℓ of the enriched seawater medium. All experiments were duplicated by culturing two tanks under the same conditions. Details of culture methods are described in Chapter 2.

6.1.1 Assessment of growth of plants of Gigartina

Growth of the vegetative fronds of Gigartina was assessed by two methods: (1) by increase in fresh weight and (2) by increase in surface area. Details of the two methods are described in Chapter 2, page (28). As shown in Figure 16 the increase in fresh weight is fairly accurate and the three replicates of this experiment show no significant differences. Furthermore, it is a quicker method of manipulation and does no harm to the plants. On the other hand assessment of the growth by increase in surface area as shown in Figure 17 was not only difficult to manipulate but also the replicates varied appreciably and the growth was not steady. Therefore in all future experiments the growth was assessed by the increase in fresh weight.

Figure 16: Shows growth rate of Gigartina fronds assessed by an increase in fresh weight.
Bars represent S.D. of the three replicates in duplicate experiments.

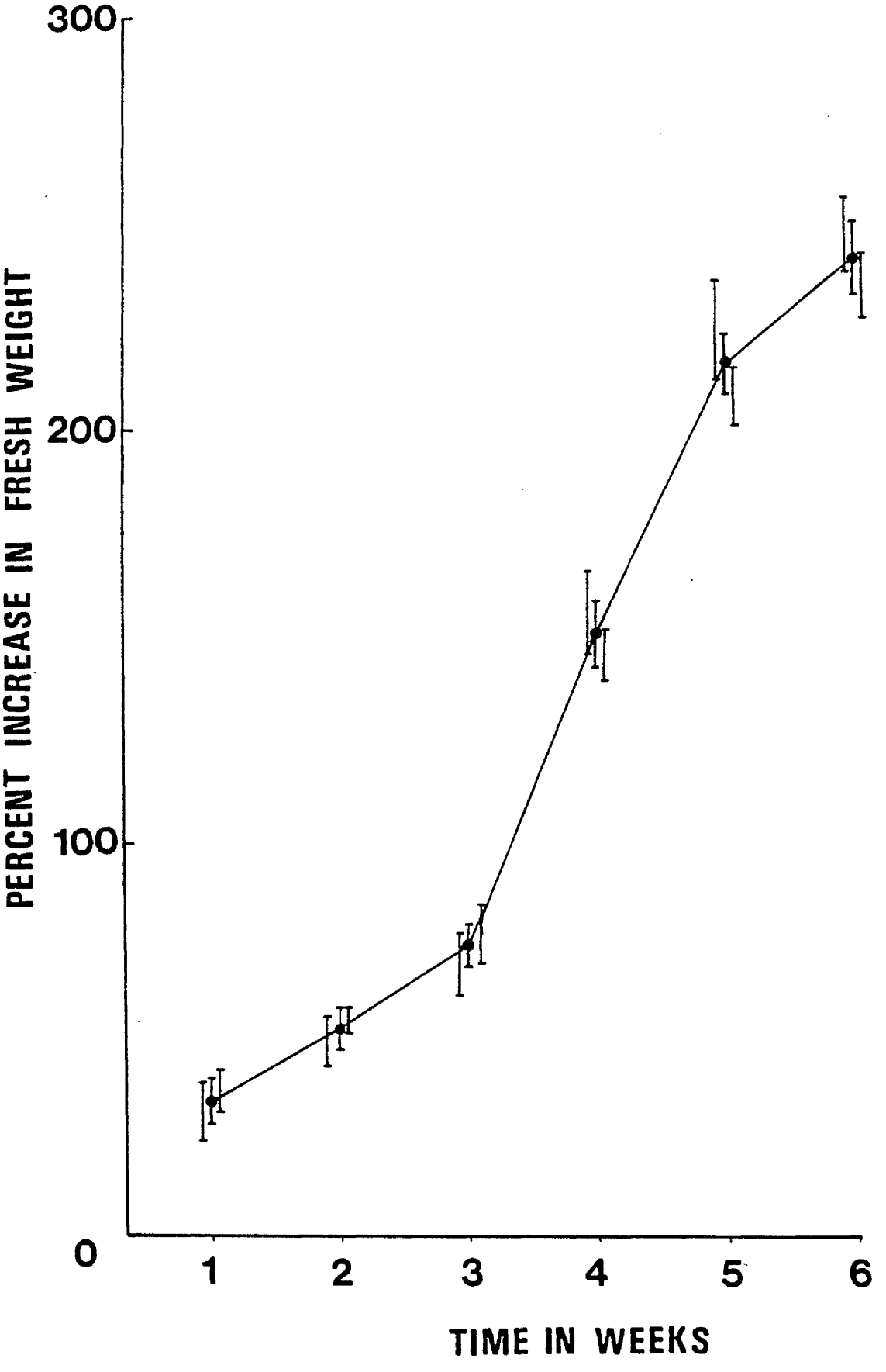
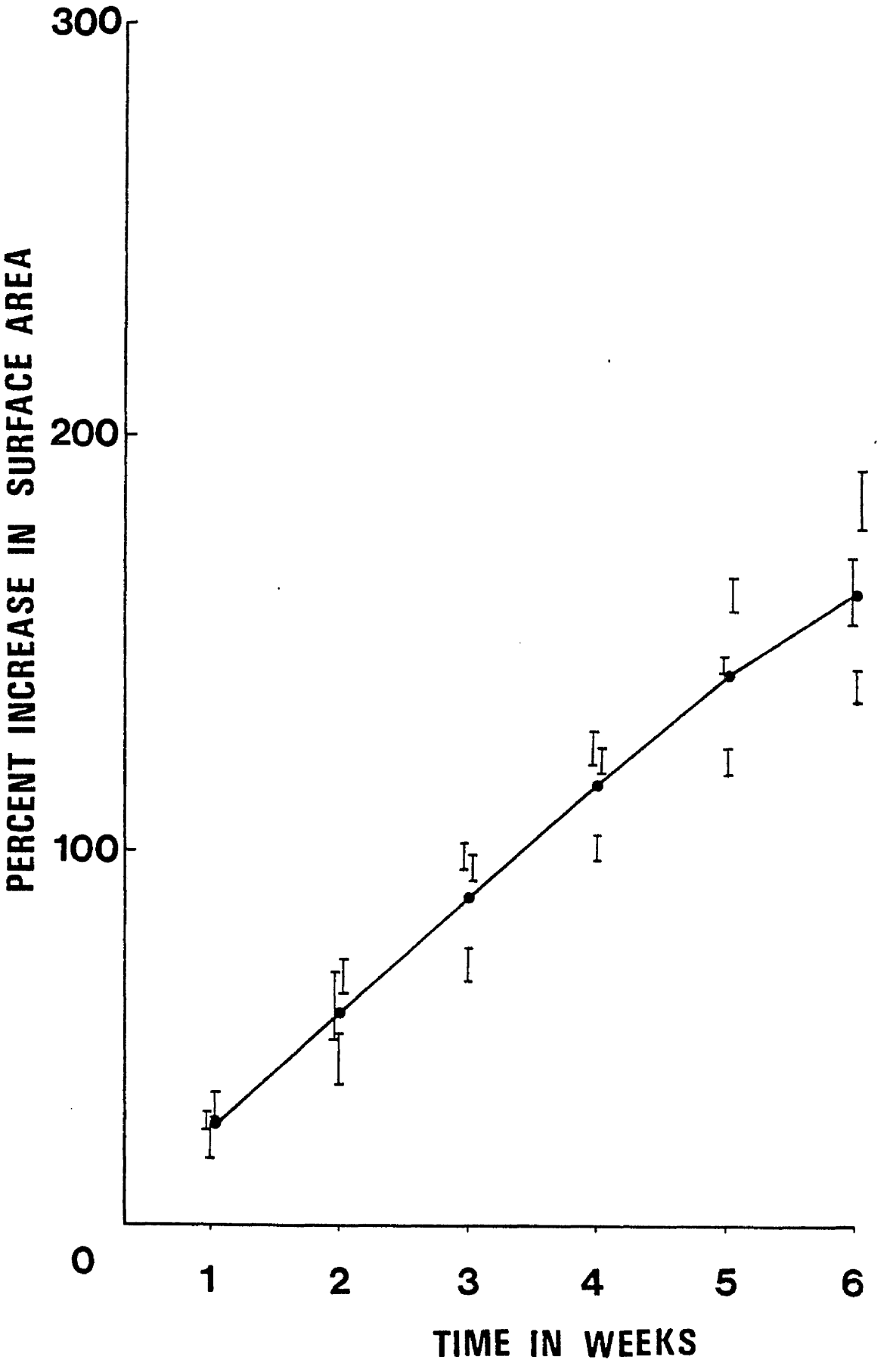

300
200
100
0
PERCENT INCREASE IN FRESH WEIGHT
1
2
3
4
5
6
TIME IN WEEKS

Figure 17: Shows growth rate of Gigartina fronds assessed by an increase in surface area.
Bars represent the S.D. of the three replicates in duplicate experiments.


300
200
100
0
PERCENT INCREASE IN SURFACE AREA
1
2
3
4
5
6
TIME IN WEEKS

6.1.2 Culture of *Gigartina* collected from the different localities under the same conditions:

The aim of this experiment was to see if plants of *Gigartina* collected from the different localities show the same growth responses when cultured under similar conditions, i.e. if there is any genetic variability in plants from different localities.

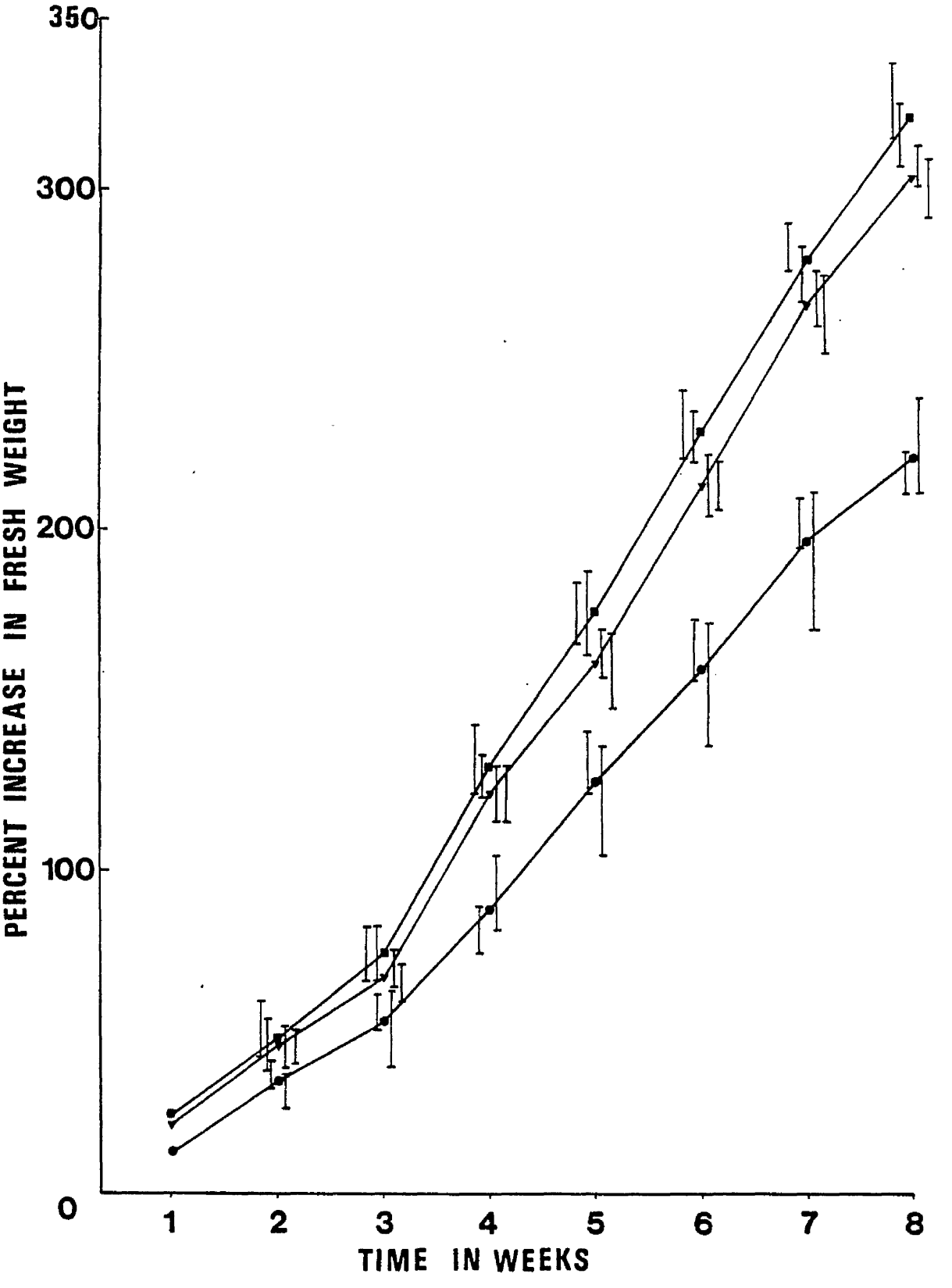
For this purpose, fronds of plants of *Gigartina*, collected from Cumbrae Island, Loch Long and Portencross, were cultured under the same laboratory conditions at 12°C, 2400 lux, daylength of 12 hours, salinity of 32‰ and fully submerged in the enriched seawater medium.

Results of this experiment which are summarised in Figure 18 show that plants from Loch Long have slower growth rates than plants collected from the other localities. Plants collected from Cumbrae Island and Portencross did not show any significant differences in growth rate when cultured under the same conditions. Plants from all the three localities grew healthily for the whole period of experiment.

To some extent these results agreed with the observation obtained from the field transplant experiments which showed that when plants of *Gigartina* from Cumbrae Island were transplanted to Portencross and vice versa, plants in the two localities were growing healthily and as well as the undisturbed plants of the two localities. When plants from Cumbrae Island and Portencross were transplanted to Loch Long, although they survived they came to resemble to 'native' plants of the new habitat much sooner. On the otherhand, when plants from Loch Long were transplanted to Cumbrae Island or Portencross, although

Figure 18: Shows growth rates of plants of Gigartina collected from different localities and cultured under the same conditions. Bars represent the S.D. of the duplicate experiments.

- ● plants collected from Loch Long
- ▼ plants collected from Cumbrae Island
- ■ plants collected from Portencross


350
300
200
100
0
PERCENT INCREASE IN FRESH WEIGHT
1
2
3
4
5
6
7
8
TIME IN WEEKS

many have been washed away by heavy seas, the remaining plants (especially at Cumbrae Island) have resembled the plants in their original locality. The results of this experiment suggest a possible genetic variability in plants from Loch Long.

To test the role of environmental conditions on growth of fronds of Gigartina, a series of experiments were carried out in which fronds of Gigartina collected from the one locality (Cumbrae Island) were cultured under different laboratory conditions:

(a) Culture of Gigartina fronds under different salinities

Five different salinities (4‰, 8‰, 16‰, 24‰, and 32‰) were prepared by diluting 'normal' seawater (32‰) with distilled water. Under each of these salinities fronds of Gigartina were cultured at 12°C, 2400 lux, a daylength of 12 hours and fully submerged in the enriched seawater medium.

Results of this experiment are illustrated in Table 1 and Figure 19. As shown from these data, increases in growth rate of fronds of Gigartina were obtained in all salinities from 8‰ - 32‰. At the very reduced salinity (4‰) fronds became pale in colour and many of the fronds lost their rigidity and started to disintegrate after only three weeks. The growth rate was also slow at a salinity of 8‰, and although fronds became pale in colour, they survived this low salinity for a longer period. After 8 weeks, some had lost their rigidity and started to disintegrate. Whilst the plants tolerated immersion in media of 16‰ for the whole period of the experiment, growth was much slower than at any other higher salinity. The results showed also that there were no significant differences

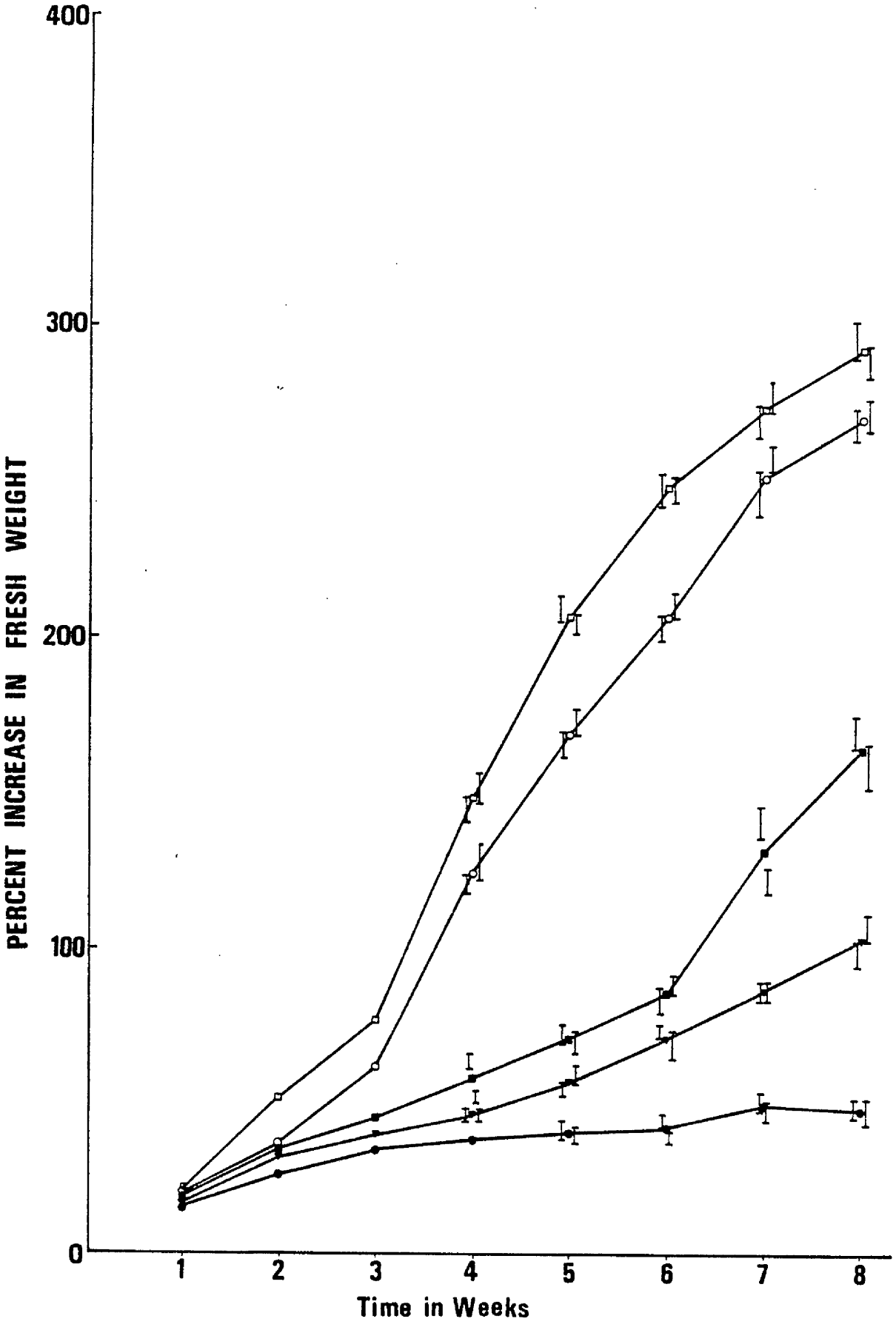
Table 1: Effect of various salinities on the growth of fronds of Gigartina stellata, collected from Davy's Rock, Cumbrae Island and grown fully submerged in Boney's medium at 12°C, 2400 lux, and daylength of 12 hours.

Salinity ‰ \ Time in weeks	Initial weight in grams	Time in weeks							
		1	2	3	4	5	6	7	8
4	0.30 – 0.9	* 0.35 ** 15% + 1.5	0.38 27% 1.8	0.40 35% 1.5	0.41 38% 2.0	0.42 40% 3.5	0.42 41% 2.5	0.45 49% 3.6	0.44 47% 4.0
8	0.25 – 0.4	0.29 18% 1.0	0.33 32% 1.4	0.35 40% 2.0	0.37 47% 2.5	0.39 55% 3.4	0.42 70% 4.0	0.46 86% 3.0	0.5 102% 4.0
16	0.22 – 1.0	0.25 20% 1.8	0.29 35% 1.5	0.31 45% 1.4	0.33 57% 2.5	0.36 70% 3.6	0.39 85% 3.8	0.49 130% 4.5	0.56 163% 6.5
24	0.28 – 0.8	0.34 22% 1.6	0.38 37% 1.2	0.45 61% 2.0	0.62 122% 4.5	0.75 168% 4.0	0.85 205% 4.0	0.97 250% 5.5	1.02 268% 5.0
32	0.20 – 0.9	0.23 23% 1.5	0.29 52% 1.8	0.34 76% 1.4	0.48 147% 4.0	0.59 205% 3.6	0.67 245% 4.5	0.72 270% 5.0	0.75 290% 7.0

* = Mean value of increase in fresh weight of 20 fronds in grams.
** = % increase in fresh weight.
\+ = Standard deviation

Figure 19: Shows growth rates of Gigartina fronds cultured at various salinities.
Bars represent the S.D. of the duplicate experiments.

● at 4‰
▼ at 8‰
■ at 16‰
○ at 24‰
□ at 32‰


400
300
200
100
0
PERCENT INCREASE IN FRESH WEIGHT
1
2
3
4
5
6
7
8
Time in Weeks

in growth rates of Gigartina at 24‰ and 32‰.

These results suggest that Gigartina plants are able to grow at relatively low salinities, but not in brakish water situations. At Loch Long the plants may be covered with water of very low salinity but only for short periods.

(b) Culture of Gigartina fronds under different illuminations

The various illuminations tested were 400 lux, 600 lux, 800 lux, 1200 lux, 2400 lux and 3000 lux at 12°C, a daylength of 12 hours, a salinity of 32‰ and the fronds fully submerged in the enriched seawater medium.

Results of these experiments are illustrated in Figure 20, and summarised in Table 2. As is clear from these data, fronds of Gigartina exhibited an increase in growth correlated with the increase in illuminations from 400 lux to 3000 lux. In spite of the variation in growth rate, Gigartina seemed to grow healthily at all levels of illumination. The maximum growth (320% increase in fresh weight) was obtained at 2400 - 3000 lux. At half this illumination level (1200 lux) fronds of Gigartina exhibited an increase of about 260% in their fresh weight., therefore growth did not show a proportional increase related to the amount of illumination. At 1200 lux, after 5 weeks, the two replicates, as shown in Figure 20 , did not overlap with each other, and some significant differences in growth rate in the two replicates were obtained.

Under illuminations of 800 lux and below, fronds not only grew slower but became paler in colour and by the end of the period of experiment many fronds especially those at 400 lux and 600 lux, lost

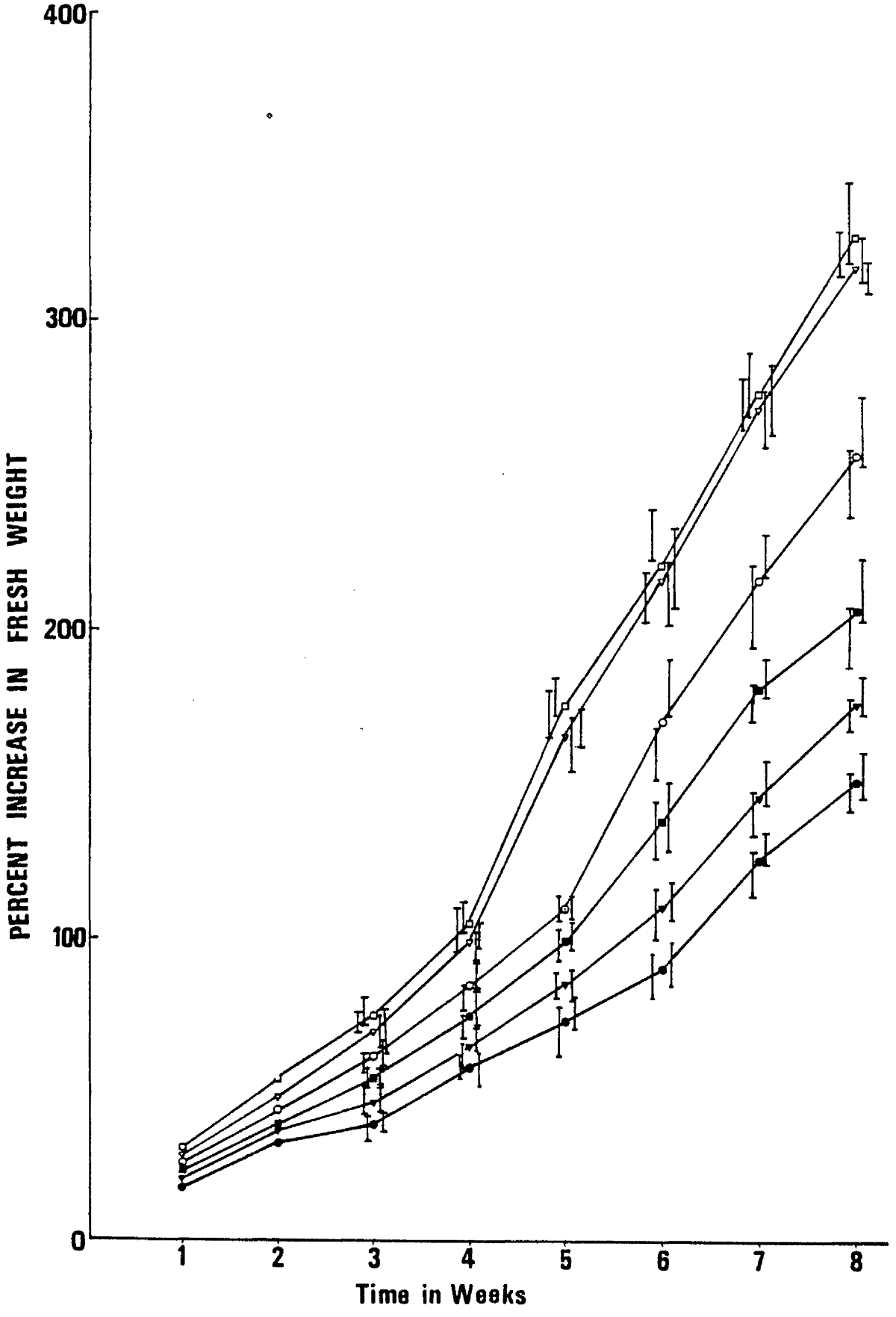
Table 2: Effect of various illuminations on the growth of fronds of Gigartina stellata, collected from Davy's Rock, Cumbrae Island and grown fully submerged in Boney's medium at 12°C, daylength of 12 hours and 32 ‰ salinity

Time in weeks / Illumination lux	Initial weight in grams	Time in weeks							
		1	2	3	4	5	6	7	8
400	0.21	* 0.25	0.28	0.29	0.33	0.35	0.40	0.47	0.53
	-	** 19%	34%	40%	58%	73%	90%	125%	150%
	1.4	+ 2.5	2.0	4.3	6.3	6.5	7.5	6.8	7.5
600	0.27	0.32	0.37	0.40	0.45	0.50	0.57	0.67	0.75
	-	22%	38%	47%	65%	85%	110%	145%	175%
	1.3	2.0	2.3	5.0	5.0	5.5	7.7	7.5	6.8
800	0.31	0.38	0.44	0.48	0.54	0.62	0.74	0.87	0.95
	-	25%	40%	55%	75%	99%	138%	180%	205%
	1.0	2.3	2.1	3.5	6.5	5.5	9.0	6.5	8.5
1200	0.20	0.26	0.30	0.34	0.39	0.44	0.56	0.66	0.74
	-	25%	45%	62%	85%	110%	170%	215%	255%
	1.2	2.0	1.8	4.0	6.0	4.5	9.5	8.8	10.0
2400	0.30	0.39	0.46	0.52	0.61	0.81	0.97	1.13	1.27
	-	26%	49%	70%	99%	165%	215%	270%	315%
	1.3	1.6	2.2	6.0	5.5	9.5	8.0	9.0	7.0
3000	0.19	0.25	0.31	0.35	0.41	0.55	0.63	0.74	0.84
	-	28%	55%	75%	105%	175%	220%	275%	375%
	1.1	1.5	2.0	4.5	5.6	9.0	11.0	9.5	9.0

* = Mean value of increase in fresh weight of 20 fronds in grams
** = % increase in fresh weight
\+ = Standard deviation.

Figure 20: Shows growth rates of Gigartina fronds cultured at various illuminations.
Bars represent the S.D. of the duplicate experiments.

- ● at 400 lux
- ▼ at 600 lux
- ■ at 800 lux
- ○ at 1200 lux
- ▽ at 2400 lux
- □ at 3000 lux


400
300
200
100
0
PERCENT INCREASE IN FRESH WEIGHT
1
2
3
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6
7
8
Time in Weeks

their rigidity and started to disintegrate. The results showed also that there were no significant differences in growth rates of Gigartina fronds at 2400 lux and 3000 lux.

(c) Culture of Gigartina fronds under various daylength regimes

The various daylength regimes tested were light/dark times of $8:\overline{16}$, $12:\overline{12}$ and $16:\overline{8}$. These daylength regimes were controlled by an automatic time switch. Under each of these daylength regimes fronds of Gigartina were cultured at 12°C, 2400 lux, a salinity of 32‰ and fully submerged in the enriched seawater medium.

Results are illustrated in Figure 21 and summarised in Table 3. Details of the results are as follows:

1. The maximum growth rate was obtained in daylength of $16:\overline{8}$ and the minimum growth rate was obtained in the shortest daylength regimes.
2. At the shortest daylength regime ($8:\overline{16}$) the growth was much slower for the first 5 weeks and then growth showed a rapid increase, and by the end of the period of the experiment growth under this short day reached the same level as in the daylength of 12 hours. In fact as shown in Figure 21, there were no significant differences in the growth rates under daylength of $12:\overline{12}$ and $8:\overline{16}$.
3. Under all different daylength regimes, good growth of Gigartina was obtained. Thus light duration does not seem to be a crucial factor controlling the growth of Gigartina.

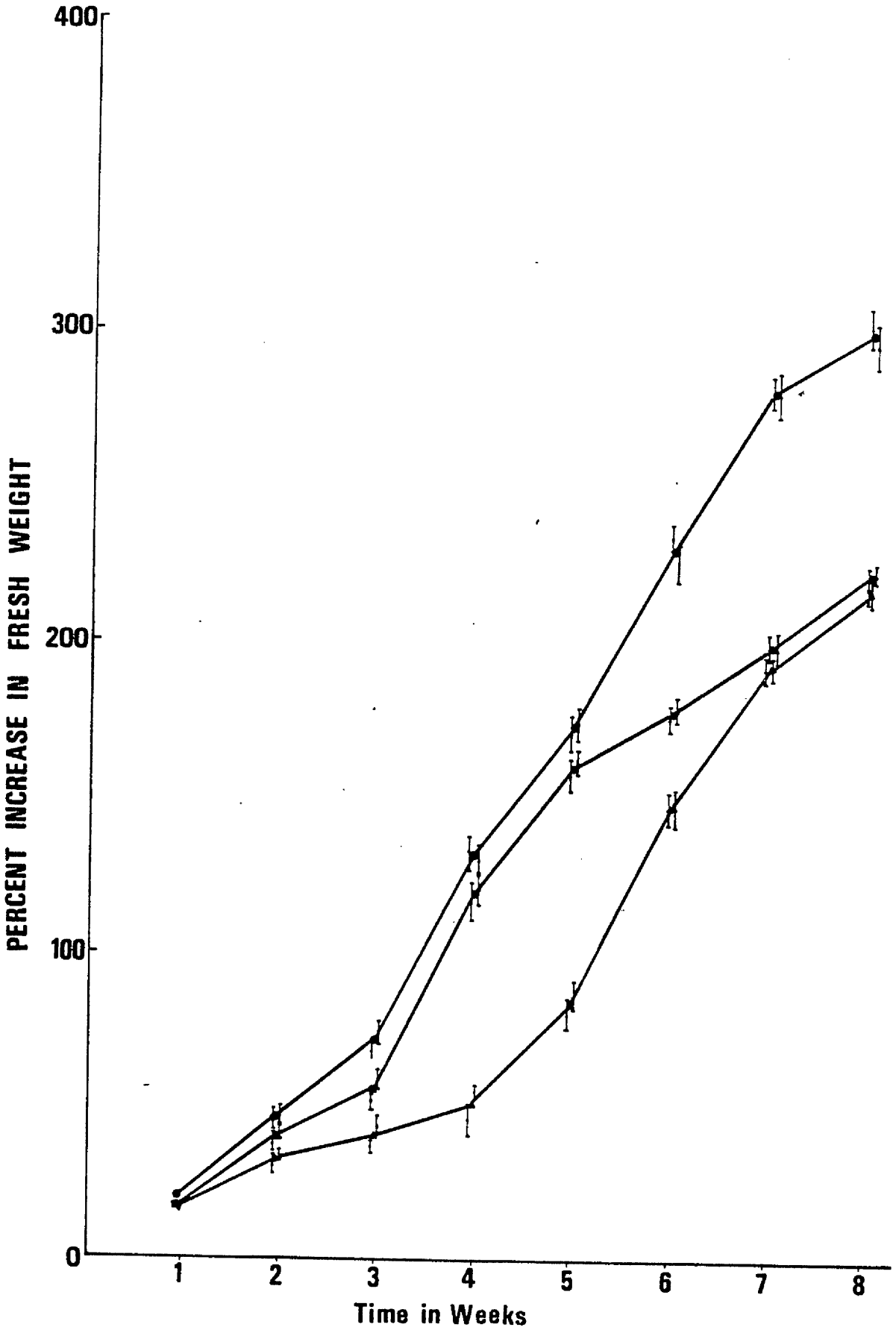
Table 3: Effect of various daylength regimes on the growth of fronds of Gigartina stellata collected from Davy's Rock, Cumbrae Island and grown fully submerged in Boney's medium at 12°C, 2400 lux and 32 ‰ salinity.

Photoperiod L/D \ Time in weeks	Initial weight in grams	Time in weeks							
		1	2	3	4	5	6	7	8
8/$\overline{16}$	0.25 – 1.2	* 0.29 ** 18% + 2.0	0.33 34% 2.6	0.35 42% 3.0	0.37 51% 4.2	0.46 85% 5.0	0.61 147% 6.5	0.73 193% 4.0	0.78 215% 4.5
12/$\overline{12}$	0.46 – 1.3	0.54 19% 1.8	0.64 41% 3.0	0.71 56% 3.5	1.00 120% 5.0	1.18 160% 7.5	1.26 178% 4.0	1.37 200% 5.5	1.47 222% 4.0
16/$\overline{8}$	0.30 – 0.7	0.36 22% 1.2	0.43 47% 3.5	0.50 72% 3.0	0.68 132% 5.5	0.79 172% 6.5	0.96 230% 6.0	1.11 280% 6.5	1.16 300% 7.0

* = Mean value of increase in fresh weight of 20 fronds in grams
** = % increase in fresh weight
\+ = Standard deviation

Figure 21: Shows growth rates of Gigartina fronds cultured under various daylength regimes.
Bars represent the S.D. of the duplicate experiments.

- ▼ under Light/dark; 8:$\overline{16}$
- ■ under Light/dark; 12:$\overline{12}$
- ● under Light/dark; 16: $\overline{8}$


400
300
200
100
0
PERCENT INCREASE IN FRESH WEIGHT
1
2
3
4
5
6
7
8
Time in Weeks

Maximum growth of fronds of Gigartina is clearly obtained in the long day regime. This equates with the observed patterns of growth in nature, in that the maximum growth is observed in spring and summer months where the day is longer. Similar field observations were made by Burns (1971) and Pybus (1977) for Gigartina stellata. Over 4-5 weeks the 12:$\overline{12}$ regime is similar but then seem to fall off. Under the shortest day regime growth is slower over 5 weeks then starts to catch up. Is this a mobilization of reserves?

(d) Culture of Gigartina frond under various periods of submergence in seawater.

The various periods of submergence tested were: 4 hours per day, 8 h/d, 12 h/d, 18 h/d and fully submerged. These periods of submergence were obtained by the tide machine as described in Chapter 2, on page (30).

Under each of these periods, fronds of Gigartina were cultured at 12°C, an illumination of 2400 lux, a salinity of 32‰ and, when fully submerged, in the enriched seawater medium.

Results of this experiment are shown in Table 4 and Figure 22. As is clear from these results good growth of Gigartina fronds was obtained in all periods of submergence. However, there was a gradual increase in growth rate from 4 h/d to full submergence. Although there was no significant differences in growth rate under 18 h/d and full submergence, the best growth was observed at 18 h/d. Generally, these results agreed with field observation in many ways:

1. The top shore plants of Gigartina in all localities are smaller in size than plants growing on the lower shore.
2. In the exposed localities (Portencross and Cumbrae Island) Gigartina are much larger in size than plants growing in the sheltered locality (Loch Long).

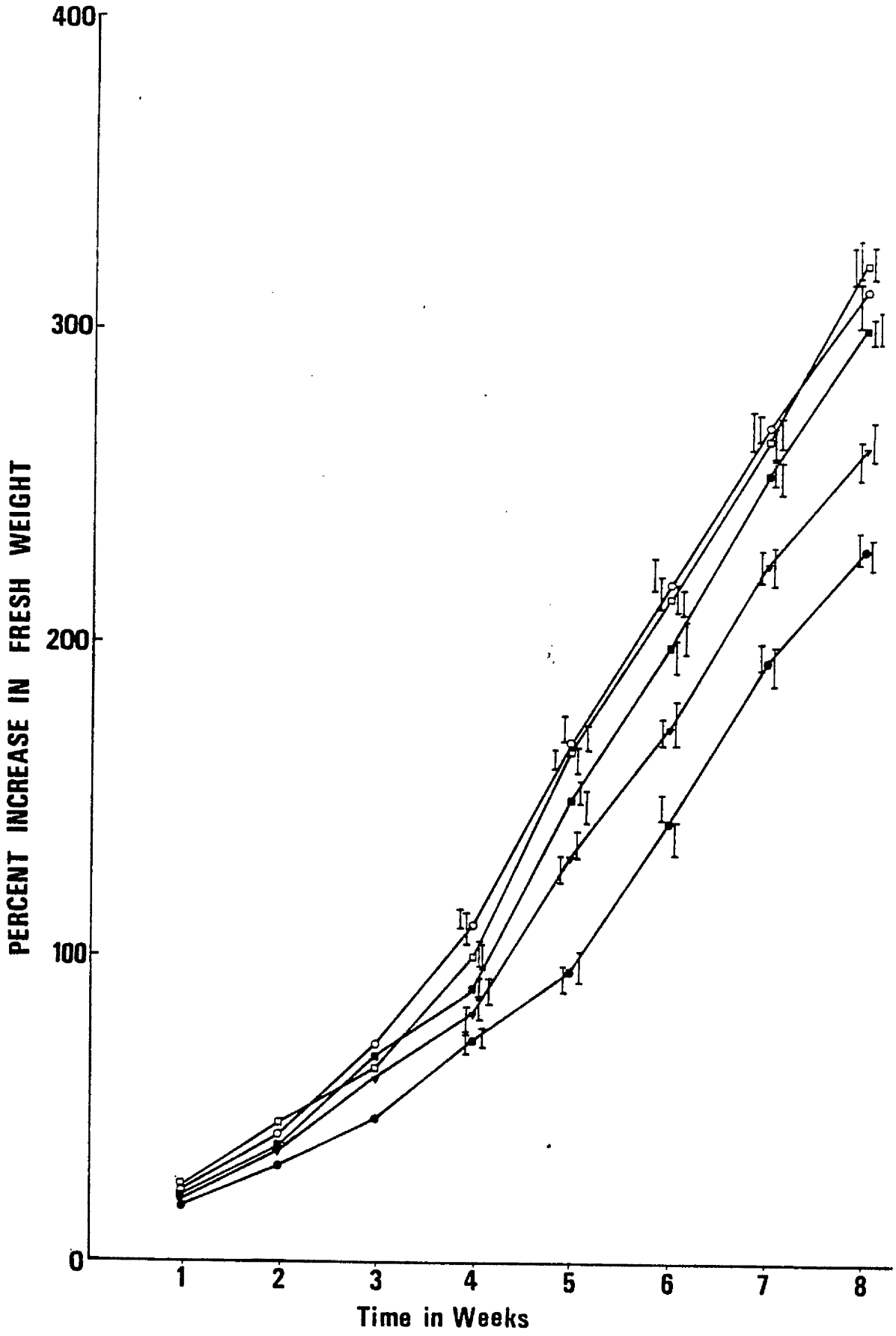
Table 4: Effect of various periods of submergence in seawater on the growth of fronds of Gigartina stellata collected from Davy's Rock, Cumbrae Island and grown in Boney's medium at 12°C, 2400 lux, daylength of 12 hours and in 32‰ salinity.

Time in weeks / Submergence h/d	Initial weight in grams	Time in weeks							
		1	2	3	4	5	6	7	8
4	0.32 - 1.5	* 0.38 ** 20% + 2.0	0.43 33% 1.8	0.47 48% 1.6	0.55 73% 3.5	0.63 95% 4.5	0.78 142% 5.0	0.95 195% 5.0	1.06 230% 4.5
8	0.24 - 1.7	0.29 22% 2.3	0.33 37% 1.8	0.38 60% 1.5	0.44 81% 4.0	0.56 132% 4.0	0.66 173% 5.5	0.78 225% 5.5	0.87 262% 6.0
12	0.19 - 1.0	0.21 24% 1.4	0.24 40% 1.2	0.27 58% 2.1	0.32 90% 4.0	0.42 150% 4.5	0.51 200% 5.5	0.60 257% 5.0	0.68 300% 4.5
18	0.20 - 1.3	0.26 25% 2.0	0.30 43% 2.2	0.36 72% 1.6	0.44 110% 4.5	0.56 170% 4.0	0.66 220% 4.0	0.76 270% 5.0	0.85 312% 6.5
24	0.30 - 0.8	0.38 27% 1.5	0.45 47% 1.8	0.51 65% 1.2	0.61 100% 4.0	0.81 165% 4.0	0.97 215% 5.5	1.12 265% 5.0	1.29 320% 6.0

* = Mean value of increase in fresh weight of 20 fronds in grams
** = % increase in fresh weight
\+ = Standard deviation

Figure 22: Shows growth rates of Gigartina fronds cultured under various periods of submergence in seawater.
Bars represent the S.D. of the duplicate experiments.

- ● submerged for 4 hours/day
- ▼ submerged for 8 hours/day
- ■ submerged for 12 hours/day
- ○ submerged for 18 hours/day
- □ fully submerged


400
300
200
100
0
PERCENT INCREASE IN FRESH WEIGHT
1
2
3
4
5
6
7
8
Time in Weeks

6.2 Culture of sporelings of Gigartina

According to Marshall et al (1949), Edelstein, Chen and McLachlan (1974) and Rueness (1978) only sexual plants of Gigartina stellata have been found in northern temperate waters and most of the records of the male plants are doubtful and because of the lack of a tetrasporic phase, reproduction was assumed to be only by means of carpospores. The aim of these culture experiments was to study the effects of laboratory induced environmental factors on growth of sporelings of Gigartina stellata. For this purpose spores of Gigartina stellata obtained from fertile papillae cut from mature plants freshly collected from the shore, were cultured on glass slides. Details of culture methods are described in Chapter 2.

Glass slides bearing attached spores were placed in culture tanks containing 5 ℓ of enriched seawater medium. Sporelings were grown at 12°C, illumination of 2400 lux, daylength of 12 hours and at salinity of 32‰ . The medium was changed at first twice a week and later every week.

As shown in plate (13) sporelings of Gigartina grew healthily in the culture medium for a period of 5 months, at which time the mean diameter of sporelings reached 0.5 mm. The disc-like sporelings then became detached from the glass slides and lost into the surrounding medium. In no case did these disc-like sporelings give rise to erect branches. The sporelings obtained from carpospores of Gigartina stellata have been considered the tetrasporophyte. Thus when West (1972) cultured the tetraspores of Petrocelis fransiscana from California, he obtained fertile gametophytes which resembled Gigartina agardhii. West and Polanshek (1972) cultured

the carpospores of Gigartina papillata from California, they found some spores formed crustose plants which resembled Petrocelis anatomically. West, Polanshek and Guiry (1977) cultured the tetraspores of Petrocelis cruenta from Ireland, and obtained gametophytes. Carpospores from the gametophyte gave rise to Petrocelis-like crustose growths. They also stated that Gigartina stellata represents the naturally occurring gametophyte of Petrocelis cruenta in Ireland and possibly elsewhere. Marshall et al (1949) obtained only basal discs from the carpospores of Gigartina stellata and they were unsuccessful in associating a crustose stage with G. stellata. Rueness (1978) found that the carpospores of Gigartina stellata from the west coast of Norway germinated as crustose discs at 12°C, and 17°C, 200-300 lux and 1500 lux and under a photoperiod of L/D 16:8. Under these conditions the crustose discs increased in diameter through marginal growth, attaining diameters of about 25 mm. after 4 years. At the higher levels of temperature and light intensity, erect axes were formed and grew to reproductive maturity in about 12 months with only slight increase in the disc diameters after establishment of erect axes. Tveter and Mathieson (1976) observed a high frequency of sporeling coalescence when they cultured the sporelings of Gigartina stellata. They stated that the sporeling coalescence is associated with an earlier initiation and growth of erect fronds.

Sporelings of Gigartina were cultured on ground glass slides under the same culture conditions as previously described. Sporelings grew healthily and reached 0.5 mm after 5 months(plate 13)and then became detached from the glass slides with no sign of forming erect branches. The same results were obtained when sporelings of

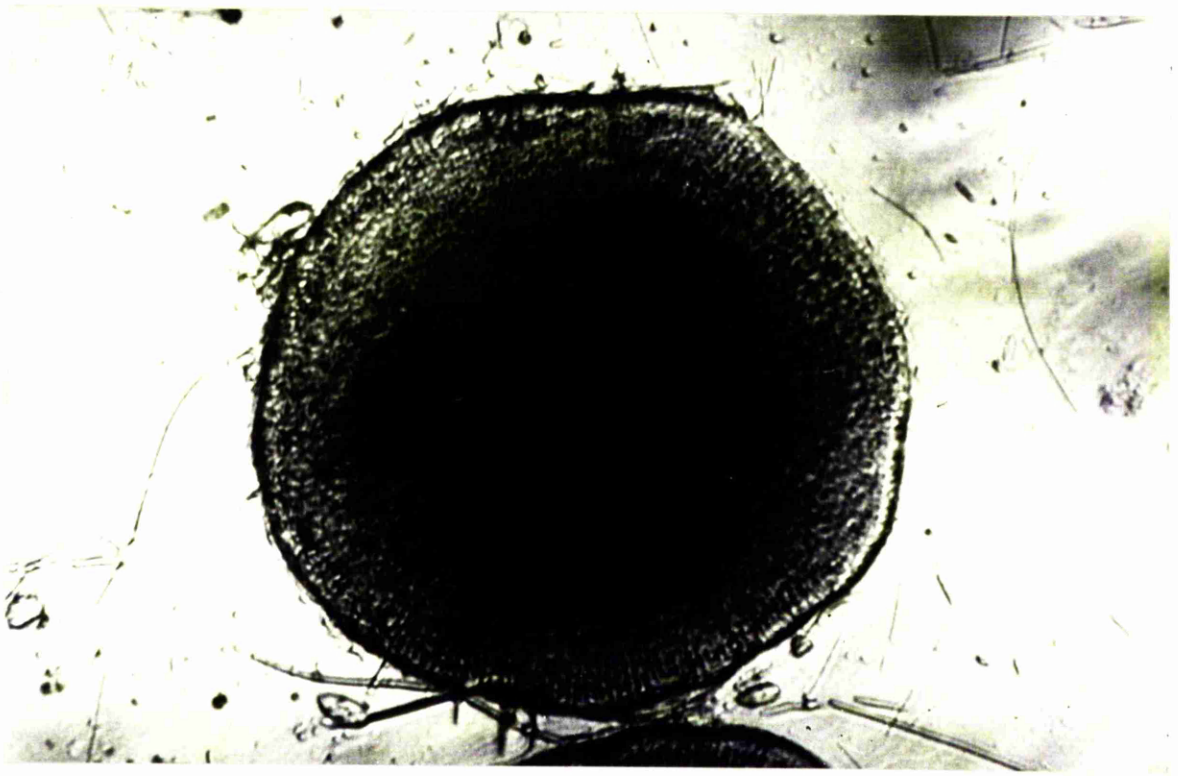


Plate 13: Showing the 5 months old disc-like sporelings of Gigartina stellata.
X 170.

Gigartina stellata collected from other localities in the Firth of Clyde (Loch Long and Portencross) were grown under the same culture conditions as described above.

Contamination with bacteria and diatoms is a common experience in all cultures, despite the careful cleaning of fertile fronds with camel-hair brushes prior to spore settlements and also using the cleaned, brushed papillae instead of the fertile fronds. An antibiotic mixture consisting of 200 µg/ℓ Streptomycin and 800 µg/ℓ Penicillin (1625 units/mg) was found to prevent the growth of bacteria. Also the growth of benthic diatoms was eliminated by using GeO_2 at concentration of 5 mg/ℓ of culture medium. Sporelings of Gigartina stellata were not affected by the use of the antibiotic mixture and GeO_2. Sporelings grew as healthily as the control, reaching 0.5 mm in diameter after 5 months.

Studies on the life history of the related species Chondrus crispus were established three-quarters of a century ago when Derbyshire (1902) described the germination of tetraspores and carpospores and the early stages of development of the basal disc. Since then an enormous amount of work has been published about Chondrus crispus and enough information is available on the life history of this alga. Therefore, the culture of sporelings of Chondrus is not only useful in comparing the results with those already obtained by Gigartina but also to check the culture technique.

Sporelings from carpospores of Chondrus crispus collected from Portencross, were cultured using the same culture method and under the same culture conditions as previously described. The sporelings were found to grow much faster than those of Gigartina stellata. After only 3.5 months the disc-like sporelings reached a mean diameter

of 0.8 mm and formed the first cylindrical erect branches (plate 14).

In the present experiments sporelings of Chondrus crispus were grown both in media with GeO_2 (2 mg/ℓ of culture medium) and in media without addition of the diatom inhibitor as controls. It was in these control cultures that contaminating benthic diatoms showed profuse growth on the slides except in the vicinity of the Chondrus sporelings, around each of which clear zones could be observed after 6 weeks. With older crusts (after 2.5 months) these clear zones were prominent features of the culture slides, (plate 15), sometimes forming an interlinked series of cleared areas when a number of juvenile plants were growing in close proximity. The inhibition zone would seem to be a complete barrier against invasion by the diatoms. Crustose sporelings of the related red alga Gigartina stellata of similar age and size and grown under identical conditions did not show this clear radial zone in the presence of an accompanying population of benthic diatoms.

The antimicrobial effects of marine algae have been reviewed by Sieburth (1964) and Burkholder and Sharma (1969). The concept of a "chemical claws" mechanism of interspecific competition between macroscopic marine algae was proposed by Walker and Smith (1948) in explanation of the antibiotic effects of exudates of Ascophyllum nodosum on zoospores of Laminaria hyperborea, and the dominance of Ascophyllum nodosum in mid-shore regions of the fucoid covered shores. The active libration of polyphenols (Tannins) from certain marine algae has been shown to induce severe antibiotic effects on bacteria and algae (Lewin, 1962; Conover and Sieburth, 1966; McLachlan and Craigie, 1964, 1966). Antibacterial activity, with some seasonal variation, has been reported for 34 species of red algae (including

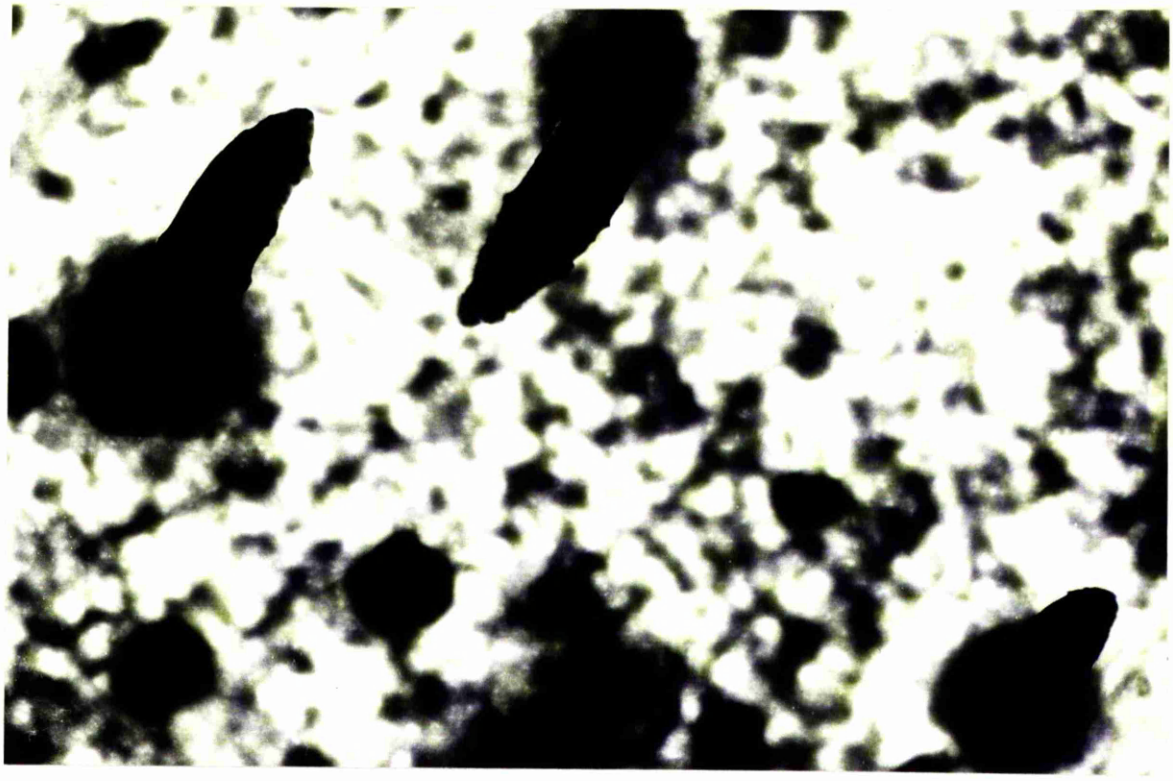


Plate 14: Showing the 3.5 months old sporelings of Chondrus crispus, with the cylindrical erect branches.

X25

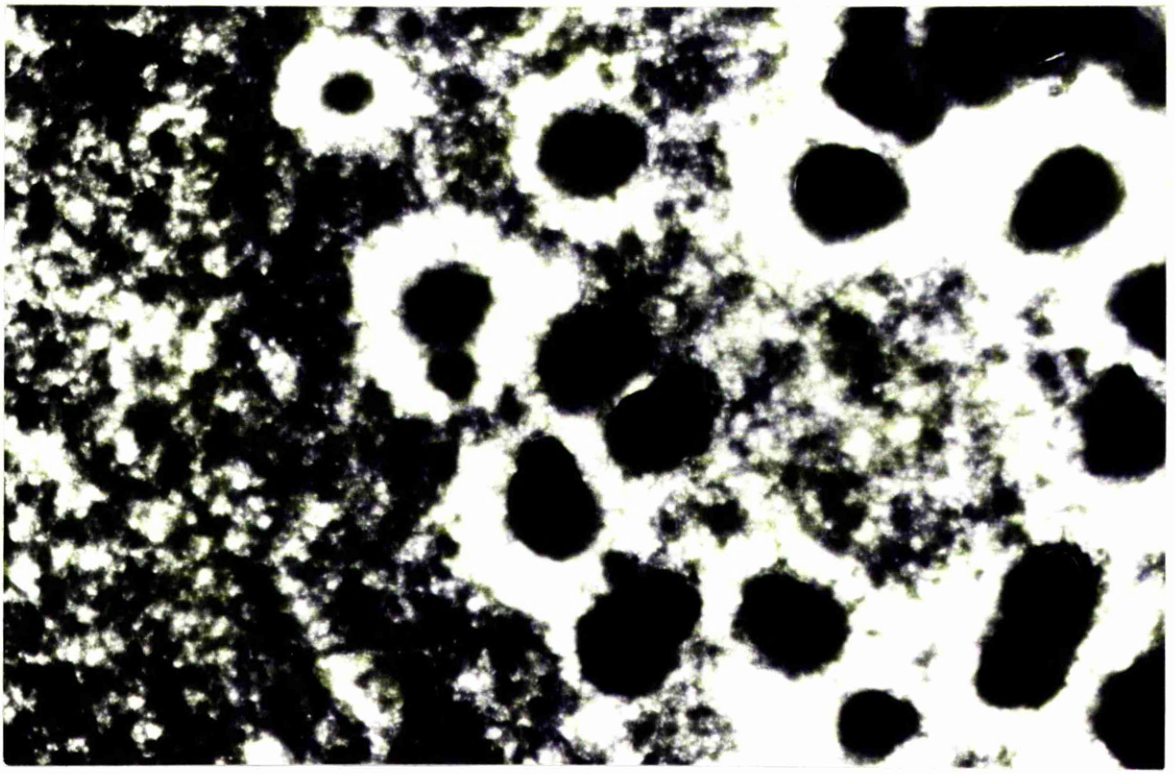


Plate 15: Showing sporeling of Chondrus crispus with the clear zones around each sporeling.
X25

Chondrus crispus), five species of green algae and 15 species of brown algae (Hornsey and Hide, 1974). With Chondrus a winter peak of antibacterial activity was observed (Hornsey and Hide, 1976a). Variations were also observed in activities with different regions of thalli, and maximum antibacterial activity was obtained from the meristematic apices of Chondrus (Hornsey and Hide 1976b). Fletcher (1975) observed that growth of the crustose red algae Porphyrodiscus simulans Batt. and Rhondophysema elegans (Crovan frat. ex J. Ag.) Dixon in culture was inhibited by ectocrines of Ralfsia spongiocarpa Batt., a species known to produce tannins in considerable quantities in tide pools (Conover and Sieburth 1966). Hornsey and Hide (1974) reported that thalli of Gigartina stellata did not show antibacterial activity in contrast to the marked activity of Chondrus crispus. With Chondrus this antibacterial activity was greater in meristematic region of the thalli. The crustose germlings also show that this antibacterial activity of the meristematic areas of Chondrus occurs at all stages in the plant's life. By contrast, Gigartina sporelings failed to show this antibiotic activity.

THE EFFECTS OF LABORATORY INDUCED ENVIRONMENTAL FACTORS ON GROWTH OF SPORELINGS OF GIGARTINA STELLATA.

(a) Effect of salinities on growth of sporelings.

Sporelings of Gigartina stellata collected from Cumbrae Island were cultured under various salinities (4 ‰ , 8 ‰ , 16 ‰ , 24 ‰ , and 32 ‰) at 12°C, 2400 lux, a daylength of 12 hours and sporelings were grown in the enriched medium.

Results are summarised in Table 5 and Figure 23. Details of the results are as follows:

1. At salinity of 4 ‰ , the growth of sporelings (expressed as increase in diameter) was very slow. The sporelings became very pale in colour and many became detached and lost into the surrounding medium after the first few weeks.

2. Growth of sporelings was also very slow in media of salinity of 8 ‰ . Although many sporelings became pale in colour and detached from the slides, a large number continued to grow for the whole period of the experiment.

3. There was a good healthy growth of sporelings in media of salinity of 16 ‰ , but with a slightly slower growth rate than at 24 ‰ and 32 ‰ .

4. Results showed that there were no significant differences in the growth rate of sporelings at salinities of 24 ‰ and 32 ‰ .

These results agreed with the results obtained by Burns (1971) who found that sporelings of Gigartina stellata exhibited maximum growth in salinities of 25, 30 and 35 ‰ , above and below these levels growth was substantially reduced.

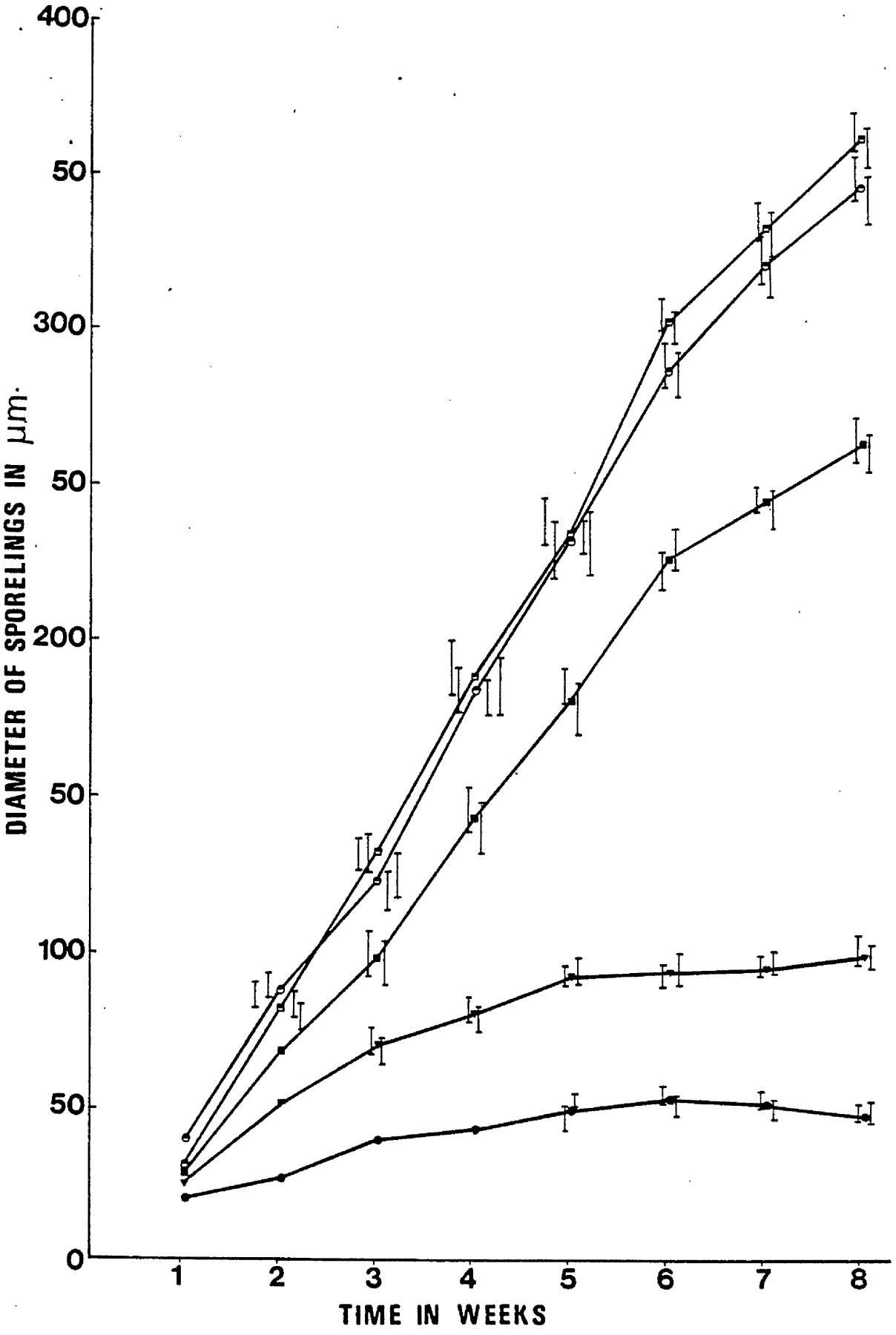
Table 5: Effect of various salinities on the growth of sporelings of Gigartina stellata collected from Davy's Rock, Cumbrae Island, grown fully submerged in Boney's medium at 12°C, 2400 lux and daylength of 12 hours.

Salinity ‰ \ Time in weeks	1	2	3	4	5	6	7	8
4	* 19.4 + 2.3	25.6 2.3	38.7 2.7	42.4 4.5	48.0 5.3	51.2 6.8	50.3 5.7	46.0 7.0
8	26.0 2.6	51.7 6.2	68.2 4.4	79.8 4.7	90.3 7.0	92.9 5.6	93.0 9.8	97.8 9.8
16	27.4 3.7	67.0 6.7	96.6 9.4	140.0 12.7	178.0 12.4	221.2 10.2	243.0 3.7	261.0 7.8
24	30.2 2.3	81.0 6.8	130.8 10.3	187.6 11.8	233.2 9.2	300.0 14.4	330.0 12.7	358.0 13.6
32	39.3 7.9	86.0 8.3	120.6 10.0	181.8 10.7	229.6 12.7	284.4 12.4	318.8 12.0	342.4 9.5

* = The mean value of increase in diameter of 50 sporelings in µm.
\+ = Standard deviation

Figure 23: Shows growth rates of Gigartina sporelings cultured at various salinities.
Bars represent the S.D. of the duplicate experiments.

- ● at 4 %
- ▼ at 8 %
- ■ at 16 %
- ▣ at 24 %
- ⊖ at 32 %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TIME IN WEEKS

(b) Effect of illuminations on growth of sporelings.

Sporelings of Gigartina stellata collected from the Isle of Cumbrae were cultured under various levels of illumination (400 lux, 600 lux, 800 lux, 1200 lux, 2400 lux and 3000 lux). Details about illumination is described in Chapter 2, page (27). Under each of these illuminations sporelings were cultured at 12°C, a daylength of 12 hours and in the enriched seawater medium at salinity of 32‰ . Previous studies have indicated that sporelings of littoral algae have higher light requirements than sublittoral plants (Boney and Corner 1962, 1963).

Results of this experiment are summarised in Table (6) and Figure (24).

Although good growth of sporelings was obtained in all illumination levels, there was a gradual increase in growth rates from illumination of 800 lux to 3000 lux. At illuminations of 400 lux and 600 lux very slow growth of sporelings was obtained and many sporelings became pale in colour, and detached from the slides. Good healthy growth of sporelings was obtained at illumination of 1200 lux. However, growth was slightly slower than at 2400 lux and 3000 lux. Results also show that there were no significant differences in growth rates at illuminations of 2400 lux and 3000 lux. Burns (1971) observed a gradual increase in growth of sporelings of Gigartina stellata from 1300 lux to 7700 lux.

(c) Effect of daylength regimes on growth of sporelings.

Sporelings of Gigartina stellata were cultured under three daylength regimes; light/dark times; 8:$\overline{16}$, 12:$\overline{12}$ and 16:$\overline{8}$ at 12°C, 2400 lux and in the enriched seawater medium at salinity of 32‰ .

Table 6: Effect of various illuminations on the growth of sporelings of Gigartina stellata collected from Davy's Rock, Cumbrae Island, grow fully submerged in Boney's medium at 12°C, daylength of 12 hours and in 32 ‰ salinity.

Time in weeks / illumination lux	1	2	3	4	5	6	7	8
400	* 17.2 + 2.0	28.0 2.6	35.0 3.8	50.0 4.6	58.0 3.0	65.0 5.3	76.0 7.6	85.0 8.3
600	18.6 2.8	35.0 3.1	45.0 3.5	60.0 5.3	75.0 6.6	86.0 9.0	90.0 6.6	99.0 8.3
800	25.8 2.6	40.0 3.5	60.0 2.8	85.0 4.4	100.0 4.3	125.0 6.5	140.0 6.7	155.0 9.8
1200	30.0 2.0	45.0 3.5	80.0 7.6	120.0 9.0	175.0 10.4	200.0 11.2	240.0 9.0	265.0 13.0
2400	34.9 3.0	75.0 5.2	110.0 4.7	168.0 4.8	225.0 7.9	270.0 10.6	300.0 16.9	355.0 13.0
3000	40.0 2.8	77.0 6.9	115.0 5.6	175.0 9.1	235.0 12.0	280.0 8.4	308.0 12.8	368.0 19.7

* = The mean value of increase in diameter of 50 sporelings in µm.
\+ = Standard deviation

Figure 24: Shows growth rates of Gigartina sporelings cultured under various illuminations. Bars represent the S.D. of the duplicate experiments.

● under 400 Lux
▼ " 600 "
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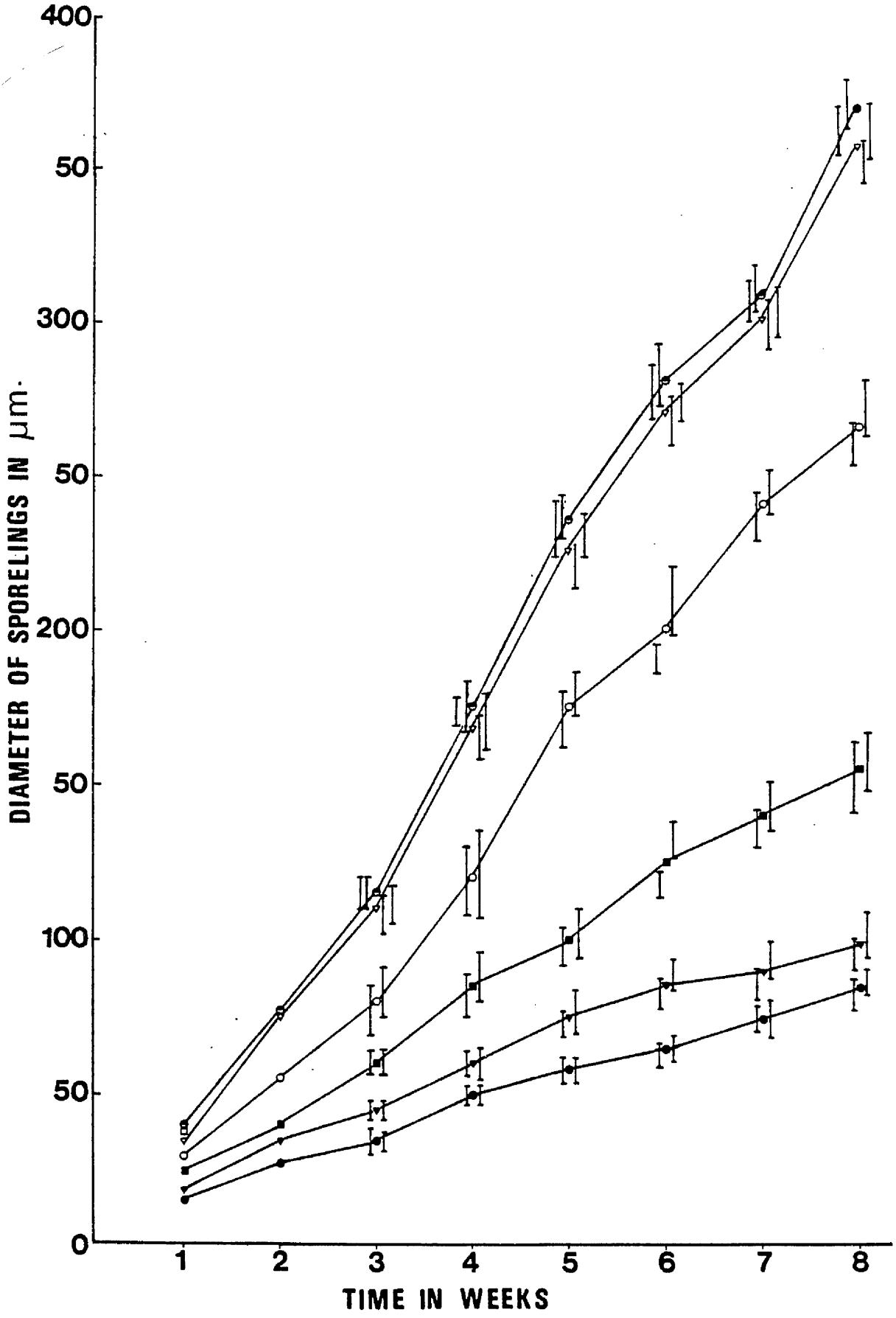
○ " 1200 "
▽ " 2400 "
◓ " 3000 "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TIME IN WEEKS

Results of this experiment which are summarised in Table (7) and Figure (25) showed a good healthy growth of sporelings was obtained in all daylength regimes. Maximum growth rate was obtained in the longer day regime (16:$\overline{8}$) and minimum growth rate was observed in the shortest day regime (8:$\overline{16}$).

Light duration does not seem to be a crucial factor controlling the growth of sporeling of Gigartina stellata. Arasaki (1953) found that spores of some red algae germinated and developed more rapidly in the longer light duration.

(d) Effect of various periods of submergence in seawater on growth of sporelings.

The different periods of submergence in seawater were provided by the tide machine as described in Chapter 2, page (30). Sporelings of Gigartina stellata were cultured under 4 hours/day, 8 h/d, 12h/d, 18 h/d and fully submerged in the enriched seawater medium at 12^{o}C, 2400 lux, daylength of 12 hours and salinity of 32‰ .

Results are summarised in Table (8) and illustrated in Figure (26). As shown from these data, sporelings of Gigartina grew healthily under all periods of submergence in seawater medium. There was a positive correlation between growth rate of sporelings and the periods of submergence in seawater medium, i.e. better growth was obtained in the longer submergence period and vice versa. The results also showed that there were no significant differences in growth rates of sporelings under periods of 12 hours/day and above this period. Thus Gigartina sporelings showed a broad tolerance to the different periods of submergence in seawater medium which is reflected in the local abundance of the plant on lower shore rock faces.

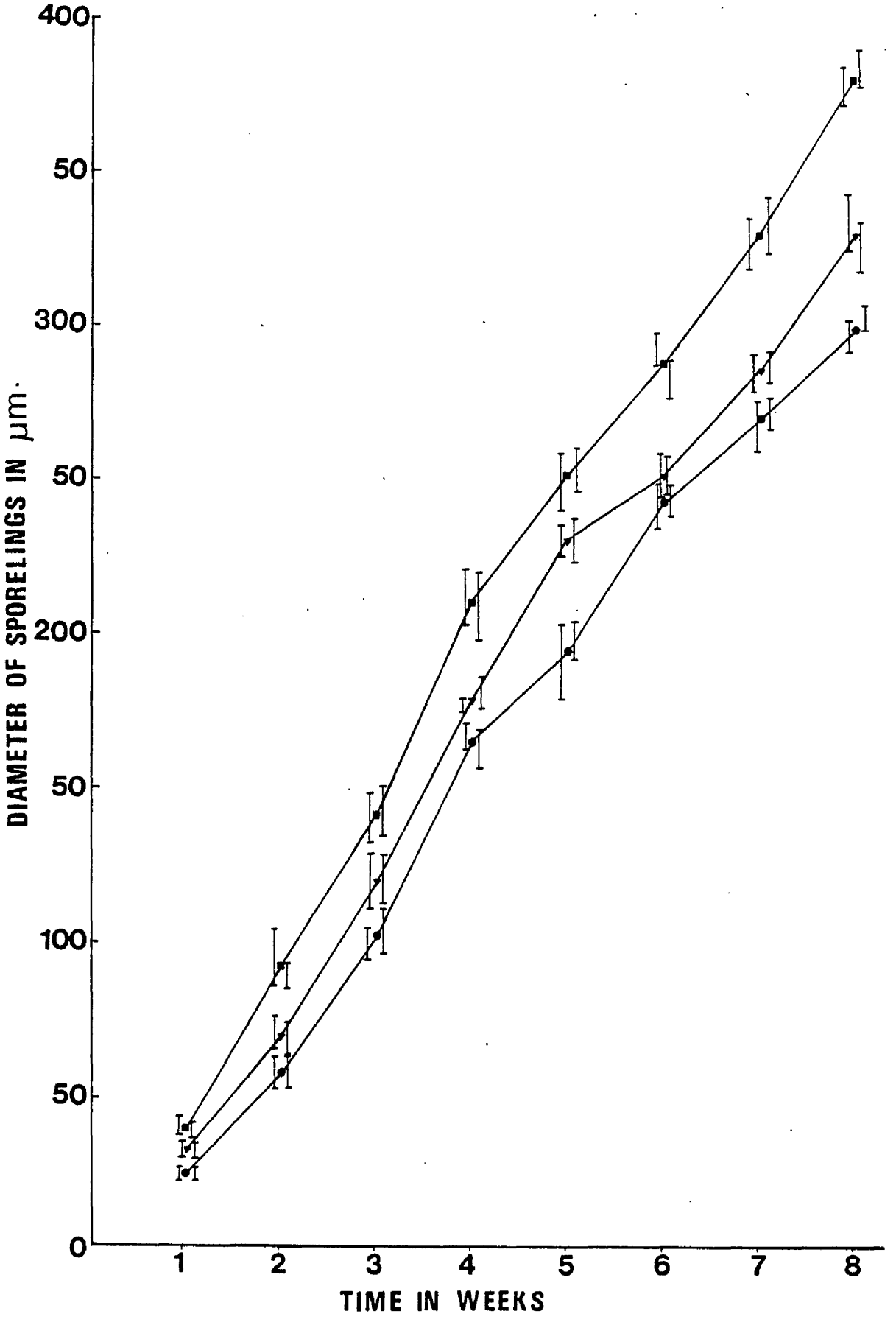
Table 7: Effect of various daylength regimes on the growth of sporelings of Gigartina stellata collected from Davy's Rock, Cumbrae Island and grown fully submerged in Boney's medium at 12°C, 2400 lux and 32 ‰ salinity.

Daylength L/D \ Time in weeks	1	2	3	4	5	6	7	8
$8/\overline{16}$	* 25.6 + 2.3	58.0 4.0	102.0 4.0	163.6 5.0	193.0 6.8	241.6 14.8	268.0 12.3	297.0 17.0
$12/\overline{12}$	32.5 2.3	70.0 4.3	118.5 7.6	177.0 11.6	227.8 10.2	249.8 9.6	283.8 14.4	328.0 13.4
$16/\overline{8}$	40.2 2.2	92.0 6.8	140.8 8.4	208.5 9.7	250.0 12.0	286.0 14.0	328.0 17.0	375.0 16.6

* = The mean value if increase in diameter of 50 sporelings in µm.
\+ = Standard deviation

Figure 25. Shows growth rates of Gigartina sporelings cultured under various daylength regimes. Bars represent the S.D. of the duplicate experiments.

● under $8:\overline{16}$
▼ " $12:\overline{12}$
■ " $16:\overline{8}$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TIME IN WEEKS

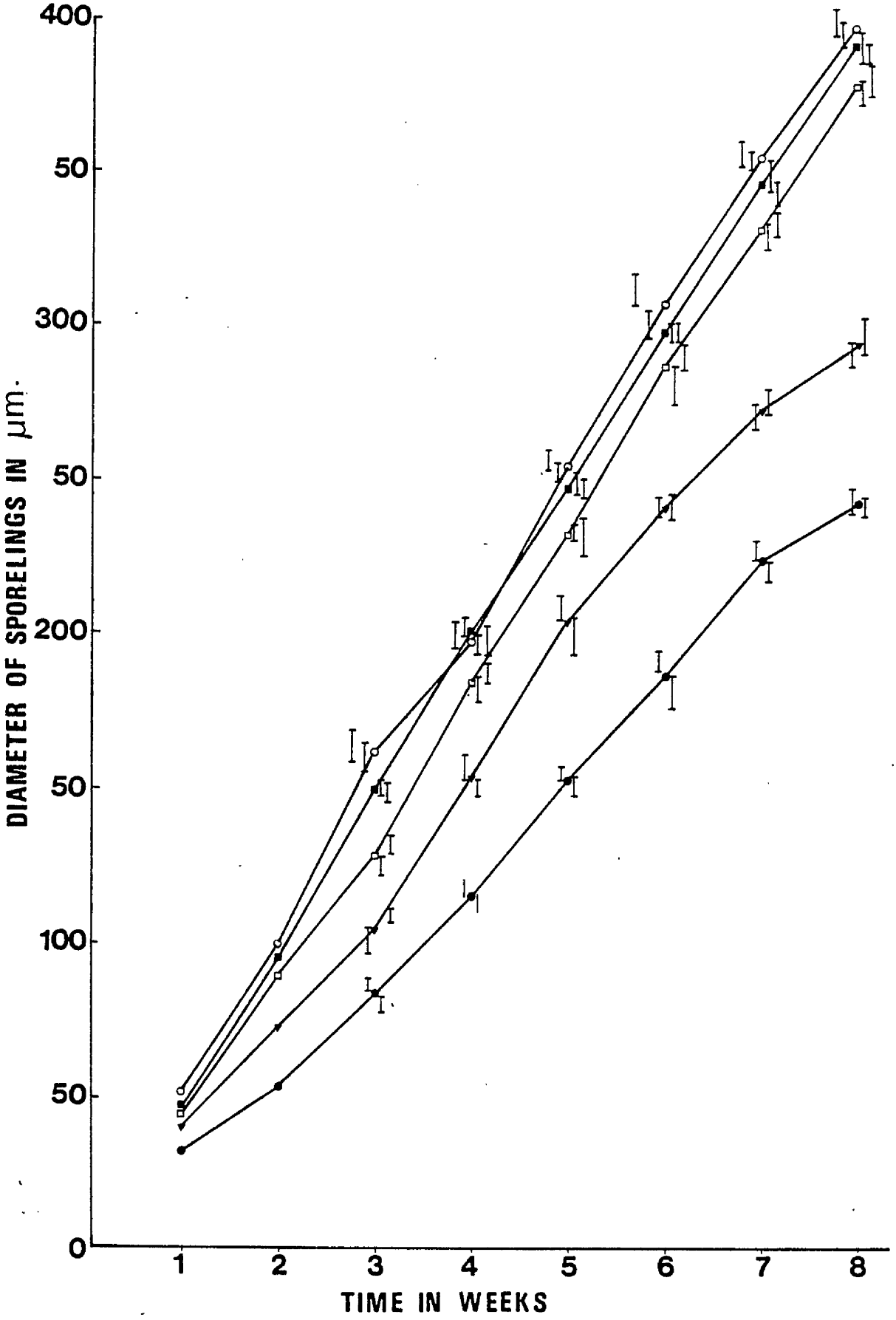
Table 8: Effect of various periods of submergence in seawater on the growth of sporelings of Gigartina stellata collected from Davy's Rock and grown in Boney's medium at 12°C, 2400 lux, daylength of 12 hours and 32‰ salinity.

Submergence h/d \ Time in weeks	1	2	3	4	5	6	7	8
4	* 33.0 + 7.6	53.0 4.4	83.7 5.0	114.8 10.6	152.0 11.3	185.0 9.5	225.0 15.5	243.0 11.6
8	40.6 2.7	74.0 4.2	103.7 9.2	154.0 9.9	203.8 15.0	240.0 14.6	271.6 13.7	293.0 22.5
12	45.0 4.8	89.5 9.5	128.0 10.5	184.0 11.0	231.0 9.5	285.0 10.8	330.0 10.0	376.0 11.0
18	47.5 5.4	95.0 10.4	150.0 9.9	200.0 11.0	246.0 11.0	295.0 13.3	345.0 12.7	390.0 9.9
24	51.6 5.3	100.0 10.7	162.0 8.9	197.0 8.9	253.0 9.6	306.0 12.9	353.0 15.5	396.0 13.0

* = The mean value of increase in diameter of 50 sporelings in µm.
+ = Standard deviation.

Figure 26: Shows growth rates of Gigartina sporelings cultured under various periods of submergence in seawater.
Bars represent the S.D. of the duplicate experiments.

● submerged for 4 hours/day
▼ " " 8 " "
□ " " 12 " "
■ " " 18 " "
○ fully submerged.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TIME IN WEEKS

CHAPTER 7

STUDIES ON CARRAGEENAN CONTENTS AND GEL STRENGTHS OF GIGARTINA IN NATURE AND UNDER EXPERIMENTAL CONDITIONS:

The red alga Gigartina stellata is one of the major sources of the economically important phycocolloid Carrageenan. During the last few decades many papers have been published which have clarified our knowledge of the structure and properties of Carrageenan, yet a lot to be known about its biology. Carrageenan has usually been fractioned into a KCl- insoluble fraction (k-Carrageenan) and KCl- soluble fraction (λ-Carrageenan). Black et al (1955) stated that Carrageenan from Gigartina stellata gave poor separation into k- and λ- fractions. Waaland (1975) and Pickmere et al (1975) found that the gametophytic stages of other Gigartina species have only k- Carrageenan while the tetrasporophytic stages have only λ- Carrageenan.

7.1 Seasonal observation on Carrageenan contents of Gigartina and gel strengths of Gigartina from different localities

The freshly collected plants of Gigartina from Loch Long, Cumbrae Island and Portencross were cleaned by removing all epiphytes and epizoa. Plants were then immersed very quickly in distilled water to remove excess of salt, and were then dried in an oven at 80-90°C until constant weight was obtained (over night). The quantity of Carrageenan in the dried seaweed and the gel strength of Carrageenan were measured from monthly collections of plants from the three localities as described in Chapter 2, page (31).

This study was commenced in June 1976 and terminated in November 1977. The results of this study are shown in Tables 9a, b, c, 10 a, b, c, Figures 27 and 28. Details of the results are as follows:

Carrageenan:

(a) As shown from Tables 9a, b, c, and Figure 27 the highest value of Carrageenan was recorded in plants collected from Portencross and the lowest value of Carrageenan in plants collected from Loch Long. Plants collected from Cumbrae Island showed an intermediate value. This might well be related to the fact that plants from Portencross have much thicker fronds while the fronds of plants from Loch Long are much thinner, and plants from Cumbrae Island have an intermediate frond thickness. However, Fuller (1971) in his study on Chondrus crispus from New Hampshire, U.S.A., stated that the quantity of Carrageenan was usually greater in plants of coastal than of estuarine locations.

Table 9a: Monthly measurements of Carrageenan as % of dry weight of plants of Gigartina from Cumbrae Island.

Time in months / Carrageenan as % of dry Wt. of seaweed	1976							1977										
	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N
a.	39.5	40.5	33.5	31	30	31	29	32.5	40	44.8	50	44.5	39.5	35.5	31	27.5	27.5	28.5
b.	41.8	42	32.6	29	30	29	28	33	39.5	56	50.5	46.8	40.8	36	28.5	27	27	26
c.	37.5	36.9	37	32.8	34	33.5	28	36	44.5	51	57	41.5	42.2	40.5	36.5	35	31.5	34.5
Mean =	39.6	39.5	34.4	30.9	31.3	31.2	28.3	33.8	41.3	50.6	52.5	44.3	40.8	37.3	32	29.8	28.7	29.7
S.D. =	2.2	2.6	2.3	1.9	2.3	2.3	0.6	1.9	2.8	5.6	3.9	2.7	1.4	2.8	4.0	4.5	2.5	4.4

Table 9b: Monthly measurements of Carrageenan as % of dry weight of plants of Gigartina from Portencross.

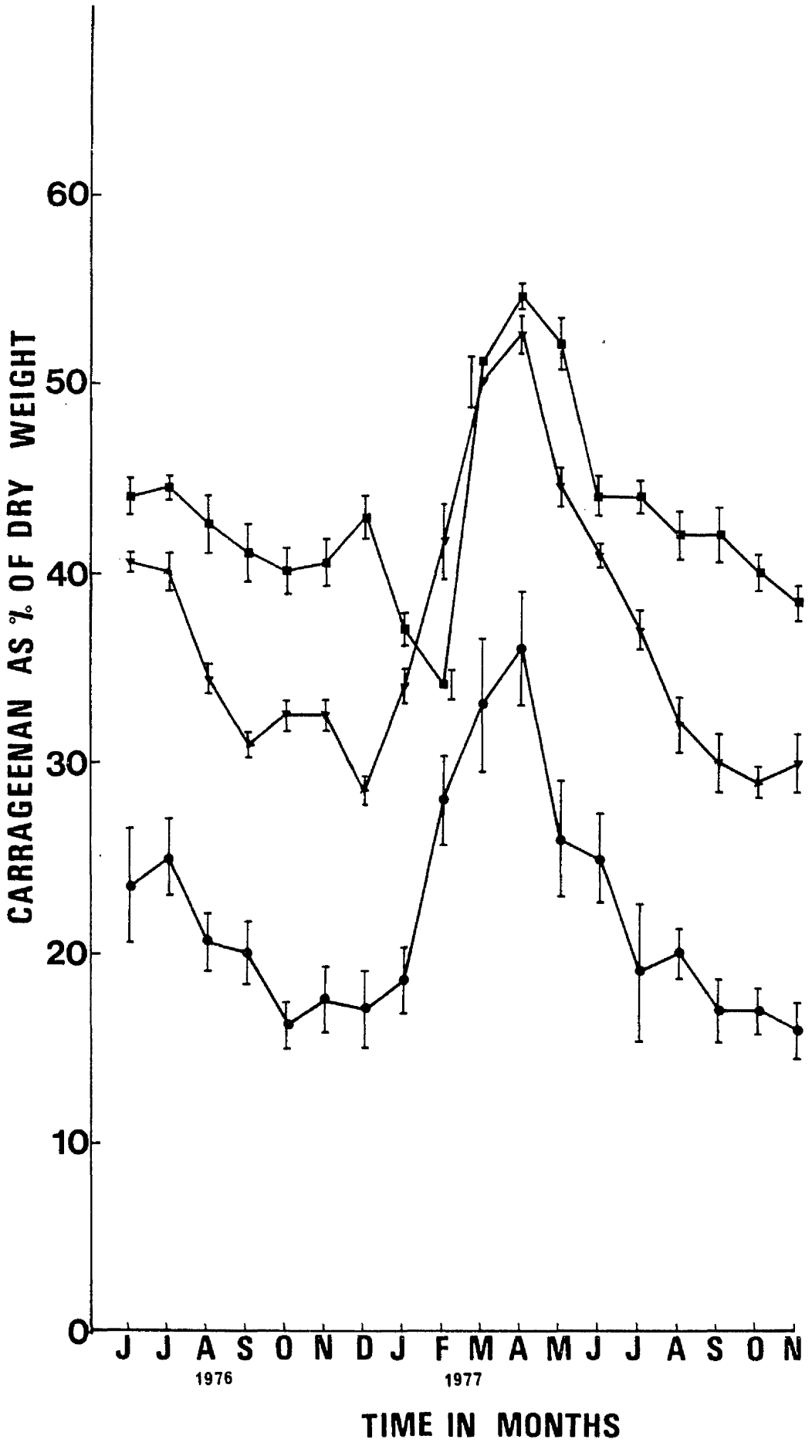
Carrageenan as % of dry wt. of seaweed \ Time in months	1976 J	J	A	S	O	N	D	1977 J	F	M	A	M	J	J	A	S	O	N
a.	43	44	40	40	43.5	39	43	38	32.5	50.5	53	51.5	46.5	45.5	39.5	41	39	41
b.	47	43.5	40	46	37	44.5	46	35	35.5	52.5	56.5	56.5	41	42	45.4	47	38	37.5
c.	42	46	47.5	37.5	38.5	37.5	40	38.5	34.5	50	53.5	49	42.5	42.5	40	39	42	37
Mean =	44	44.5	42.5	41.2	39.7	40.3	43	37.2	34.2	50.8	54.3	52.3	43.3	43.3	41.7	42.3	39.7	38.5
S.D. =	2.7	1.3	4.3	4.4	3.4	3.7	3.0	1.9	1.5	1.0	1.9	3.8	2.9	1.9	3.3	4.2	2.0	2.2

Table 9c: Monthly measurements of Carrageenan as % of dry weight of plants of Gigartina from Loch Long.

Time in months / Carrageenan as % of dry wt of seaweed	1976 J	J	A	S	O	N	D	1977 J	F	M	A	M	J	J	A	S	O	N
a.	21.5	23	19.5	18	18.5	17.5	14.5	15	19	25	30	31.5	23	23	21.5	18.5	16	19.5
b.	20	29	23.5	23	15	14.5	15	20.5	17.5	24	30	34	30	20	18	18	16	16
c.	28.5	23	18.5	18.5	15	20.5	21	16	25	35	39	41.5	23.5	32	17.5	23	19.5	15
Mean =	23.3	25	20.5	19.8	16.2	17.5	16.8	17.2	20.5	28.0	33.0	35.7	25.5	25.5	19.0	19.8	17.2	16.8
S.D. =	4.5	3.5	2.6	2.8	2.0	3.0	3.6	2.9	4.0	6.0	5.2	5.0	3.9	3.9	2.2	2.8	2.0	2.4

Figure 27: Showing the seasonal variation in Carrageenan contents of plants of Gigartina stellata from the three localities in the Firth of Clyde. Bars represent the S.D. in 3 replicates.

- ● plants from Loch Long
- ▼ plants from Cumbrae Island
- ■ plants from Portencross


CARRAGEENAN AS % OF DRY WEIGHT
60
50
40
30
20
10
0
J J A S O N D J F M A M J J A S O N
1976
1977
TIME IN MONTHS

(b) The results also show the seasonal variation in Carrageenan contents. The maximum yield of Carrageenan was recorded in the spring months, between March and May and the minimum yield was recorded in the autumn months between September and December. This was observed in two successive years with plants from the three localities. These observations agreed with the results of Butler (1936) working on Chondrus crispus who found that maximum Carrageenan concentration occurred during the warmer months. Pickmere et al (1975) studied the variation in Carrageenan levels and composition in three New Zealand species of Gigartina reported that no significant seasonal effects on Carrageenan levels were noted. They found a slight depression in the total Carrageenan content of all three species during the winter months. Marshall et al (1949) also found that the maximum yield of Carrageenan from Gigartina stellata was recorded in the spring months and the minimum yield was recorded in the winter months. The results of the present work showed a complete disagreement with the results of Fuller (1971) working on Chondrus crispus from New Hampshire, who found that Carrageenan concentration was highest in late fall-early winter while the lowest value was recorded during the spring-early summer.

Gel strength:

The gel strength of Carrageenan of Gigartina from the different localities was measured by the gelometer described in Chapter 2. Results of this study are summarised in Table 10a,b,c and Figure 28. Details are as follows:

(a) The strongest gel was recorded in plants from Loch Long.

The lowest Carrageenan gel strength was recorded in plants from

Table 10a: Monthly measurements of gel strength of Carrageenan of plants of Gigartina from Cumbrae Island

Time in months / Gel strength in g.	1976							1977										
	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N
a.	25	25	23	27	27	26	29.5	32.5	25	25	23.5	21	23	27	25	22	26	28
b.	26	25	24	27	27	26	29	32.5	26	25	23.5	22	23	29	25	23	26	28
c.	26	25	25	27	26	26.5	29	32	26	25	24	21	23.5	29	25	23	25	28
Mean =	25.7	25	24	27	26.7	26.2	29.2	32.3	25.7	25	23.7	21.3	23.2	28.3	25	22.7	25.7	28
S.D. =	0.6	0	1.0	0	0.6	0.3	0.3	0.3	0.6	0	0.3	0.6	0.3	1.2	0	0.6	0.6	0

Table 10b: Monthly measurements of gel strength of Carrageenan of plants of Gigartina from Portencross.

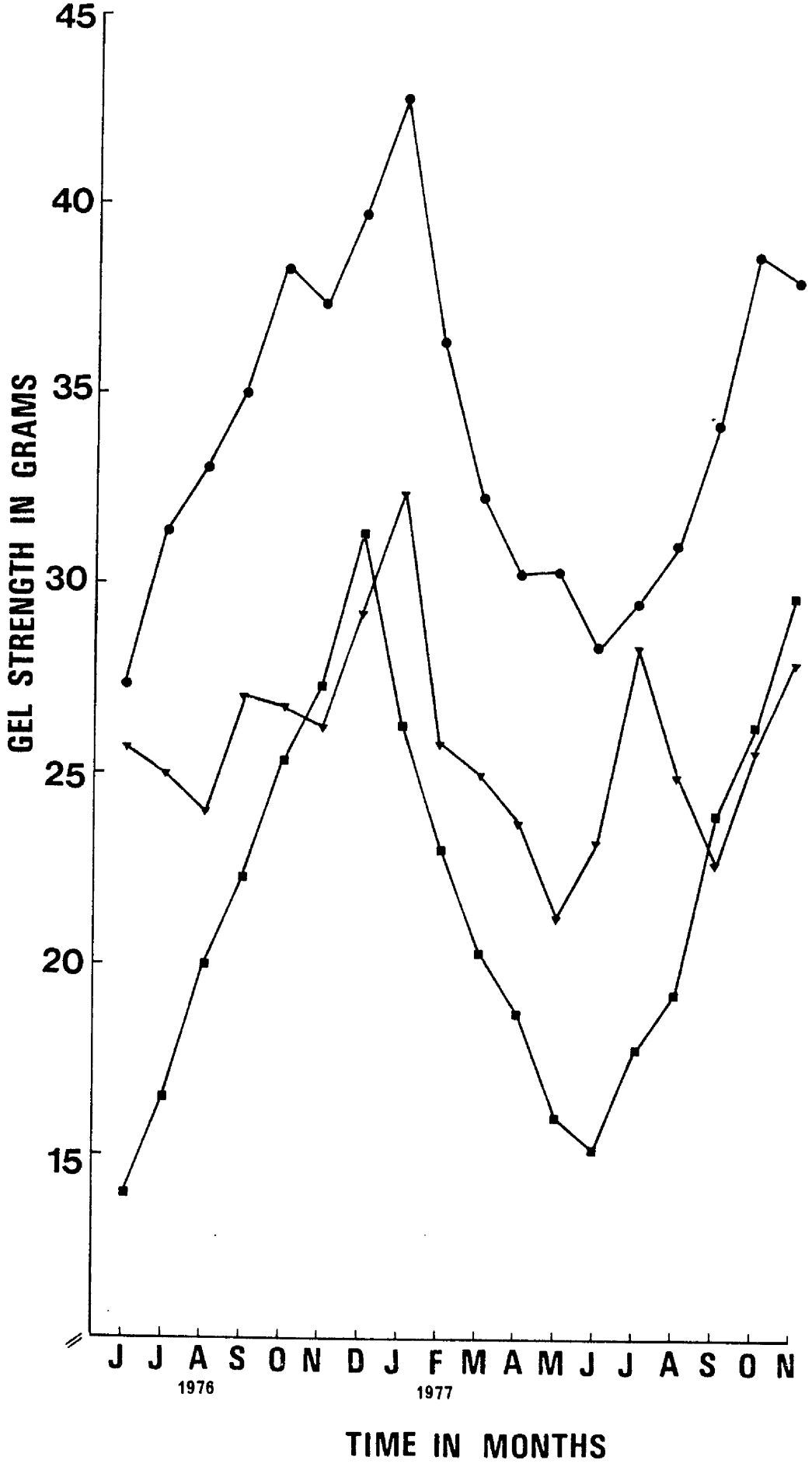
Time in months / Gel strength in g.	1976 J	J	A	S	O	N	D	1977 J	F	M	A	M	J	J	A	S	O	N
a.	14	16	20	23	25	27.5	31	26	23	20	19	16	15.5	17	19	24	26.5	29
b.	14	17	20	22	26	27.5	31	27	23	21	19	16	15	18	19	24	26.5	30
c.	14	16.5	20	22	25	27	32	26	23	20	18	16	15	18	20	24	26	30
Mean =	14	16.5	20	22.3	25.3	27.3	31.3	26.3	23	20.3	18.7	16	15.2	17.7	19.3	24	26.3	29.7
S.D. =	0	0.5	0	0.6	0.6	0.3	0.6	0.6	0	0.6	0.6	0	0.3	0.6	0.6	0	0.3	0.6

Table 10c: Monthly measurements of gel strength of Carrageenan of plants of Gigartina from Loch Long.

Gel strength in g. \ Time in months	1976							1977										
	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N
a.	27.5	31	33	36	38	38	39	42	37	32	30.5	30	28	29.5	31	34.5	39	38
b.	27.5	32	33	35	38	37	40	43	36	33	30	31	29	29.5	31	34	39	38
c.	27	31	33	34	39	37	40	43.5	36	32	30	30	28	29.5	31	34	38	38
Mean =	27.3	31.3	33	35	38.3	37.3	39.7	42.7	36.3	32.3	30.2	30.3	28.3	29.5	31	24.2	38.7	38
S.D. =	0.3	0.6	0	1.0	0.6	0.6	0.3	0.3	0.6	0.6	0.3	0.6	0.6	0	0	0.3	0.3	0

Figure 28: Showing the seasonal variation in gel strength of Carrageenan of Gigartina stellata from the three localities in the Firth of Clyde. S.D. is very small value and difficult to represent on the figure.

- ● plants from Loch Long
- ▼ plants from Cumbrae Island
- ■ plants from Portencross


45
40
35
30
25
20
15
GEL STRENGTH IN GRAMS
J J A S O N D J F M A M J J A S O N
1976
1977
TIME IN MONTHS

Portencross and again plants from Cumbrae Island had an intermediate value. As previously explained, plants of Gigartina at Loch Long may be covered with water of low salinity at low tide. Marshall et al (1949) found that preliminary washing of the seaweed up to 12 hours in running water increased the hardness of the gel. This will be discussed separately later on.

(b) The gel strength of Carrageenan of Gigartina from all localities showed a seasonal variation with the strongest gels obtained in winter months between December and March, whilst the weakest gel was obtained in spring and summer, between March and August. Fuller (1971) found that the strongest gel of Chondrus crispus was recorded in late fall-early winter. Marshall et al (1949) reported that the highest value of gel strength was recorded in autumn and winter. Both these studies used different methods of measuring gel strength.

The various replicates of gel strength measurement showed no significant differences between them. The standard deviation was a very small value and in all cases difficult to represent on the figure.

7.2 Carrageenan and gel strength measurements of Gigartina after being transplanted throughout the different localities in the Firth of Clyde area:

As shown above, the different populations of Gigartina showed significant differences in the quantity and quality of Carrageenan. This following experiment was designed to see if these variations in Carrageenan quantity and quality are due to environmental conditions or to inherited characters. For this purpose, plants of Gigartina were transplanted throughout the three localities in the Firth of Clyde, i.e. Loch Long, Cumbrae Island and Portencross in reciprocal fashion. Details of transplant methods are described in Chapter 2, page (24). Large quantities of plants of Gigartina were used to avoid the loss of plants in heavy seas. The period of transplant was three months, although it would have been better to transplant the plants for more than 3 months. This was impossible however, because plants transplanted from Loch Long to Portencross disappear very quickly.

This experiment was initiated in February 1978 and terminated in May 1978.

Table 11 summarises the Carrageenan content in the transplanted Gigartina and Table 12 summarises the gel strength measurements in the transplanted Gigartina; as shown from these data:

(a) Carrageenan content in plants from Loch Long increased when plants were transplanted to Cumbrae Island, but it did not reach the same seasonal level as the undisturbed plants of Cumbrae Island and Portencross.

Table 11: Shows Carrageenan as % of dry weight of Gigartina after being transplanted throughout the different localities in the Firth of Clyde.

Locality receiving transplant	Source of transplant		
	Loch Long	Cumbrae Island	Portencross
Loch Long	34.0% 29.0% 24.5%	30.6% 34.8% 33.0%	34.0% 39.6% 37.5%
Cumbrae Island	31.0% 37.5% 44.0%	48.0% 45.0% 42.0%	48.0% 45.0% 50.0%
Portencross	41.0% 31.5% 36.5%	47.0% 46.5% 44.0%	53.5% 55.0% 51.5%

Table 12: Shows gel strength of Carrageenan in plants of Gigartina after being transplanted throughout the different localities in the Firth of Clyde:

Localities receiving transplant	Source of transplant		
	Loch Long	Cumbrae Island	Portencross
Loch Long	29.0 g 31.5 g 30.0 g	25.0 g 25.5 g 26.0 g	22.0 g 22.0 g 24.0 g
Cumbrae Island	24.5 g 25.0 g 27.0 g	21.5 g 23.0 g 24.5 g	24.0 g 23.0 g 23.0 g
Portencross	26.5 g 27.5 g 25.0 g	22.5 g 21.5 g 23.0 g	20.0 g 19.0 g 20.0 g

(b) The value of Carrageenan content of plants from Portencross decreased when plants were transplanted to Loch Long and Cumbrae Island, and it was very similar to the undisturbed plants of Cumbrae Island and Loch Long at the same time of year.

(c) When plants from Cumbrae Island were transplanted to Portencross the Carrageenan increased.and reached a higher level than that of the undisturbed plants on Cumbrae Island. When Cumbrae Island plants transplanted to Loch Long the Carrageenan content decreased but was higher than in plants growing naturally in Loch Long.

Gel strength in the transplants as shown in Table (12) shows a slight increase in its value when plants from Portencross and Cumbrae Island were transplanted to Loch Long but it did not reach the same level as in the "native" plants of Loch Long. It showed a slight reduction in its value when plants were transplanted from Loch Long to Cumbrae Island and Portencross, but again it did not reach the same level as the "native" plants of Cumbrae Island and Portencross.

These results show very clearly the important role of environmental conditions in governing the quantity and quality of Carrageenan. A very sharp increase in gel strength was recorded when plants from Cumbrae Island and Portencross were left to grow at Loch Long for about 6 months. Experiments carried out over 3 months were not really long enough to significantly affect the gel strengths.

7.3 . Effect of environmental conditions on the quantity and quality of Carrageenan.

Results of the transplant experiments together with the seasonal variations in the quantity and quality of Carrageenan indicated the important role of environmental conditions on Carrageenan. This next study was made to throw some more light on the effect of laboratory induced environmental factors on the quantity and quality of Carrageenan.

About 100 g of cleaned healthy fronds of Gigartina collected from Cumbrae Island were kept for two weeks in the laboratory under a variety of environmental factors. Carrageen contents and gel strength of Carrageenan were measured as previous described. Three replicates were carried out under each of the following conditions:

(a) Salinity:

Five different salinities of seawater were tested; 4‰ , 8‰ , 16‰ , 24‰ and 32‰ . Under each of these salinities plants of Gigartina, obtained from Cumbrae Island, were kept fully submerged for a period of two weeks (May 14 - 27) at 12°C, 2400 lux and a daylength of 12 hours, then the carrageenan contents and the gel strengths were measured as previously described. Plants could not be kept for a longer period not only because the very low salinities would harm the plants but also for problems of time and space, however, clear differences in carrageenan contents and gel strengths were observed even after this short period.

Results of this experiment are summarised in Table (13) and illustrated in Figure 29(a) and (b).

Carrageenan contents:

Results showed that salinity is an important factor governing the carrageenan contents. There was a gradual increase in carrageenan content from salinities of 4‰ to 32‰ . At the lowest salinities (4‰ and 8‰) when plants became very pale in colour and many started to loose their rigidity, carrageenan was in its lowest level. Even at 24% when plants grew healthily, there was a big difference in carrageenan contents when compared with carrageenan measurement at salinity of 32‰ .

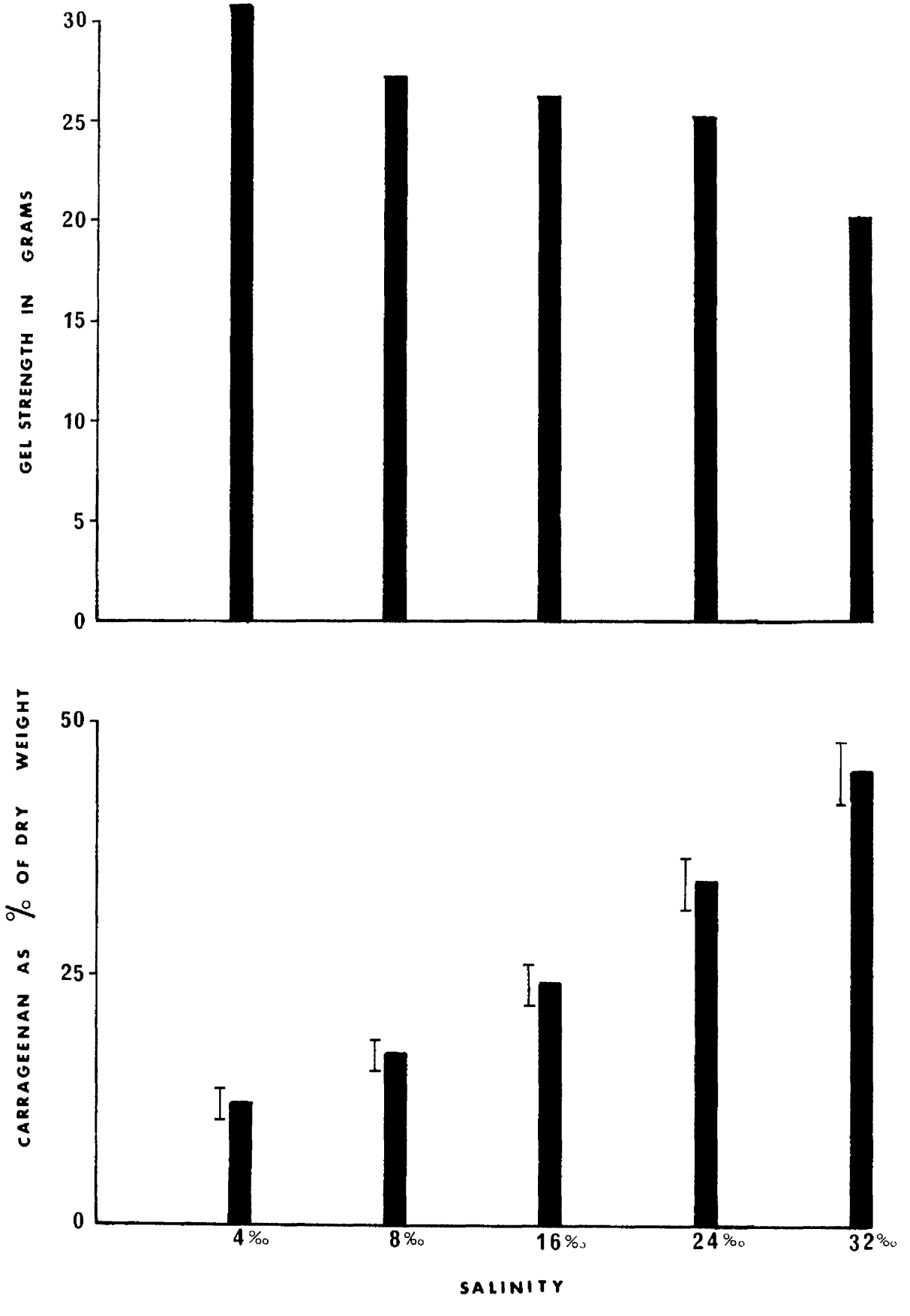
These results might explain the fact that plants of Loch Long, which grow in estuarine habitats and may be covered with water of low salinity for sometime during the tidal cycle, have lower carrageenan contents. Similar results were obtained by Fuller (1971) who reported that the percent of carrageenan was usually greater in plants of coastal than of estuarine locations.

Table (13): Effects of various salinities on carrageenan content and gel strength of plants of Gigartina kept for two weeks fully submerged in seawater of various salinities at 12°C, 2400 lux and daylength of 12 hours. Plants collected from Cumbrae Island in May 1977.

Properties measured \ Salinities	4‰	8‰	16‰	24‰	32‰
Carrageenan as % of dry weight	12.0 17.5 10.5	17.0 13.5 20.5	24.0 27.5 22.0	34.0 30.5 35.0	45.0 40.5 50.5
Gel strength in grams	30.5 31.0 30.0	27.0 29.0 25.5	26.0 23.5 28.0	25.0 23.5 26.0	20.0 23.0 18.5

Figure 29(a): Shows gel strengths of carrageenan in plants of Gigartina collected from Cumbrae Island and kept at various salinities.
S.D. is small value and difficult to represent on the figure.

Figure 29(b): Shows carrageenan contents in plants of Gigartina collected from Cumbrae Island and kept at various salinities.
Bars represent the S.D. of 3 replicates


GEL STRENGTH IN GRAMS
30
25
20
15
10
5
0
CARRAGEENAN AS % OF DRY WEIGHT
50
25
0
4‰
8‰
16‰
24‰
32‰
SALINITY

Gel strength:

As shown in Table 13 and Figure 28, there was a negative correlation between salinity and gel strength, i.e. the value of gel strength increased at low salinities and vice versa. These results might explain once more the fact that plants of Loch Long, which grow in estuarine habitats and may be covered with water of low salinity for sometime during the tidal cycle, have stronger gel than plants collected from Portencross and Cumbrae Island. Similar results were found by Marshall et al (1949) who stated that washing of the seaweed with running water (up to 12 hours) removed readily diffusible salts as well as a certain amount of organic matter with undesirable properties and the effect of this washing on the final gel was to increase its hardness.

(b) Illumination:

Five different levels of illumination were tested; 400 lux, 600 lux, 800 lux, 1200 lux and 2400 lux. Under each of these illuminations plants of Gigartina collected from Cumbrae Island were kept fully submerged for two weeks (24 April - 7 May) at 12°C, daylength of 12 hours and at a salinity of 32‰ and then the carrageenan contents and gel strengths were measured as described before.

Results are summarised in Table 14 and Figure 30(a) and (b).

Carrageenan contents:

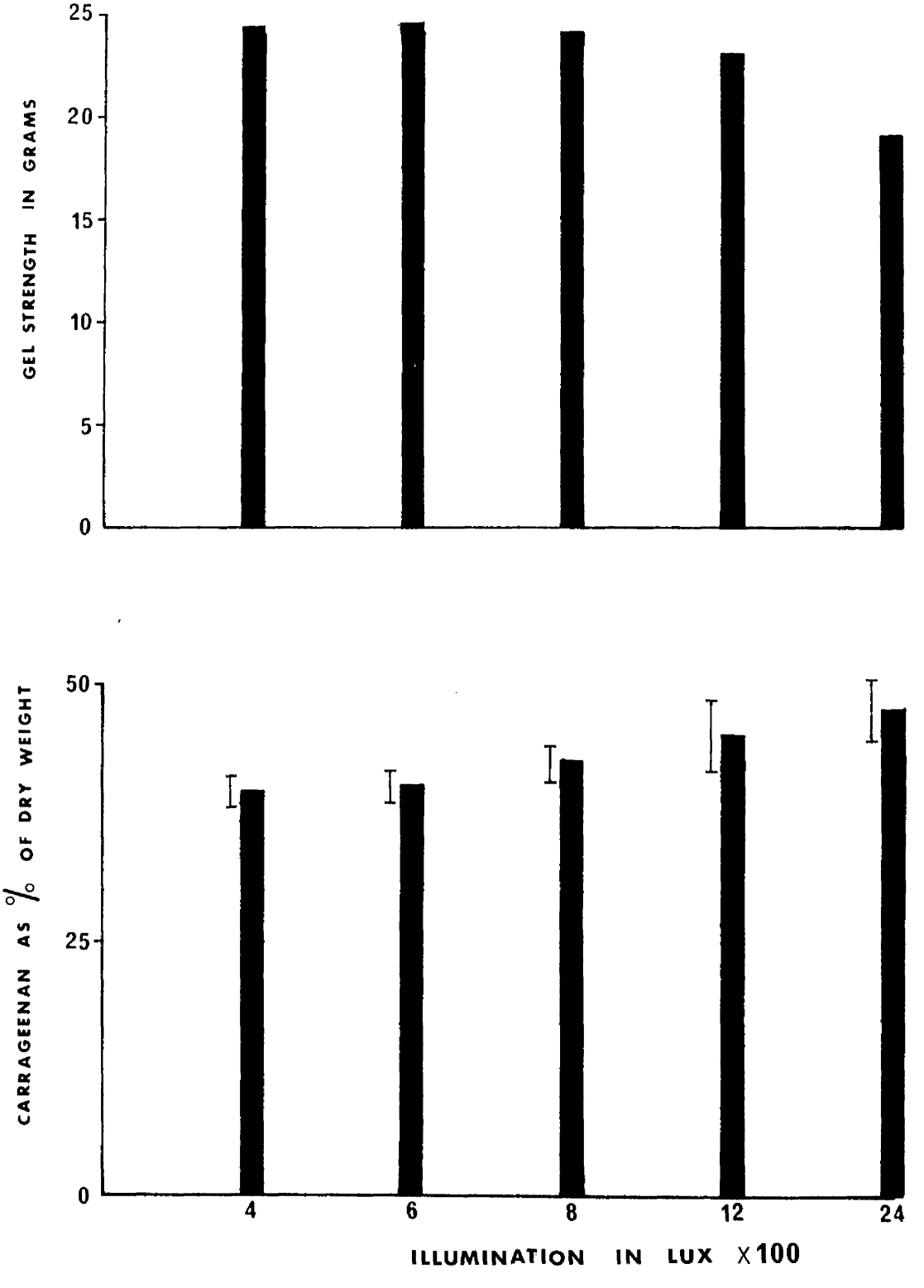
Carrageenan contents were not affected very much by different levels of illumination. A slight reduction in carrageenan content was observed with plants kept under illuminations of 400 lux and 600 lux. The highest value of carrageenan was recorded with plants kept at 2400 lux for two weeks.

Table (14): Effect of various levels of illumination on Carrageenan content and gel strength of plants of Gigartina kept for two weeks fully submerged in seawater at 12°C, daylength of 12 hours and salinity of 32‰ . Plants collected from Cumbrae Island in April 1977.

Illuminations / Properties measured	400 lux	600 lux	800 lux	1200 lux	2400 lux
Carrageenan as	39.5	40.0	40.5	42.5	44.5
% of dry weight	34.0	42.5	44.5	38.5	42.5
	43.5	36.5	37.0	47.0	49.0
Gel strength	23.5	24.0	24.0	23.0	20.0
in grams	24.0	22.5	24.0	25.0	18.5
	23.5	25.0	24.5	20.5	20.5

Figure 30(a): Shows gel strengths of carrageenan of plants of Gigartina collected from Cumbrae Island and kept under various illuminations.
S.D. is very small value and difficult to represent on the Figure.

Figure 30(b): Shows carrageenan of plants of Gigartina collected from Cumbrae Island and kept under various illuminations.
Bars represent the S.D. of 3 replicates


GEL STRENGTH IN GRAMS
25
20
15
10
5
0
CARRAGEENAN AS % OF DRY WEIGHT
50
25
0
4
6
8
12
24
ILLUMINATION IN LUX X100

Gel strength:

Again illuminations showed slight effects on the gel strength. As shown in Table 14 and Figure 28, there were no clear differences in gel strength at all levels of illumination except at 2400 lux where slightly weaker gel was obtained.

(c) Daylength regimes:

Plants of Gigartina collected from Cumbrae Island were kept fully submerged for two weeks (4 - 17 March) under three different daylength regimes; L/D; 8:$\overline{16}$, 12:$\overline{12}$ and 16:$\overline{8}$ at 12°C, 2400 lux and salinity of 32‰ , and then the carrageenan contents and gel strengths were measured as described before.

Results are summarised in Table 15 and Figure 31(a) and (b). It is clear from these data that light duration is not a crucial factor controlling the quantity and quality of carrageenan. A slight increase in carrageenan content was observed from daylength of 8:16 to 16: 8, but even under the shortest daylength regime carrageenan contents were little changed. These results disagreed with previous observations which showed that carrageenan in all localities exhibited maximum value in spring months where the day is longer, this may be due to the short period of experiment (two weeks).

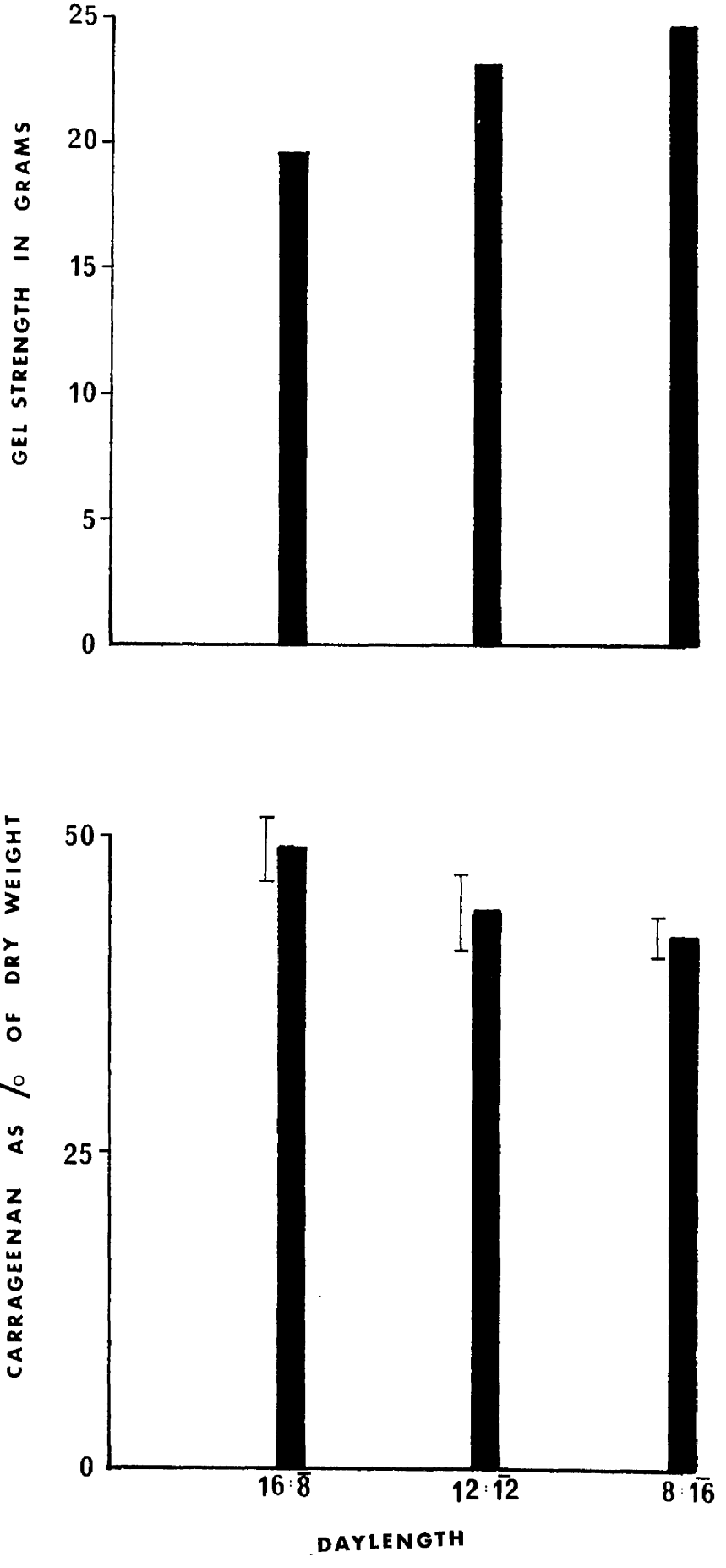
Gel strength as shown in Table 15 and Figure 30(b) as usual showed an opposite trend to the carrageenan content, and the longer light period is connected with weaker gel while the short daylength regime is connected with stronger gel.

Table (15): Effect of daylength regimes on carrageenan content and gel strength of plants of *Gigartina* kept for two weeks fully submerged in seawater at 12°C, 2400 lux and salinity of 32‰ . Plants collected from Cumbrae Island in March 1977.

Properties measured \ Daylength regimes	8:$\overline{16}$	12:$\overline{12}$	16:$\overline{8}$
Carrageenan as	42.0	44.0	49.0
% of dry weight	47.5	46.0	53.5
	39.0	43.0	44.5
Gel strength	24.5	23.0	19.5
in grams	23.5	23.5	20.0
	24.5	24.5	20.0

Figure 31(a): Shows gel strength of carrageenan of plants of Gigartina collected from Cumbrae Island and kept under various daylength regimes. S.D. is very small value and difficult to represent on the Figure.

Figure 31(b): Shows carrageenan contents of plants of Gigartina collected from Cumbrae Island and kept under various daylength regimes. Bars represent the S.D. of 3 replicates.


GEL STRENGTH IN GRAMS
25
20
15
10
5
0
CARRAGEENAN AS % OF DRY WEIGHT
50
25
0
16 : 8
12 : 12
8 : 16
DAYLENGTH

(d) Submergence in seawater:

Five different periods of submergence in seawater were obtained by the tide machine as described in Chapter 2, page (30); 4 hours/day, 8 h/d, 12 h/d, 18 h/d and full submergence. Under each of these periods plants of Gigartina collected from Cumbrae Island were kept for two weeks (22 June - 5 July) at 12°C, 2400 lux, salinity of 32‰ and a daylength of 12 hours, and then the carrageenan contents and gel strengths were measured as described before.

Results of this experiment are summarised in Table 16 and Figure 32(a) and (b). These results showed clearly that both carrageenan contents and gel strengths were affected by the different periods of submergence. Carrageenan content showed a negative correlation with periods of submergence, the higher carrageenan contents were obtained under the short period of submergence and lower carrageenan contents obtained under longer periods of submergence and at full submergence.

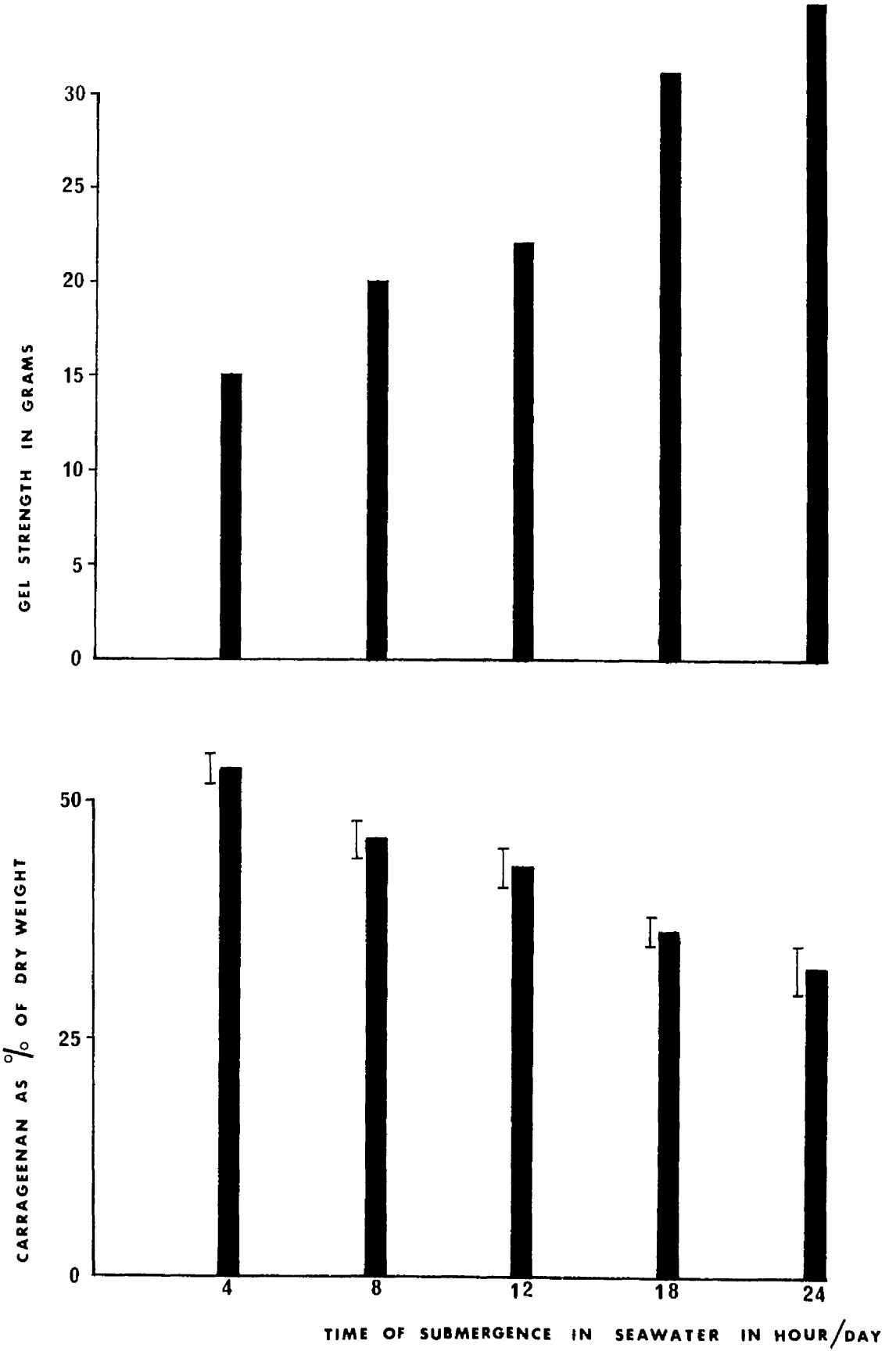
Gel strength showed a positive correlation with period of submergence. A stronger gel obtained with longer periods of submergence and vice versa.

Table (16): Effect of different periods of submergence on carrageenan content and gel strength of plants of Gigartina kept for two weeks at 12°C, 2400 lux, daylength of 12 hours and salinity of 32‰ . Plants collected from Cumbrae Island in June 1977.

periods of submergence / properties measured	4 h/d	8 h/d	12 h/d	18 h/d	24 h/d
Carrageenan as % of dry weight	59.0	46.5	43.0	36.5	32.5
	53.5	48.0	39.5	41.5	30.5
	50.5	45.0	48.0	32.0	34.5
Gel strength in grams	15.0	20.5	22.0	31.0	36.5
	15.5	20.0	21.5	30.0	35.0
	16.0	20.0	22.0	31.0	35.0

Figure 32(a): Shows gel strengths of carrageenan of plants of Gigartina collected from Cumbrae Island and kept for different periods of submergence in seawater.
S.D. is very small value and difficult to represent on the Figure.

Figure 32(b): Shows carrageenan contents of plants of Gigartina collected from Cumbrae Island and kept for different periods of submergence in seawater.
Bars represent the S.D. of 3 replicates.


GEL STRENGTH IN GRAMS
30
25
20
15
10
5
0
CARRAGEENAN AS % OF DRY WEIGHT
50
25
0
4
8
12
18
24
TIME OF SUBMERGENCE IN SEAWATER IN HOUR/DAY

7.4 Determination of Carrageenan contents and gel strengths in plants of Gigartina of different stages of maturity.

Figure (15) in Chapter 5, shows that the maximum spore discharge of plants of Gigartina from the three localities in the Firth of Clyde was recorded in September through December. This period as shown in Figure 27 is connected with lower carrageenan contents and stronger gel. On the other hand Figure 15 also shows that the minimum spore discharge (sterile plants) is in April through July. This period, as shown in Figure 27, is connected with higher carrageenan content and weaker gel.

The aim of this experiment was to compare the carrageenan content and gel strengths of the most fertile plants of Gigartina and of the sterile plants of Gigartina. For this purpose, the carrageenan contents and gel strengths were measured in 5 different stages of maturity of plants of Gigartina as described in Chapter 2, page (25). This was done with plants from all three localities.

Results are summarised in Tables (17) and (18) and illustrated in Figures (33) and (34). As shown from these results, carrageenan contents of plants of Gigartina from all three localities was higher when measured in the sterile plants and it is always lower when measured in the most fertile plants. Gel strength showed an opposite trend, i.e. the most fertile plants have stronger gel and the sterile plants have weaker gel.

Therefore, there results confirm the fact that it should be a seasonal variation in both the carrageenan contents and gel strengths. These results disagreed with the results of Fuller (1971) who studied carrageenan of Chondrus crispus from New Hampshire, U.S.A. and stated

Table 17: Measurement of carrageenan content of plants of different stages of maturity collected from the different localities in the Firth of Clyde area.

CUMBRAE ISLAND

Stage 1	Stage 2	Stage 3	Stage 4	Stage 5
a. 73.3%	75.2%	49.0%	40.0%	41.7%
b. 80.9%	78.2%	51.8%	42.8%	49.2%
c. 75.6%	76.9%	49.6%	47.0%	50.0%
Mean = 76.6%	76.8%	50.1%	43.3%	47.0%
S.D. = 3.9	1.5	1.5	3.5	4.6

PORTENCROSS

Stage 1	Stage 2	Stage 3	Stage 4	Stage 5
a. 73.6%	76.0%	49.0%	46.0%	47.8%
b. 81.5%	80.5%	53.0%	50.5%	53.2%
c. 80.2%	79.5%	51.0%	49.5%	51.0%
Mean = 78.4	78.7%	51.2%	48.7%	50.7%
S.D. = 4.2	2.4	2.0	2.4	2.7

LOCH LONG

Stage 1	Stage 2	Stage 3	Stage 4	Stage 5
a. 42.5%	42.9%	37.5%	20.8%	18.0%
b. 46.2%	34.8%	31.5%	24.7%	17.3%
c. 44.2%	38.9%	34.3%	27.2%	21.3%
Mean = 44.3%	38.9%	34.4%	24.2%	18.9%
S.D. = 1.9	4.0	3.0	3.2	2.2

Table 18: Measurements of the gel strength in the different stages of maturity of plants of Gigartina stellata collected from the different localities in the Firth of Clyde.

CUMBRAE ISLAND

Stage 1	Stage 2	Stage 3	Stage 4	Stage 5
20.0g	20.0g	23.0g	24.0g	29.0g
21.5g	19.0g	23.0g	25.0g	29.0g
20.0g	22.0g	24.0g	23.5g	29.0g
Mean = 20.5g	20.3g	23.3g	24.2g	29.0g

PORTENCROSS

Stage 1	Stage 2	Stage 3	Stage 4	Stage 5
15.0g	18.0g	18.0g	17.0g	19.0g
14.5g	18.5g	18.0g	17.5g	17.5g
14.0g	17.0g	19.0g	16.5g	20.0g
Mean = 14.3g	17.8g	18.3g	17.0g	18.8g

LOCH LONG

Stage 1	Stage 2	Stage 3	Stage 4	Stage 5
33.5g	40.0g	40.0g	44.0g	48.0g
34.0g	38.5g	41.0g	45.0g	47.0g
35.0g	39.5g	41.0g	43.5g	50.0g
Mean = 34.2g	39.3g	40.7g	44.2g	48.7g

Figure 33: Shows the carrageenan contents in different stages of maturity of plants of Gigartina from all three localities.

Stage 1: fronds without any papillae

" 2: fronds with initiation of papillae

" 3: fronds with many immature papillae

" 4: fronds with some mature papillae

" 5: fronds with all papillae fertile

A = Loch Long

B = Cumbrae Island

C = Portencross

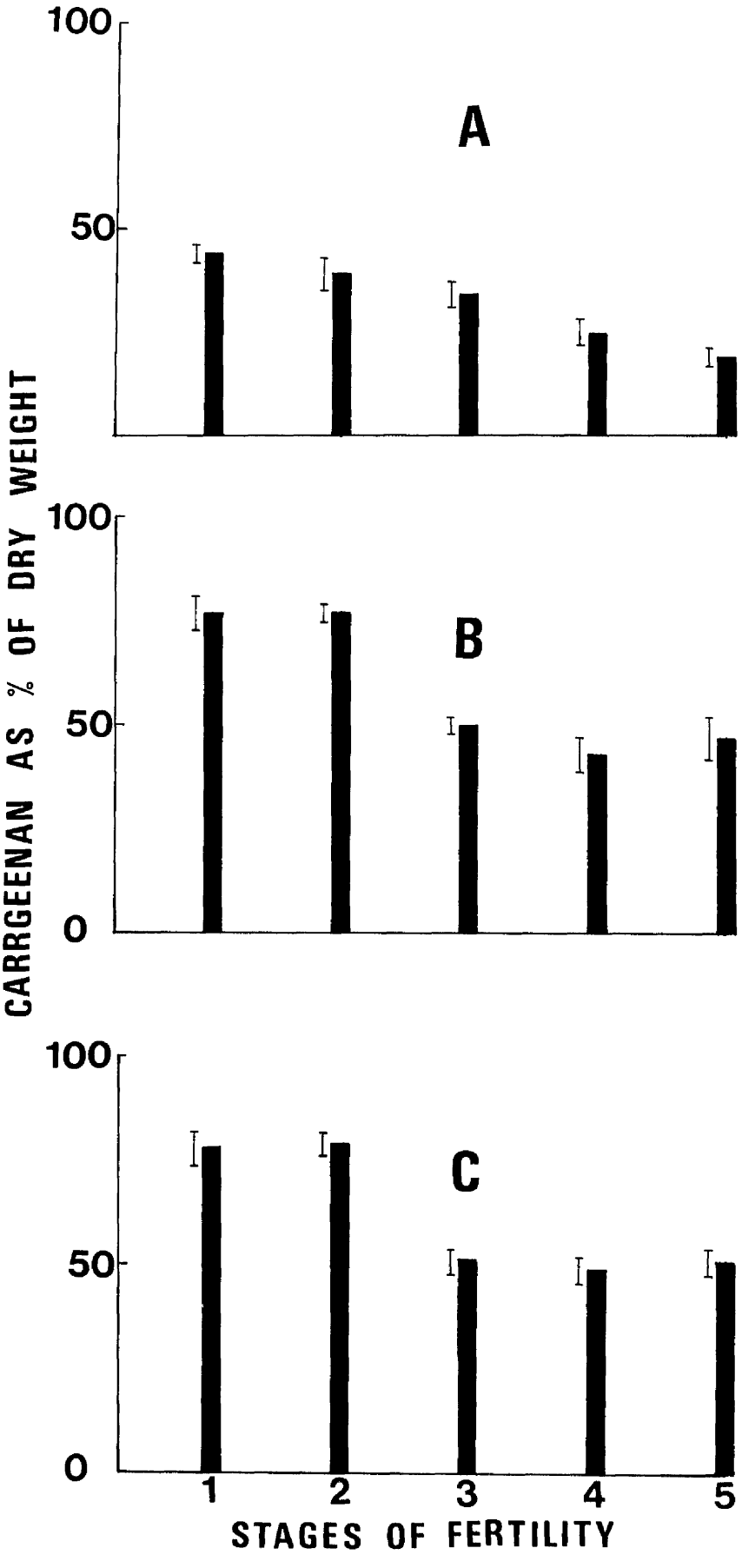
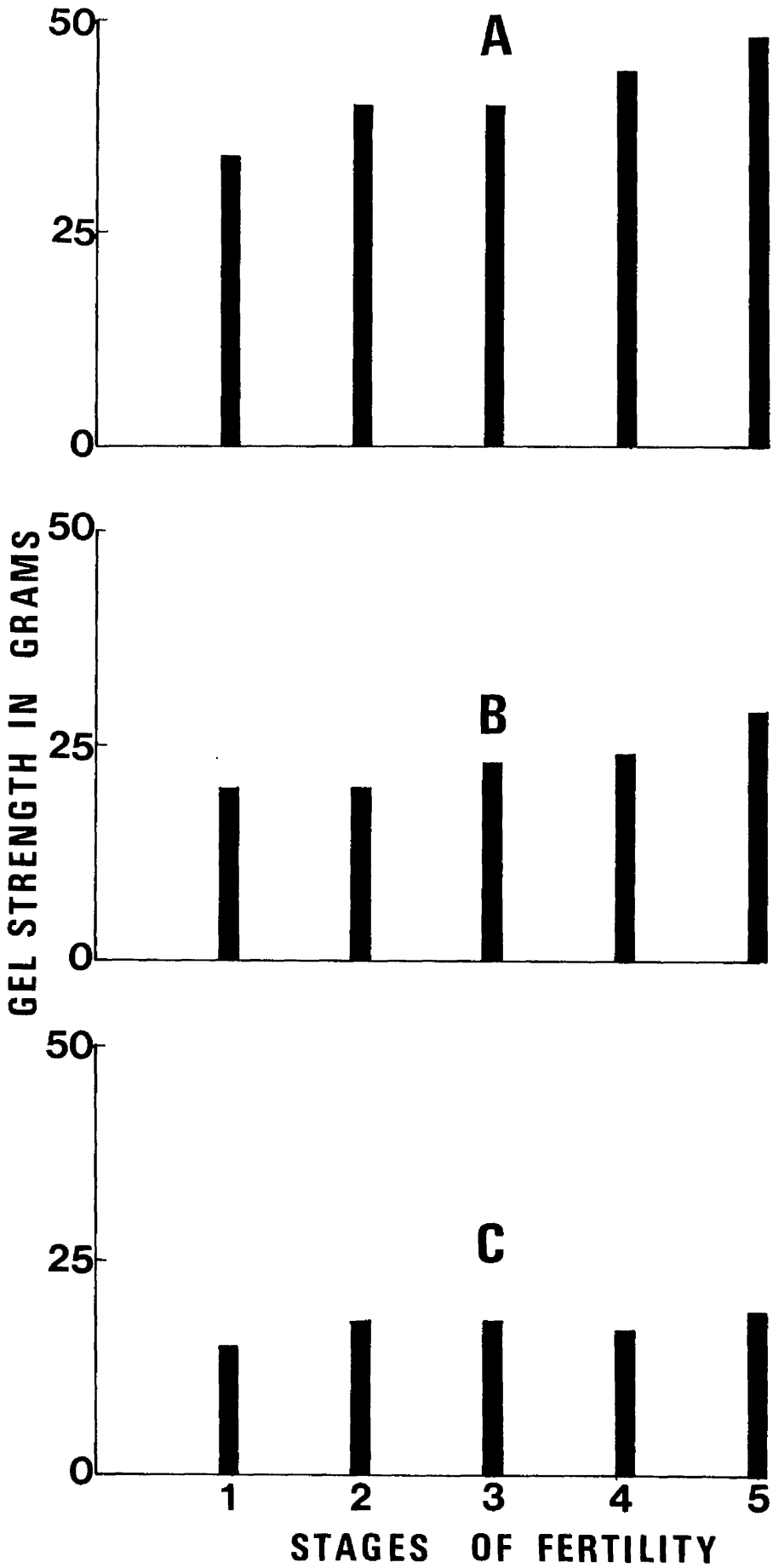

100
50
A
100
50
0
B
100
50
0
C
1
2
3
4
5
CARRGEENAN AS % OF DRY WEIGHT
STAGES OF FERTILITY

Figure 34: Shows the gel strength in different stages of maturity of plants of Gigartina from all three localities.


50
25
0
A
50
25
0
B
50
25
0
C
GEL STRENGTH IN GRAMS
1
2
3
4
5
STAGES OF FERTILITY

that plant age, reproduction, vertical position and exposure to wave action had no effect on the quantity or properties of carrageenan.

CHAPTER 8.

FURTHER EXPERIMENTAL STUDIES ON GIGARTINA STELLATA IN RELATION TO HABITAT CONDITIONS:

The tough fleshy fronds of Gigartina from Portencross contain more carrageenan but with a weaker gel strength compared with the thin papery fronds of plants from Loch Long with their lower carrageenan contents and stronger gel. Plants from Cumbrae Island with an intermediate fronds thickness have an intermediate carrageenan content and gel strength.

It has been suggested that thick cell walls and intracellular matrices in intertidal marine algae (of which carrageenan is a typical example) are of functional significance for the following reasons:

(a) They act as a cell 'cushion' against physical damage due to wave shock.

(b) They act as a 'buffer system' to prevent excessive water loss from the vicinity of cells during intertidal drought periods.

(c) They act as a 'free space' to prevent a rapid loss of inorganic ions when plants are immersed in fresh or brackish water.

From the results of the present studies on carrageenan, certain questions can be asked; Do the Gigartina plants from these different localities have the same ability to withstand dehydration?; Do the dried plants of Gigartina from the three localities have the same ability to recover when put back into seawater; and why does the carrageenan of plants from the different localities have different values of gel strength?

The answers to these questions need to be obtained by means of experimental studies.

8.1 The rates of water loss of Gigartina from the different localities.

The ability of plants of Gigartina to postpone the onset of tissue dehydration was initially assessed by measuring desiccation rates in individual young plants using the method outlined in Chapter 2, page (34). Drying was carried out in the laboratory in which temperature and relative humidity fluctuated somewhat. However, since the samples were dried over the same period of time, they underwent the same variations of atmospheric conditions, and a direct comparison is valid. The importance of the sample's size in determining its relative dehydration was demonstrated by Schonbeck (1976). In the present experiment the desiccation rates of the different plants were obtained only with plants of similar size. The course of desiccation was compared in three sets of plants, each set consisting of 10 plants from Loch Long, 10 plants from Cumbrae Island and 10 plants from Portencross. Plants were weighed at intervals of 2 hours over a drying period of 12 hours. The final drying was carried out by placing plants in desiccator for 3 days.

Results are summarised in Tables 19, 20 and 21 and illustrated in Figure 35. Details of the results are as follows:

(a) A sharp decrease in weight of plants from Loch Long was observed after the first two hours (only 50% of the initial fresh weight remained). A slow gradual decrease in weight of plants was recorded during the following 10 hours. The plants lost 65% of their fresh weight in the 12 hours drying period. When plants were put

Table 19: Rates of water loss of plants of Gigartina from Loch Long

	Drying time / Initial weight in gram		2 hours	4 hours	6 hours	8 hours	10 hours	12 hours	72 hours
1	0.2665	*	0.1235	0.1035	0.0925	0.0905	0.0905	0.0905	0.0885
		+	53.7%	61.2%	65.3%	66.0%	66.0%	66.0%	66.8%
2	0.2935		0.1400	0.1325	0.1200	0.1180	0.1155	0.1100	0.0925
			52.3%	54.9%	59.1%	59.8%	60.7%	62.5%	68.5%
3	0.2905		0.1445	0.1280	0.1200	0.1150	0.1100	0.1025	0.0975
			50.3%	55.9%	58.7%	60.4%	62.1%	64.7%	66.5%
4	0.2825		0.1400	0.1315	0.1185	0.1100	0.1050	0.1000	0.0850
			50.3%	53.5%	58.0%	61.0%	62.8%	64.6%	69.7%
5	0.3145		0.1800	0.1500	0.1375	0.1285	0.1200	0.1080	0.1000
			42.8%	52.3%	56.3%	59.1%	61.8%	65.6%	68.2%
6	0.2340		0.1250	0.1050	0.1020	0.1000	0.0900	0.0880	0.0775
			53.2%	55.1%	56.4%	57.3%	61.5%	62.4%	66.9%
7	0.5120		0.2385	0.2100	0.1900	0.1855	0.1800	0.1750	0.1600
			46.0%	59.0%	62.9%	63.8%	64.8%	65.8%	68.8%
8	0.4475		0.2100	0.1925	0.1755	0.1600	0.1520	0.1425	0.1375
			46.5%	57.0%	60.8%	64.3%	66.0%	68.2%	69.3%
9	0.3565		0.1505	0.1400	0.1325	0.1300	0.1255	0.1200	0.1155
			57.8%	60.7%	62.8%	63.5%	64.8%	66.3%	67.5%
10	0.4465		0.2085	0.1900	0.1775	0.1625	0.1555	0.1500	0.1425
			53.3%	57.5%	60.2%	63.6%	65.2%	66.4%	68.0%

* = Latest weight of plants in gram.
\+ = Loss of weight as % of initial fresh weight.

Table 20 : Rates of water loss of plants of Gigartina from Cumbrae Island

	Drying time / Initial weight in gram		2 hours	4 hours	6 hours	8 hours	10 hours	12 hours	72 hours
1	0.3060	*	0.1600	0.1350	0.1300	0.1185	0.1125	0.1100	0.0950
		+	47.7%	55.9%	57.0%	61.3%	63.2%	64.0%	69.0%
2	0.3015		0.1615	0.1315	0.1255	0.1120	0.1075	0.1070	0.0885
			46.4%	56.4%	58.4%	62.9%	64.4%	64.5%	70.7%
3	0.2540		0.1485	0.1275	0.1200	0.1125	0.1100	0.1105	0.0725
			41.5%	49.8%	52.8%	55.7%	56.7%	56.5%	71.5%
4	0.3980		0.2650	0.1925	0.1700	0.1680	0.1670	0.1655	0.1200
			33.4%	51.6%	57.3%	57.8%	58.0%	58.4%	70.0%
5	0.3435		0.1900	0.1800	0.1585	0.1555	0.1500	0.1500	0.1055
			44.7%	47.6%	53.9%	54.7%	56.3%	56.3%	69.3%
6	0.3920		0.2415	0.2000	0.1850	0.1785	0.1725	0.1700	0.1185
			38.4%	49.0%	52.8%	54.5%	56.0%	56.6%	69.8%
7	0.3180		0.1865	0.1650	0.1500	0.1435	0.1400	0.1405	0.0885
			41.4%	48.1%	52.8%	54.9%	56.0%	55.8%	72.2%
8	0.2725		0.1535	0.1300	0.1155	0.1050	0.0950	0.0900	0.0775
			43.7%	52.3%	57.6%	61.5%	65.1%	67.0%	71.6%
9	0.3425		0.1895	0.1600	0.1475	0.1380	0.1295	0.1300	0.1000
			44.7%	53.3%	56.9%	59.7%	62.2%	62.0%	70.8%
10	0.3785		0.2310	0.2115	0.2055	0.1885	0.1800	0.1800	0.1100
			39.0%	44.1%	45.7%	50.2%	52.4%	52.4%	71.0%

* = Latest weight of plants in gram.
+ = Loss of weight as % of initial fresh weight.

Table 21: Rates of water loss of plants of Gigartina from Portencross.

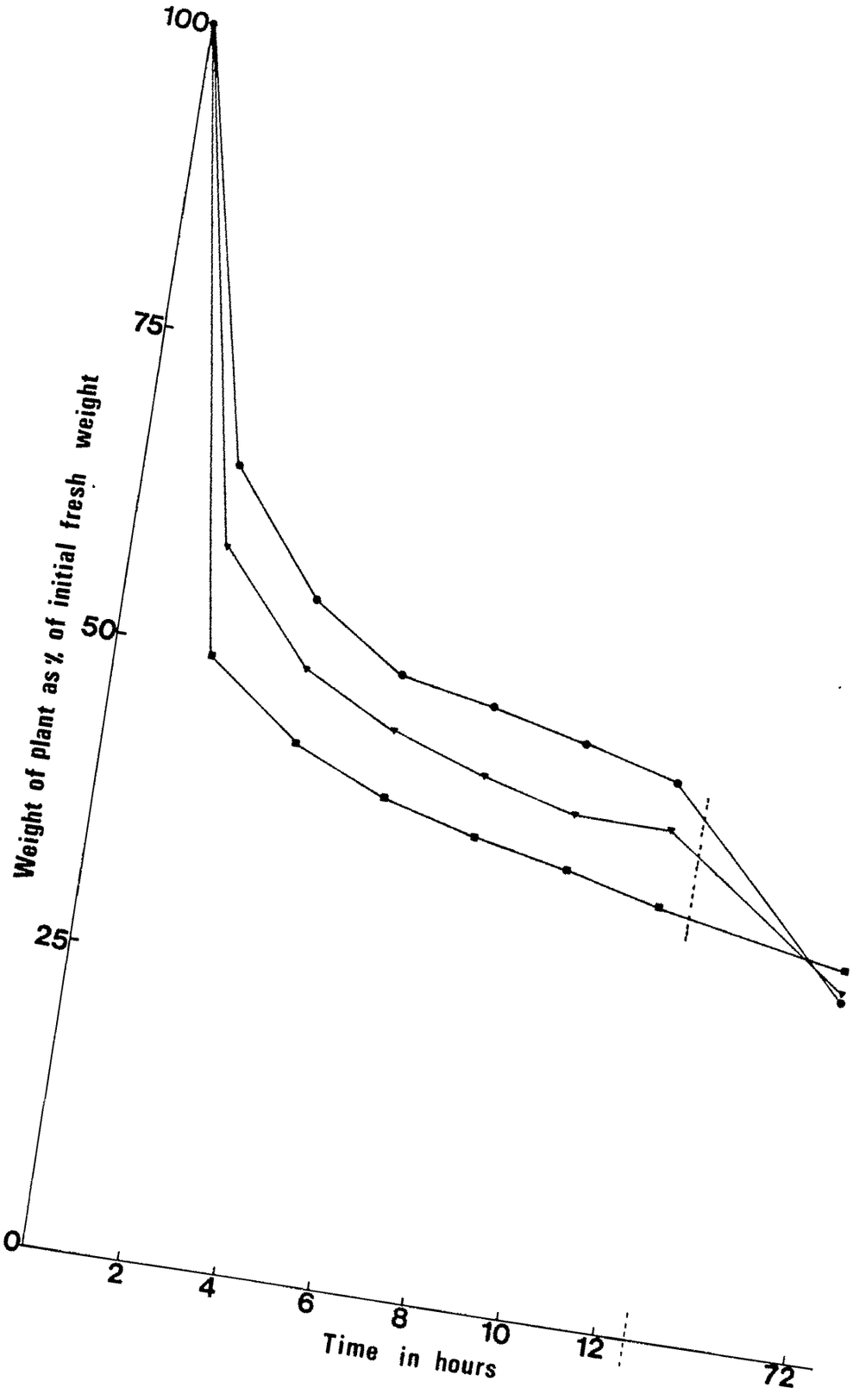
	Drying time / Initial weight in gram		2 hours	4 hours	6 hours	8 hours	10 hours	12 hours	72 hours
1	0.2565	*	0.1575	0.1370	0.1300	0.1225	0.1100	0.1000	0.0775
		+	38.6%	46.6%	49.3%	52.3%	57.1%	61.0%	69.8%
2	0.4600		0.2800	0.2385	0.2300	0.2225	0.2000	0.1850	0.1450
			39.1%	48.2%	50.0%	51.6%	56.5%	59.8%	68.5%
3	0.2595		0.1575	0.1325	0.1300	0.1275	0.1205	0.1125	0.0785
			38.9%	48.5%	49.5%	50.5%	53.2%	56.3%	69.5%
4	0.2890		0.1550	0.1255	0.1225	0.1220	0.1220	0.1220	0.0800
			46.4%	56.5%	57.6%	57.8%	57.8%	57.8%	72.3%
5	0.5930		0.4000	0.3650	0.3500	0.3500	0.3450	0.3350	0.1825
			32.6%	38.5%	41.0%	41.0%	41.8%	43.5%	69.2%
6	0.5020		0.3385	0.3050	0.2785	0.2715	0.2700	0.2700	0.1445
			32.6%	39.2%	44.5%	45.9%	46.2%	46.2%	71.2%
7	0.3985		0.2460	0.1950	0.1855	0.1800	0.1800	0.1745	0.1250
			38.3%	51.0%	53.5%	54.8%	54.8%	56.2%	68.6%
8	0.4090		0.2850	0.2300	0.1865	0.1820	0.1800	0.1800	0.1155
			30.3%	43.8%	54.4%	55.5%	56.0%	56.0%	71.8%
9	0.5220		0.4000	0.3480	0.2750	0.2650	0.2600	0.2650	0.1500
			23.4%	33.3%	47.3%	49.2%	50.2%	59.2%	71.3%
10	0.3945		0.2500	0.2150	0.2000	0.1880	0.1800	0.1800	0.1150
			36.6%	45.5%	49.3%	52.4%	54.4%	54.4%	70.9%

* = Latest weight of plants in gram.

+ = Loss of weight as % of initial fresh weight.

Figure 35: Rates of water loss of plants of *Gigartina* from the three localities in the Firth of Clyde.

- ● Plants from Portencross.
- ▼ Plants from Cumbrae Island.
- ■ Plants from Loch Long.


100
75
50
25
0
Weight of plant as % of initial fresh weight
2
4
6
8
10
12
72
Time in hours

in desiccator for 3 days, they only lost 3% in the 3 days, and the final dry weight was 32% of the initial fresh weight.

(b) Plants from Portencross seem to be able to postpone the tissue dehydration more than plants from Loch Long, since plants lost only 35% of their initial fresh weight after two hours, again slow gradual decrease in weight of plants was observed, and after 12 hours drying period plants lost 55% of the initial fresh weight. An interesting result was observed when plants were put in the desiccator for 3 days. A sharp decrease in the weight was recorded and the final dry weight was 29.7% of the initial fresh weight.

(c) Plants from Cumbrae Island were also able to delay the rate of water loss from their tissue. Plants lost only 42% of their weight in the first two hours, and a gradual decrease in weight of plants was observed in the following 10 hours and about 41% of the initial weight remained after 12 hours drying period. Again a sharp decrease in the weight of plants was recorded when plants were placed in a desiccator for 3 days and the final dry weight was 30% of the initial fresh weight.

8.2 Rates of ions loss of plants of Gigartina.

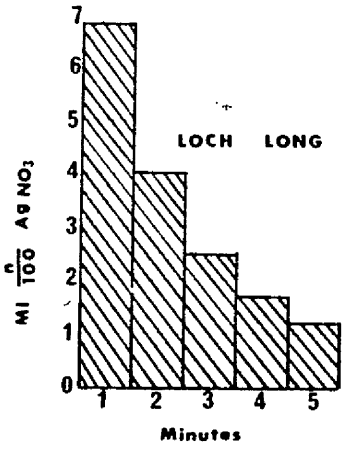
Plants of Gigartina growing in the estuarine habitat of Loch Long may be covered with water of very low salinity for some periods at low tide. These plants have a lower carrageenan content but of higher gel strength. Furthermore, by keeping plants of Gigartina from Cumbrae Island in water of reduced salinity a similar result was obtained, i.e. lower carrageenan content and stronger gel(p.95). Marshall et al (1949) found that the preliminary washing of seaweed (up to 12 hours in running

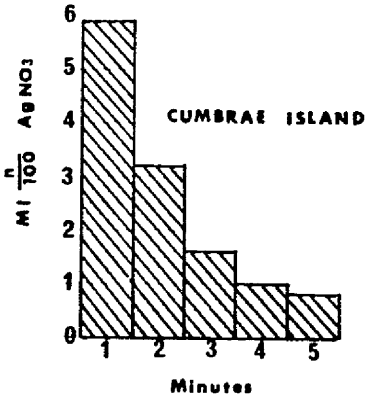
Figure 36: Rates of chloride ions loss in plants of Gigartina from the three localities in the Firth of Clyde.

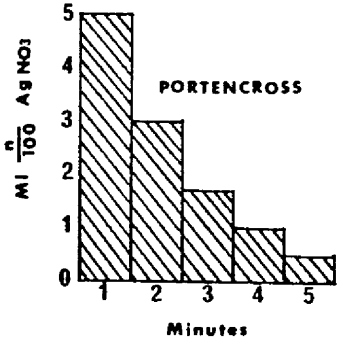
A. Rates of chloride ions loss in plants freshly collected from the shore.

B. Rates of chloride ions loss in plants from previous treatment after being placed in normal seawater for 48 hours.

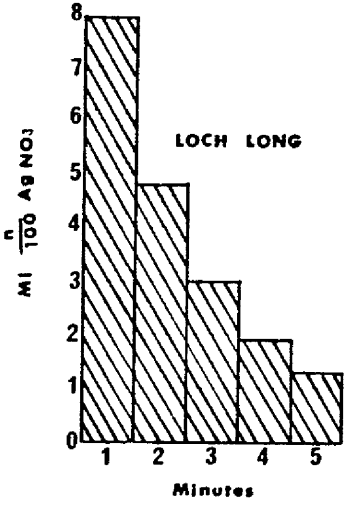
A

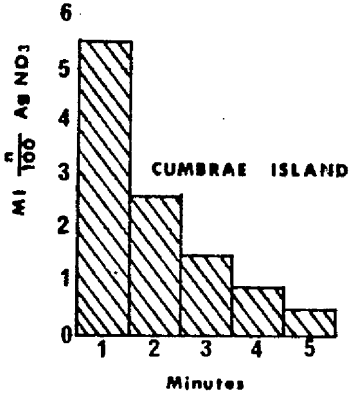

LOCH LONG
Ml n/100 Ag NO3
Minutes

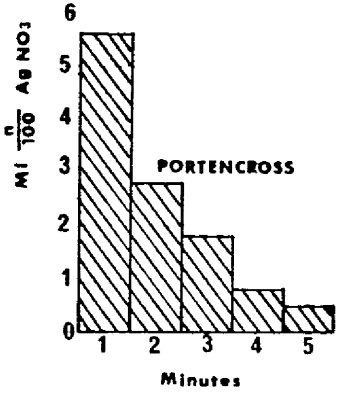

CUMBRAE ISLAND
Ml n/100 Ag NO3
Minutes


PORTENCROSS
Ml n/100 Ag NO3
Minutes

B


LOCH LONG
Ml n/100 Ag NO3
Minutes


CUMBRAE ISLAND
Ml n/100 Ag NO3
Minutes


PORTENCROSS
Ml n/100 Ag NO3
Minutes

water) removed readily diffusible salts as well as a certain amount of salt with undesirable properties and the effect of this washing on the final gel was to increase its hardness. Zabik and Aldrich (1968) reported that ion-exchanged kappa-carrageenan with $A\ell^{3+}$, Fe^{2+}, Fe^{3+} and Sn^{2+} prevented gel formation. The other ion-exchanged carrageenan had significantly lower gel strength than the non-exchanged carrageenan. Furthermore, they found that the Mg^{2+} and Ca^{2+} exchanged carrageenan had greater gel strength than the NH_4^{1+} and Na^{1+} - carrageenan. The use of KCl with the NH_4^{+1}, Ca^{2+}, Mg^{2+} and Na^{1+} - exchanged resulted in increased gel strength.

Gessner and Hammer (1968) stated that in distilled water, marine benthic algae immediately lose ions. In the present experiment this phenomenon was demonstrated by means of chloride titration. Details of the method are outlined in Chapter 2, page (34).

Results are illustrated in Figure 36(a) and (b). As is clear from Figure 36(a), plants of Gigartina from all different localities showed the same pattern of the chloride ion loss, i.e. maximum loss was observed in the first minute, then loss declined during the following minutes. Gessner and Hammer (1968) suggested that ion movements out and into the free space are involved. Through its free space the alga establishes an ionic equilibrium with its external medium. If the algae are killed or injured the extent of chloride loss is much higher. At all times fronds of Gigartina from Loch Long lost more chloride ions than plants from other localities, this however may be explained by the fact that the rate of loss of chloride ions was related to the weight of the thallus and not to its surface area. Since plants of Loch Long of the same weight have

substantially larger surface area than plants from other localities, the rate of ion loss would be expected to be higher when compared on a fresh weight basis.

When the fronds of Gigartina from the previous experiment were put back into normal seawater for 48 hours and then the rates of loss of chloride ions again measured, fronds of Gigartina from Loch Long as shown in Figure 36(b) lost more chloride ions than plants from the other localities, and this time the differences were more obvious. At all times a considerable amount of chloride ions was lost. On the other hand plants from Cumbrae Island and Portencross, when put back into seawater for 48 hours, did not show any significant differences in the rates of chloride ions from previous results. This may be realted to the fact that the fronds of Gigartina from Loch Long are more sensitive than fronds of plants from Cumbrae Island and Portencross which contain more carrageenans which prevent the rapid loss of chloride ions.

8.3 Studies on photosynthesis of Gigartina.

Photosynthesis in the red alga Gigartina harveyana was studied by Emerson and Green (1934) who used pieces of G. harveyana weighing less than 150 mg, suspended in 8 ml of ordinary seawater. They stated that the rather primitive methods used in measuring photosynthesis in G. harveyana do not permit much control of external conditions. Photosynthesis rises to a maximum in about 15 minutes and soon afterwards enters an almost linear decline. This decline in rate of photosynthesis was not due to irreversible injury from the experimental conditions, but to a steady fall in the carbon dioxide concentration

because of its removal in photosynthesis. This was overcome by saturating the ordinary seawater with 5% CO_2 in air. Burns (1971) and Burns and Mathieson (1972) measured the rates of photosynthesis of plants of Gigartina stellata in 6 mm discs removed from the thin flattened portions of the thalli. The rates of oxygen exchange in the sample were recorded in a Gilsen Differential Respirometer. Results of this study showed that light saturation intensity for photosynthesis of G. stellata is higher than that for many sublittoral plants. Maximum photosynthesis/respiration ratios were recorded at 20°C and at 40‰.

It is assumed that the measure of oxygen liberated is an indirect indication of carbon fixed, and thus represents photosynthetic rates. In the present study, the rate of oxygen liberation of plants of Gigartina stellata were measured by the method outlined in Chapter 2, page (32).

(a) Measurement of oxygen liberation rates of plants of Gigartina from the different localities in the Firth of Clyde.

Rates of oxygen liberation were compared in plants from the three localities. Ten plants from each locality were incubated for 2 hours at 12°C, 2400 lux, the rates of oxygen liberation were measured by the method outlined in Chapter 2, page (32). Results are summarised in Table 22. Rates of oxygen liberation, calculated on a wet weight basis were essentially similar in plants from all 3 localities. This was a surprising result considering the tough, fleshy, large plants of Portencross compared with the thin papery fronds of plants of Loch Long. However plants of Loch Long have a higher percent dry matter than plants from other localities, and must fix more carbon than plants

from Portencross and Cumbrae Island to produce a given amount of new tissue. Therefore Gigartina from Loch Long would be expected to produce somewhat less new tissue per unit existing tissue per day, and would show a slower weight gain. This was found to be true in the culture experiments. It has been shown that Gigartina in Loch Long have a more bushy habit of growth than plants from the other localities. Plants from Loch Long and other localities appear to differ not in photosynthetic rate, but in how they allocate their photosynthate; i.e. their growth strategy. The Loch Long plants produce a bushy thallus composed of tissue with high dry matter content while Gigartina from Portencross and Cumbrae Island utilize their photosynthate to produce a fleshy tough and large plants.

Table 22: Comparison of oxygen liberation rates ($\mu g.\ O_2/g/h$) of Gigartina stellata from different localities:

	Loch Long	Cumbrae Island	Portencross
	14.0	14.1	15.8
	11.8	11.7	14.3
	9.0	14.9	18.8
	8.6	15.6	8.9
	14.3	16.2	8.5
	16.0	14.8	14.6
	10.6	11.5	14.0
	8.3	12.4	9.2
	9.7	12.3	15.1
	10.0	14.7	11.0
Mean:	11.25	13.8	13.0
S.D.	2.6	1.7	3.4

(b) Comparison of rates of oxygen liberation in plants from the 3 localities after being dried for 12 hours.

The rates of oxygen liberation were compared in 5 plants from each of the three localities after being dried for 12 hours under the laboratory conditions. Plants were put back into seawater for 48 hours and then they were incubated for 2 hours at 12°C and 2400 lux. As shown in Table 23, plants from Loch Long showed very low rates of oxygen liberation after 12 hours drying. Plants from Cumbrae Island showed slightly higher rates of oxygen liberation than the Loch Long plants. Surprisingly plants from Portencross showed much higher rates of oxygen liberation when dried for 12 hours than plants from other localities. This however, indicated clearly that plants from Portencross which have fleshy, tough fronds were better able to recover after being dried for 12 hours. The ability of plants from Portencross to recover can be explained by the previous results which showed that the Portencross plants lost only 45% of their weight when dried for 12 hours, compared with 66% of weight loss in plants from Loch Long and 52% of weight loss in plants from Cumbrae Island.

Table 23: Comparison of oxygen evolution rates ($\mu g\ O_2/g/h$) in Gigartina from different localities after being dried for 12 hours

	Loch Long	Cumbrae Island	Portencross
	2.6	3.5	7.0
	1.8	3.8	6.8
	2.2	2.7	5.7
	2.0	3.3	7.1
	2.2	2.5	7.7
Mean:	2.2	3.2	6.9
S.D.:	0.3	0.6	0.7

(c) Comparison of rates of oxygen liberation of plants of Gigartina at different salinities.

The rates of oxygen liberation in plants of Gigartina collected from Cumbrae Island were compared in plants incubated for 2 hours in 5 different salinities; (4‰, 8‰, 16‰, 24‰, and 32‰) at 12°C and 2400 lux. At each salinity three replicates were carried out.

Results which are summarised in Table 24 showed a gradual increase in rates of oxygen liberation from salinity of 4‰ to 32‰. At the very low salinities 4‰ and 8‰ the rate of oxygen libration was very slow, this may explain the failure of Gigartina frond to grow continuously submerged when cultured under these low salinities. At a salinity of 16‰, although the culture experiments showed that Gigartina grew healthily with slightly slower growth rates than under normal salinities, the rates of oxygen liberation are significantly slower. This may be due to the short time of incubation which did not allow any adaptation for the plants when kept in this low salinity. There were no significant differences in the rates of oxygen libration when plants incubated at salinities of 24‰ and 32‰, in agreement with the results obtained from culture experiment which showed similar growth rates under these two salinities.

Table 24: Comparison of oxygen libration rates ($\mu g\ O_2/g/h$) in Gigartina collected from Cumbrae Island and treated with different salinities:-

	4‰	8‰	16‰	24‰	32‰
	4.7	7.2	7.9	18.7	13.8
	4.8	7.3	9.7	15.3	14.4
	4.5	6.6	8.9	14.5	14.1
Mean:	4.7	7.0	8.8	16.2	14.1
S.D.:	0.2	0.4	0.9	2.2	0.3

Thus the different plants of Gigartina which vary in their carrageenan contents were found also to have different abilities to postpone tissue dehydration. Plants from Portencross with more carrangeenan were better able to withstand the dehydration, whilst plants from Loch Long (lower quantities of carrageenan) were least able to delay the tissue dehydration. Furthermore, it was found that the Portencross plants (which did not loose as much of their water content after the 12 hour drying period) had a better ability to recover metabolic activity when put back into seawater.

There are various possibilities for the variations in gel strength of the carrageenan from the different plants:-

1. The reduced salinities, as at Loch Long, which are connected with higher gel strength, may contain greater quantities of Ca^{2+} and Mg^{2+}, and these ions were found to increase the gel strength (Zabik and Aldrich, 1968). The carrageenan of lower gel strengths (as from the Portencross plants)

(a) have better water retaining properties than the harder gel. Hence the plants are more able to postpone tissue dehydration.

(b) the carrageenan of softer gel is more elastic in its response to wave shock, and so form a better cell 'cushion'.

DISCUSSION:

Three distinct localities with Gigartina were identified in a series of preliminary surveys of the Firth of Clyde. These localities principally differed in the degree of exposure to and shelter from wave action, salinity of seawater and the substrata. The polymorphic nature of both Chondrus crispus and Gigartina stellata are well known phenomenon with data from both the U.K. and U.S.A. (see page 1-6). The variable morphology would seem to be a noticeable feature of these and related genera, and would seem also to be well shown in the three morphological types described in the present work. Are these stable morphological variants?, Transplant experiments in which plants of Gigartina were transplanted throughout the three localities in reciprocal fashion have shown that in all cases the transplants did not show any changes in their morphological characters for up to 3 months, then gradually the changes became noticeable. After one year it was impossible to distinguish between the transplants and the undisturbed (native) plants. This was mainly due to fronds newly emerged from basal crust which came to resemble the 'native' plants. At Cumbrae Island all the three morphological forms (including the two transplants) could be easily distinguished even after 6 months. Plants transplanted from Loch Long to Portencross were lost in heavy seas, and it would have been much informative if they could have survived for a longer period. The loss of transplants from Loch Long could be either because of the small area of basal crusts (see page 39), or because of the type of substratum on which Gigartina grows. Although the transplants responded very slowly to the new habitat conditions, the morphological

variability of Gigartina seems to be mainly due to environmental conditions. The association of morphological variability of marine algae with habitat conditions has been suggested by many phycologists. For example Moss (1948) stated that the variability in the morphological characters of Fucus vesiculosus is associated with the degree of exposure and shelter conditions. Knight and Parke (1950) related the variability in the morphological characters of Fucus vesiculosus and Fucus serratus to the salinity of seawater. Burns (1971) associated the morphological variability of Gigartina stellata with the degree of exposure and shelter conditions. Finally, Taylor and Chen (1973) related the morphological variability of Chondrus crispus to the degree of exposure and shelter conditions.

Would these 3 morphological forms respond in the same ways if they were cultured under the same laboratory conditions?. This was answered by the first culture experiment in which the 3 morphological forms of Gigartina were cultured under the same laboratory conditions. All plants grew healthily for the whole period of the experiment, but with slower growth rate of plants from Loch Long. There were no significant differences in growth rates of plants from Cumbrae Island and Portencross. The slow growth rate of plants from Loch Long may suggest that these plants have not got the same ability to adapt themselves to the full submergence in normal seawater.

As was described before, the three localities are different in the salinity of seawater as well as the degree of exposure and shelter conditions, and this latter factor might affect the illumination and the time of submergence during the tidal cycle. Neish and Fox (1971) have shown that in culture frond colour of Chondrus crispus is controllable by light and nutrient.

Are there any differences in the morphological behaviour of Gigartina when cultured under various laboratory induced environmental conditions? Results of these experiments suggest very clearly that Gigartina showed different responses under all different culture conditions. Plants were able to grow at relatively low salinity, but not in "brackish" water. At Loch Long, the plants may be covered with water of very low salinity but only for a short period during the tidal cycle. Although, Gigartina seems able to grow healthily at all levels of illumination there was a gradual increase in growth rates from 400 lux to 3000 lux, with the low levels of illumination affecting the colour of the fronds. A similar affect was obtained in water of low salinity. There were no significant differences in growth rates of Gigartina under 2400 lux and 3000 lux, therefore it was not necessary to grow Gigartina under higher illumination than 3000 lux. Daylength regimes affect the growth of Gigartina. Maximum growth was recorded in longer days. This agreed with the field observations which show that the maximum growth in nature occurred in spring months where the daylength is increasing. Similar results was found by Marshall et al (1949); Burns (1971) and Pybus (1977). Over 4-5 weeks, the growth at 12:$\overline{12}$ regime is similar to the longer day regimes, but then seems to fall off. Under the shortest day regime growth is slower over 5 weeks then starts to catch up, and this might be due to a mobilization of reserves. There was a positive correlation between growth rates and the periods of submergence in seawater. This may explain why the top shore plants are smaller in size than plants growing on lower shore, and why Portencross, which is more exposed than other localities, generally has the larger plants.

Recolonization studies of the denuded and sterlized areas of the rocky shore would help to elucidate the life history of Gigartina stellata, and indicate whether cultivation on artificial substrata placed in the sea would be commercially practicable, and also throw some light on the morphological development of sporelings of Gigartina from the spore "rain" on the rock. In these experiments complete sterilization was carried out as described on page (49). Also the clearing of strips at different times of the year was made in order to follow any seasonal variability in recolonization.

After 2·5 years, all strips were covered by Fucus plants with no sign of the Gigartina plants or the red crust described as a Petrocelis spp. Burns (1971) found similar results when he stated that Gigartina stellata in New Hampshire required at least 3 years to establish themselves on denuded and sterilized rocky surfaces. The results of the present work disagreed with the results obtained by Marshall et al (1949) and Hruby (1977), who stated that Gigartina stellata needs only 6 months before they appear on denuded quadrats. Marshall et al (1949) made their experiment in July and sterilized small quadrats using mercuric chloride after scraping the surface of the rocks. Hruby (1977) scraped, brushed and burned small areas at Tomont End in the northern side of Cumbrae Island. This result was checked by clearing two successive strips at Tomont End in July 1977. On one strip the area was cleared only by scraping and brushing the surface of the rock, whilst the other strip was cleared as described before. After only 4 months a carpet of young plants of Gigartina covered the first strip while the other strip was still bare. This clearly confirmed the previous results and showed that by not sterilizing the strip Gigartina appeared as new growths from the perennial basal

crusts lodged in protected areas of rock and when these basal crusts were destroyed by sterilizing the strip, Gigartina failed to reappear.

Since the recolonization studies failed to produce any results, regeneration of plants of Gigartina was examined by cutting plants of small quadrats at the middle part of the stalk, about 2 cm from the basal crust, leaving the basal crust with part of the stalk to regenerate. Results of this experiment showed that the quadrats became covered by Enteromorpha spp and Cladophora spp but without Gigartina regeneration. After 6 months the cut fronds of Gigartina had disintegrated and disappeared while the quadrats remained covered by green algae. New growths of Gigartina produced by the basal crust appeared underneath the green carpet. These results not only indicate that the stalks of this length of Gigartina were not able to regenerate, but also that they were unable to prevent the development of a dense cover of green algae. Newly formed young branches were able to compete with the green carpet that covered the quadrat. The question remains why Gigartina sporelings did not appear underneath the Fucus on the cleared strip. It might be that the spores and sporelings were unable to establish themselves under the brown algal cover. In experiments where plants were cut a higher position of their fronds leaving part of the flattened fronds as well as the stalk to regenerate, then after 7 months the average length of the new growths from the cut surface was about 2 cm, but without the dense green carpet. This shows how slowly Gigartina regenerates in the field. For some reason, some plants formed numerous proliferations from the margin of the fronds, whilst the cut surface was healed but did not show any sign of regeneration.

Carpospore cultures of Gigartina papillata from California and Gigartina stellata from Ireland gave rise to plants resembling Petrocelis fransiscana and P. cruenta respectively which are regarded as tetrasporophytes (West, 1972; West and Polanshek, 1972 and West, Polanshek and Guiry, 1977). Would similar results be obtained with the carpospores of Gigartina stellata from the Firth of Clyde? and what would be the environmental tolerances of the spores and sporelings of Gigartina in relation to the habitat conditions studied?.

The failure of the sporelings to develop erect branches under culture conditions was disappointing, especially when Chondrus sporelings successfully formed the erect branches over the same time and under the same conditions. Attempts to culture the tetraspores of Petrocelis cruenta from the Firth of Clyde were equally disappointing. As far as results were obtained, the Gigartina life history for the Firth of Clyde appears similar to that obtained by Marshall et al (1949) who obtained only basal crusts from the carpospores of Gigartina stellata and were unsuccessful in associating a crustose stage with Gigartina stellata. Similarly Rueness (1978) on the west coast of Norway could not obtain the erect branches from germlings of Gigartina stellata unless he grew the carpospores at temperatures over 17^{o}C and this temperature would not be obtained in the Firth of Clyde. Moreover, Burns (1971) stated that the rate of increase in growth of sporelings of Gigartina stellata was greater between 3 and 11^{o}C. than between 11 and 19^{o} C.

An unexpected result was the antibiotic activity of Chondrus sporelings compared with those of Gigartina. Does this bare any ecological significance? It might be that the diatom growth helped

detach the Gigartina sporelings. It is possible that growth of isolated Gigartina sporelings was a mistake, since aggregations of sporelings of both Chondrus crispus and Gigartina stellata have been shown to develop more rapidly (Tveter and Mathieson, 1976). Certainly, the fate of Gigartina sporelings needs more critical study.

Gigartina sporelings responded to the laboratory induced environmental conditions in similar ways as the fronds did. It would have been more useful, however to study the effect of environmental conditions on sporelings with their erect branches.

Studies on carrageenan contents and gel strengths of plants of Gigartina stellata from different populations showed clearly that the three populations varied in both their carrageenan contents and gel strength with maximum carrageenan content and weakest gel strength recorded in plants from the exposed localities and minimum carrageenan contents and stronger gel recorded in plants from Loch Long. Similar results were found by Fuller (1971) in his study on Chondrus crispus from New Hampshire in that the quantity of carrageenan was usually greater in plants from coastal than from estuarine locations. The effect of fresh water on gel strength was reported by Marshall et al (1949) who showed that preliminary washing of plants (up to 12 hours in running water) increased the hardness of the gel. These variations in the quantity and quality of carrageenan seem to be indirectly controlled by environmental factors. Results of the transplant experiments showed that after 3 months the carrageenan contents of the transplanted Gigartina began to resemble that of the carrageenan of the 'native' plants, although changes in gel strength were less obvious. The role of environmental conditions on carrageenan contents and gel strength was studied in the laboratory

by keeping a large quantity of plants of Gigartina from one locality (Cumbrae Island), under a variety of laboratory induced environmental conditions; (various salinities, illuminations, daylength regimes and periods of submergence in seawater). All these conditions were found to affect the quantity and quality of carrageenan, but the most effective factor was the salinity of seawater.

Carrageenan contents and gel strengths showed seasonal variation. The maximum yield of carrageenan was recorded between March and May and the minimum carrageenan content between September and December. Maximum gel strength was recorded in winter months between December and March, whilst the minimum value of gel strength was recorded in spring and summer, between March and August. From previous results the period of maximum spore discharge was recorded between September and December whilst the period of minimum spore discharge was recorded between April and July. Therefore, it was observed that the maximum carrageenan content, the weakest gel and the minimum spore discharge were all recorded in the same period, and the minimum carrageenan content, the strongest gel and the maximum spore discharge recorded in the same period. It was also found that the most fertile plants have the smallest carrageenan content and strongest gel whilst the least fertile plants have maximum carrageenan content and weakest gel. Is this because in the sterile plants the branches are young growths and the different carrageenan is a developmental feature? or does the formation of fruiting papillae cause a drain in the plant's energy and a change in carrageenan as a result? or is the spore discharge of the fertile plants accompanied by a considerable loss of mucilage, including

carrageenan? Alternatively the explanation may lie in the seasonal growth behaviour. The Spring and Summer are periods of more active photosynthesis and then there is more photosynthate available for carrageenan formation, with the Autumn and Winter months the period of minimum growth and photosynthate activity. It may be asked whether these seasonal changes in carrageenan contents and gel strength are no more than indications of seasonal rhythms of growing activity?

In further experimental studies fronds of plants of Gigartina from Portencross which were found to contain more carrageenan were more able to postpone the onset of tissue dehydration than plants from Loch Long which contain less carrageenan. Plants from Portencross were more able to control the loss of chloride ions from the 'free space' when kept in fresh water compared with plants from Loch Long. Although, plants from the different populations showed similar rates of oxygen liberation which represents the rates of photosynthesis, the plants from different populations seem to differ in how they allocate their photosynthates. Plants from Portencross produce more tissue and tough fronds while plants from Loch Long produce bushy forms with thin fronds. The Portencross plants were better able to recover and photosynthise when dried for 12 hours than plants from Loch Long. This was obvious because when plants from Portencross were dried for 12 hours they lost 55% of their initial fresh weight in contrast with 65% of the initial fresh weight lost in plants from Loch Long.

Thus it can be concluded that plants of Gigartina stellata from the three localities in the Firth of Clyde vary in the morphological character, and their carrageenan contents and gel strengths. These variations would seem to be due to environmental conditions, but do appear to be of some functional significance.

Despite the fact that Gigartina stellata was extensively studied by Marshall et al (1949) in the Firth of Clyde, the present experimental and ecological study has shown that there are many features of the biology of this common red alga still to be explored. Some contributions regarding morphological forms, environmental conditions and tolerances of plants and young stages have emerged in the present study. The recognition of the likely part that carrageenan plays in the environmental and seasonal life of the plant is particularly interesting.

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APPENDIX (1a)

1 - Plants from Cumbrae Island:

a - Quadrat No.1.

Length of frond in mm	Width of frond in mm	Thickness of frond in mm	Length of stalk in mm	Diameter of stalk in mm	Area of basal crust in mm^2	No. of erect fronds per basal crust	No. of papillae per sq.cm
120	25	0.40	35	1.5	64	50	23
110	20	0.45	30	1.2	100	41	27
140	20	0.40	32	1.5	40	37	31
130	17	0.40	28	0.9	40	29	25
90	18	0.42	28	0.9	80	44	18
100	20	0.42	25	1.0	60	33	24
110	18	0.35	30	1.2	84	39	20
110	15	0.30	30	1.0	32	28	20
105	15	0.40	19	1.5	42	22	27
120	17	0.40	18	0.85	60	25	31
110	20	0.40	20	0.95	48	29	34
105	22	0.42	23	0.95	81	30	41
120	20	0.40	20	1.0	12	37	25
110	21	0.32	26	1.05	49	55	27
110	17	0.43	24	0.95	12	38	19
95	16	0.45	25	1.0	84	36	33
100	15	0.40	29	1.0	13	51	36
105	15	0.40	30	1.25	10	40	22
110	15	0.30	28	1.3	40	22	24
100	17	0.35	30	1.3	49	43	27
100	20	0.25	25	1.2	72	29	20
95	21	0.40	26	1.2	14	40	26
110	18	0.42	20	1.3	10	36	38
125	18	0.45	29	1.2	112	35	43
105	19	0.40	32	1.25	18	27	40
1: 109.4	18.4	0.39	26.5	1.14	49	36.2	28
11.5	2.6	0.05	4.5	0.2	30	8.4	7.3

APPENDIX (1b)

1 - Plants from Cumbrae Island:

b - Quadrat No.2.

Length of frond in mm	Width of frond in mm	Thickness of frond in mm	Length of stalk in mm	Diameter of stalk in mm	Area of basal crust in mm^2	No. of erect fronds per basal crust	No. of papillae per sq.cm
95	15	0.40	26	1.0	80	30	26
80	16	0.40	20	1.0	30	33	24
110	15	0.40	28	1.5	28	29	19
130	15	0.45	25	1.5	30	37	20
100	15	0.32	23	2.0	64	50	30
105	15	0.45	25	1.0	72	40	27
110	15	0.43	27	1.0	30	41	35
95	18	0.40	30	0.8	64	30	26
75	20	0.40	30	0.85	9	24	34
130	20	0.30	30	0.8	130	27	41
115	16	0.30	26	1.5	54	33	45
105	15	0.45	28	1.0	130	32	19
115	17	0.42	30	0.85	45	17	25
110	15	0.45	30	0.8	64	33	23
105	12	0.45	26	0.85	36	30	32
130	12	0.45	25	0.75	32	36	34
110	12	0.35	25	0.85	56	39	17
90	17	0.38	25	0.95	81	40	22
90	15	0.40	27	0.95	80	27	28
95	15	0.40	24	1.5	16	14	30
85	15	0.42	21	1.25	24	20	30
100	14	0.35	22	1.3	21	27	36
95	17	0.30	28	1.2	48	33	35
130	18	0.40	30	0.9	18	37	27
110	14	0.40	30	1.0	81	38	20
an: 104.6	15.6	0.39	26.2	1.08	52.1	31.8	28.2
). 15.3	2	0.05	3	0.31	32.7	8	7.2

APPENDIX (1c)

1 - Plants from Cumbrae Island:

c - Quadrat No.3.

	Length of frond in mm	Width of frond in mm	Thickness of frond in mm	Length of stalk in mm	Diameter of stalk in mm	Area of basal crust mm^2	No. of erect fronds per basal crust	No. of papillae per sq.cm
	120	18	0.50	30	1.3	96	50	38
	140	20	0.40	30	1.2	50	57	21
	130	21	0.45	28	1.5	64	44	27
	120	15	0.48	25	1.0	100	47	30
	120	20	0.40	20	1.0	84	38	30
	105	20	0.60	25	1.25	96	52	36
	140	13	0.40	30	1.2	150	55	25
	130	15	0.40	35	1.3	96	50	20
	110	18	0.50	35	1.1	120	77	19
	90	10	0.55	33	1.0	80	52	18
	100	12	0.45	30	1.5	80	65	24
	110	13	0.45	27	1.25	100	55	27
	100	17	0.45	26	1.4	80	60	33
	140	19	0.45	25	1.1	48	50	19
	130	10	0.40	20	1.0	64	58	30
	130	13	0.40	35	1.3	100	29	28
	120	15	0.42	18	1.4	80	18	24
	110	11	0.35	20	1.5	120	36	23
	80	15	0.40	18	1.25	4	44	25
	125	18	0.45	28	1.25	80	40	20
	100	15	0.45	30	1.25	240	58	27
	120	17	0.40	35	1.5	144	50	23
	90	20	0.35	30	1.3	100	50	20
	140	23	0.45	30	1.5	70	38	28
	120	21	0.50	26	1.45	25	19	31
an:	116.6	16.4	0.44	27.6	1.27	90.8	47.6	25.8
D.	16.9	3.7	0.06	5.3	0.17	45	13.4	5.4

APPENDIX (2a)

2 - Plants from Portencross:

a - Quadrat No.1.

	Length of frond in mm	Width of frond in mm	Thickness frond in mm	Length of stalk in mm	Diameter of stalk in mm	Area of basal crust in mm^2	No. of erect fronds per basal crust	No. of papillae per sq.cm.
	100	20	0.40	25	2	35	16	30
	95	20	0.42	20	1.5	21	14	21
	110	22	0.45	30	1.4	64	22	17
	100	19	0.40	25	1.5	120	11	12
	130	25	0.45	20	1.25	80	30	22
	100	20	0.60	28	1.15	49	17	20
	110	25	0.42	30	1.15	45	19	27
	95	20	0.45	30	1.2	150	9	30
	100	20	0.50	18	1.2	54	16	29
	115	19	0.50	19	1.25	168	15	20
	135	23	0.45	28	1.2	100	12	21
	110	25	0.45	20	1.25	120	12	23
	120	25	0.45	25	1.2	21	9	30
	115	25	0.43	23	1.25	64	13	20
	130	20	0.40	26	1.5	40	17	19
	145	22	0.42	25	1.5	14	22	17
	105	20	0.45	28	1.2	150	20	31
	110	21	0.40	30	1.2	14	21	36
	90	25	0.55	30	1.15	24	26	27
	105	18	0.45	32	1.2	40	18	20
	110	20	0.50	31	1.25	80	14	21
	85	20	0.55	30	1.2	100	12	25
	110	23	0.50	30	1.3	16	13	20
	100	24	0.40	25	1.2	32	14	21
	110	22	0.45	26	1.25	36	19	26
an:	109.5	21.7	0.46	26.2	1.23	65.5	16.4	23.4
D.	14.2	2.3	0.06	4.2	0.24	46.6	5.1	5.5

APPENDIX (2b)

2 - Plants from Portencross:

b - Quadrat No.2.

	Length of frond in mm	Width of frond in mm	Thickness frond in mm	Length of stalk in mm	Diameter of stalk in mm	Area of basal crust in mm^2	No. of erect frondsper basal crust	No. of papillae per sq.cm
	105	25	0.45	28	1.5	80	9	40
	100	22	0.42	25	1.25	50	11	22
	120	20	0.40	30	1.25	40	14	30
	110	26	0.45	30	1.4	54	20	36
	95	24	0.42	30	1.5	100	21	29
	125	25	0.50	27	1.8	15	12	21
	105	25	0.43	26	1.5	16	14	18
	130	24	0.50	25	1.2	16	17	20
	110	25	0.45	20	1.25	35	10	21
	90	22	0.40	28	1.2	18	11	14
	115	24	0.45	30	1.5	64	11	30
	105	20	0.43	31	1.2	60	19	38
	120	27	0.42	26	1.2	25	24	26
	95	25	0.55	30	1.5	10	23	25
	100	26	0.60	30	1.4	20	20	20
	130	27	0.42	25	1.5	16	7	31
	120	27	0.45	25	1.25	50	12	30
	105	26	0.43	24	1.5	100	16	18
	110	25	0.42	22	1.5	40	18	24
	120	27	0.53	28	1.3	30	11	22
	110	25	0.55	25	1.5	56	10	30
	105	20	0.52	25	1.5	25	31	30
	110	24	0.43	24	1.25	9	29	25
	100	26	0.45	20	1.7	40	29	20
	120	23	0.40	25	1.55	24	20	23
an:	110.2	24.4	0.45	26.4	1.4	39.7	16.8	25.3
).	10.9	2.2	0.05	3.1	0.17	26	6.7	6

APPENDIX (2c)

2 - Plants from Portencross:

c - Quadrat No.3.

	Length of frond in mm	Width of frond in mm	Thickness of frond in mm	Length of stalk in mm	Diameter of stalk in mm	Area of basal crust in mm^2	No. of erect fronds per basal crust	No. of papillae per sq.cm
	130	20	0.45	30	1.5	240	26	44
	110	18	0.35	25	1.4	120	28	29
	90	23	0.45	30	1.25	120	14	33
	100	25	0.50	24	1.18	150	10	30
	95	25	0.50	25	1.75	240	21	36
	145	24	0.45	28	1.15	48	25	30
	100	25	0.47	30	1.6	50	29	30
	90	22	0.48	30	1.25	54	20	29
	115	22	0.35	32	1.25	25	35	25
	100	20	0.45	25	1.8	96	19	28
	130	20	0.48	25	1.6	81	28	29
	130	19	0.50	30	2.0	50	23	30
	115	20	0.60	25	1.25	48	21	36
	120	20	0.48	30	1.18	100	15	38
	95	22	0.35	28	1.60	64	27	40
	100	25	0.70	28	1.5	45	24	42
	100	20	0.40	30	1.55	32	32	29
	105	23	0.30	25	1.5	56	17	30
	120	25	0.55	30	1.25	60	27	35
	140	20	0.70	28	1.2	20	28	30
	130	25	0.45	25	1.5	36	20	40
	95	23	0.55	25	1.25	40	27	28
	110	22	0.70	28	1.25	18	29	22
	125	20	0.40	32	1.2	240	31	25
	100	20	0.45	30	1.3	135	15	32
an:	111.6	21.9	0.48	27.9	1.4	78.9	23.6	32
D.	16	2.3	0.06	2.6	0.23	60	6.3	5.7

APPENDIX (3a)

3 - Plants from Loch Long:

a - Quadrat No.1.

Length of frond in mm	Width of frond in mm	Thickness of frond in mm	Length of stalk in mm	Diameter of stalk in mm	Area of basal crust in mm^2	No. of erect fronds per basal crust	No. of papillae per sq.cm
50	16	0.4	9	0.6	56	27	25
50	17	0.4	10	0.5	50	29	33
75	16	0.3	15	0.5	20	33	20
80	17	0.3	12	0.55	30	14	30
70	15	0.2	10	0.7	24	30	18
70	15	0.3	8	0.8	50	36	32
65	18	0.3	9	0.85	15	30	25
55	15	0.3	7	0.35	15	42	20
55	16	0.4	10	0.75	40	21	23
60	17	0.2	12	0.75	12	18	27
70	17	0.3	10	0.75	64	23	21
75	18	0.4	8	0.7	30	20	25
80	15	0.3	8	0.3	24	26	22
60	14	0.3	9	0.35	20	18	20
50	14	0.4	7	0.3	25	19	27
55	15	0.3	4	0.3	16	24	24
60	14	0.3	11	0.4	16	29	22
55	15	0.3	3	0.45	6	31	26
70	14	0.25	9	0.35	64	40	19
65	17	0.3	10	0.55	50	22	18
60	15	0.4	8	0.55	50	20	35
50	16	0.3	7	0.55	48	30	35
55	15	0.3	10	0.45	120	36	24
50	17	0.4	12	0.5	15	14	20
70	17	0.35	6	0.55	32	15	27
n: 62.2	15.8	0.32	9	0.54	35.7	25.9	24.7
. 9.8	1.3	0.06	2.6	0.17	24	7.9	5

APPENDIX (3b)

3 - Plants from Loch Long:

b - Quadrat No.2

	Length of frond in mm	Width of frond in mm	Thickness of frond in mm	Length of stalk in mm	Diameter of stalk in mm	Area of basal crust in mm^2	No. of erect frondsper basal crust	No. of papillae per sq.cm
	55	15	0.3	5	0.6	20	29	17
	70	15	0.35	8	0.3	25	21	14
	50	17	0.4	12	0.45	9	27	24
	65	14	0.3	10	0.25	5	23	27
	75	15	0.25	13	0.35	36	36	30
	50	15	0.3	11	0.8	3	31	25
	70	15	0.3	10	0.85	4	34	30
	60	18	0.3	8	0.35	6	19	22
	55	18	0.4	8	0.75	25	27	20
	50	17	0.4	6	0.65	36	28	23
	70	16	0.4	5	0.5	10	36	19
	75	15	0.4	5	0.55	14	40	18
	75	16	0.3	7	0.45	30	40	24
	80	16	0.35	9	0.45	40	30	26
	60	16	0.3	15	0.40	35	35	30
	65	17	0.3	10	0.40	16	42	33
	75	15	0.3	12	0.35	36	14	30
	55	15	0.25	6	0.35	25	15	32
	60	18	0.4	5	0.45	9	18	33
	60	15	0.3	5	0.45	6	24	21
	55	17	0.35	5	0.35	10	31	23
	50	16	0.3	4	0.6	30	32	25
	70	17	0.4	3	0.4	15	29	20
	75	18	0.25	6	0.55	16	32	23
	70	17	0.35	5	0.45	8	25	25
an:	63.8	16.1	0.35	7.7	0.48	18.8	28.7	24.6
D.	9.6	1.2	0.05	3.2	0.15	12	7.6	5.2

APPENDIX (3c)

3 - Plants from Loch Long:

c - Quadrat No.3.

	Length of frond in mm	Width of frond in mm	Thickness of frond in mm	Length of stalk in mm	Diameter of stalk in mm	Area of basal crust in mm^2	No. of erect frondsper basal crust	No. of papillae per sq.cm
	70	16	0.3	10	0.55	15	29	11
	65	15	0.4	8	0.7	8	21	14
	75	15	0.25	12	0.75	25	19	16
	60	14	0.4	15	0.35	9	18	10
	70	14	0.3	8	0.45	12	30	8
	60	17	0.3	9	0.60	20	34	16
	70	16	0.25	10	0.35	18	19	22
	80	15	0.25	10	0.45	12	22	25
	70	15	0.3	12	0.45	42	25	11
	55	15	0.3	10	0.35	35	21	13
	50	15	0.3	8	0.65	27	18	12
	55	14	0.35	8	0.8	18	25	16
	60	14	0.3	6	0.7	21	30	9
	70	18	0.4	5	0.45	20	22	8
	65	17	0.4	10	0.45	6	20	14
	55	16	0.25	10	0.45	16	23	19
	60	15	0.3	10	0.45	16	21	10
	70	14	0.2	10	0.40	6	26	20
	55	14	0.2	13	0.60	18	25	16
	65	14	0.4	8	0.75	10	17	15
	75	17	0.3	5	0.35	18	15	15
	70	15	0.3	11	0.8	15	18	9
	75	15	0.4	10	0.7	16	16	16
	65	15	0.3	5	0.4	25	14	14
	50	18	0.3	10	0.45	20	21	13
n:	64.6	15.3	0.308	9.3	0.45	17.9	22	13.9
).	8.3	1.3	0.06	2.5	0.15	8.4	5	4.5

APPENDIX 4:

Antibiotic effects of crustose germlines of the red alga *Chondrus crispus* on benthic diatoms.

Attention has recently been drawn to the lack of experimental data on interspecific competition between marine organisms[1]. Heteroantagonistic interactions between microbial planktonic algae in culture have been described for numerous species[2-8]. The concept of a 'chemical claws' mechanism of interspecific competition between macroscopic marine algae has been postulated for some years[9], and the active liberation of polyphenols ('tannins') from certain brown algae has been shown to induce severe antibiotic effects on bacteria and algae[10-15]. Anti bacterial activity, with some seasonal variation, has been reported for 34 species of red algae (including *Chondrus crispus*), 5 species of green algae and 15 species of brown algae[16]. A winter peak of antimicrobial activity was observed[17]. Variations in antimicrobial activity were also obtained with different regions of thalli, with maximum activity in the meristematic apices of *Chondrus*[18]. The growth of the crustose red alga *Porphyrodiscus simulans* Batt. and *Rhodophysema elegans* (Crouan frat ex J.Ag.) Dixon has been found to be inhibited by secretions of the brown alga *Ralfsia spongiocarpa* Batt. in culture[19], a genus known to produce tannins in appreciable quantities in tide pools[20]. There seem to be no reports to date on antibiotic production by crustose red algae, or by a crustose juvenile stage. We describe here some observations made with germlings of the red alga *Chondrus crispus* in culture.

Carpospore settlements on cleaned glass slides were obtained using fertile plants collected from intertidal habitats on the Island

of Great Cumbrae in the Firth of Clyde. Slides were placed in an enriched sea water medium under 12 h illumination per day at 2400 lux and 12°C. After $2\frac{1}{2}$ months the crustose germlings attained a mean diameter of 0.5 mm, and after $3\frac{1}{2}$ months the crusts were 0.8mm in mean diameter and produced the first erect branches. It was observed that contaminating benthic diatoms in cultures not treated with GeO_2 showed profuse growth on the slides except in the vicinity of the Chondrus germlings, around which clear zones could be observed after 6 weeks. With older crusts (Fig.1a) these clear zones were prominent features on the culture slides, sometimes forming an interlinked series of cleared areas when a number of juvenile plants were growing in close proximity (Fig.1b). The inhibition zone appears to be a complete barrier against invasion by the diatoms. The peripheral zones of cells in the crusts are the meristematic regions, and this antibiotic activity would seem to be a noticeable feature of the growth centres of Chondrus[18]. Crustose germlings of the related red alga Gigartina stellata of similar age and size did not show this clear radial zone in the presence of an accompanying population of benthic diatoms when grown under identical conditions.

Attention has been drawn to the possible involvement of antibiotics in the competitive growths of crustose algae and to the ecological implications[19]. Whilst it is accepted that a charged and smooth glass surface is an unnatural substratum for algal growth, we have noticed that the crustose germlings of C. crispus are more firmly attached than those of G. stellata, which are easily dislodged after 3 months in culture. In the case of G. stellata the benthic diatom growth came close to the advancing border of the crust. One effect of this competitive growth may be the loosening of the chemical bond

between the underside of the Gigartina crust and the glass surface - possibly by means of extracellular products released from the diatoms. The inhibition zones around the Chondrus germlings appear to be effective barriers against competitive encroachment of the space by the diatoms, and the bonding of the germling adhesive substance appears unaffected.

K. Khfaji

A.D. Boney

Department of Botany,
University of Glasgow,
Glasgow, G12 8QQ.

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