THE INTERACTIONS OF CERTAIN LITTORAL DIATOMS

WITH YOUNG PLANTS OF MARINE ALGAE

Rang Huang, B.Sc., M.Sc.

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Department of Botany,
University of Glasgow,
Scotland, U.K.

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To my dear late father

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Summary

The interactions of certain littoral diatoms with young algal plants were studied from various aspects. The distribution of littoral diatoms on the shores of the Isle of Cumbrae, Scotland was also investigated.

Diatom community in the littoral area is probably related largely to the temperature and length of sunshine period. Both the association and interactions of diatoms with macroalgae would seem to involve complex physico-chemical actions. The similarity of epiphytic diatom assemblages on various macroalgae varies with the seasons. For laboratory studies, certain common diatoms in the littoral area were isolated from natural materials.

The results of experimental studies indicated that interactions did occur when diatoms and young plants of marine algae associated, particularly in the very early stage of spore and zygote attachment on the substratum. The principal features of these interactions vary greatly with algal species studied, and are affected by physico-chemical factors. The various interactions would seem to be due mainly to algal extracellular substances and to the mechanical attachment of diatom cells on the germinated spores and zygotes. The antidiatom activities of fucoid germlings and red algal sporelings, as well as the effects of diatom mucilaginous material on the morphogenesis and growth rate of algal sporelings and germlings, were examined in detail. Transformation of photosynthetic products (as dissolved organic matter) between certain littoral diatoms and red algal sporelings was demonstrated by ^{14}C labelled carbon compounds under different experimental conditions. The diatom interactions are of ecological importance in view of the wide distributions of these organisms in nature.

CHAPTER 1

General Introduction

It is a well known fact that with any new non-toxic surface introduced into the sea, or with any natural marine substratum cleaned of the existing cover of living organisms, there follows a series of competitive interactions involving a sequence of settlements of microbial organisms, including bacteria, algae and invertebrate larvae. These mechanisms lead to the formation of colonies of macro-organisms, and are now well known from many marine habitats in different parts of the world and are clearly of great significance in both the fouling and bio-deterioration of man-made structures. Whilst the colonizing sequences have been frequently described, much less is known about the interactions occurring at the microbial level. Diatoms are common early colonizers of such habitats, followed by settlement of stages of macroalgae. Whilst there are occasional reports which indicate that some competitive interactions do occur between sporelings and germlings stages of marine algae and littoral diatoms e.g. (Khfaji and Boney, 1979; Schonbeck and Norton, 1979), the nature and significance of these interactions have not been studied in detail. The researches described in this thesis represent attempts to examine in greater depth the extent to which the presence of diatoms influence the attachment and early development of sporelings and germlings of marine algae.

The researches to be described are the results of a series of experiments, set out in the following chapters. The study was initiated by a seasonal survey of the littoral diatoms. This survey provided fundamental information on the common diatom taxa in littoral habitats and their distributional patterns. To examine which of these diatoms had the more significant effects on young plants of marine algae, some taxa included in 31 diatom clones isolated from nature were

grown in unialgal cultures (Chapter 3). These diatoms were subsequently tested for their effects on the growth and viability of the young plants (Chapter 4), and from these experiments certain diatoms were identified as being of particular significance. The sporelings and germlings used in the experiments were of those common intertidal marine algae with relatively long fertile periods in nature. These species ensured a supply of healthy young plants at most times of the year, and enabled adequate replications of the experiments (Chapter 4).

Most interactions between diatoms and young plants of marine algae occur when they are attached and close to each other on the substratum. The algal interactions measured in this thesis were thus based on the growth phenomena of both diatoms and macroalgae growing in the same cultures. Since algal interactions are closely related to the given environmental conditions it is of interest to know whether and to what extent the change of environmental parameters can influence the features of these interactions. This was further examined with certain significant algal taxa. One might expect that the features of interactions between organisms would vary greatly with the species investigated. The question then arises as to what features of interactions would be of greater ecological importance. The main themes investigated and reported in this thesis have been concerned with a search for the causes of biotic effects of marine algae on diatoms, and vice versa; and to investigate the extent to which photosynthate transferance occurs between diatoms and young plants and vice versa, especially with those diatoms which significantly show particular affinities as epiphytes; and to study the effects of mucilage producing diatoms on the growth and morphogenesis of young plants.

CHAPTER 2

A SURVEY OF LITTORAL DIATOMS ON THE ISLE OF CUMBRAE

2.1. Introduction

The distribution of littoral diatoms in the British Isles has been much investigated. Ghazzawi (1933) described the diatoms in the littoral area of Liverpool and on Post Erin shores in the Isle of Man. Carter (1933) and Hopkins (1964 a, b) studied the estuarine diatoms in Sussex, England. Hustedt and Aleem (1951) gave systematic account of mud-flat diatoms at the Salstone, near Plymouth, and Salah (1952) at Blakeney Point, Norfolk. The detailed work of Aleem (1950a,b,1969) had contributed much to our knowledge of the littoral diatom communities on various substrata as well as their zonal and periodical distributions in relation to macroalgae and tidal levels. At present, very little is known about the seasonal occurrence of littoral diatoms in the Firth of Clyde. The earliest report of Gregory (1857) described some "new" marine diatoms from the deposits of the Firth of Clyde, but relevant studies have rarely been mentioned in the literature since then. Brief comments on certain littoral diatoms of the Isle of Cumbrae have been included in some annual reports of the Scottish Marine Biological Association (summary in Marshall and Boney, 1974). Droop (1955) and Hannah (1979) surveyed microscopic organisms in certain supra-littoral pools of the Isle of Cumbrae, but they failed to show the diatom inhabitants in the pools. Gow and McLean (1982) have given a preliminary account of the benthic diatom communities of the Clyde Estuary. The marine macroalgae and unicellular algae excluding diatoms have been investigated extensively in the past years (Gibb, 1939; Norton, 1974; Clokie and Boney, 1979, 1980). Consequently, a seasonal survey of littoral diatoms in the Firth of Clyde would seem

to be necessary.

Studies on the epiphytic diatoms of macroalgae have led to the identification of certain associations between diatoms and macroalgae (host-epiphyte specificity). To date this feature is not well understood, and there are a number of interpretations (Aleem, 1950a; 1969 Edsbagge, 1966a; Hopkins, 1964b; Round, 1971). Epipelic and epilithic diatoms have appeared frequently in patches and varied greatly with the localities, substrata and even animal grazing (Castenholz, 1961). Various types of artificial or non-artificial substrata have been introduced for diatom attachment in nature either exposed to air or submerged (Gumtow, 1955; Sládeček and Sládečková, 1964; Wetzel, 1964; Hanic and Pringle, 1978; Tuchman and Stevenson, 1980). Of these, glass is the one mostly used for various purposes in situ, such as the study of attached community structure and of primary productivity or production (Sládeček and Sládečková, 1964; Riznyk, 1973; Tuchman and Blinn, 1979). Wohlschlag and Hasler (1951) and Admiraal(1977), however, transferred natural sediments to the laboratory and examined diatom development under cultural conditions.

The attachment and abundance of littoral diatoms on various substrata are much subject to environmental conditions. Most ecological studies of marine littoral diatoms, however, are still limited in the descriptive, qualitative stage of investigation (McIntire and Moore, 1977). The question arises as to how far these environmental factors might have affected the change of diatom communities when two or more diatom assemblages are compared. Further, the maximum diatom diversity and abundance of diatom entities in nature are not easily measured when based on collected materials. Estimations of community structures and variations of collected assemblages by

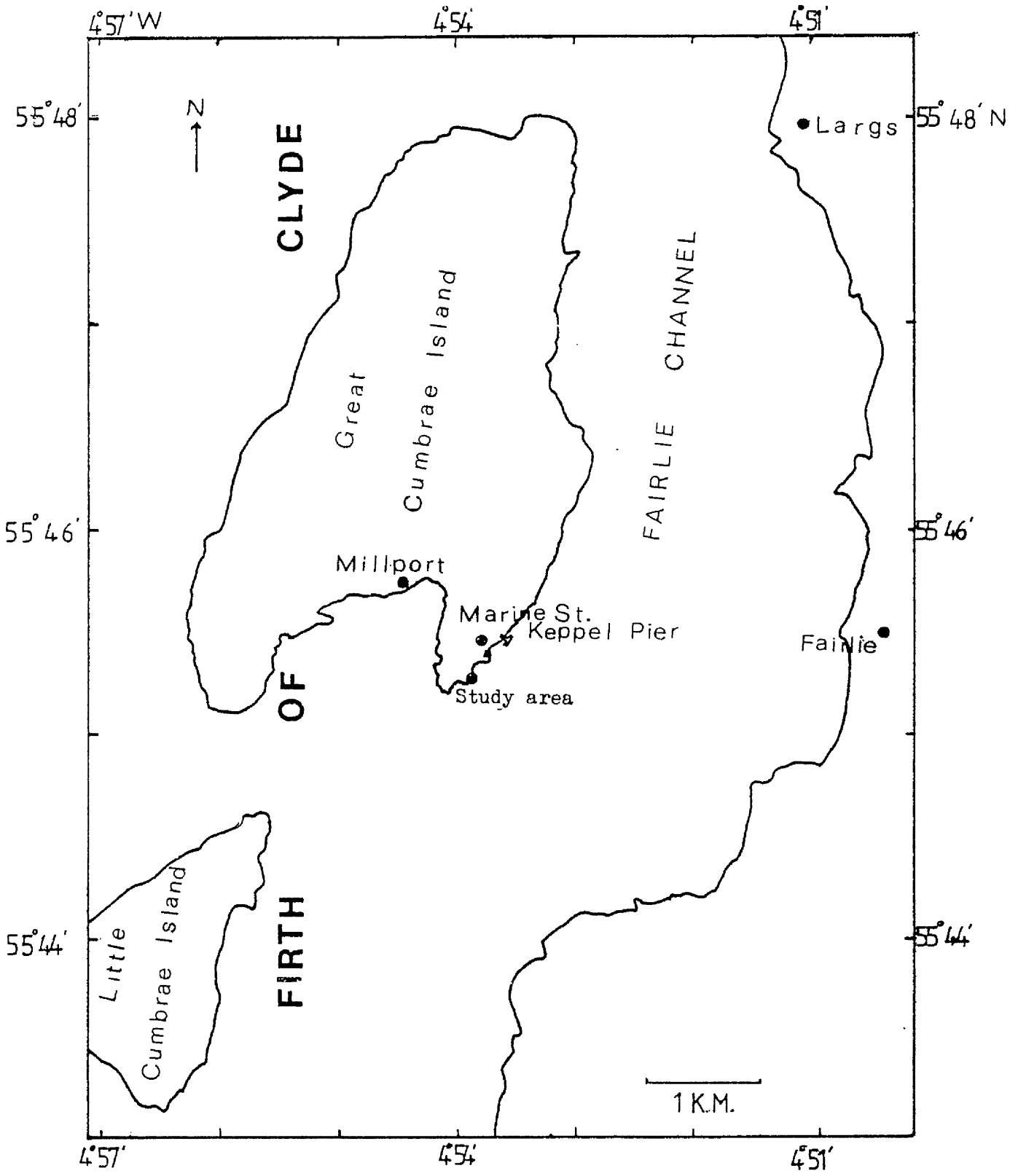
numerical analyses are used increasingly by many authors (Aleem, 1971; Main and McIntire, 1974; Tuchman and Stevenson, 1980; Stevenson and Stoermer, 1981). Such data analyses, undoubtedly, provide more valuable information about the nature of both attached diatom communities and their substrata, and are much more ecologically significant. In the present study, the diatoms in the littoral area, mostly on seaweeds, as well as those in seawater in a locality of Great Cumbrae Isle, Firth of Clyde were investigated. Results regarding the distributional patterns and their characters were also obtained. These studies were considered an essential preliminary to the main theme of this research, i.e., the interactions of certain littoral diatoms with young plants of marine algae.

2.2. Principal study area

As shown in Fig.1. the study area is situated at the southeastern coast of Great Cumbrae Isle in the Firth of Clyde, some 3200m from the western coast of Scotland. In the vicinity of this area are a rubbish heap, the University Marine Biological Station and Keppel Pier, some 100, 350 and 450m respectively to the north. Millport is more than 1000m to the north-north west of the studied area.

The area studied is about 20m long and 15m wide extending from the Laminaria zone to the Pelvetia zone. It is dominated by hard rocks of red sandstone in the upper and spray zones. A few rocks in a small part of the lower shore form a small cliff-like feature. Large boulders and stones are scattered in most parts of the area and there are several small tide pools in the middle shore. The areas of pebbles and sand grains are restricted mostly in the lower shore and separated by the rocks. Behind the shore is a wide grass area extending to the main road of the Great Cumbrae

Figure 1 Map showing the localities of study area, Marine Biological Station, Keppel Pier and Millport on the Great Cumbrae Isle.


4°57'W
4°54'
4°51'
55°48'
55°46'
55°44'
N
CLYDE
Great Cumbrae Island
FAIRLIE CHANNEL
Largs
Millport
Marine St.
Keppel Pier
Fairlie
Study area
FIRTH OF
Little Cumbrae Island
1 K.M.

Isle some 50m to the west. The whole shore of the study area inclines moderately toward the sea.

The marine algal communities present are characteristic of sheltered shores (Gibb, 1939). The algae displayed in this area are considerably clear in the formation of algal zones by certain common seaweeds. With a lower growth rate and the highest capacity to withstand desiccation and high air temperature, Pelvetia canaliculata (L.) Dene, et Thur. and Fucus spiralis L. occurs in the upper-most shore region, mostly from 2.73m above chart datum. They are then followed immediately in a seaward direction by rapidly growing fucoid algae , Ascophyllum nodosum (L.) Le Jol. and Fucus vesiculosus L. The densely tufted red alga Polysiphonia lanosa (L.) Tandy attaches to and grows on the fronds of Ascophyllum nodosum throughout the year. These three major taxa occupy a broad gently sloping area from M.H.W.L. to M.L.W.L. The serrated wrack, Fucus serratus L., flourishes in the study area between lower shore and a small part of middle shore, where F. vesiculosus is growing below 2.3m (mainly between M.L.W.N. and M.L.W.S.). Its prolonged immersion in seawater results in certain animals and plants becoming attached to the fronds of this alga , e.g., the coiled Serpulid tube-worm, Spirorbis borealis Daudin., Enteromorpha compressa Grev., Ectocarpus spp. and Palmaria palmata (L.) O. Kuntze particularly in spring and summer. The area inhabitated by F. serratus is also covered by the abundant red alga, Gigartina stellata (Stackh.) Batt., particularly on the relatively exposed, steeper rocks. In seaward direction, a large and more or less flat band of the barnacle Balanus spp. rises from the F. serratus area and inclines gently toward the sea, more than 10m away from the lower part of the F. serratus zone. The top of this band is inhabited by plants of G. stellata, F. serratus and Porphyra umbilicalis

(L.)J.Ag. etc. The lower shore is dominated by Laminaria spp., Himanthalia elongata(L.)S.F.Gray, Ulva lactuca L. and P. palmata. The red alga Chondrus crispus is present in small numbers. Algae in tide pools are mainly represented by filamentous algal plants, i.e., Cladophora rupestris(L.) Kutz. and Ectocarpus spp.; other members of red and green algae are also common. In the spray zone above the high tide mark, Enteromorpha intestinalis(L.) Link commonly appears in small patches and is most conspicuous in summer.

The annual growth feature of macroalgae in the studied area is rather regular with an increase of algal taxa in spring and summer and a gradual decrease after summer. In winter only those main taxa described above remain growing and dominant in the study area. Therefore, for the study on epiphytic diatoms only those common and dominant algae were collected and examined.

2.3. Materials and Methods

2.3.1 Sampling

The diatoms in the studied area were collected from different substrata, i.e., macroalgae, artificial substrata and the barnacles. The diatoms in the plankton of the seawater along the shore were also recorded. The times of collections are listed in Appendix 1.

2.3.1.1 Diatoms on macroalgae

The epiphytic diatoms (also including endophytic diatoms) on the ten most dominant macroalgae in the study area were examined in different months. These macroalgae were: Enteromorpha intestinalis, Cladophora rupestris (Chlorophyta), Pelvetia canaliculata, Ascophyllum

nodosum, Fucus spiralis, F. vesiculosus, F.serratus (Phaeophyta), Gigartina stellata, Polysiphonia lanosa and Palmaria palmata (Rhodophyta). The algal fronds which looked free of epiphytes and sediment with the naked eye were excised randomly from the study area, and placed separately in plastic bags by their species. In the laboratory digestion of the excised fronds was done by placing 5gm algal fronds (wet weight) in boiled concentrated nitric acid (Main and McIntire, 1974 ; Sullivan and Reimer, 1975) in a beaker. After digestion, the acid solution was diluted with distilled water and the diatom frustules were allowed to settle down on the bottom of a glass cylinder for more than 4 hr. for each occasion. Then the supernatant was decanted and more distilled water was added. This procedure was repeated more than five times until the acid remaining in the solution was washed off. Finally, the frustules were concentrated to 5ml in distilled water and kept in a small vial with several drops of alcohol to prevent fungi from forming in the preserved material. For mounting diatom frustules, three drops of frustule suspension were added onto a cleaned microscopic cover slip and dried gently on a hot plate under the dust-free condition. Then the cover slip was mounted on a glass slide in Pleurax mounting medium (Hanna, 1949). Two replicates were made for each sample of macroalgae.

2.3.1.2 Diatoms on artificial substratum

For the collection of non-epiphytic diatoms in the study area microscopic glass slides were used as an artificial substratum for diatom attachment. The slide was fixed in a slide tray similar to that used by Hruby (1977). On the seashore the trays bearing slides were either fixed directly on the surface of rocks chosen randomly from the studied localities with the aid of an adhesive

material, Araldite (Quick-setting Epoxy Resin CIBA-GEIGY, Plastic and Additives Company, Cambridge), or on large stones and brought to the localities after fixation during the neap tide. The localities for the fixed slides were divided into four sites according to the macroalgae: (i) F. spiralis zone (S1); (ii) upper A. nodosum-F. vesiculosus zone (S2); (iii) lower A.nodosum-F. vesiculosus zone (S3); (iv) F. serratus zone (S4). In each site five to eight slides were fixed, and the times of fixation are shown in Appendix 1. During the investigation, it was seen, however, the slides fixed in S1 were much more exposed to sunlight during the daytime owing to the less density and smaller plants of F.spiralis; while those in S2, S3, and S4 were frequently covered by dense and large fucoid fronds and they received comparatively less sunlight every day. However, this was considered relevant since this study was mainly concerned with the natural development of littoral diatoms in the study area. After 14 - 27 days, the slides were harvested for the determination of diatom cell density and dominant species on the fixed slides. Unfortunately, most of the slides fixed disappeared or were broken, particularly in S1 and S4,possibly due to the strong tidal force. For diatom materials the slides were detached from the trays and replaced with clean slides for the later attachment. The slides were then placed separately in slide boxes (17.2cm x 8.8cm x 0.9cm), which contained some moistened tissue paper to avoid drying during transportation to the laboratory about 2-3 hr. after collection. Preliminarily determinations of diatom cell density and dominant species were carried out under the Vickers Patholux Microscope at 10 x 1.3 x 40 magnitude; 40 observed fields were examined from each slide, and the numbers as well as the taxa were recorded. After determination, the diatoms on the slides were detached with a soft

brush and washed into distilled water in a beaker. Then the diatoms were treated with 5% Hydrogen peroxide solution (ca. 27.5%) and boiled for 10 minutes. Finally, the cleaned frustules were mounted on slides with Pleurax as described for epiphytic diatoms.

2.3.1.3 Diatoms in barnacles

In the study area, the barnacle bed was situated in the lower littoral zone, where G. stellata, Porphyra spp. and Palmaria palmata were growing. Since the upper part of this bed was composed of a soft layer of fragmented shells of barnacles and Mytilus, dead plants and other organic detritus, the yellow-brown debris (Ba) beneath the shells was collected randomly from five places of the barnacle bed with a pointed tool and a brush. The collected samples were combined and suspended in distilled water in a small vial. In the laboratory, acid treatment and mounting procedures for this debris were the same as those for slide-attached diatoms.

2.3.1.4 Diatoms in seawater

The seawater samples were collected monthly from the Keppel Pier of the Marine Biological Station, Millport at full tide. 5 litre plastic bottles were used for the collection. The seawater samples were brought to the laboratory and centrifuged at 15^{o}C and 10,000 rpm with a continuous centrifuge, MSE High Speed 18. Then the concentrated diatom materials were cleaned with acid and finally mounted on slides.

All pertinent slides of this study are now stored in the Department of Botany, University of Glasgow.

2.3.2. Diatom taxa identification and abundance

The collected diatom samples mounted on slides were examined with a microscope at 10 x 1.3 x 100 magnitude. For each of the duplicated slides of an individual sample, 250-300 diatom frustules were identified and counted; however, if, in some occasions, the total numbers of frustules were less than 200 (mostly in F.spiralis and P.canaliculate samples), then all the frustules mounted on the slides were examined. Thus each sample collected consisted of the pooled data from two counts of generally more than 200 frustules and each sample had total number of generally greater than 400. During the data analysis the two counts were combined and considered as a diatom assemblage of the individual sample. Identification and terminology of diatom taxa used in the present study were based mainly on Hendey (1964), Hustedt (1930, 1931-59); Van Heurck (1896) and Smith (1853-56). Some diatom frustules which could not be identified to species level were classified only into genera and those uncertain diatoms were placed in "others". Identification below the species rank was not made because of the considerable confusion and questionable validity of many of the subspecific taxa listed in the literature (DeFelice and Lynts, 1978). The lists of all identified taxa with authorities are stored in the Library of Botany Department, University of Glasgow. Those diatoms listed in fact represent only a part of the diatoms in the study area. Some diatoms with weakly silicified cell walls are easily destroyed during acid treatments or they were very difficult to see under the microscope (Hendey, 1964).

2.3.3 Data analysis

The analysis of data followed those used by McIntire and

Overton (1971), Main and McIntire (1974) and Amspoker (1977).

2.3.3.1 Species composition parameters

Species information measure (H") (Shannon and Weaver, 1949).

$$H'' = - \sum_{i=1}^{S} \left(\frac{N_i}{N}\right) \log_2 \left(\frac{N_i}{N}\right)$$

Species diversity index (SDI) (Simpson, 1949).

$$SDI = 1 - \sum_{i=1}^{S} \left(\frac{N_i}{N}\right)^2$$

Where N_i is the number of individuals in the i-th species, N is the total number of individuals in the sample, and S is the total number of species in the sample. H" is a biased estimator of H'. According to the above equations, the larger the value, the less the dominance of individual species, and therefore, diatom species were more evenly distributed in the samples.

2.3.3.2 Redundancy index (RI)

$$RI = \frac{H_{max} - H''}{H_{max} - H_{min}}$$

and $H_{max} = \log_2 S$, $\quad H_{min} = - \left[\log_2 N - \left(\frac{N-S+1}{N}\right) \log_2 (N-S+1) \right]$

Where S is the total number of taxa in the sample and N is the total number of individuals in the sample. RI is a useful measure which indicates the relative degree of species dominance in the samples. The RI value ranges from 0 to 1, and the smaller the value, the less dominance of the individual taxon and the diatom species are more evenly distributed.

2.3.3.3 Similarity index (SIMI) (Stander, 1970 c.f.Amspoker, 1977).

$$SIMI\ (1,2) = \frac{SIM\ (1,2)}{SD(1) \times SD(2)}$$

$$\text{and} \quad SIM(1,2) = \sum_{i=1}^{S} \left(\frac{n_{1i}}{N}\right)\left(\frac{n_{2i}}{N}\right)$$

$$\text{and} \quad SD(1) = \sqrt{\sum_{i=1}^{S} \left(\frac{n_{1i}}{N_1}\right)^2} \qquad SD(2) = \sqrt{\sum_{i=1}^{S} \left(\frac{n_{2i}}{N_2}\right)^2}$$

Where n_{1i} is the number of i-th taxon in the sample 1 and N_{2i} is the number of individuals in sample 2. The similarity index indicates the degree of similarity between two populations of diatom assemblages (used in the present study). The values range from 0 to 1; the greater the value, the greater the similarity between two populations.

2.3.3.4 Niche breadth (Bi) (Levins, 1968)

$$Bi = \exp\left[-\sum_{r=1}^{Q} \left(\frac{n_{ir}}{Ni}\right) \log_e \left(\frac{n_{ir}}{Ni}\right)\right]$$

Where n_{ir} is the number of individuals of the i-th taxon found in the r-th sample, and Ni is the summation of individuals of the i-th taxon found in all Q samples. The value of Bi can range from 1 to Q, and it indicates the tendency of a taxon to be evenly distributed among the samples considered. The larger the value, the greater the tendency of a taxon to be evenly distributed in the samples collected.

2.3.4 Environmental parameters

2.3.4.1 Meteorological information

2.3.4.1.1 Sunshine hours

The records of sunshine hours for Millport, Great Cumbrae Isle, are not available. However, such records from Prestwick Meteorological Office, some 20 miles to the southeast of Great Cumbrae Isle, were obtained. Barnes (1955) and Hannah (1979) indicated

that there is no great variation over the coast of outer Firth and the nearest station on the southwest coast of the mainland.

2.3.4.1.2 Rainfall and wind characters

Rainfall data were also recorded in Prestwick Station. The wind characters, i.e., speed and direction, described by Barnes (1955) and Hannah (1979) were used in this study.

2.3.4.2 Air and seawater temperatures

Air temperature readings were available from the Prestwick Station, while surface seawater temperature was recorded daily at Keppel Pier, Marine Station at 09.00 G.M.T. However, both air and water temperatures at the times of collections were also recorded and read to 0.1^{o}C with a thermometer.

2.3.4.3 Tidal data

The tidal levels were recorded continuously with a Negretti and Zambra tide gauge at Keppel Pier by the University Marine Biological Station. Maximum and minimum tidal levels were obtained.

2.3.4.4 Determination of seawater salinity

50ml seawater sample was collected from the study area with a clean plastic bottle during the collections of macroalgae. Salinity was determined by following the method of Martin (1972), where the salinity (%o) was almost numerically equal to the volume(ml) of silver nitrate solution needed to titrate a 10ml sample and the final value of salinity was obtained after a small correction to the reading from the burette.

2.3.4.5 Determination of pH

The determination of hydrogen ion concentration (pH) in the seawater was done with a pH Meter 17020, Electronic Instruments Limited in the laboratory after collection.

2.3.4.6 Nutrients

Recently, Hannah (1979) had determined the concentrations of nitrate, phosphate and silicate in the seawater of Fairlie Channel of Firth of Clyde which is close to the study area. These data are used for the present study.

2.4 Results

2.4.1 Environmental parameters

2.4.1.1 Meteorological data (see Table 1)

Generally, the length of daily bright sunshine was increased greatly from winter to spring. In December to February, the mean monthly length of sunshine was less than 2hr. day^{-1}, whilst it reached a maximum of more than 7 hr. day^{-1} in April and May, 1980. The mean daily lengths for 1981 (only those records listed in Table 1) was much similar to those in the former year except May, where sunshine was only 5.17hr. day^{-1} compared to 9.84hr. day^{-1} in May, 1980.

Based on the records of total rainfall, 1980 was a somewhat dry year; in most months of 1980 the amounts of rainfall were less than those recorded between 1941-1970. The records in Table 1 showed that in the early part of the year the rainfall dropped from 81mm in January to 52-58mm in March and April, and thereafter throughout the summer months the total fall increased and reached a maximum of 101mm in September. Then the amount of rainfall decreased to 87-97mm

Table 1 Monthly air temperatures, sunshine, rainfall, snow and sleet recorded at Prestwick station (by courtesy of Meteorological Office, Edinburgh)

		J	F	M	A	M	J	J	A	S	O	N	D	Ann.
Air temperature (°C), Mean	(1980)	2.4	5.1	4.1	8.5	11.1	12.3	13.5	14.1	13.5	8.5	6.4	5.7	8.7
Sunshine (h), Mean	(1980)	1.68	1.53	3.77	7.07	9.84	4.14	4.65	4.13	2.64	2.46	2.18	1.00	3.72
	(1981)	1.18	2.84	3.08	6.95	5.17	5.15	4.60	–	–	–	–	–	–
Rainfall (mm), Total	(1980)	81	54	52	3	58	62	83	93	101	97	87	91	912
	(1941–70)	68	53	60	55	22	101	163	148	118	172	96	131	1137
Snow and Sleet (day) Total	(1980)	7	4	9	0	0	0	0	0	0	0	2	5	27

to the end of the year. Snow and sleet occurred from January to March and from November to December. Within these two periods the amount of rainfall increased as described above.

Barnes (1955) indicated that there was only a little change in the mean hourly wind speed at Millport throughout the years investigated. The maximum speed, 13.22 knots, which appeared in October was only 5 knots greater than the minimum in June, while the wind speeds recorded at Prestwick Station in 1977 were high in March and April, generally above 11 knots (Hannah, 1979). The wind directions at Millport were mostly in south-west quadrant. In March and May the north-east wind was prevalent, while Hannah (1979) indicated that only a small part of the wind directions was in south-west quadrant in most of the months recorded at Prestwick Station. In March and May the north-east wind was also not apparent.

2.4.1.2 Air and water temperatures

The fluctuation of mean monthly air temperature recorded at Prestwick Station (Table 1) was quite similar to those recorded during sample collections, (Table 2). The temperature increased from May to September with the higher mean temperatures, 13.5 - 14.1 C^{o} appeared in the late summer and early autumn. On one occasion during the collection the air temperature rose to a maximum of 24.12^{o}C on 12th May. Surface seawater temperatures (Table 2) recorded both at Keppel Pier and in the study area were quite similar with an increase of temperature from late April to early November. The temperatures recorded in the study area ranged from 5.6 to 16.5^{o}C with a maximum value of 16.5^{o}C on 8th August. In autumn and winter, the mean monthly seawater temperatures were very low and ranged

Table 2 Air and surface temperatures, pH values and salinities determined in study area and at Keppel Pier (* recorded at Keppel Pier by courtesy of University Marine Biological Station, Millport).

	Date		Air	Temperature (C^{o}) Water	pH	S(‰)	Temperature* (C^{o}) water
1980	J	11	7.8	7.3	7.44	30.99	7.57
		25	4.5	6.8	7.46	31.73	
	F	26	8.7	7.3	7.40	31.12	7.12
	M	13	6.8	8.2	7.50	31.00	6.97
		27	6.5	6.8	7.72	31.20	
	A	12	9.8	8.0	7.72	31.32	7.90
		29	13.2	9.5	7.48	30.83	
	M	12	24.12	10.3	7.63	31.01	9.75
		29	14.7	10.6	7.62	30.81	
	J	12	19.2	13.5	7.63	31.65	11.70
		25	18.0	12.0	7.48	30.64	
	J	12	19.3	15.3	7.50	29.95	12.50
	A	8	15.8	16.5	7.75	29.55	14.10
	S	7	14.0	14.3	8.00	34.70	13.69
	O	3	8.3	13.3	7.50	30.30	11.69
	N	4	8.0	10.5	7.55	30.75	10.56
	D	13	7.6	7.9	7.48	29.40	8.47
1981	J	12	3.5	6.7	7.57	28.60	6.88
		27	8.0	7.5	7.50	32.50	
	F	4	5.0	7.0	7.70	31.25	6.29
	M	3	4.5	5.6	7.65	32.00	7.17
		17	6.5	6.5	7.92	31.35	
	A	13	11.0	8.5	7.85	31.60	8.91
		28		7.40	7.86	32.53	
	Mean		10.64	9.47	7.62	31.11	9.45

from 6.29 to 11.69°C. Hannah (1979) recorded a minimum surface seawater temperatures of 7.59°C in March, 1976 and 6.99°C in February, 1977. The highest temperatures in July and August, 1976-7 were 14-14.8°C.

2.4.1.3 pH and salinities

The hydrogen ion concentration (pH) in the collected seawater changed irregularly in the year investigated, (Table 2). Therefore, its seasonal variation was not apparent in the results. The pH values ranged from 7.40 to 8.00 with a mean value of 7.62.

The grand mean for the surface seawater salinity recorded at Keppel Pier within 1949-1953 was 32.10‰, (Barnes, 1955), which was very little different from the values obtained in this study, i.e. 31.11‰, (Table 2). The salinities of the study area ranged from 28.60 in January 1981 to 32.53‰ in April of the same year. However, the variation of salinities was not regular in the times of collections as shown in the pH values.

2.4.1.4 Tidal data

Except September, October and November both mean monthly high tide and low tide levels were rather similar in the months of 1980, whereas there was a slight increase in March and May, 1981 when compared to those recorded in the former year, (Table 3). The mean monthly high tide levels ranged from 2.89m in January, 1981 to 3.21m in September, 1980, and the low tide ranged from 0.35m in April, 1981 to 0.91m in September, 1980. The mid-tide level was also slightly higher in autumn months (1.89 - 2.06m); the lowest values of 1.65m and 1.66m were found in April and January, 1981 respectively. Further, the range of distance between mean high

Table 3 Mean monthly tidal levels above the chart datum (m)

	J	F	M	A	M	J	J	A	S	O	N	D
						1980						
Maximum	3.00	3.13	3.07	2.98	3.00	3.06	3.02	3.03	3.21	3.18	3.18	3.05
Minimum	0.50	0.46	0.48	0.44	0.45	0.54	0.52	0.51	0.91	0.74	0.61	0.62
Average	1.75	1.79	1.77	1.71	1.72	1.80	1.77	1.77	2.06	1.96	1.89	1.83
						1981						
Maximum	2.89	2.95	3.16	2.96	3.20	3.19	2.89	3.02	—	—	—	—
Minimum	0.43	0.48	0.63	0.35	0.72	0.58	0.55	0.58	—	—	—	—
Average	1.66	1.71	1.89	1.65	1.96	1.88	1.72	1.80	—	—	—	—

and low tides in 1980 was increased from 2.5m in January to the maximum of 2.67m and 2.59m in February and March respectively, and thereafter it was decreased gradually to 2.30m in September and 2.43m in December. Generally, the tidal rhythms recorded were rather stable over the period of investigation.

2.4.1.5 Nutrients

Hannah (1979) found that the concentrations of nitrate, phosphate and silicate were generally increased from autumn to a maximum in winter, then they were decreased largely in spring to a minimum in summer. The maximum and minimum values were 21.9 and 1.24; 1.32 and 0.31; 12.33 and 0.8µg at 1^{-1} respectively.

2.4.2 Vertical distribution of diatoms in relation to zonation of macroalgae

In the study area, the zonation of macroalgae was rather clear. Therefore, the vertical distribution of littoral diatoms is described according to the distributional patterns of macroalgae from spray zone to lower littoral zone.

2.4.2.1 Enteromorpha intestinalis zone

Achnanthes delicatula Kutz. was the dominant epiphytic diatom from late autumn to the spring of next year (October to April). This taxon was always associated with a large naviculoid diatom, Navicula elegans W. Smith, particularly in November. During the period of April to October, Cocconeis scutellum Ehr., Hantzschia amphioxys (Ehr.) Grun. and Synedra affinis Kutz. were common representatives. In May to June, Cocconeis scutellum, Hantzchia amphioxys, Rhabdonema minutum Kutz. and Amphora ovalis Kutz. became abundant on the Enteromorpha fronds. Other representatives, Achnanthes parvula Kutz, Cocconeis stauroneiformis

(Van Heruck) Okuno, Achnanthes brevipes Ag., Navicula ramosissima (Ag.) Cleve and N. grevilleana Hendey, were also present in the samples examined.

2.4.2.2 Pelvetia canaliculata-Fucus spiralis zone

The uppermost P.canaliculata and F.spiralis were much exposed to light and high air temperature during the daytime of neap tide particularly in summer. The plants bore relatively fewer diatoms than other macroalgae in the study area, and most of the diatom taxa appeared during the spring and summer (April to August); the common diatoms were similar to those on E.intestinalis fronds. In April there were considerably large amounts of Licmophora gracilis Grun. and its related species, whilst Achnanthes brevipes was the most common taxon in December. The endophytic diatom, Navicula endophytica Halse, was common but not abundant on F. spiralis fronds. Its occurrence in P.canaliculata samples, however, was much suspected and probably due to the contamination from fucoid plants. Navicula cancellata Donkin was the common diatom to both brown algae.

The attached diatoms on the glass slides fixed near F.spiralis were largely different from those on algal fronds. Of 27 species (not including centric forms) identified in March only 7 taxa were recognized from F.spiralis samples. The diatom community on the slides was represented mainly by Navicula ramosissima, Achnanthes brevipes, A.longipes (Ag.) Cleve and Rhabdonema minutum.

2.4.2.3 Ascophyllum nodosum-Fucus vesiculosus zone

This zone received a rather stable tidal rhythm daily. The diatom assemblages collected from these two plants were dominated by Cocconeis scutellum, Navicula endophytica and Achnanthes parvula;

the former occupied approximately 65%* of total individuals observed on A.nodosum (April) and F.vesiculosus (March). It is very interesting that the period of relative abundance of Navicula endophytica differed for each host plant ; In October it was associated with A.nodosum and April to May with F.vesiculosus. Cocconeis stauroneiformis was common on both brown algae during June to November. Synedra investiens W. Smith was a common taxon on F.vesiculosus but not A.nodosum. Other diatom taxa appeared mainly from June to November on A.nodosum and March to August on F.vesiculosus fronds.

The red macroalga, Polysiphonia lanosa, attached to A.nodosum was rich in diatoms. The diatom assemblages were also dominated by Cocconeis scutellum and its related species throughout the year representing about 95% of total individuals examined. Achnanthes parvula, Gomphonema species No. 1 and 2, Navicula ramosissima, Synedra affinis and S.investiens were common in the assemblages. There were 27 identified taxa on either P.lanosa or A.nodosum and 9 taxa for both macroalgae in July, while there were 25 and 2 taxa respectively in August. Similarity values of diatoms from P.lanosa or A.nodosum or in sample collections were not significant.

The diatom flora attached to slides in this zone also differed in its species composition from those on macroalgae.

The diatoms attached to the slides of the upper (S2) and lower (S3) sites were represented by the following taxa which were not abundant in epiphytic assemblages: Navicula ramosissima, N.grevilleana, Amphipleura rutilans (Trent.) Cleve, Achnanthes brevipes and A.subsessilis, Kutz. However, during April to June, a large quantity of Amphora spp., Biddulphia spp., Nitschia closterium W.Smith appeared prevalently on the slides,

* see supplement to this thesis "Diatoms on the Isle of Cumbrae, Scotland" for the quantities measured.

Table 4 The abundance (cells mm^{-2}) of dominant diatoms and numbers (n) of slides collected in different time periods

Localities	14-28 Apr. Mean ± S.E.	n	26 Apr.-19 May Mean ± S.E.	n	19 May-15 Jun. Mean ± S.E.	n	10-27 Jul. Mean ± S.E.	n
S1	-		16.36	1	80.91	1	-	
S2	16.36	1	268.41 ± 218.72	4	2372.66 ± 715.53	3	37.49 ± 8.56	4
S3	-		670.60 ± 587.04	3	1195.91 ± 761.36	2	163.86 ±134.97	4
S4	2293.64	1	56.72	1	-		-	
Dominat diatoms	Navicula spp. Achnanthes longipes Amphora spp. N.closterium Biddulphia spp.		Amphora spp. Biddulphia spp. Navicula spp. N.closterium Cocconeis scutellum Skeletonema spp.		Achnanthes spp. Biddulphia spp. N.closterium			

(Table 4). The endophytic diatom, Navicula endophytica which was the main taxon in fucoid samples was not found on slides. Other common but not abundant diatoms were Nitzschia fonticola Grun., Rhabdonema spp., Synedra affinis, S.investiens, Melosira moniliformis, (O.F.Muller) Ag., Amphora ovalis, Licmophora spp. and Cocconeis scutellum and its related species.

Several shallow water pools were found in the A.nodosum-F.vesiculosus zone. The pools were inhabited mainly by Cladophora, Ectocarpus and other macroalgae as described previously. Cladophora rupestris, a green alga, was collected for the investigation of the tide-pool diatom flora. Its diatom assemblages were dominated by Cocconeis scutellum and C. stauroneiformis, whilst in May and June, Grammatophora oceanica Ehr, Synedra investiens, Roicosphenia curvuta (Kutz.) Grun., Rhabdonema minutum and Navicula shared equally the dominance with the former diatoms. Amphora ovalis was common mostly in November through May of the next year. Other minor entities, e.g., Achnanthes parvula, Cocconeis discul-oides Hust., Navicula ramosissima and Gomphonema spp. frequently appeared in tide-pools. The planktonic forms, e.g., Coscinodiscus spp. and Thalassiosira spp. were also found on some occasions.

2.4.2.4 Fucus serratus zone

Apart from a large quantity of Navicula endophytica, which occup-ied 47.8 to 99.1% of total individuals, F.serratus bore a few diatoms in June and August. Those diatoms were Synedra investiens, Rhabdonema minutum, R.adriaticum, Cocconeis scutellum and C.stauroneiformis. In March, cell numbers of C.scutellum and Navicula arenarica Donkin in-creased and both species were common in the samples.

Beneath the F.serratus plants, the diatoms attached to the glass

slides predominantly consisted of mucilagineous tube-forming diatoms, i.e. Amphipleura rutilans, Navicula grevilleana and N.ramosissima; those diatoms had generally larger cell size. Some other diatoms were also common in various months of collections, e.g. Stauroneis constricta (W.Smith) Cleve. in spring, Achnanthes brevipes, A.subsessilis and Rhabdonema minutum in summer.

2.4.2.5 Gigartina stellata zone

This red alga in the lower littoral zone bore a great variety of diatom taxa. The diatom assemblages collected were ccnstantly dominated by the coccoid-form diatom , Cocconeis; the cell number of this taxon increased largely in November to a maximum in May (85.6% of total individuals) and then decreased to the minimum in October (26.3% of total individuals). However, the major components of the diatoms changed seasonally according to their relative abundance, C.scutellum in early spring (March and April), C.placentula throughout the summer, and C.stauroneiformis was prevalent in November. Achnanthes, Navicula, Rhabdonema and Synedra also showed considerable numbers of cells in various months; their representatives were A.brevipes, A.parvula, A.groenlandica Cleve, N.cancellata, R.minutum, R.adriaticum, S.affinis, S.investiens, Licmophora gracilis (Ehr.) Grun. and L.flabellata (Grev.) Ag. Other minor diatoms, e.g., Fragilaria and Grammatophora,had fewer cell numbers in the collected samples.

On Balanus the diatom flora consisted principally of the tube-forming diatom, Amphipleura rutilans, and chain-forming diatom, Rhabdonema minutum; they represented up to 83.9% of total individuals examined. Both Licmophora gracilis and Stauroneis sp. were the common entities in March and July. Whilst Cocconeis scutellum and its related species were abundant on macroalgae they were comparatively fewer on

Balanus.

2.4.2.6 Laminaria zone

In this zone, P.palmata was collected for diatom examinations. P.palmata attached itself either to the rocks or other large algae, e.g., F.serratus,and is immersed in seawater most of the time. Like G.stellata, P.palmata fronds harboured a large quantity of tiny diatoms, and its major taxa varied with the months of collection. In the early spring (March), the diatom flora was dominated by Achnanthes groenlandica, greater than 87.3% of total individuals examined; however, its cell number decreased to only 14.4% of total individuals in April. Cocconeis scutellum, Rhabdonema minutum and Gomphonema species No.2 became relatively abundant in April. In May, considerable amounts of Nitzschia vitrea Norman and L.gracilis occurred in the investigated samples. During the summer these dominant species were replaced by Cocconeis placentula Ehr. and C.distans Greg.;the latter two diatoms occupied approximately 76.5% and 83.3% of total individuals respectively. Diatoms of Navicula and Nitzschia appeared in smaller numbers in the assemblages. Nevertheless the total diatom cell number attached to P.palmata decreased gradually after summer.

2.4.3 Periodical abundance of littoral diatoms

Most of diatom taxa collected randomly from the study area did not have sufficient cell numbers for the assessment of monthly variation on the abundance. However, there were, generally, two periods of abundance during the collections, one in the early spring (April-May) and the other in autumn (October).

2.4.3.1 Achnanthes spp.

In the study area, most of the Achnanthes species appeared as

minor entities in the epiphytic assemblages except A.brevipes, A.delicatula, A.parvula and A.groenlandica. A.brevipes appeared mainly on large algal plants above the G.stellata zone particularly in summer and winter. In March and July, this diatom appeared on slides as an important associate of Amphipleura rutilans-Navicula ramosissima-N.grevilleana community. In the spray zone, A.delicatula was the most important diatom on the fronds of Enteromorpha particularly in spring and autumn, whilst it rarely developed in summer. The maximum development of this diatom usually coincided with that of Navicula elegans, a large and beautiful diatom, on the collected fronds. Another common diatom, A.parvula, occurred mainly in the lower littoral zone, i.e. on G.stellata and P.palmata fronds, from August to December except a large number (up to 25% of total individuals) occurred on A.nodosum in April. By contrast, the small diatom, A.groenlandica, appeared mainly in the Laminaria zone, and had a maximum growth in the early spring (March), i.e., more than 87% of the total individual cells of assemblage on P.palmata fronds. However, its cell numbers decreased greatly afterwards, i.e. less than 15% of total individuals in April.

2.4.3.2 Amphipleura rutilans

The cells of this diatom always appeared as a colony enclosed by mucous material on the solid substratum. In the study area, it was extremely abundant on the fixed slides as well as on Balanus in March and July. In the latter it occupied more than 80% of total individuals in the samples observed. Periodical variations of abundance were not clear due to the unsufficient samples and the difficulty of observation under the microscope. However, Hendey (1964) indicated that this diatom and its related taxa were mostly common in winter from January to March.

2.4.3.3 Cocconeis spp.

Cocconeis was the most important component in the epiphytic diatom assemblages. It usually formed a Cocconeis community or association in the littoral area particularly on red algal plants. In the studied area, the major taxa were of C.scutellum, C.plancentula and C.stauroneiformis Whilst on the three red algae investigated,these three diatom taxa showed different growth regimes in the sampling months based on their relative abundance. C.scutellum was abundant in March and April, and the cell numbers then decreased gradually to the lowest values in August and November. This decrease occurred after April and was compensated by the increased cell number of C.placentula in May and June and by C.stauroneiformis in August and November. In October, C.scutellum started to increase in numbers and then dominated in the coming spring. The commonly found minor taxa, C.disculoides, C.disculus (Seh.) Cleve., C.distans Greg., C.costata Greg., C.pinnata Greg. and C.californica Grun. appeared on various occasions.

2.4.3.4 Grammatophora spp.

This diatom was one of the most common epiphytic diatoms of marine habitat. It usually appeared in the lower littoral zone and formed a Grammatophora - Cladophora community (Aleem, 1950a,1969). This community was also seen in the study area, with large numbers of Grammatophora cells attached on the tips of Cladophora rupestris fronds in tide pools. The maximum development occurred in May and November, comprising 64.4% and 24.5% of total individuals respectively. The main representatives were G.oceanica and G.marina, (Lyngb .) Kutz. G.serpentina Ehr. was less common in the investigated samples.

2.4.3.5 Licmophora spp.

Licmophora is a widely distributed epiphyte in the lower littoral zone. Although this diatom was never abundant in the study area, some relatively larger cell numbers were found on Palmaria palmata and Polysihonia lanosa (20.48%,14.26% of total individuals respectively) during the spring and summer, i.e., March and August, whilst the diatoms found on Pelvetia canaliculata was probably contaminated from fucoid plants through tidal water. The Licmophora diatom community was composed mainly of L.gracilis and L.flabellata. Other relevant taxa, i.e., L.ehrenbergii (Kutz.) Grun. and L.juergensii Ag., were also frequently found in the collected samples.

2.4.3.6 Navicula endophytica

Except the difference of relative abundance with host plants described previously this unique intercellular diatom also showed seasonal variation in abundance in fucoid plants. In the study area, a large quantity of N.endophytica was found in Fucus serratus and F.vesiculosus. Based on the mean percentage of this diatom in total individuals from F.serratus and F.vesiculosus, N.endophytica had different growth periods with peaks in spring and autumn. In June and August, its relative abundance decreased in contrast to the increase of associated taxa in the algal samples.

2.4.3.7 Navicula ramosissima and N.grevilleana

Both diatoms growing in mucous tubes were widely distributed from the upper to lower littoral zones. However, they did not become dominant or common taxa on algal plants except on P.lanosa in the late autumn. On the glass slides, both taxa developed largely from the F.spiralis zone to the F.serratus zone in March and July. In other months, both diatoms were few in the collected samples.

2.4.3.8 Rhabdonema spp.

The cells of this diatom are usually in a ribbon-like filamentous form and attach themselves to large algal plants with a stalk extruded from the basal cell. R.minutum and R.adriaticum were the main taxa on the investigated macroalgae. Both diatoms appeared mostly in the lower littoral zone and tide pools. Mean monthly abundance showed that Rhabdonema, particularly R.minutum, developed in March and from June to August. In November and December this diatom, though not disappear completely, had fewer cells in algal samples.

2.4.3.9 Synedra spp.

Synedra also appeared frequently as an epiphytic diatom on macroalgae throughout the year investigated. The most important component was S.investiens. This diatom developed well on Cladophora rupestris, G.stellata and Polysiphonia lanosa in June where cell numbers ranged from 5.28 to 32.13% of total numbers in the samples. Some other species, e.g. S.affinis and S.gailonii (Bory) Ehr., were also found in the collected samples.

2.4.4 Diatoms suspended in seawater

The monthly fluctuation of occurrence and some major genera in seawater was shown in Table 5. Generally, diatom cell numbers increased in spring (March to May) and autumn (September to October), similar to the littoral area. In July and November only a few were found in the samples. Seasonal variation of abundance was also demonstrated clearly in some diatoms. In the early spring, the common true planktonic diatoms were Thalassiosira condensata Cleve, T.nordenskioldii Cleve and T.baltica (Grun.) Ostenfeld, whilst the chain-forming diatom, Skeletonema costatum (Grun.) Cleve, was most abundant in April, i.e., greater than

Diatom Genus	1980 M	A	M	J	J	A	S	O	N	1981 J	F
Achnanthes	-	+	+	+	+	+	+	+	+	++	+
Actinocyclus	-	-	++	-	-	-	-	-	-	+	-
Amphiprora	-	-	-	-	-	-	-	-	-	+	+
Amphileura	-	-	-	++	-	++	-	+	-	-	-
Amphora	+	+	+	+	+	++	+	+	-	+	+
Bacillaria	+	-	-	+	-	-	-	-	-	-	-
Chaetoceros	-	-	+	-	+	-	+	+	-	+	+
Cocconeis	-	-	+	+	+	++	++	++	+	+	++
Coscinodiscus	++	+	++	+	-	+	++	+	+	+	+
Cyclotella	-	-	+	+	-	-	-	+	-	+	-
Denticula	+	-	-	-	-	-	-	+	-	-	+
Diploneis	-	-	-	-	-	+	+	-	-	+	-
Ditylum	+	-	-	-	-	-	+	-	-	-	-
Fragilaria	-	-	+	-	+	+	-	+	-	-	-
Gomphonema	-	-	-	-	-	-	+	-	-	+	+
Grammatophora	-	-	-	-	-	-	-	-	-	-	+
Hantzschia		-	-	+	+	-	+	-	-	-	-
Licmophora	-	+	+	++	-	+	+	-	+	-	-
Melosira	+	+	-	+	-	+	++	+	-	-	+
Navicula	+	+	+	++	+	++	++	++	+	+	++
Nitzschia	+	+	+	+	+	+	+	+	+	+	+
Opephora	-	+	+	-	-	-	-	-	-	+	+
Pleurosigma	-	-	-	-	-	-	+	+	+	+	+
Rhabdonema	-	-	-	-	-	-	+	+	-	-	++
Rhizosolenia	-	-	-	+	-	+	-	+	-	+	+
Rhoicosphenia	-	-	-	-	-	-	-	-	-	-	-
Skeletonema	+	++	-	-	-	-	-	-	-	-	+
Stauroneis	-	-	-	-	-	+	-	-	-	-	-
Thalassiosira	+++	-	-	-	-	+	+	++	+	+	+
Tropidoneis	-	+	+	-	-	-	-	-	-	-	-

Table 5 The monthly distributions of common diatoms suspended in seawater

(- absent; + present; ++ frequent to common; +++ abundant)

90% of total diatoms observed based on the frustule counts. In the late spring, the dominance of S.costatum was replaced by Actinocyclus, Chaetoceros and Thalassiosira. Actinocyclus subtilis (Greg.) Ralfs appeare with an amount of more than 15% of total frustule counts in the samples. The common pennate diatom in the North Sea and the coast of Scotland, Tropidoneis (Hendey, 1964), was obtained in spring. Other planktonic forms, e.g., Coscinodiscus spp. and Melosira spp., were frequently observed in the collected samples.

As shown in the Appendix 2, the majority of diatom taxa distributed in seashore water overlapped with those found in littoral habitats. They were dominated by species of Navicula, Nitzchia, Cocconeis and Achnanthes from August through October and into February. The major representatives were N.ramosissima, C.scutellum, C.disculus, C.staurneiformis, N.fonticola Grun. and Achnanthes brevipes. Grammatophora marina was much less common in the samples.

2.4.5 Diatom flora analyses

A total of 39472 diatom frustules collected from the study area were examined and identified, of which 29588 diatoms were on macroalgae, 4783 diatoms on fixed slides and in barnacles and 5101 diatoms in seawater samples. Diatoms which were not classified even to genus level represented less than 0.5% of grand total cell number. Therefore, these unclassified diatoms were not considered as significant source for the evaluation of the nature of diatom communities. Centric diatoms consisted of less than 1% of total diatoms collected from the littoral area, whilst they occupied more than 40% of planktonic diatoms collected from the seawater. There were 7 genera found only in phytoplankton , 13 found only in littoral habitats and 26 were common to both (Appendix 2). Navicula, Nitzschia and Cocconeis were most abundant in both taxa and number. Other genera, Achnanthes, Rhabdonema, Rhoicosphenia and Licmorphora, were also common

in the collections.

2.4.5.1 Diatom community structure

2.4.5.1.1 Niche breadth

The seven most abundant taxa of epiphytic assemblages which had a total frustule number greater than 100 in at least two collections (by month) are used for analyses. Generally, the niche breadths (Bi) shown in Table 6 were rather small and varied greatly with diatom species and times of collections, their mean values ranged from 1.103 (A.delicatula) to 5.477 (N.cancellata) by species, and 1.074 (December) to 3.229 (June) by month (Table 6), N.endophytica and C.scutellum were constantly abundant throughout the year of investigation: however, the latter had a greater niche breadth than the former in the distribution. The niche breadths for pennate diatoms attached to glass slides and in Balanus debris were similar to those on macroalgae. The values ranged from 1.311 (N.grevilleana) to 3.314 (N.ramosissima) with a mean value of 2.259 (Table 7).

Table 7 Niche breadths (Bi) and total numbers of frustules (in parentheses) of most abundant diatoms on slides and in barnacle debris.

	3/3-17/3	10/7-27/7	Mean
Achnanthes brevipes	2.530	2.100	2.315
	(109)	(234)	
Amphipleura rutilans	2.950	2.135	2.542
	(482)	(860)	
Navicula grevilleana	1.311	2.237	1.774
	(104)	(367)	
N.ramosissima	3.314	2.605	2.959
	(382)	(125)	

2.4.5.1.2 Parameters of species diversity

Based on 32 most common epiphytic diatoms in the collected samples,

Table 6 Niche breadths (Bi) and total numbers of frustules (in parentheses) of most abundant diatom taxa in macroalgal samples of various months

	M	A	M	J	A	O	N	D	Mean
Achnanthes delicatula	1.012	1.228	–	–	–	1.138	1.000	1.138	1.103
	(571)	(627)				(139)	(376)	(460)	
A. groenlandica	1.154	2.860	–	–	–	–	–	–	2.007
	(554)	(169)							
Cocconeis placentula	1.184	–	1.568	2.284	1.206	–	–	–	1.560
	(116)		(413)	(939)	(129)				
C.scutellum	4.987	4.428	4.604	4.627	3.750	3.882	3.383	–	4.237
	(1516)	(1790)	(625)	(856)	(226)	(558)	(389)		
C. stauroneiformis	–	–	1.000	3.861	2.106	–	4.889	1.084	2.588
			(157)	(241)	(787)		(593)	(147)	
Navicula cancellata	–	–	–	–	6.468	4.486	–	–	5.477
					(127)	(122)			
N.endophytica	2.372	2.054	2.011	2.146	1.313	3.013	1.632	1.000	1.942
	(152)	(1120)	(1156)	(945)	(567)	(1341)	(687)	(270)	
Mean	2.141	2.642	2.295	3.229	2.968	3.129	2.726	1.074	

(Appendix 3), the community structure of diatom assemblages showed a great variety along with the species and localities of host plants as well as the sampling months. In Table 8, the species diversity indexes (SDI) and information parameter (H") of diatom assemblages of both F.vesiculosus and F.serratus were comparatively lower than those on other host plants; the values were 0.190 to 0.393 and 0.891 to 1.497 respectively. Higher values were obtained from C.rupestris, P.palmata and G.stellata, and therefore, their diatom taxa were more evenly distributed than those of F.vesiculosus and F.serratus. While the higher values for brown algae, P.canaliculata and F.spiralis were due to the scarcity of diatoms attached during the sample collections. For redundancy (RI) (Table 8) the highest value was for F.serratus and the lowest for C.rupestris. This indicated that diatom assemblages in the former was dominated by certain taxon, i.e., N.endophytica, while such a character was less obvious with Cladophora plants.

2.4.5.2 Similarity of diatom assemblages

2.4.5.2.1 On macroalgae

Degree of similarity of those diatom assemblages on different macrophytic hosts is shown in Table 9. The values of similarity (SIMI) demonstrated a great variation over the host plants and months investigated. In general, the values were higher during April and June and decreased in August and October, and the similarity tended to increase when two host plants were grown closer to each other though it was not necessarily so. The diatom community of Enteromorpha was quite different from the other macroalgae. In April, the community structures of diatom assemblages of red algae, P.lanosa, G. stellata and P.palmata, were identical (> 0.99), while after June, the similarity was less obvious. The results also indicated that assemblage structure of F.vesiculosus was coincided with that of

Table 8 The numbers of samples(n), taxa(s), total individuals (TN) and mean ± S.E. of species diversity indexes (SDI), information parameters (H") and redundancy indexes (RI) of 32 selected diatoms on various macroalgae

Macroalgae	n	s	TN	SDI	H"	RI
E. intestinalis	8	25	3401	0.455 ± 0.122	0.966 ± 0.251	0.520 ± 0.130
C. rupestris	8	29	3477	0.626 ± 0.276	1.445 ± 0.191	0.399 ± 0.063
P. canaliculata	6	23	409	0.666 ± 0.046	1.369 ± 0.186	0.424 ± 0.254
A. nodosum	8	21	1168	0.504 ± 0.079	0.940 ± 0.177	0.360 ± 0.072
F. spiralis	4	22	215	0.688 ± 0.090	1.497 ± 0.393	0.554 ± 0.433
F. vesiculosus	7	25	2859	0.374 ± 0.107	0.891 ± 0.258	0.576 ± 0.097
F. serratus	8	25	4095	0.190 ± 0.080	0.393 ± 0.120	0.760 ± 0.066
P. lanosa	8	32	3442	0.536 ± 0.086	1.273 ± 0.224	0.484 ± 0.067
G. stellata	7	27	3462	0.596 ± 0.085	1.486 ± 0.264	0.423 ± 0.722
P. palmata	7	27	2112	0.617 ± 0.114	1.531 ± 0.286	0.355 ± 0.109

	Ei	Cr	Pc	An	Fs	Fv	Fe	Pl	Gs	Pp
Ei		0.000	0.587	0.000	-	0.000	0.000	0.000	0.000	0.000
Cr	0.587		0.755	0.920	-	0.066	0.579	0.978	0.974	0.867
Pc	0.816	0.480		0.770	-	0.109	0.109	0.758	0.759	0.752
An	-	-	-		-	0.068	0.557	0.940	0.931	0.836
Fs	0.692	0.415	0.695	-		-	-	-	-	-
Fv	0.096	0.079	0.072	-	0.527		0.843	0.069	0.071	0.073
Fe	0.024	0.058	0.033	-	0.481	0.993		0.589	0.586	0.521
Pl	0.931	0.623	0.953	-	0.727	0.088	0.033		0.997	0.909
Gs	0.033	0.249	0.026	-	0.071	0.006	0.009	0.041		0.917
Pp	0.018	0.168	0.011	-	0.059	0.002	0.006	0.014	0.993	

Table 9 The similarity values of diatom assemblages on macroalgae in April (upper) and June (lower), 1980.

(Ei = E. intestinalis; Cr = C. rupestris; Pc = P. canaliculata; Fs = F. spiralis; An = A. nodosum; Fv = F. vesiculosus; Fe = F. serratus; Pl = P. lanosa; Gs = G. stellata; Pp = P. palmata)

Continued: August (upper) and October (lower), 1980

	Ei	Cr	Pc	An	Fs	Fv	Fe	Pl	Gs	Pp
Ei		0.041	0.245	0.274	0.256	0.273	0.017	0.018	0.239	0.424
Cr	0.402		0.360	0.596	0.397	0.213	0.007	0.859	0.121	0.076
Pc	0.014	0.886		0.633	0.612	0.613	0.207	0.010	0.169	0.318
An	0.157	0.057	0.061		0.841	0.625	0.235	0.235	0.229	0.218
Fs	0.106	0.000	0.000	0.704		0.543	0.047	0.011	0.214	0.145
Fv	0.152	0.013	0.016	0.997	0.705		0.745	0.035	0.217	0.312
Fe	0.151	0.001	0.005	0.993	0.704	0.996		0.002	0.036	0.015
Pl	0.139	0.098	0.790	0.070	0.015	0.035	0.023		0.067	0.066
Gs	0.061	0.408	0.435	0.066	0.087	0.038	0.042	0.363		0.429
Pp	0.077	0.659	0.224	0.060	0.139	0.010	0.106	0.537	0.642	

F. serratus but not F.spiralis. The generally low values of similarity in the collection during August and October indicated that the diatom distribution was much dependent on environmental conditions .

2.4.5.2.2 On slides and in Balanus debris

The diatom assemblages attached to fixed glass slides and in Balanus debris were also analysed (Table 10). In March, the similarity value decreased from 0.906 between S1 and S2 to 0.160 between S1 and Ba; it was higher between Ba and S4, than between Ba and S3 and S2.

Table 10 The similarity values of diatom assemblages in barnacles and on slides of different localities in March (upper) and July (lower), 1981.

	S1	S2	S3	S4	Ba
S1		0.906	0.765	0.661	0.160
S2	-----		0.670	0.824	0.276
S3	-----	0.159		0.445	0.015
S4	-----	0.174	0.996		0.675
Ba	-----	0.295	0.350	0.407	

During summer (July), the similarity between the collected samples was not apparent except that between S3 and S4. The dominant taxa and abundance of attached diatoms in the studied localities were shown in Table 4. The diatom cell density in localities S1, S2 and S3 were increased from April to May and June and then decreased in late July though the slides were fixed longer in the littoral area in May and June. The values ranged from 16.36 in S1 (April - May) to 2372.66 cells mm^{-2} in S2 (May - June). Nevertheless the cell density varied greatly from slide to slide at the same locality (i.e. high S.E.values). Microscopic observations indicated that the dominant attached diatom taxa were quite similar in the three consecutive months of collections. However, Achnanthes was most dominant in April and June, Biddulphia in May and

July, and Navicula and Amphora in April and May respectively. The weakly silicified cells of N.closterium appeared consistently in a large quantity on the fixed slides. The most common epiphytic diatom , Cocconeis, also appeared in a considerable amount on the slides in the spring.

2.5. Discussion

The periodical development of diatoms on various macroalgae investigated is clearly shown and is similar to that described for the south coast of England (Aleem, 1950a). The two peaks of diatom abundance in the year investigated coincided with the increase and decrease of temperatures in April and October respectively. Both higher and lower temperatures, generally, allow fewer diatoms to develope (Aleem, 1950a). Hopkins (1964a,b) proposed that high temperatures together with desiccation on the shore cause the death of epiphytic diatoms and secretion of mucilage of mud-flat diatoms when their movement is limited. Wulff and McIntire (1972) indicated that the vertical distribution of estuarine diatoms is closely related to light intensity and period of desiccation. Therefore, the rapid increase of length of sunshine hours in April and May probably have had a stimulating effect on the diatom development (Castenholz,1967; Admiraal and Peletier, (1980). Hannah (1979) found that the greatly decreased concentrations of nitrate, phosphate and silicate in spring coincided with the outburst of planktonic diatoms in seawater. However, the relationship between nutrients and the periodical development of littoral diatoms in the present study is doubtful since mineral nutrients can be either diffused or brought into seawater by the tidal force from the sediments(for diatom growth) (Hendey, 1964; Admiraal, 1977). Other environmental factors, e.g. salinity and pH might affect diatom development to a much less

significant level with no dramatical change taking place during the investigation.

Zonal distribution of epiphytic diatoms along the axis of tidal level is closely related to the tidal conditions (Aleem, 1969). In the study area, such zonation is not very clear. Aleem (1950a, 1969) concluded that filamentous algae afford more suitable substrata for attached diatoms than foliose, globose and papillose phytal surfaces and Round's review (1971) indicated that mucilage-producing algae bear relatively few diatoms. However, this was not always true for the investigated algae. In the present study, except for the fucoids, the mucilage producing algae Gigartina stellata and Palmaria palmata also supported greater numbers of diatom cells and their mean species diversity index (SDI) and redundancy (RI) are similar to those of the filamentous algae Cladophora rupestris and Polysiphonia lanosa. Moreover, Main and McIntire (1974) found that the epiphytic diatom flora varies with both the species and individual plants of "host" algae.

The degree of similarity between diatom assemblages varies greatly with seasons. This is mainly attributable to the change of abundance of certain common taxa in response to the physical environments in which they are living. From the results, the difference of community structures between those in upper and lower littoral zones tends to increase with the increase of temperature, i.e. in June and August. However, except in Enteromorpha, Fucus vesiculosus and F. serratus, there is a general trend of increased similarity values when there is a maximum development. The importance of temperature on the selection of dominant species of periphyton on various substrata is also shown (Tuchman and Blinn, 1979). The diatom community appearing on Enteromorpha in the spray zone is rather isolated and extremely different from those of other zones covered regularly by

tidal movements. Main and McIntire (1974) also indicated the variability of estuarine diatom assemblages in correlation with different salinities and exposure periods.

The epiphytic diatom communities in the study area are characterized by a rather low diversity and high dominance of commonly attached taxa. In fact, the most common diatoms in the study area have a considerably small niche breadth in distribution which is limited mainly in certain host plants of the lower littoral zone. Many reports indicated the necessity of living substrata for epiphytic diatom attachment (e.g. DeFelice and Lynts, 1978). Thus it is not surprising that diatoms which appeared abundantly or commonly on algal plants are not so often found on non-living substrata, (i.e. glass and barnacle debris) in the present study. Consequently the nature of phytal substrata play a decisive role in diatom attachment.

Two hypotheses have been proposed regarding epiphyte-macrophyte associations, either physical condition of macrophytes is considered important (Aleem, 1950a, 1969; Hopkins, 1964b; Main and McIntire, 1974)

, or biochemical interactions occur between epiphytes and host plants (e.g. Harlin, 1978). However, in view of the seasonal abundance of the most dominant taxa, i.e. Cocconeis spp. (mostly epiphytes) and Navicula endophytica (endophyte) in the present study, the dependence of association probably involves a physico-chemical complex. This is why the diatom flora often varies between the plants nearby or even on the different parts of the same plant as shown by Hopkins (1964b); Janson (c.f. Round, 1971) and Main and McIntire, (1974). The importance of the physico-chemical complex is supported by the following observations. Firstly, the abundance of N. endophytica in the non-receptacle-bearing fronds of F. serratus and F. vesiculosus but not F. spiralis and Ascophyllum nodosum; the latter appeared to be rich in this taxon in the receptacles (Baardseth, 1966; Häsle, 1968). Secondly, there are different periods of dominance for

the three most common Cocconeis species on red algal fronds, i.e. C.scutellum., C.placentula and C.stauroneiformis. Lastly, the abundance of Cocconeis spp. and N.endophytica are always associated with relatively fewer cells of other taxa. It is of considerable interest to know whether there are biotic interactions between epiphytes or between epiphyte and host plants. Edsbagge (1966a) showed that rapid development of epiphytic diatoms occurs when most of the seaweeds become less active. In observations of Tansen (1972), the relative numbers of both N.endophytica and C.scutellum in fucoid plants vary , and the former is absent when the latter becomes endophytic in Fucus plants, while Tuchman and Blinn (1979) found interactions between attached diatoms in the selection for living host plants at suitable temperatures. Metabolic interactions between periphyton and host macrophytes are well understood (Allanson, 1973).

The diatom flora attached to non-living substrata, i.e., glass and barnacle debris, demonstrates many differences from that on macro-algae. Some epiphytic entities observed in the non-epiphytic assemblages are probably contaminated by thanatocoenoses of dead frustules (Round, 1971; DeFelice and Lynts, 1978). As shown in the results, the similarity of diatom assemblages on substrata increases when their localities are close. This reflects a rather stable distributional pattern in respect to physical gradients along the axis of tidal level.

Although local physical environments affect greatly the diatom development, distributional patterns of most of the taxa observed in the study area of Firth of Clyde are rather similar to those in other coastal areas of British Isles (Ghazzawi, 1933; Aleem, 1950a,1969; Hendey, 1951; Hopkins, 1964a). Therefore, those common diatoms have a capability to survive environmental fluctuations within a certain range. Littoral diatoms

appearing in plankton in large numbers may contribute to the productivity of coastal water to a considerable extent, in addition to serving as food for grazing animals (Castenholz, 1961; Hendey, 1964; Round, 1971).

McIntire and Moore (1977) indicated that the ecological significance of interactions among diatom taxa associated in an assemblage or between littoral diatoms and other plants and animals nearby, has rarely been studied. The present investigation has demonstrated clearly the difference of distributional patterns of common littoral diatoms on substrata in both the abundance and associated taxa present in nature. Diatoms growing either as epiphytes or on non-living substrata were isolated for more detailed studies on their interactions with algal sporelings and germlings, so that the ecological significance of their interactions could be assessed from the laboratory experiments to be described in the following chapters.

CHAPTER 3

ISOLATION OF CERTAIN LITTORAL DIATOMS

3.1. Introduction

During the investigation of littoral diatoms as described in Chapter 2, diatoms of thirty-one clones from various sources of materials were isolated in the laboratory. The dates of collections and the sources of materials are listed in Appendix 4. For isolation, small segments of fronds excised from the tips of macroalgae were pressed slightly into enriched agar medium (Appendix 5): suspensions of scraped materials from rock surfaces, attached glass slides and barnacle debris were streaked directly on the agar surface. The diatoms in the above materials were allowed to grow at 15 ± 1°C, 3000 lux and light-dark regime = 14-10 h. After 7-14 days, the developed diatom colonies were picked off with a sterilized needle under a dissecting microscope and streaked again on fresh agar plates. Afterwards, the diatoms were kept growing on the agar plates under the same conditions as described above. This procedure was repeated several times until diatoms of single species were obtained. Finally, the isolated diatoms were maintained growing in both liquid and solid enriched seawater media for the use of the studies described in the later chapters. Identification of these isolated diatoms was done after acid cleaning and mounting of frustules on microscopic glass slides as described in the previous chapter. Photomicrographs of diatom taxa were taken from the mounted slides using ILFORD Pan F 35mm high contrast films. Some important characters of frustules of certain unknown species are described. Frequency of occurrence described below, each isolated taxon is based on the observations on the materials collected from the study area in Chapter 2. The following systematic account of isolated diatoms is arranged in an alphabetical order of genera and species.

3.2 Systematic account

Achnanthes Bory

Achnanthes linearis (W. Smith) Grun. GB 7*

Cleve-Euler, 1953, p.41, Fig.569.

Remarks: Common but few in number in the littoral area of various tidal levels.

Achnanthes sp. GB 29

Cells form long ribbon-like filaments. Valves broadly lanceolate with apices attenuate. Central area narrowly linear. Striae finely distributed, not distinct, somewhat straight in the centre and slightly convergent towards the apices. Length 19-20μm, breadth 7-8μm.

Remarks: Rarely found in the study area.

Amphora Ehrenberg

A. acutiuscula Kutz. GB 6.

Kutzing, 1844, p.108, pt.5, Fig.32. Van Heurck, 1896, p.134, pt. 1, Fig.5.

Remarks: Found occasionally in both seawater and littoral area.

A. perpusilla Grun. GB 5.

Van Heurck, 1896, p.127-8, pt.1, Fig.12. Hustedt, 1930, p.343, Fig.627. Cleve-Euler, 1953, p.88, Fig.664a-c.

Remarks: Very rare in the study area.

Cocconeis Ehrenberg

C. stauroneiformis (Van Heurck) Okuno GB 9, 31, pt.7, Fig.1.

Hendey, 1964, p.180.

Cocconeis scutellum var. stauroneiformis Van Heurck, Van Heurck, 1896, p.257, Hustedt, 1930, p.339, Fig.792. Hendey, 1951, p.44.

Cocconeis scutellum var. stauroneiformis W. Smith, Cleve-Euler, 1953, p.6, Fig.489e.

* GB etc. represents clone reference numbers.

Remarks: A major epiphytic diatom; appears abundantly and found in various seasons particularly on the red algae, G. stellata, P. lanosa and P. palmata. Abnormal markings were frequently observed in cultures.

Fragilaria Lyngbye.

F. pinnata Ehr. GB 8

Hustedt, 1930, p.142, Fig.141; 1959, p.160, Fig.671. Hendey, 1951, p.36; 1964, p.153.

Remarks: Not uncommon. Frustules extremely variable in both outline and markings.

Fragilaria tabulata (C.A.Agard) Horst · GB 4

Lang-Bertalot,H. , 1980, p.750, Fig.173.

Remarks: Frequently found in few numbers in the samples of littoral area.

Fragilaria sp. GB 10, pt.7, Fig.6.

Cells usually in filaments. Cleaned frustules variable in outline, generally have one or more notches in each frustule. Valves central area narrow, striae seem to be parallel throughout the valves, not distinct.

Remarks: Rarely found in the littoral area.

Guinardia H. Peragallo.

Guinardia flaccida (Castracane) Peragallo. GB 26.

Hendey, 1964, p.141, pt. 5, Fig.5.

Remarks: Rarely found in the collected samples.

Navicula Bory

N. directa (W.Smith). Ralfs. GB 21

Hendey, 1964, p.202.

Pinnularia directa W. Smith. W. Smith, 1853, p.56, pt.18, Fig.172.

Navicula directa W. Smith. Van Heurck, 1896, p.189, pt.25, Fig.722.

Remarks: Frequently found at all tidal levels. Appears mostly in the spring.

N. grevilleana Hendey, GB 25, 30, Pt.7, Fig.3.

Hendey, 1964, p.191, pt.30, Fig.1, pt.40, Fig.2.

Schizonema grevillei W. Smith, W. Smith, 1856, p.77, pt.58, Fig.364.

Navicula grevillei (Ag.) Cleve, Hendey, 1951, p.46, pt.4, Figs.1-6, pt.5, Figs. 1-4, pt.6, Figs.1-13, pt.7, Figs.1-5.

Remarks: Cells form mucilaginous colonies. Very common and widely distributed at all tidal levels, mostly in spring and early summer.

N. seminulum Grun. GB 1,2

Lund, 1946, p.68-71, Fig.5.

Remarks: Common in the littoral area. A common soil diatom of Lund (1946). Its habitat of distribution is probably from fresh water to brackish water.

Navicula sp. GB 22, 23, 24, pt.7, Fig.4.

Cells solitary, Valves lanceolated, slightly inflated in the middle. Central area narrow. Terminal nodules distinct and slightly remote. Striae radiate towards the apices. Two central striae on both sides of the valves shortened. Striae 12-14 in 10μm.

Remarks: Commonly found in littoral area and seawater.

Nitzschia Hassall

N. angularis W. Smith GB 15.

W. Smith, 1853, p.40. Hendey, 1951, p.74, pt.11, Fig.5, 1964, p.281, pt.39, Fig.6.

Remarks: Few in number but frequently found in the littoral area and seawater.

N. closterium (Ehr.) W. Smith GB 16. Pt.3, Fig. A-D.

W. Smith, 1853, p.42, pt.15, Fig.120. Hendey, 1951, p.72, 1964, p.283, pt.21, Fig.8.

Remarks: Very common, sometimes abundant on solid surfaces other than plants. Appears mainly in the spring.

N. fonticola Grun. GB 13, 18

Hustedt, 1930, p.415, Fig.800. Cleve-Euler, 1952, p.88, Fig.1500.

Nitzschia palea var. fontica Grun. Van Heurck, 1896, p.402, pt.17, Fig.557.

Remarks: A small diatom, commonly appears in upper and spray zones in all seasons.

N. lanceolata var. incrustans Grun. GB 19

V. Heurck, 1896, p.401, pt.17, Fig.551. Hendey, 1974, p.291.

Remarks: Found occasionally in the collected samples.

Nitzschia sp. GB 14, pt.7, Fig.5.

Valves feebly arcuate and constricted in the media portion. Apices slightly prolonged and rostrate. Keel central narrow, with small dots, 14-18 in 10µm, the two median sometimes distant, striae obscure. Girdle face narrowly lanceolate. Length 22-24 µm, breadth 3-4 µm.

Remarks: Common but in few numbers in all intertidal levels and in seawater.

Pleurosigma Greville.

P. strigosum W. Smith, GB 17.

W. Smith, 1853, p.64, pt.21, Fig.203. Hendey, 1951, p .63, Pt.11, Fig.7, 1964, p.246, pt.36, Fig.7. Cleve-Euler, 1952, p.22, Fig.1369a.

Pleurosigma angulatum var. strigosum Van Heurck, 1896, p.251, pt.6, Fig.261.

Remarks: Not common. Appears mostly in summer and autumn in littoral area and seawater.

Rhabdonema Kutzing.

R. minutum Kutz. GB 28.

Kutzing, 1844, p.126, pt.21, Fig.II,4. Van Heurck, 1896, p.361, pt.12, Fig.488a. Hendey, 1964, p.172.

Remarks: Very common epiphytic diatom. Appears mainly in the lower littoral zone and tide pools in spring.

Stauroneis Ehrenberg

S. constricta (W. Smith) Cleve GB 3, pt.7, Fig.2.

Hustedt, 1931-59, p.825, Fig.1168.

Remarks: Commonly appears on the fixed glass slides and Balanus debris. Very rare on macroalgae.

Synedra Ehrenberg.

S. affinis Kutz. GB 11, 12

W. Smith, 1853, p.73, pt.12, Fig.97. Hendey, 1951, p.35, 1964, p.162, pt.41, Fig.4.

Remarks: Common epiphytic diatom, particularly in and near the lower littoral zone. Appears frequently with S. investiens W. Smith. Cells usually in ribbon-like filaments. Abnormal outlines and markings of frustules are very common in the cultures.

S. investiens W. Smith, GB 20.

W. Smith, 1853, p.98. Hendey, 1964, p.162.

Remarks: Very common epiphytic taxon, particularly on filamentous algae, e.g., Cladophora and Polysiphonia. Abnormal outlines and markings of frustules also occur in the cultures.

Surirella Turpin.

S. ovata Kutz. GB 27

Kutzing, 1844, p. 62, pt.7, Fig.1-4.

Remarks: Cells very small. Valves length 8-10μm, breadth 3-4μm. Common in the lower littoral zone.

CHAPTER 4

INTERACTIONS BETWEEN CERTAIN LITTORAL DIATOMS ON THE GROWTH AND SURVIVAL OF YOUNG PLANTS OF MARINE ALGAE

4.1. Introduction

The effects of physical and chemical factors on algal growth have been well documented (Knight and Parke, 1950; Provasoli, 1958; Jones and Dent, 1971; Moss and Sheader, 1973; Soeder and Stengel, 1974; Sheader and Moss, 1975; Boney, 1978; Terry and Moss, 1981). Little is known about the interactions between the growth rates of macroalgae and unicellular algae in the environment in which they are associated. The toxicity of the extracellular phenolic substances of the brown alga Fucus vesiculosus to unicellular algae, including diatoms, was reported by McLachlan and Craigie (1964). Crustose plants of brown and red algae also demonstrate antibiotic activity (Fletcher, 1975; Khfaji and Boney, 1979). Polyphenols of brown algae may serve as chelators and subsequently induce diatom growth in the cultures (Ragan et al., 1980). Schonbeck and Norton (1979a) observed an inhibitory effect of diatoms on the early growth of Fucus spiralis.

To date there is a general lack of work to elucidate the nature of these heterogenous interactions, particularly between littoral diatoms and germinated algal spores and zygotes. In the present study, the effects of certain littoral diatoms on the growth of algal sporelings and germlings of marine algae were investigated.

4.2 Materials and Methods

4.2.1 Sporelings and germlings of algae

Fertile fronds of Ulva lactuca L.(Chlorophyta), Gigartina stellata (Stackh.) Batt., Chondrus crispus Stackh. (Rhodophyta), Ascophyllum nodosum L. Le Jol., Fucus vesiculosus L. and F.spiralis L.(Phaeophyta)

were collected from the littoral area near the University Marine Biological Station, Millport, Isle of Cumbrae, Scotland (Fig.1) at different dates (Appendix 7). After collection, the excised fronds were stored for 1-3 days in a cold room at 5°C before the release of reproductive cells.

4.2.2 Release and attachment of reproductive cells

The methods for obtaining diatom-free reproductive cells varied with the species studied. With Ulva lactuca, several pieces of fertile thalli from different gametophytes were washed in cold tap water then several times in sterile seawater. They were then slightly dried on blotting paper at room temperature (13-15°C) for 20-40 minutes. The dried tissue from different plants were then placed separately in beakers with enriched seawater medium (Appendix 5) at 15 ± 1°C 3000 lux and light/dark regime = 14/10 hr per day to obtain the release of gametes. The released gametes were collected immediately according to their phototactic behaviour and mixed in a small flask. Then the zygotes were diluted with sterile seawater to a suitable cell concentration. With Gigartina stellata, the papillae bearing fertile carposporangia were picked from the fronds and placed in 30ml sterile vials with 8-10ml sterile seawater. Washing procedures were carried out by shaking the papillae with a flask shaker at a moderate speed. The seawater was subsequently decanted and replaced with fresh seawater. This procedure was repeated 8-10 times to minimize the possibility of diatom contamination. The washed papillae were then placed in 1-3 drops of enriched seawater medium in a dish under the culture conditions described above. After several hours, the carpospores were released around the papillae. They were then examined under the microscope for attached diatoms. With Chondrus crispus, small pieces of fronds with fertile carposporangia were used to obtain carpospores. The procedure for washing sporangia was the same as with G.stellata. With Fucus vesiculosus, F.spiralis, and

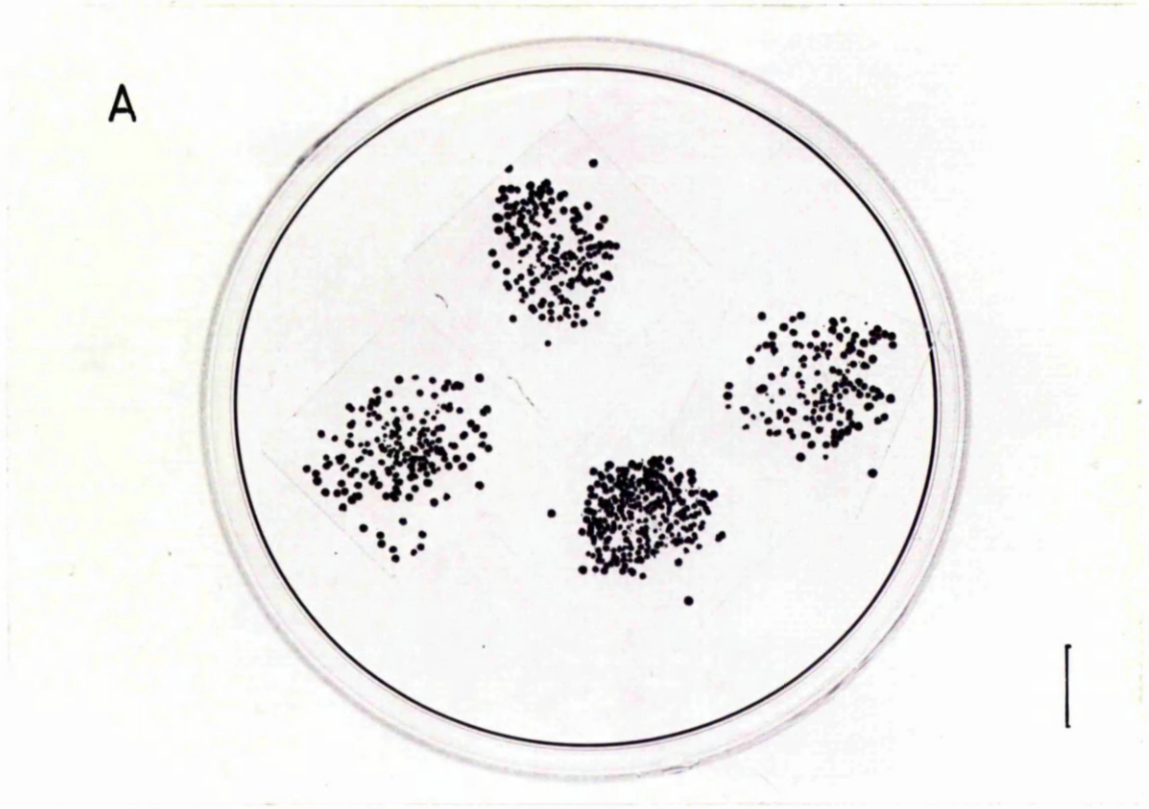
Ascophyllum nodosum, the excised thalli with fertile receptacles were washed with cool tap and sterile seawater several times. They were then slightly dried at room temperature. Afterwards, male and female receptacles were placed separately in beakers of sterile seawater under the conditions described above (separation is not necessary with F.spiralis). After several hours, oospheres and antherozoids were released into seawater and were collected and mixed thoroughly for approximately 30 minutes to facilitate fertilization. The seawater containing free antherozoids was then decanted and the fertilized oospheres were collected and re-suspended in cold sterile seawater in a smaller glass container. The zygotes were allowed to settle for an interval of 1-3 minutes (depending on the volume of suspension), then the seawater was decanted again and the sedimented zygotes were re-suspended in sterile seawater. The above procedure was repeated 8-10 times. This sedimentation method for cleaning zygotes was described by McLachlan et al. (1971).

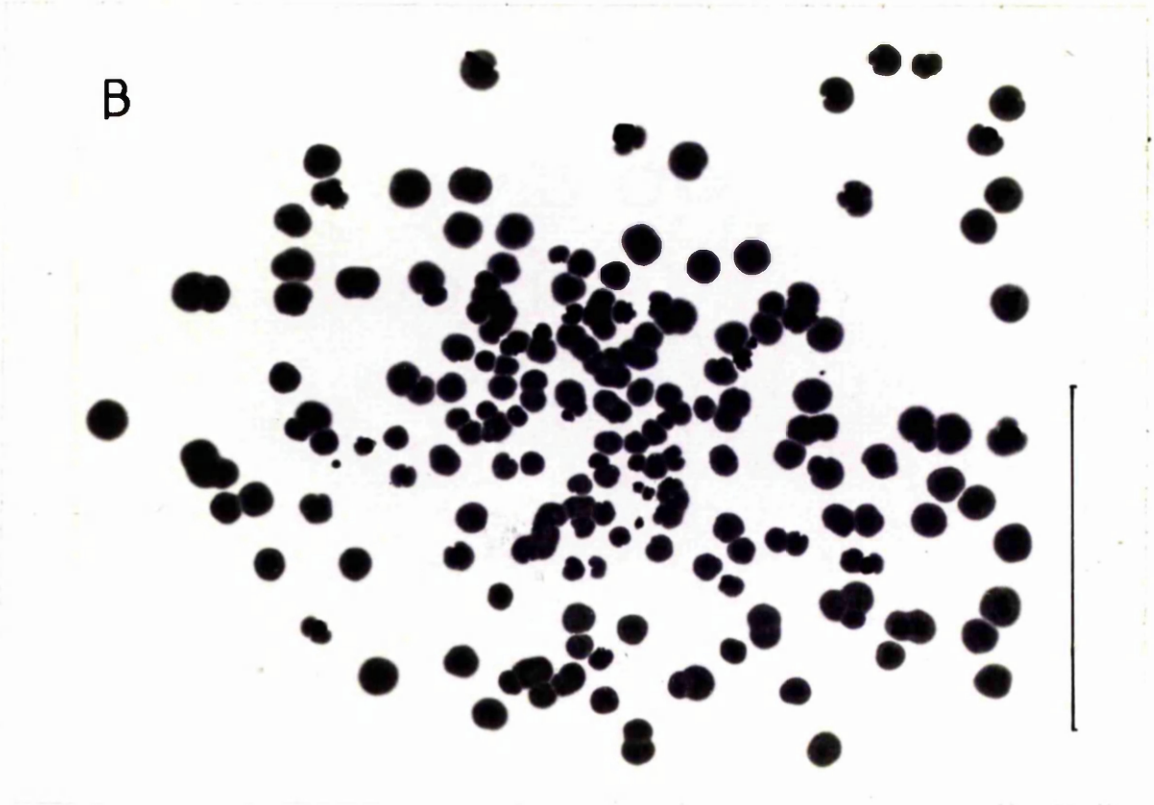
The collected algal reproductive cells described above were diluted to appropriate concentrations with sterile seawater, then 3-5 drops of diluted suspensions were added onto each of the square glass slides (25mm each side) which were submerged in the sterile enriched seawater medium in a 90mm diameter petri dish before addition. Finally, the dish was placed in a growth cabinet under the above conditions. Similar method for allowing algal reproductive cells to attach and grow on glass slides was also described by Reynolds (1950), (plate 1).

4.2.3 Diatoms

The isolated diatoms (Chapter 3) were maintained in both enriched seawater and artificial seawater media (Appendix 6) for several days (Appendix 8) before treatments with young algal plants; the latter medium was modified from that of Harrison et al. (1980).

Plate 1 The diatom-free crustose sporelings of Gigartina stellata (A, B) and Chondrus crispus (C) and germlings of Fucus serratus (D) growing on glass slides; scale = 1cm., A,B= 42 days, C=98 days, D=30 days .


A


B

C

D

4.2.4 Treatment of young algal plants

4.2.4.1 Green and red algae in artificial seawater medium

After 2-6 days of attachment the germinated zygotes of *Ulva* and carpospores of *G.stellata* and *C.crispus* (germlings and sporelings respectively) on the glass slides were washed in three consecutive beakers of sterilized distilled water and then in sterile artificial seawater medium (ASWM) to remove natural seawater. The washed plants along with the slides were placed in 100ml flasks which contained 30ml sterile artificial medium. Then aliquots of 0.1 - 2.0ml of 8-day old diatom suspensions (initially growing in ASWM) were added to the flasks with or without germinated zygotes and carpospores. The final diatom cell concentration was $1.1 - 1.3 \times 10^3$ cells ml^{-1}. Two replicates were monitored for the control and for each isolated diatom clone with or without germlings or sporelings. Finally, the mixed algal cultures were grown at $15 \pm 1^{o}C$, 3000 lux (Philips, Daylight) and light/dark regime = 14/10 hr. With *G.stellata*, the % survival was determined from the initial and final numbers of germinated spores attaching to slides (initial number was obtained from the mean number of attached spores on ten slides randomly taken from the petri dishes).

4.2.4.2 Brown algae in enriched seawater medium

Since the young fucoid plants did not grow well in the artificial seawater medium, the experiments were carried out in enriched seawater medium. Furthermore, in order to avoid luxuriant growth of either algal germlings or diatoms the medium was diluted to 1/10th strength of original medium (Schonbeck and Norton, 1979a). Newly germinated fucoid zygotes were detached easily from the slides during washing and transferring; consequently only germinated zygotes over 10 days old were used in the present study (Appendix 8). The diatom-free fucoid plants were grown in

non-enriched seawater for 2 days prior to experiments. They were then washed in the sterile seawater and placed in flasks with 30ml 1/10th enriched seawater medium. The diatoms initially growing in the same strength of enriched seawater medium were collected by centrifuging at 5000rpm and 15°C for 20 minutes, and were re-suspended in sterile seawater. Aliquots of less than 10ml of diatom suspensions were added to each flask containing 30ml experimental medium and with and without young fucoid plants. The final diatom cell concentration in the flasks were 0.5 - 0.7 x 10^3 cells ml^{-1}. The algal germlings and diatoms were cultured in the conditions as described for green and red algae.

During the experiments, the flasks were shaken carefully and moved to different positions daily so that the flasks were exposed evenly to the light source.

4.2.5 Growth rate determination

After 10-17 days (Appendix 8), the algal cultures were fixed by adding 5 drops of 10% formalin solution. Then the slide with the young algal plants attached was removed from the flask and placed in a 25mm diameter petri dish for the determinations of sizes and number of plants on glass slide under the microscope. Then the algal plants along with the glass slide were returned to the flask for the determination of total diatom cell number in the culture.

For total diatom cell number determination, some 50 glass beads were added to the flask, and the flask was then shaken for 1 minute to accelerate the separation of diatoms from algal plants and flask slides. Cell counts were made with an improved Neubauer Counting Chamber. The total diatom cell number in each flask was estimated from the average of ten counts and the final volume of culture in flask.

4.2.6 Statistical analyses of significance of treatments

Analysis of variance and the Least significant difference were used to test significance of treatments at $p < 0.05$ level. However, in statistical analyses , comparisons of significance were made only between means of control and each diatom-treatment; other comparisons between diatom-treatments were not considered in the present study, because the present study is mainly to know whether and which diatoms can significantly affect the growth of young algal plants and vice versa. In addition, the growth rates of diatoms studied varied greatly from each other under the experimental conditions.

4.3. Results

4.3.1 Effects of diatoms on the germination and growth of algal sporelings and germlings

The effects were greatly variable with the diatom treatments. In Table 11, the length of Ulva lactuca varied largely in the cultures with and without diatoms. Statistical analysis, however, indicated that four diatom species, i.e. Cocconeis stauroneiformis (GB 9), Fragilaria pinnata (GB 8), Nitzschia lanceolata sp.(GB 19) and Navicula directa (GB 21) significantly increased the growth of U.lactuca. In Chondrus crispus (Table 12), the addition of diatom suspensions generally resulted in smaller crusts; there were 16 out of 30 diatom clones investigated which inhibited significantly Chondrus growth. Among them, Nitzschia closterium demonstrated the strongest effect on this red alga, where the mean disc size was about 1/3 of the control. The reduction of % germination and survival of Gigartina stellata carpospores (Table 13) in the presence of diatoms was also indicated clearly, particularly with Stauroneis constricta (GB 3) and Navicula grevilleana (GB 30). However, Cocconeis stauroneiformis and Navicula sp. (GB 23) seemed to be unharmful to the germination and growth of attached carpospores. The effects of diatoms on the fucoid plants were different from those on red algae in that their growth was less affected, (Table

Table 11 Effects of certain littoral diatoms on the growth of *Ulva lactuca* germlings (data based on 2 replicates and 30 germlings in each replicate; data given as mean ± range).

Diatom species	Length (µm)	Diatom growth (a)
Controls	689.00 ± 44.87	
Achnanthes linearis (GB 7)	1144.75 ± 42.57	±
Amphora acutiuscula (GB 6)	931.00 ± 41.04	++
Amphora perpusilla (GB 5)	165.50 ± 13.69	±
Cocconeis stauroneiformis (GB 9)	1493.88 ± 59.45*	---
Fragilaria pinnata (GB 8)	1693.25 ± 53.05*	++++
F. tabulata (GB 4)	289.75 ± 26.76	+++
Fragilaria sp. (GB 10)	1421.00 ± 19.36	±
Guinardia flaccida (GB 26)	723.88 ± 58.59	++++
Navicula directa (GB 21)	1612.38 ± 61.85*	±
N. seminulum (GB 1)	1447.38 ± 87.11	+++
N. seminulum (GB 2)	526.00 ± 37.61	++++
Navicula sp. (GB 22)	1364.13 ± 51.17	++++
Navicula sp. (GB 23)	1269.00 ± 49.69	++++
Navicula sp. (GB 24)	1223.00 ± 40.95	++++
Nitzschia fonticola (GB 13)	524.88 ± 34.40	±
N. lanceolata (GB 19)	1490.13 ± 56.08*	+++
Nitzschia sp. (GB 14)	1083.75 ± 66.37	±
Pleurosigma strigosum (GB 17)	273.40 ± 11.50	++++
Stauroneis constricta (GB 3)	334.63 ± 15.66	+++
Synedra affinis (GB 11)	1310.88 ± 50.67	±
Synedra affinis (GB 12)	831.00 ± 27.41	±
S. investiens (GB 20)	999.00 ± 41.80	+
Surirella ovata (GB 27)	1271.25 ± 36.45	++++
$F = 2.947$ ($p < 0.01$)		

(a) = total cell No. with germlings/total cell No. without germlings x 100%; ++++ = > 200%, +++ = 150–200% ++ = ≥ 130 and < 150% + = ≥ 110 and < 130%, ± = ≥ 90% and < 110%, - = < 90 and > 70%, -- = < 70 and ≥ 50%, --- = < 50%; * significance level at 95%, ** at 99%, *** at 99.9%.

Table 12 The effects of certain littoral diatoms on the growth of Chondrus crispus sporelings (data based on 2 replicates and 30 sporelings in each replicate, data given as mean±range of diameters of crusts; (a) referred to Table 11).

Diatom species	Diameter (μm)	Diatom growth (a)
Controls	257.71 ± 13.00	
Achnanthes linearis (GB 7)	227.54 ± 6.59	±
Achnanthes sp. (GB 29)	169.87 ± 16.34**	++++
Amphora acutiuscula (GB 6)	217.09 ± 8.93	+++
A. perpusilla (GB 5)	187.54 ± 7.34*	+
Cocconeis stauroneiformis (GB 9)	231.65 ± 8.86	±
Fragilaria pinnata (GB 8)	194.60 ± 7.54*	-
F. tabulata (GB 4)	154.94 ± 6.69***	++
Fragilaria sp. (GB 10)	199.75 ± 8.08*	++++
Guinardia flaccida (GB 26)	181.02 ± 6.66**	-
Navicula directa (GB 21)	203.34 ± 5.61*	++
N. grevilleana (GB 25)	170.15 ± 10.28**	---
N. grevilleana (GB 30)	234.91 ± 11.16	---
N. seminulum (GB 1)	193.50 ± 16.43*	-
N. seminulum (GB 2)	200.45 ± 7.05*	-
Navicula sp. (GB 22)	246.99 ± 8.96	++
Navicula sp. (GB 23)	211.60 ± 7.22	-
Navicula sp. (GB 24)	226.37 ± 8.62	++++
Nitzschia angularis (GB 15)	212.67 ± 13.94	-
N. closterium (GB 16)	97.71 ± 3.93***	±
N. fonticola (GB 13)	236.22 ± 1.09	-
N. fonticola (GB 18)	225.28 ± 8.67	--
N. lanceolata (GB 19)	230.98 ± 6.87	±
Nitzschia sp. (GB 14)	167.43 ± 7.05**	--
Pleurosigma strigosum (GB 17)	135.02 ± 3.14***	--
Rhabdonema minutum (GB 28)	270.30 ± 8.67	--
Stauroneis constricta (GB 3)	191.48 ± 8.92*	+
Synedra affinis (GB 11)	202.98 ± 8.54*	±
Synedra affinis (GB 12)	211.98 ± 8.54	±
S. investiens (GB 20)	196.60 ± 7.51*	-
Surirella ovata (GB 27)	256.98 ± 10.64	++++

$F = 3.877$ ($P < 0.01$)

Table 13 Effects of certain littoral diatoms on the % survival of *Gigartina stellata* carpospores (data given as mean ± range; (a) referred to Table 11)

Diatom species	% germination	Diatom growth (a)
Controls	31.60 ± 10.60	
Achnanthes linearis (GB 7)	6.49 ± 3.90	++
Achnanthes sp. (GB 29)	4.76 ± 0.62	±
Amphora acutiuscula (GB 6)	3.03 ± 1.30	-
A. perpusilla (GB 5)	6.28 ± 6.28	++
Cocconeis stauroneiformis (GB 9)	33.77 ± 5.63	+++
Fragilaria pinnata (GB 8)	6.93 ± 3.90	+
F. tabulata (GB 4)	5.84 ± 1.51	++++
Fragilaria sp. (GB 10)	9.31 ± 2.38	±
Guinardia flaccida (GB 26)	2.81 ± 2.81	++++
Navicula directa (GB 21)	7.58 ± 1.95	++++
N. grevilleana (GB 25)	2.16 ± 1.24	---
N. grevilleana (GB 30)	0.00 ± 0.00	++++
N. seminulum (GB 1)	5.63 ± 0.43	±
N. seminulum (GB 2)	20.00 ± 3.46	++
Navicula sp. (GB 22)	8.48 ± 1.51	++++
Navicula sp. (GB 23)	30.74 ± 5.14	+++
Navicula sp. (GB 24)	7.36 ± 3.43	++++
Nitzschia angularis (GB 15)	2.38 ± 1.95	++++
N. fonticola (GB 13)	5.19 ± 1.73	++++
N. fonticola (GB 18)	4.76 ± 3.90	++
N. closterium (GB 16)	4.55 ± 3.68	++
N. lanceolata (GB 19)	4.98 ± 0.65	+++
Nitzschia sp. (GB 14)	5.84 ± 0.21	±
Pleurosigma strigosum (GB 17)	9.74 ± 3.68	++
Rhabdonema minutum (GB 28)	6.71 ± 2.02	+
Stauroneis constricta (GB 3)	0.00 ± 0.00	++++
Synedra affinis (GB 12)	24.24 ± 1.64	---
Synedra investiens (GB 20)	5.40 ± 4.11	+++
Surirella ovata (GB 27)	0.22 ± 0.22	+++

Table 14 Effects of certain littoral diatoms on the growths of 2 replicates and 30 germlings in each replicate : data

Diatom species	*F. spiralis* Length (μm)	*F. spiralis* Width (μm)	Diatom growth (a)
Controls	650.40±30.50	200.14± 7.19	
Achnanthes linearis (GB 7)	554.52±23.53	197.64± 5.92	++++
Achnanthes sp. (GB 29)	515.39±26.34	187.03± 6.07	++++
Amphora acutiuscula (GB 6)	625.58±33.53	211.55± 7.72	++++
A. perpusilla (GB 5)	560.45±33.53	197.64± 5.92	++++
Cocconeis stauroneiformis(GB 9)	589.67±27.85	204.75± 9.39	++++
C. stauroneiformis (GB 31)	604.51±26.83	209.86±11.77	++++
Fragilaria pinnata (GB 8)	600.98±30.00	207.63± 7.00	++++
F. tabulata (GB 4)	546.61±23.00	187.33± 5.77	++++
Fragilaria sp. (GB 10)	640.19±31.35	209.10± 8.19	++++
Guinardia flaccida (GB 26)	587.52±25.68	210.56± 6.96	++++
Navicula directa (GB 21)	707.48±35.13	224.24± 8.03	++++
N. grevilleana (GB 25)	638.48±26.85	195.56± 6.34	±
N. grevilleana (GB 30)	607.90±29.90	199.17± 5.46	++++
N. seminulum (GB 1)	572.76± 6.31	195.10± 6.31	++++
N. seminulum (GB 2)	599.28± 5.92	190.56± 5.92	++++
Navicula sp. (GB 22)	665.65±31.63	218.55± 7.08	++++
Navicula sp. (GB 23)	706.33±35.37	223.24± 8.09	++++
Navicula sp. (GB 24)	628.27±35.34	207.71± 5.54	+
Nitzschia angularis (GB 15)	574.45±25.30	196.52± 6.66	++++
N. closterium (GB 16)	561.38±29.22	193.52± 5.66	++
N. fonticola (GB 13)	470.09±26.61	180.49± 5.28	++++
N. fonticola (GB 18)	585.29±35.16	202.93± 9.58	-
N. lanceolata (GB 19)	645.50±38.50	220.55± 8.11	++++
Nitzschia sp. (GB 14)	735.78±34.44	236.93± 8.01	±
Pleurosigma strigosum(GB 17)	398.19±21.50	151.30± 4.64	++++
Rhabdonema minutum (GB 28)	711.17±32.95	225.32± 9.62	+++
Stauroneis constricta (GB 3)	589.67±18.92	194.33± 9.61	++++
Synedra affinis (GB 11)	682.34±22.34	304.01± 6.80*	++++
S. affinis (GB 12)	686.53±32.74	223.70± 6.36	++++
S. investiens (GB 20)	530.76±33.29	192.10± 6.57	±
Suriella ovata (GB 27)	692.19±30.27	226.47± 6.31	++++
F	1.28(NS)	3.36**	

.fucoid germlings (data based on

given as mean ± range : (a) = referred to Table 11)

F. vesiculosus		Diatom growth (a)	*A. nodosum*		Diatom growth (a)
Length (µm)	Width (µm)		Length (µm)	Width (µm)	
1011.24±39.83	291.84± 7.23		617.43±33.92	237.08± 9.23	
644.04±77.59***	228.47±15.84**	++++	623.74±19.69	244.47± 5.23	−
898.65±51.98	276.69±10.15	++	642.91±23.84	244.39± 5.38	±
652.88±91.51***	232.32±16.46**	++++	617.52±19.60	237.39± 5.38	±
571.37±64.98***	224.24±12.84***	++	670.11±18.54	252.69± 4.15	±
835.14±31.23*	246.23± 4.59*	++++	597.69±28.38	252.85± 7.15	+++
923.65±35.14+	282.07± 8.23	−	637.50±21.97	252.62± 4.54	+
876.27±51.37	264.97± 9.07	++++	573.83±21.83	223.47± 7.84	+
685.18±61.06***	235.70±11.15**	+++	618.43±16.85	237.32± 4.69	+++
829.88±44.29*	250.70± 7.54*	±	680.34±24.45	253.85± 5.92	−
900.73±44.09	266.38± 7.07	+	597.59±20.84	234.92± 6.38	+++
941.48±39.83	275.69± 6.38	+	667.80±28.53	260.38± 8.00*	+++
1018.92±48.68	277.69±10.15	−−	629.99±18.76	245.23± 3.77	−−
968.25±50.98	289.99±13.30	±	553.30±20.38	229.70± 4.00	+
1078.76±42.68	287.91± 8.07	++	588.44±19.92	233.34± 3.77	±
916.88±44.53	273.46± 7.54	+++	606.24±21.38	226.62± 4.15	+++
915.11±40.53	264.07± 8.46	++	586.44±20.61	244.54± 6.61	++++
915.57±42.06	279.22± 8.38	++++	559.30±22.15	237.01± 4.31	+
865.97±30.45	264.15± 6.92	++++	436.48±15.07***	201.32± 1.97***	−−
944.41±41.76	283.30± 8.15	+++	627.35±19.84	236.39± 7.15	+++
836.52±40.68*	247.93± 6.27*	±	618.35±18.07	234.62± 4.46	+
919.57±38.60	282.30± 7.46	++	729.24±25.07**	269.71± 5.84**	+
987.01±39.07	289.61± 8.92	+	639.04±23.30	254.54± 6.92	++
942.80±44.76	276.46± 6.77	+++	687.79±21.69*	250.92± 3.77	++
967.78±38.30	279.47± 7.77	+++	657.42±19.30	252.54± 6.31	−
851.51±43.68	255.85± 8.15	+	597.13±16.84	230.78± 6.92	++
870.12±39.53	266.07± 7.54	++	639.97±22.07	251.23± 4.23	±
734.24±45.37**	258.92± 9.46	++++	589.13±15.69	237.31± 5.31	−
828.21±40.99*	246.08±6.61*	++++	610.28±22.45	244.00± 4.77	±
874.97±39.14	252.39± 6.92*	−−	704.90±17.73*	253.15±13.00	+
947.79±44.76	287.99± 9.30	++++	635.74±17.61	228.09± 6.61	+
868.20±42.68	272.53± 9.92	+	617.13±17.61	240.24± 4.69	±
F = 17.37***	2.19*		3.80***	2.58**	

14). The growth rate of Fucus vesiculosus was inhibited by the diatoms, e.g. Amphora perpusilla (GB 5), A. acutiuscula (GB 6) and A. linearis (GB 7). Growth rates of F. spiralis and Ascophyllum nodosum were rarely inhibited by the isolated diatoms except Navicula sp. (GB 24) with A. nodosum. There were in fact some comparatively larger algal plants observed in diatom-treated cultures, e.g., Navicula sp.(GB13) with A.nodosum and Synedra affinis (GB 11) with F. spiralis.

Whether there was a correlation between growth rates (based on the diameters of crusts and lengths and widths of germlings) and densities of algal plants in the cultures was also determined statistically. The results showed that the correlation coefficient(r) fell between -0.014 to 0.342. Thus, clearly, the growth rates of algal sporelings and germlings showed clear correlations with the diatom treatments, and not with their densities in the cultures.

4.3.2 Effects of algal sporelings and germlings on the growth of diatoms

Most diatoms grew well in both enriched seawater and artificial seawater media whereas, a few diatoms grew slowly in either artificial or enriched medium. Nevertheless the effects of macroalgae on diatom growth were quite apparent in the results. Total cell numbers of most diatoms investigated were greatly increased when they were grown with macro-algae in the cultures, particularly with Fucus spiralis and Gigartina stellata. The majority of diatoms grown with fucoid plants showed growth rates of more than two orders of magnitudes over that of the controls. With Chondrus, however, the growth rate of some 40% of the diatom clones studied was reduced significantly to less than 90% of the controls on the basis of total cell number.

4.4 Discussion

The present results demonstrate clearly that interactions between

benthic diatoms and macroalge do occur, influencing the growth rates of both organisms. The presence of macroalgae in the cultures enhances greatly diatom growth, but the effects of diatoms on the growth of young algal plants are less apparent. However, the interactions of both micro- and macroalgae vary greatly between and within algal species investigated.

In mixed cultures, it is well known that algal growth is, to a great extent, governed by the properties of extracellular substances (Proctor, 1957; Fletcher, 1975). The production of extracellular substances from the algae varies considerably with the species and strains of algae studied. The substances may be either inhibitors or stimulators of algal growth (Lefèvre, 1964). Early reports indicated that the antibiotic substances are released mainly from the growth regions of brown and red algae (Sieburth and Conover, 1965; Khfaji and Boney, 1979). McLachlan and Craigie (1964) found that growth of many unicellular algae, including diatoms, was inhibited by phenolic substances released from Fucus vesiculosus. However, in the present study, the diatom growth was enhanced greatly by fucoid plants, particularly in F. spiralis cultures where the growth rate of the diatoms exceeded that in the control by up to 2 orders of magnitude. It is assumed that, firstly, the toxic organic substances, e.g., phenolic compounds, released from fucoid algae are less toxic to diatoms either due to the inadequate quantities present or to the greater resistance of the diatoms investigated. Secondly, the toxic organic substances from both diatoms and macroalgae might be chelated and neutralized by metal ions in the cultures. The chelation of metal ions by organic substances has been considered as essential in maintaining normal diatom growth in culture studies (Saunders, 1957; Droop, 1961; Davies, 1970). Ragan et al.(1980) also pointed out that the brown algal polyphenols had chelating and detoxicating capabilities. Thirdly, in

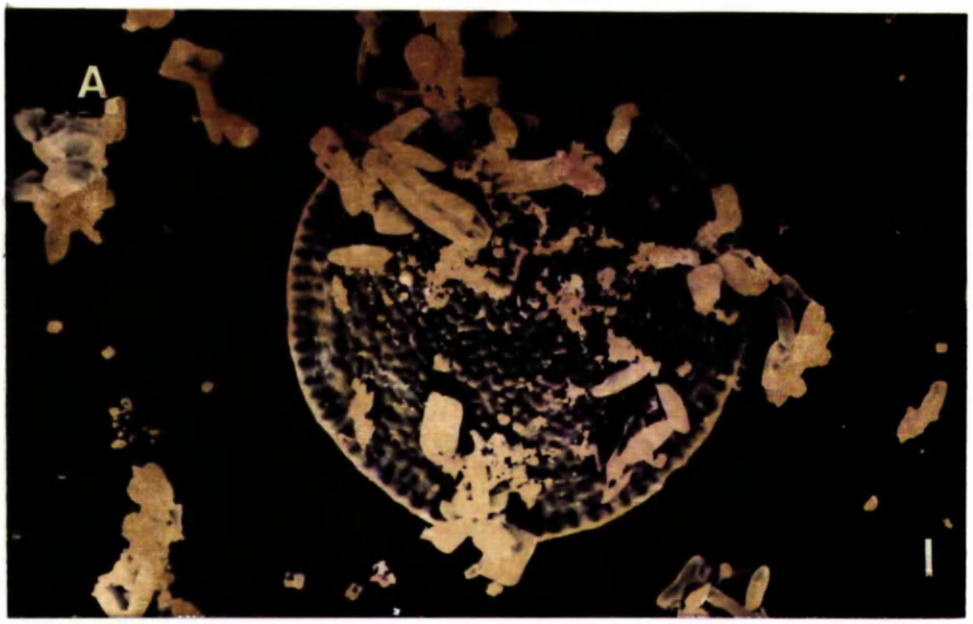
comparison with the control, the increase of diatom cell number in the cultures with young algal plants suggests that certain organic substances, most likely derived from macroalgae, could serve as nutrient substitutes or active agents in the acceleration of diatom growth. In fact, heterotrophic nutrition in marine benthic diatoms is known to be common (Lewin and Lewin, 1960; Round, 1971; Admiraal and Peletier, 1979). The results are different from those observed by Schonbeck and Norton (1979a); they found that diatom numbers were reduced or eliminated in diluted enriched seawater media after being cultured with F. spiralis germlings for about 4 weeks. Possibly the different cultural period (15 days in the present study) are the main cause of the different results. The higher percentage of diatom clones inhibited by C. crispus in artificial seawater medium suggests the antibiotic nature of algal substances (Hornsey and Hide, 1974; Khfaji and Boney, 1979), which are possibly involved in biotic interactions to some extent with diatoms in limited nutrients of artificial seawater.

Although some algal spores and zygotes have the ability to tolerate a wide range of salinities, temperatures, and light intensities (Boney, 1978; Terry and Moss, 1981), some biotic factors, the presence of e.g. diatoms, should not be ignored in the consideration of growth rate of macroalgae in nature. Clearly, as seen in the present results, some isolated diatoms alter the growth rate of young algal plants, particularly red algae, and their effects are exhibited in growth inhibition rather than enhancement. Among the macroalgae studied, F. spiralis demonstrates the greatest growth capacity in the presence of diatoms. On the contrary, germination and survival of red algal carpospores of G. stellata are retarded significantly by diatoms with the exception of Cocconeis stauroneiformis. The question arises as to whether the inhibitory effect is more significantly correlated to age or species of macroalgae since the fucoid algae used were slightly older than red algae. It is quite possible that such reduction in survival

and growth rate caused by associated diatoms in red algal cultures will also occur in fucoid zygotes in the very early stage after germination. Further, the growth rate as well as the morphology of individual algal plants could be of important factors involving in the biotic interactions. For example, the fucoid plants normally grow upright and fast; thus, they decrease largely the diatom stress on the competition for nutrients and space in the cultures. In red algae, on the other hand, the spores germinate and grow relatively slowly, and their discoid encrusting form encourages diatoms to attach. These might cause some physical disadvantages to algal growth, e.g., reduction light intensity by the diatom cover which appears on the discoid growths as well as the possible damage of surface cells by attached diatom mucilaginous materials (Plate 2, B).

Although the diatoms Amphora, Nitzschia and Stauroneis appear more inhibitory to the growth rate of young algal plants than other diatoms investigated in the algal mixed cultures, the results clearly indicated that diatoms which inhibit or stimulate growth of a particular macroalgal species may not have the same effects on other species. Indeed, interactions of algal growth rate vary enormously with environmental conditions which subsequently influence the physiological state of individual cells or plants. Nevertheless, it seems likely that, in general, macroalgae can enhance benthic diatom growth, whilst diatoms affect macroalgae immediately after spores and zygotes attach to the substratum. Possibly some external physical and chemical factors of the cells or plants, as described above, play some important roles in the interactions between micro- and macroalgae. This physico-chemical effect on the interactions between diatoms and young algal plants is further described in Chapter 5.

Plate 2 SEM photographs showing the attachment of diatoms Stauroneis constricta (A) and Cocconeis stauroneiformis (C) on Gigartina crusts and the damaged crust (B) (preparation of algal materials for SEM , see Appendix 11); scale = 10 μm.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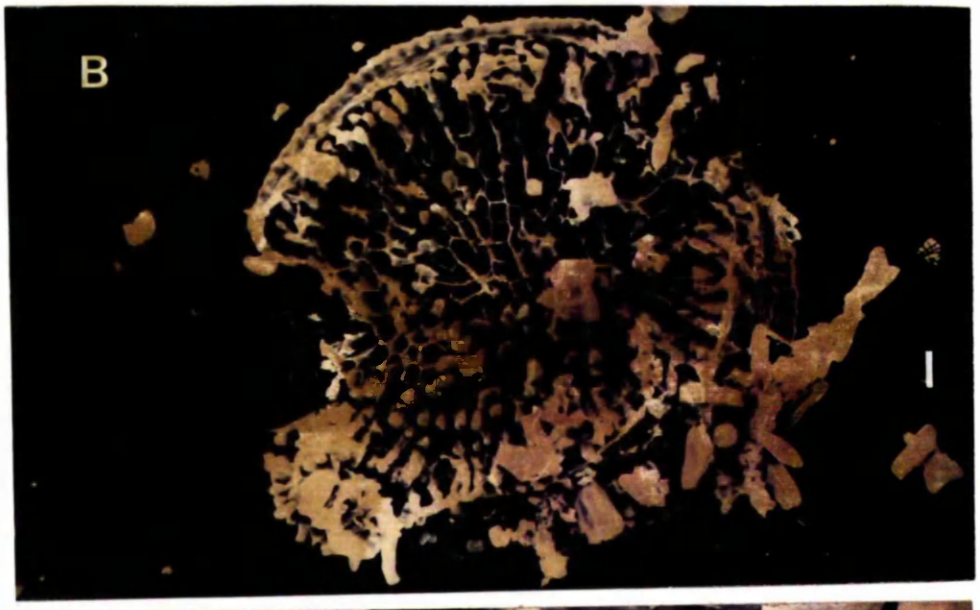
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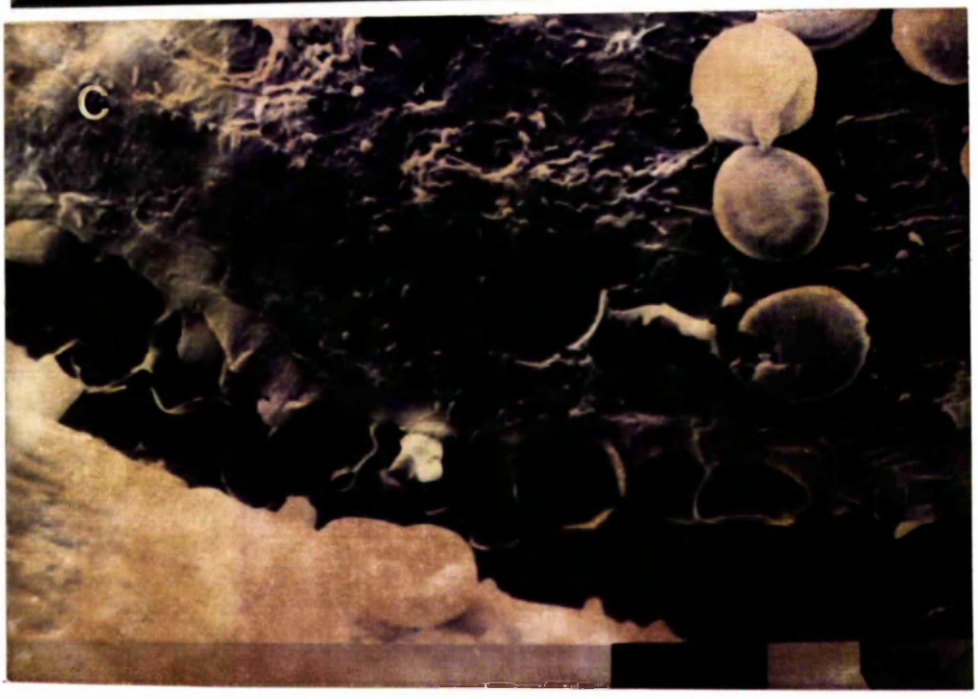
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CHAPTER 5

INTERACTIONS BETWEEN CERTAIN LITTORAL DIATOMS AND YOUNG PLANTS OF MARINE ALGAE: SOME PHYSICO-CHEMICAL FACTORS

5.1. Introduction

Many authors studied the physical and chemical conditions for the growth of algal sporelings and germlings (Jones and Dent, 1971; Mathieson and Burns, 1971; McLachlan et al., 1971; Chen and McLachlan, 1972; Terry and Moss, 1981). Their results showed that a great variety of growth conditions affected the algal species investigated. The possible role of microalgae in affecting growth of algal sporelings and germlings remains little known. The previous chapter indicated that some littoral diatoms significantly affected the growth rate and survival of young macroalgae under constant cultural conditions. Therefore, such effects of associated organisms, i.e., diatoms, should be considered as equally important as those abiotic factors in the evaluation of macroalgal growth in nature. It is quite interesting to know whether and to what extent the diatom effects can be altered by the changes of physico-chemical parameters. In this chapter, more attention is paid to certain isolated diatoms (GB 3,16, (GB 3,9,16) and macroalgae which showed most significant results described previously. The effects of diatoms on the young algal sporelings and germlings were studied under various physico-chemical conditions.

5.2. Materials and Methods

5.2.1 Algal materials

The macroalgae, Gigartina stellata, Chondrus crispus and Fucus vesiculosus, were collected from the same locality as described in previous chapters on several occasions. Methods for cleaning, releasing, and settlement of the reproductive cells, i.e., carpospores (G. stellata and

C. crispus) and zygotes (F. vesiculosus), were described in Chapter 4. According to the previous results, the red alga , G. stellata, was inhibited greatly by isolated diatoms, especially Stauroneis constricta. Therefore, both algae were treated in most of the experiments in the present study. Before experiments, all macroalgae were grown in non-enriched seawater, whilst diatoms were kept continuously in both artificial and enriched seawater media.

5.2.2 Experimental methods

5.2.2.1 Nutrients

5.2.2.1.1 Different nutrient strengths of culture media

Since bacteria-free cultures of macroalgae were not successfully established, initial study by eliminating certain nutrients from the defined medium was not considered in the present study. However, the enriched seawater medium (Appendix 5) was diluted with sterile filtered seawater to different strengths of culture media: full strength, 1/2th, 1/5th, 1/10th and 1/20 enriched seawater media. In this experiment, Gigartina sporelings and the diatom, S. constricta , were grown together in 100ml flasks which contained 30ml of each of the above media.

5.2.2.1.2 Diatom filtrates

The diatom, Stauroneis constricta, initially grown in artificial seawater medium was inoculated into fresh artificial medium during its exponential growth stage, and kept at the conditions of 15 ± 1°C , 3000 lux and light/dark regime = 14/10 hr. After 5, 9 and 18 days of exponential stationary and declining growth phases respectively, 100ml diatom cultures were collected and passed separately through a HA Millipore membrane (0.45μ pore size) in a sterilized filter apparatus. Then the filtrates

of the above cultures were diluted separately with sterile artificial medium (1/2 = v/v). Diatom cell concentrations in the cultures of different growth phases were also determined before experiment. Finally, disc-form sporelings of G.stellata were introduced into the flasks which contained 30ml of the above diluted filtrates, and cultured at the same conditions described above.

5.2.2.2 Light intensities

Gigartina sporelings were also treated with the diatom, S.constricta, in artificial seawater medium. F.vesiculosus germlings were treated in 1/10th enriched seawater medium. Both cultures were placed separately at different light intensities i.e. 1000 lux, 2000 lux, 3000 lux, 4000 lux and 5000 lux under the photoperiod and temperature regimes already described.

5.2.2.3 Temperatures

G.stellata and C.crispus were grown in an artificial seawater medium with or without the diatom S. constricta and other diatoms, and placed separately at temperatures of 15 ± 1°C and 10 ± 1°C. Other experimental conditions were 3000 lux and light/dark regime = 14/10h.

5.2.2.4 Salinities

G.stellata sporelings were grown in artificial seawater media of different salinities prepared by increasing sodium chloride concentration in the defined medium. With F.vesiculosus, the different salinities of 1/10th enriched seawater medium were prepared by allowing seawater to evaporate to 1/2 of its initial volume at 50-60°C. Then the concentrated seawater was diluted with distilled water to required salinities. Nutrient solutions of suitable aliquots were added to each diluted seawater sample to give an identical strength of cultural media, i.e., 1/10th basic medium. Gigartina sporelings and Fucus germlings were treated separately with the diatom, S.constricta, in 30ml culture media under the conditions of 15 ± 1°C, 3000 lux and light/dark regime = 14/10h.

5.2.2.5 Shaking

G.stellata crustose sporelings were placed in 100ml flasks which

contained 30ml artificial seawater medium with or without diatom, S. constricta. Then the cultures were shaken continuously with a Griffin Flask Shaker at lower speed during the experiment for 13 days. The shaking experiment was carried out at the same conditions as described in 5.2.2.4.

5.2.2.6 Determination of algal growth

With the exception of the experiment on temperature effects, the rhizoid-form sporelings of both G. stellata and C. crispus were removed carefully from the slides with a needle, and the disc-form sporelings only were used for the experiments. While in the experiment with temperature, both rhizoid and disc-form crusts were used. Initial and final numbers of crusts were counted and the diameters of disc-form crusts were measured for the determination of % survival and growth rate (assessed by the increased diameter of crusts). The initial and final diatom cell concentrations in some cultures were also determined (Appendix 9).

5.3 Results

5.3.1 Nutrients

Table 15 indicates that the size of the discoid sporelings of Gigartina stellata as well as final diatom cell number was decreased with a decrease of nutrient strength in the culture media. The diameters of disc crusts in both diatom-free artificial seawater and in diatom-treated enriched seawater media were not significantly different. In the presence of diatoms, average diameters in the cultures with nutrient strength less than 1/5th basic medium were less than 1/2 of that in the full strength. In addition, in diluted cultures, the crusts appeared pale in color and unhealthy when compared with those in full strength medium.

The growth rate of Gigartina crustose sporelings was not significantly affected by diatom filtrate of exponential phase, but inhibited

Table 15 Effects of the diatom Stauroneis constricta on crust diameter size of Gigartina stellata in artificial seawater medium (ASWM) and different strengths of enriched seawater medium (BSWM) (Data given as net increases in diameters; data based on 2 replicates and 30 crusts in each replicate).*: Duncan's multiple range test.

Medium	Diameter (μm) Mean ± range	Multiple range test*	Growth assessment	Final diatom cell number (10^5 cells) Mean ± range
BSWM	226.54 ± 7.29		Red, healthy	
ASWM	192.99 ± 7.43		Red, healthy	
BSWM	177.51 ± 4.75		Red, healthy	20.97 ± 1.30
1/2th BSWM	109.13 ± 1.91		Mostly pale, unhealthy	21.48 ± 4.80
1/5th BSWM	83.14 ± 3.59		Mostly pale, unhealthy	16.77 ± 2.21
1/10th BSWM	74.01 ± 2.12		All pale	13.86 ±14.11

Analysis of variance

Source of variance	d.f.	S.S.	M.S.	F
Between media	5	40311.65	8062.33	159.18 ($P < 0.001$)
Error	6	303.89	50.65	
Total	11	40615.54		

Table 16 The crust diameters of *Gigartina stellata* in artificial seawater medium (ASWM) and with diatom filtrates of various growth phases (Exp = exponential phase, Sta = stationary phase, Dec = declining phase; data based on 3 replicates and 40 crusts in each replicate : data given as net increases in diameters).

Medium	Diameter (μm) (Mean ± S.E.)	Multiple range test	Growth assessment	Diatom cell concentration (10^4 cells ml^{-1}) in filtrate
ASWM	109.62 ± 5.25		healthy	
ASWM + Exp.	100.46 ± 5.33		healthy	22.30
ASWM + Sta.	90.63 ± 3.00		healthy	37.50
ASWM + Dec	95.30 ± 2.25		healthy	18.20

Analysis of variance

Source of variance	d.f.	S.S.	M.S.	F
Diatom filtrate	3	345.68	115.22	4.05 ($0.1 > P > 0.05$)
Error	8	227.55	28.44	
Total	11	573.23		

significantly by those of stationary and declining growth phases (Table 16), ($0.01 > P > 0.05$). However, all crustose sporelings grown in both treated and non-treated cultures were healthy and normal in color.

5.3.2 Light intensities

In the presence of diatoms, the inhibited growth rates of Gigartina crusts were quite apparent at higher light intensities up to 5000 lux, and at 5000 lux, the growth rate was 1/4 to 1/3 of those at lower light intensities (Table 17). Correlation between increased diameters of crusts and reduced light intensities was highly significant ($r = -0.71$; $p < 0.005$), whereas there was no significant correlation between crustose diameters and diatom cell numbers in the cultures ($r = -0.042$; $P > 0.1$). With F. vesiculosus, the growth rate was increased with an increase of light intensity both with and without diatom cultures (Table 18). However, average length and width of algal embryos with diatoms were decreased significantly 10-30% of the control. Interactions between light intensities and diatom treatments were not significantly present.

5.3.3 Temperatures

Table 19 showed that the growth rate of G. stellata was also affected greatly by the changes of temperatures. Generally, % survival and growth rate of crustose sporelings were higher at 15°C than at 10°C. However, at higher temperature, i.e., 15°C %, survival as well as growth rate was retarded by associated diatoms, S. constricta and N. closterium, by more than 20% of those in diatom-free cultures.

% survival of algal crustose sporelings also varied with temperatures and diatom species studied. Correlation between numbers and diameter of disc-crusts was not significantly present at 15°C but at 10°C. (at 15°C. $r = 0.31$; $p > 0.1$;

Table 17 The effects of light intensities on the crust diameter size increases of Gigartina stellata in the cultures with or without the diatom Stauroneis constricta (data based on 3 replicates and 30 crusts in each replicate; data given as net increases in diameters).

Light intensity (lux)	% survival	Diameter (μm) (Mean ± S.E.)	Final diatom cell number (10^5 cells) Mean ± S.E.
1000	100.00	165.13 ± 3.67	33.44 ± 8.02
2000	100.00	230.02 ± 13.28	33.41 ± 10.34
3000	100.00	151.50 ± 18.11	33.23 ± 2.42
4000	100.00	137.20 ± 52.50	48.32 ± 1.74
5000	100.00	52.23 ± 17.94	27.85 ± 7.75

Analysis of variance

Source of variance	d.f.	S.S.	M.S.	F
Light intensity	4	48957.05	12239.26	11.34 ($P < 0.01$)
Error	10	10792.50	1079.25	
Total	14	59749.55		

Table 18 Effects of light intensities on the lengths and widths of Fucus vesiculosus germlings in the cultures with or without the diatom Stauroneis constricta (data based on 3 replicates and 30 germlings in each replicate).

Light intensity (lux)	Without diatom		With diatom	
	Length (μm) Mean ± S.E.	Width (μm) Mean ± S.E.	Length (μm) Mean ± S.E.	Width (μm) Mean ± S.E.
1000	272.58 ± 30.28	77.99 ± 6.54	234.82 ± 16.25	65.27 ± 1.35
2000	439.05 ± 10.53	116.96 ± 3.53	352.25 ± 31.19	85.00 ± 7.06
3000	427.53 ± 18.41	114.37 ± 6.12	325.82 ± 50.68	97.66 ± 15.51
4000	525.54 ± 37.67	136.11 ± 4.79	390.46 ± 37.77	125.75 ± 8.97
5000	487.16 ± 56.51	150.00 ± 13.71	337.76 ± 70.76	109.15 ± 13.88

Table 18 (continued). Analysis of variance

Source of variance	d.f.	S.S.	M.S.	F
Length				
Replicate	2	4126.46	2063.23	0.83 (N.S.)
With or without diatom	1	78258.64	78258.64	31.62 ($P < 0.001$)
Light intensity	4	140059.90	35014.97	14.14 ($P < 0.001$)
Interaction	4	11546.32	2886.58	1.16 (N.S.)
Error	18	44547.36	2474.85	
Total	29	278538.68		
Width				
Replicate	2	212.22	106.11	0.81 (N.S.)
With or without diatom	1	3804.52	3804.52	29.02 ($P < 0.001$)
Light intensity	4	14202.40	3550.60	27.08 ($P < 0.001$)
Interaction	4	1054.80	263.70	2.01 (N.S.)
Error	18	2359.56	131.08	
Total	29	21633.50		

at 10°C, r = -0.564; $p < 0.01$).

With C. crispus (Table 20), the effects of temperatures on the growth rate were rather similar to those for G. stellata; crust diameters were invariably larger at 15°C. However, inhibitory effect by diatoms was not significant. Microscopic observations indicated that all crustose growths were healthy except those treated with the diatoms Stauroneis constricta and Nitzschia closterium. Initial and final numbers of both rhizoid and disc-form crusts varied in the cultures. Nevertheless the patterns of % survival of crustose growths in diatom-treated cultures were similar at both temperatures studied.

5.3.4 Salinities

From the results (Table 21), the average increase of diameter over all salinites studied of Gigartina stellata sporelings was consistently greater in the diatom-free cultures than those with diatoms by two orders of magnitude.

The crustose growths appeared pale in colour and unhealthy compared with those in the controls. Under the microscope, the diatoms appeared in large numbers around and on the crusts as shown in the experiments with nutrient variations, light intensities and temperatures. Nevertheless the crust sizes in cultures with and without diatoms were generally larger at lower salinities. Statistical analysis showed that the presence of diatoms and salinity changes significantly affected Gigartina growth. There was no correlation between diameter of crusts and salinities or diatom cell number (r = -0.14; $P > 0.1$). Treatment with diatoms also caused smaller germlings of F. vesiculosus in different salinity regimes (Table 22). There was a correlation between algal growth rate (length and width of embryos) and number of germlings in the cultures (r = 0.58 to 0.62; $P < 0.005$).

Table 19 Effects of diatoms on the percentage survivals and crust diameters of Gigartina stellata at different temperatures (data based on 2 replicates and 30 crusts in each replicate).

Diatom species	15°C		10°C	
	% survival Mean ± range	Diameter (μm) Mean ± range	% survival Mean ± range	Diameter (μm) Range ± range
Control	97.24 ± 2.76	119.15 ± 2.83	93.03 ± 0.31	54.10 ± 6.29
Stauroneis constricta	64.80 ± 1.37	91.34 ± 2.02	35.25 ± 3.68	53.29 ± 2.74
Nitzschia closterium	42.33 ± 16.00	69.25 ± 3.53	60.33 ± 9.68	51.30 ± 1.65

Analysis of variance of mean diameters

Source of variance	d.f.	S.S.	M.S.	F
Replicate	1	61.42	61.42	3.52 (N.S.)
Diatom	2	1391.82	695.91	39.88 ($P < 0.001$)
Temperature	1	4884.77	4884.77	279.92 ($P < 0.001$)
Interaction	2	1116.91	558.45	32.00 ($0.001 < P < 0.01$)
Error	5	87.25	17.45	
Total	11	7542.17		

Table 20 Effects of diatoms on the percentage survivals and crust diameters of Chondrus crispus at different temperatures (data based on 2 replicates and 30 crusts in each replicate).

Diatom species	15°C		10°C	
	% survival Mean ± range	Diameter (μm) Mean ± range	% survival Mean ± range	Diameter (μm) Mean ± range
Control	87.96 ± 3.95	134.25 ± 8.63	80.49 ± 19.51	70.25 ± 0.56
Stauroneis constricta	56.26 ± 27.46	78.91 ± 13.55	47.90 ± 47.30	71.01 ± 0.51
Nitzschia closterium	93.78 ± 2.48	97.05 ± 13.20	79.50 ± 3.58	79.44 ± 6.25

Analysis of variance of mean diameters

Source of variance	d.g.	S.S.	M.S.	F
Replicate	1	2.57	2.57	0.01 (N.S.)
Diatom	2	1545.24	772.62	4.10 (N.S.)
Temperature	1	2637.96	2637.96	14.00 ($0.01 < P < 0.05$)
Interaction	2	1719.71	859.85	4.56 ($0.05 < P < 0.1$)
Error	5	941.79	188.35	
Total	11	6847.27		

Table 21 Effects of salinities on crust diameters of *Gigartina stellata* in the cultures with or without the diatom *Stauroneis constricta* (data based on 2 replicates and 30 crusts in each replicate).

Salinities (‰)	Without diatom		With diatom	
	% survival	Diameter (μm) (Mean ± range)	% survival	Diameter (μm) (Mean ± range)
49.60	100.00	116.04 ± 0.16	100.00	60.77 ± 2.20
*		++		--
42.50	100.00	159.02 ± 7.41	100.00	64.31 ± 2.58
*		++		--
36.07	100.00	180.22 ± 2.29	100.00	80.43 ± 0.71
*		++		-
33.25	100.00	175.60 ± 0.16	100.00	98.92 ± 5.04
*		++		++
28.20	100.00	206.00 ± 0.92	87.83	70.43 ± 0.21
*		++		-
24.11	100.00	199.05 ± 8.21	90.43	90.71 ± 4.58
*		++		-
21.78	100.00	202.84 ± 7.83	100.00	131.49 ± 9.54
*		++		--
16.84	100.00	194.21 ± 4.04	100.00	94.92 ±13.37
*		++		--

*: ++ all red, healthy; + mostly red and healthy; - mostly pale and unhealthy; -- all pale, unhealthy, crustose sporelings.

Analysis of variance

Source of variance	d.f.	S.S.	M.S.	F
Replicate	1	13.49	13.49	0.19 (N.S.)
Salinity	7	15754.68	2250.66	31.99 ($p < 0.001$)
Diatom	1	68633.27	68633.27	975.80 ($p < 0.001$)
Interaction	7	4293.52	631.36	8.72 ($p < 0.001$)
Error	15	1055.02	70.33	
Total	31	89749.98		

Table 22 Effects of salinities on the lengths and widths of Fucus vesiculosus germlings in the cultures with or without the diatom Stauroneis constricta (data based on 2 replicates and 20 germlings in each replicate).

Salinities (‰)	Without diatoms		With diatoms	
	Length (μm) Mean ± range	Width (μm) Mean ± range	Length (μm) Mean ± range	Width (μm) Mean ± range
16.30	186.09 ± 11.15	70.98 ± 0.62	158.79 ± 5.69	47.99 ± 7.46
21.63	907.03 ± 66.90	230.40 ± 8.08	575.98 ± 24.23	181.79 ± 5.16
26.20	900.03 ± 26.38	196.32 ± 4.00	715.39 ± 17.53	186.40 ± 8.36
30.85	930.48 ± 10.38	204.63 ± 3.08	730.09 ± 18.69	182.02 ± 2.62
35.10	847.51 ± 36.60	195.02 ± 7.76	735.62 ± 15.76	185.87 ± 2.93
39.35	651.41 ± 94.89	159.56 ± 4.46	652.10 ± 1.92	174.72 ±11.62
44.32	771.30 ± 48.83	183.10 ± 0.93	575.05 ± 19.76	174.11 ± 4.85
49.18	611.20 ± 72.52	162.88 ±17.92	458.71 ± 10.77	145.65 ± 4.08

Table 22 (continued), analysis of variance

	Source of variance	d.f.	S.S.	M.S.	F
Length	Replicate	1	1540.53	1540.53	0.46 (N.S.)
	Diatom	1	180992.85	180992.85	55.05 ($P < 0.001$)
	Salinity	7	1310514.90	187216.41	56.95 ($P < 0.001$)
	Interaction	7	77877.82	11125.40	3.38 ($P < 0.05$)
	Error	15	49308.56	3287.23	
	Total	31	1620234.66		
Width	Replicate	1	169.50	169.50	1.69 (N.S.)
	Diatom	1	1932.70	1932.70	19.30 ($P < 0.001$)
	Salinity	7	60874.24	8696.32	86.84 ($P < 0.001$)
	Interaction	7	2259.47	322.78	3.22 ($P < 0.05$)
	Error	15	1502.18	100.15	
	Total	31	66738.09		

Table 23 Effects of diatom Stauroneis constricta on the increases of diameters of Gigartina stellata crusts in the continuously shaken cultures with or without diatoms (data based on 3 replicates and 20 crusts in each replicate)

Diameter (μm) (Mean ± S.E.)	
Without diatom	With diatom
135.61 ± 2.48	123.20 ± 13.37

Analysis of variance (based on the means of replicates)

Source of variance	d.f.	S.S.	M.S.	F
Diatoms	1	296.10	296.10	1.68
Error	4	703.51	175.87	(N.S.)
Total	5	999.61		

5.3.5 Shaking

The mechanical shaking considerably reduced diatom numbers on the Gigartina crusts and their effects on growth of crusts. From the results (Table 23), the growth rates of crusts in both diatom-treated and diatom-free cultures were not significantly different.

5.4 Discussion

Many culture studies have indicated the physico-chemical conditions for the growth of algal sporelings and germlings (Jones and Dent, 1971; Mathieson and Burns, 1971; McLachlan et al., 1971; Chen and McLachlan, 1972; Chen et al., 1974; Moss, 1975; Khfaji, 1978; Terry and Moss, 1981; Mathieson, 1982). Generally, the growth rates of young algal plants increase with an increase of temperature and light intensity to certain maximal values, It is less affected by the change of salinity in the present study. Change of physico-chemical environments do not alter significantly the effects of diatoms on the growth of macroalgae. This is not surprising since the growth rates of benthicl diatoms are influenced by similar environmental conditions to those young macroalgae.

Results show that the nutritional conditions have a profound influence on diatom and young plant interactions. With regard to the considerably large numbers of diatom cells and algal sporelings in the cultures of limited volume (ca.30ml), competition for nutrients and the extracellular substances from both diatoms and sporelings cannot be ruled out. However, whether they are the major causes of diatom inhibitory effects is doubtful. Firstly, both the diameters of Gigartina crusts and total diatom cell numbers are increased with an increase of nutrient concentrations of the culture media. Therefore, in sufficiently or continuously enriched cultures the effects of diatoms on algal sporelings could be decreased significantly or negligible. Decrease of nutrient concentrations

in the cultures might stimulate significantly the capability of diatoms regarding nutrient uptake and this would possibly increase diatom mobility and attachment behaviour toward the algal crusts, presumably for nutrient substitutes, organic matter etc., Secondly, the correlation between sizes and numbers of sporelings and diatom cell numbers is rarely shown in the results. Consequently the effects of nutrient deficiency and associated competition between the algae are quite limited and somehow questionable. Thirdly, whilst the supplemented diatom filtrates collected from the stationary and declining growth phases, but not the exponential phase, did significantly inhibit crust growth, all crustose sporelings appeared healthy and red in colour. This further suggests that the significance of algal extracellular substances instead of nutrient deficiency in affecting growth of algal sporelings. Diatoms excrete metabolic products largely during the stationary and declining growth phases. Many authors indicated that these algal extracellular substances are of ecological importance (Proctor, 1957; Fogg, 1962; 1966; Pratt, 1966; Paul et al., 1977; Eminson, 1978; Khfaji and Boney, 1979). The effects of excreted substances possibly vary with algal species involved in the interactions. Thus Talling (1957) did not find evidence of growth-regulating substances in mixed diatom cultures. Nevertheless inhibitory effects are closely related to the physiological state of the diatoms.

Halldahl (1958) and Jones (1967) found marked effects of light intensity and temperature on the pigmentation of blue-green algae. Jones and Dent (1971) indicated that the growth rates of sporelings of intertidal red algae are increased with an increase of light intensity; at extremely high light intensity the sporelings appeared pale-yellow in colour (loss of phycoerythrin), but their growth was not inhibited due to the presence of associated pigments which protect sporelings from inhibitory green light

(Boney and Corner, 1962, 1963). In the present study, pale and unhealthy crustose sporelings of G. stellata appeared after they were covered by large numbers of diatom cells of both Nitzschia closterium and Stauroneis constricta. Such diatom aggression undoutedly creates unfavourable growth conditions for algal sporelings. These include, (a) reduction of light intensity and therefore photosynthetic rate (b) decrease of intimate contact of crustose surface with nutrients in the surrounding water and therefore nutrient uptake efficiency (c) increase in diatom extracellular substances in the immediate vicinity of the algal crusts (d) change of hydrogen ion concentration of the surrounding water resulting from microbial activities (bacteria-free cultures were not used in the present study), (e) mechanical damage of crustose surface cells by attaching mucilaginous materials of diatoms (Plate 2.B). Damaged cells caused by attached organisms were also demonstrated in macroalgae (Sieburth and Tootle, 1981). Paul et al., (1977) indicated that colonized organisms can modify structurally the surface substances of substrata into complex system which may support more variable organisms. Biodeteriorations of wood and other materials by micro-organisms in aquatic environments have been well documented (Fazzani et al., 1975; Lewis, 1975). The pale and unhealthy Gigartina crusts in the cultures were the results of permanent damage by attached diatoms instead of temporary loss of red pigment (phycoerythrin), and they were not able to grow even after exposure to suitable conditions. Diatom shading and its associated effects are the main causes accounting for the inhibitory effects on the growth and survival of red algal sporelings. This is further emphasized by the results of the continuous shaking experiment in which there were similar increases in the diameters of disc-form crusts in both diatom-free and diatom-treated cultures.

The present results obviously reveal some important roles of benthic diatoms in controlling survival and growth rate of attached germinated

spores and zygotes of macroalgae. Evidently, the external physico-chemical parameters are not adequate in determining growth characters of germinated plants in natural conditions; other effects i.e. internal micro-environments caused by associated diatoms; are necessary and are equally important or even serve as limiting factors in the determination. It is noteworthy that such diatom effects are much dependent on the species and growth features of both micro- and macroalgae in the given physico-chemical conditions. The diatoms used in the present study were mono-species which is unlikely to occur in nature. Therefore, further studies with some more diatom species are needed from the ecological point of view, and the interactions between these remain of potential interest in the evaluation of algal growth and distribution as shown in the result of the next chapter.

CHAPTER 6

EFFECTS OF CERTAIN LITTORAL DIATOMS, APPLIED SINGLY OR COMBINED, ON THE GROWTH AND SURVIVAL OF YOUNG PLANTS OF MARINE ALGAE

6.1. Introduction

In fouling and succession of marine organisms, macroalgae usually occur after diatom colonization (Wilson, 1925; Huve, 1953; Fletcher, 1974, 1980). Studies of this mainly concern the qualitative and quantitative characters of investigated algal communities. Little is known about the inter-relationships between diatoms and young plants of macroalgae. The results in the previous chapters demonstrated that certain benthic diatoms affected significantly the growth of germinated spores and zygotes in both constant and various physico-chemical conditions. Some benthic diatom communities are characterized by great varieties of species, growth features and habitats in the littoral zone. Their species distributions and interactions can be of much ecological importance to the growth of associated algal plants on the substratum.

In addition to physico-chemical factors (Round, 1957, 1960; Douglas, 1958; Cattaneo, 1978; Tuchman and Blinn, 1979) the organic matter of the substrata may also play a major role in the attachment and growth of non-periphytic benthic diatoms (Round, 1957; Lewin and Lewin, 1960; Admiraal and Peletier, 1979; Blinn et al., 1980). Stimulatory effects of Enteromorpha extracts on the diversity and colonization of epiphytic diatom communities were reported (Lee et al., 1975). The causes of attachment of diatoms to macroalgae are much debated (Edsbagge, 1966a). Recently, Eminson (1978) indicated that the surface layer of host plants is not metabolically inert and the released organic matter by macrophytes may cause the diversity of attached diatom species. Nutrient translocation from living host plants to their eiphytes was recognized by Cattaneo and Klaff (1979). Yet Tuchman and Blinn (1979)

found that the dominance of Cocconeis placentula v. lineata over Amphora coffeiformis on natural substrata was closely related to their metabolic competition at suitable temperatures. In mixed cultures of unicellular algae, inhibitory effects of one organism on other frequently appear (Proctor, 1957; Pratt, 1966). Antagonistic effects were also found between phytoplankton and zooplankton (Bainbridge, 1953). The ecological importance of extracellular substances of various algae has been indicated by Lucas (1947), Hellebust (1965) and Fogg (1966). Clearly, certain factors concerned with organic matter of different natures are involved in the above results. The questions arise as to whether there are interactions between benthic diatoms and whether these interactions can affect, directly or indirectly, survival and growth of associated algal sporelings and germlings. In the present study, further examinations on the growth features of certain isolated diatoms and sporelings of Gigartina stellata and Chondrus crispus in the cultures of various combinations were made.

6.2. Materials and Methods

6.2.1 Algal materials

Sporelings of the red algae Gigartina stellata and Chondrus crispus and germlings of Fucus vesiculosus and F. serratus were obtained from the fertile thalli collected from the Isle of Cumbrae (Fig. 1). Three diatoms, Cocconeis stauroneiformis, Stauroneis constricta and Nitzschia closterium, were used in the present study as the former did not inhibit Gigartina growth and the latter two diatoms showed great inhibitory effects on the sporelings in the previous chapters. The diatoms were maintained growing in the artificial seawater medium before experiments. For experiments, the diatom cells in the exponential growth phase were collected and used.

6.2.2 Experimental procedures

Prior to experiments the diatom-free algal sporelings or germlings growing on glass slides were washed in three consecutive beakers of sterilized distilled water to remove seawater remaining on algal plants. Then they were placed into experimental flasks as described below.

6.2.2.1 Interactions between Gigartina sporelings and diatoms, singly or mixed

The disc-form Gigartina crusts growing on small glass slides for 10 days were placed into 100ml flasks containing 30ml artificial seawater medium, but the numbers of crusts in the flasks were not equal. Aliquots of 0.05 - 0.40ml diatom suspensions of the above three diatoms were added separately to the flasks in different combinations; the final cell concentrations in the cultures were approximately 0.7 x 10^3 cells ml^{-1}. The experiment was carried out under the conditions of $15\pm1^{o}C$, 3000 lux and light/dark regime = 14/10h for 12 days.

6.2.2.2 Interactions between Gigartina and Chondrus sporelings, singly and in mixed cultures, and with diatoms, singly and mixed

Both rhizoid and disc-form sporelings of 11-day old Gigartina stellata and Chondrus crispus grown on separated slides were placed together in the flasks containing 30ml artificial culture medium. 0.45 - 0.60ml diatom suspensions of Nitzschia closterium and Stauroneis constricta respectively were added to the flasks in several combinations as shown in Table 25. Final cell concentrations of the above diatoms in the cultures were 5.43 and 5.54 x 10^3 cells ml^{-1} respectively. The cultures were kept in the same conditions described above for 12 days.

Seven combinations of red algal crusts (22-day old) and fucoid

germlings (6-day old) were treated with diatom S.constricta in the above experimental conditions for 16 days. The initial diatom cell concentration in the cultures was 4.66×10^3 cells ml^{-1}.

6.2.2.3 Effects of aged Gigartina crusts

46-day old disc-form sporelings of Gigartina stellata were placed in the flasks which contained 30ml artificial seawater medium. Then diatom suspensions of S. constricta were added to the flasks to give a final cell concentration of $0.58 - 0.63 \times 10^3$ cells ml^{-1}. Algal cultures were kept growing at 15±1 °C, 3000 lux and light/dark regime = 14/10h.

Growth rates of crusts were assessed by the increased diameters of crusts. While the percentage survival of algal sporelings were determined from the initial and final numbers of both rhizoid and disc-form crusts.

6.3. Results

6.3.1 Interactions between Gigartina sporelings and diatoms, singly or mixed

The growth rate of 10-day old G. stellata sporelings in the cultures with or without diatoms was slightly different ($0.05 < P < 0.1$) (Table 24). The growth rate of sporelings grown with C. stauroneiformis was not significantly different from that in the control (without diatoms), but sporelings in the cultures with Nitzschia closterium and S. constricta or with S. constricta alone were comparatively smaller. As shown in Table 24, the effects of diatoms on the survival of red algal sporelings were highly significant ($P < 0.001$). In the cultures of C. stauroneiformis, the survival of G. stellata was slightly higher than that in the control, whilst it

Table 24A Effects of diatoms on the % survivals and diameters of 10-day old Gigartina stellata crusts (data based on 2 replicates and 3-12 crusts in each replicate).

Diatom species	% survival Mean ± range	Diameter (μm) Mean ± range
Without diatoms	78.09± 9.67	103.32± 3.09
Cocconeis stauroneiformis	95.51± 4.50	101.75±12.50
C.stauroneiformis+Stauroneis constricta	36.73± 8.73	90.96± 6.28
Nitzschia closterium	58.20± 9.45	83.19± 0.10
C.stauroneiformis+N.closterium	22.18± 1.35	82.98± 5.52
C.stauroneiformis+S.constricta + N.closterium	32.50±12.50	82.94±11.31
S.constricta	16.83± 0.56	77.88± 2.64
S.constricta+N.closterium	21.05± 6.75	75.66± 0.14

Analysis of variance (based on % survivals)

Source of variance	d.f.	S.S.	M.S.	F
Diatoms	7	11865.73	1695.10	14.04
Error	8	966.03	120.75	(P<0.001)
Total	15	12831.76		

Table 24B Diatom cell numbers in the cultures with and without *G. stellata* sporelings

Diatom species	Initial cell No. (10^4 cells ml^{-1})	Final cell no.(10^4 cells ml^{-1}) Without sporelings (sp^-)	With sporelings (sp^+)	$\frac{sp^+}{sp^-}$
Stauroneis constricta	1.988	352.24	325.30	0.92
Cocconeis stauroneiformis	2.200	19.81	56.41	2.85
Nitzschia closterium	2.13	399.90	780.90	1.95
S. clotricta	1.988	113.95	152.78	1.34
C. stauroneiformis	2.20	11.85	13.73	1.16
S. constricta	1.988	290.21	306.90	1.06
N. closterium	2.125	33.94	32.30	0.95
C. stauroneiformis	2.200	22.93	25.43	1.11
N. closterium	2.125	1242.08	802.19	0.64
S. constricta	1.988	68.08	181.03	2.65
C. stauroneiformis	1.100	13.37	12.08	0.90
N. closterium	2.125	19.55	30.23	1.54

was largely decreased when cultured with either N. closterium or S. constricta. The low survival of Gigartina sporelings caused by S. constricta was slightly improved by the association with C. stauroneiformis in the cultures.

The growth rates of diatoms were generally increased in the presence of G. stellata sporelings (Chapter 4). An increase of cell numbers of C. stauroneiformis and N. closterium was also shown in the present study (Table 24B). However, as shown in Table 24B, the total cell numbers of these diatoms decreased greatly when cultured with S. constricta. On the other hand, the cell number of S. constricta was obviously smaller when cultured with both C. stauroneiformis and N. closterium in the cultures with or without G. stellata. The growth rate of N. closterium was also greatly induced by C. stauroneiformis particularly in spore-free cultures, but it was strongly inhibited by S. constricta.

6.3.2 Interactions between Gigartina and Chondrus sporelings, singly and in mixed cultures, and with diatoms, singly and mixed

In the above experiments, diatoms of S. constricta and N. closterium were greatly inhibitory to Gigartina sporelings. This also appeared in the cultures when both red algae, G. stellata and C. crispus, were grown together. Survival of C. crispus was not affected significantly by diatoms. Table 25 demonstrates that the growth rates of both G. stellata and C. crispus were inhibited significantly by both diatoms ($P < 0.001$), N. closterium and S. constricta. Obviously, under the same conditions, C. crispus grew faster than G. stellata; however, the patterns of their growth rates, corresponding to diatom species combinations, were shown equally in the results. The growth rates of both red algae in diatom cultures were reduced to approximately

60% to 87% of those in diatom-free cultures.

Growth features of the two diatoms were similar to those in the previous experiment. In Table 25, it was seen that growth of N. closterium was induced greatly by both red algae investigated, approximately 7 orders of magnitude of that in the sporeling-free cultures based on total cell number. On the contrary; it was reduced by approximately 30% to 90% when treated with S. constricta. The interaction between the effects of both diatoms and algal sporelings was not significantly present in the results ($P > 0.1$).

The growth rate of G. stellata further treated with the diatom, S. constricta and three macroalgae is shown in Table 26. As it was described in Chapter 4, fucoid plants, i.e., F. serratus and F. vesiculosus, did not grow healthy in the artificial medium (embryos became partially or completely pale-yellow in colour), whereas red algae and diatoms grew normally and were relatively healthy. Table 26 indicates that the averages of increased diameters of G. stellata crusts in all algal combinations were not significantly different. Effects of diatoms were not noticeable in this experiment.

6.3.3 Effects of aged Gigartina crusts

The previous experiments dealt with G. stellata of less than 12 days old after germination (Chapter 5). Reduction of % survival by associated diatoms consistently appeared in the results. With 46-day old Gigartina sporelings (Table 27), the survival was 100% at both 15°C and 10°C , and the diameters of crustose discs were not significantly different among diatom-treated and non-treated cultures. However, growth rate of Gigartina sporelings was higher at 15°C than at 10°C. Correlation between numbers and diameters of crustose sporelings

Table 25 Effects of diatoms on the crust diameter increases and % survivals of Gigartina stellata and Chondrus crispus in artificial seawater medium (data based on 2 replicates and 7-30 crusts in each replicate; data given as net increases of diameters of crusts).

Diatom species	G. stellata		C. crispus		Total diatom cell number (10^5) sporelings	
	% survival (Mean ± range)	Diameter (μm) (Mean ± range)	% survival (Mean ± range)	Diameter (μm) (Mean ± range)	(-)	(+)
Without diatoms	73.81 ± 26.19	106.14 ± 7.12	86.96 ± 13.09	188.67 ± 13.22		
Stauroneis constricta	30.04 ± 4.75	93.21 ± 1.02	82.86 ± 11.43	153.81 ± 2.07	17.34	20.50
Nitzschia closterium	32.90 ± 9.90	66.72 ± 1.65	85.37 ± 11.30	113.21 ± 15.28	16.45	117.00
Stauroneis constricta	14.92 ± 14.92	82.75 ± 1.05	84.74 ± 6.69	156.02 ± 12.66	20.99	17.60
Nitzschia+closterium					11.11	11.23

Analysis of variance of diameters

Source of variance	d.f.	S.S.	M.S.	F
Diatoms	3	6691.12	2230.37	14.64 ($P < 0.001$)
Algal sporelings	1	17245.60	17245.60	113.22 ($P < 0.001$)
Interaction	3	727.03	242.34	1.59 (N.S.)
Error	8	1218.51	152.31	
Total	15	25882.26		

Table 26 Effects of the diatom Stauroneis constricta on the growth of Gigartina stellata sporelings in various combinations of cultures with or without Fucus germlings and Chondrus sporelings (data based on 3 replicates and 7-30 crusts in each replicate).

Combination of macroalgae	Diameter (μm) (Mean ± S.E)	Growth assessments*				
		G. stellata	C. crispus	F. serratus	F.vesiculosus	Diatom
G. stellata	167.07 ± 15.55	H				G
G. stellata + C. crispus	145.53 ± 13.39	H	H			NG
G. stellata + F. serratus	156.95 ± 22.68	H		UH		VG
G. stellata +F. vesiculosus	129.46 ± 22.69	H			UH	VG
G. stellata + C. crispus + F. serratus	155.77 ± 14.59	H	H	UH		VG
G. stellata + C. crispus + F. vesiculosus	200.51 ± 16.61	H	H		UH	G
G. stellata + F. serratus + F. vesiculosus	155.46 ± 22.35	H		UH	UH	G

* H = healthy, UH = unhealthy, G = good, VG = very good, NG = not good.

Analysis of variance

Source of variance	d.f.	S.S.	M.S.	F
Macroalgae	6	8797.96	1466.32	2.72
Error	14	7538.13	538.43	(N.S.)
Total	20	16336.09		

Table 27 Effects of diatoms* on the survival and growth of 46-day old Gigartina stellata sporelings at different temperatures (data based on 2 replicates and 4-24 crusts in each replicate ; data given based on the net increases of crust diameters).

Diatoms	15°C		10°C	
	% survival	Diameter (μm) Mean ± range	% survival	Diameter (μm) Mean ± range
Without diatoms	100	207.07 ± 4.62	100	120.44 ± 7.51
Cocconeis stauroneiformis	100	175.65 ± 6.90	100	102.79 ± 0.67
Stauroneis constricta	100	172.96 ± 15.02	100	114.19 ± 6.36
Nitzschia closterium	100	175.63 ± 29.37	100	101.46 ± 17.93

* Initial diatom cell numbers (10^4 cells) added : C. stauroneiformis = 1.80, S. constricta = 1.88, N. closterium = 1.54

Analysis of variance of diameters of crustose sporelings

Source of variance	d.f.	S.S.	M.S.	F
Diatoms	3	1689.38	563.12	0.78 (N.S.)
Temperatures	1	21378.83	21378.83	29.93 (P < 0.001)
Interaction	3	389.48	129.82	0.18 (N.S.)
Error	8	5713.48	714.18	
Total	15	29171.17		

was also not significant at 95% confidence level ($r = 0.13$ and -0.56 at $15^{o}C$ and $10^{o}C$ respectively).

6.4. Discussion

In the present results, the inter-relationships between diatom species and between diatoms and algal sporelings are demonstrated clearly in various algal combinations. Growth rate of every single algal species concerned is governed not only by physico-chemical parameters but also by the associated diatoms.

The diatoms studied have strong affinity for G. stellata crusts. However, their different growth features probably account for the different effects on the crustose growth. The littoral diatoms, N. closterium and S. constricta, consistently appear in large numbers on the young sporelings, and thus reduce greatly, light availability and nutrient uptake of the crusts in addition to some possible mechanical damage by diatom mucilaginous material (Chapters 4 and 5). Whilst the epiphytic diatom, C. stauroneiformis has a lower growth rate, it gradually attaches (Plate 2,c) to the margin of Gigartina crusts during the growth. This could, to some extent, form a biotic barrier which protects young crusts from invasion by other diatoms. Under the microscope, the cell numbers of both N. closterium and S. constricta on crusts were decreased considerably in the presence of C. stauroneiformis. It is likely that certain ectocrines (Lucas, 1947; Lefèvre, 1964) released from C. stauroneiformis directly inhibits the growth of S. constricta, but not N. closterium (as shown in the mixed cultures of Olithodiscus luteus and Skeletonema costatum; Pratt, 1966). This is emphasized by the similar results which appeared in the cultures without Gigartina crusts. Similarly, N. closterium is inhibited by S. constricta, but enhanced by C. stauroneiformis in the cultures without associated

crusts. Since the experiments were carried out in a limited volume of culture medium with rather high algal density, spatial and nutrient competitions between diatoms and germinated spores might be persistent for long periods. Nevertheless, the above results are ecologically interesting in that they reveal that some similar interspecific antagonisms and stimulations between benthic diatoms might occur in nature and consequently ensure the successful germination and development of associated spores and zygotes. In the studies on the bottom-living diatoms in lakes, Round (1960) also indicated the possibility of interactions between species. In addition, the results could, at least partly, explain why C. scutellum and its related species commonly predominate in the littoral diatom assemblages (Edsbagge, 1966b; DeFelice and Lynts, 1978; also in Chapter 2).

In consideration of diatom-macrophyte associations, morphology of host plants (Aleem, 1969) is an important factor. Cattaneo (1978) claimed that the linkage of epiphytic diatoms and macrophytic host plants is based on the physical advantage rather than biological activity of the host plant. However, these concern the post-attachment phenomena of diatoms on fully grown plants. Studies of these aspects have not yet placed emphasis on the very young plants, i.e. algal sporelings and germlings. It is not certain whether there is a difference and to what extent of this difference will be between diatom associates on both young and old host plants. Earlier, Knudson (1957) failed to show any difference of attached Tabellaria flocculosa diatom communities on both new and old shoots of Phragmites (host plant). Harrison (1982) found a lower biomass of epiphytes on actively growing leaves which have higher potency of inhibitors. However, as shown in the present study, diatom attachment and association can occur on the

macrophytes of the very early stages of growth. Presumably, the diatoms which attach or come close to the young Gigartina crusts either by their motile ability or by chance of various mechanisms would remain in the associations until the conditions are no more suitable ("suitability", Aleem, 1950a). Thus temporary attachment or permanent association would depend on the algal activities bounded up with physico-chemical conditions. In the examination on the affinity of isolated diatoms for algal germlings and sporelings, most diatoms observed tended to increase their affinity after two weeks. It is probably related to some organic matter excreted from the young algal plants. Indeed, uptake and assimilation of organic matter are commonly found in the benthic diatoms (Lewin and Lewin, 1960; Lee et al, 1975; Admiraal and Peletier, 1979; Blinn et al, 1980). Catteneo and Kalff (1979) indicated that a small amount of nutrient was transferred from host plants to their epiphytes. The dissolved organic matter released from the macrophytes may provide the metabolic substrates and therefore niches and, eventually increase species diversity of a diatom community (Eminson, 1978). Further, radioisotope techniques provide much evidence of transformation, uptake and assimilation of organic matter between unicellular algae and macrophytes (Jones, 1967; Aleen, 1971; Smith and Penhale, 1980). Sieburth and Thomas (1973) found cuticle composition to be an important factor in microbial attachment. The relationships between biosynthetic capabilities of marine algae and the associated bacterial flora was also observed (Kong and Chan, 1979). Perhaps, certain laboratory methods as shown in the present study can provide much more knowledge about the algal associations. With regard to the effects of diatoms it seems likely that epiphytic diatoms are less harmful to germinated spores and zygotes than other benthic diatoms.

The antidiatom nature of C. crispus and fucoid plants (McLachlan and Craigie, 1964; Khfaji and Boney, 1979) is an interesting example of algal interactions. For example, survival of Chondrus sporelings is not affected by the diatoms studied, whilst the survival is largely decreased in diatom-treated Gigartina crusts. However, the presence of C. crispus in the cultures did not reduce significantly the effects of diatoms; growth of both red algae are inhibited equally in the cultures. Further combinations with fucoid plants completely reduce the effects of diatoms on Gigartina growth. This is probably attributable to the combined effects of extracellular substances excreted from both red and brown algae; possibly there are some interactions also appearing between algal sporelings and germlings in the cultures.

Many studies on algal fouling have revealed that diatom colonizations are always followed by the macroalgae, Enteromorpha, Ectocarpus and Ulva (Wilson, 1925; Huve, 1953; Aleem, 1957; Fletcher, 1974; 1980). There is no doubt that macroalgae and their progenies are added into algal communities throughout various interactions of both biotic and abiotic factors. Perhaps the algae which produce antibiotic substances and show higher growth rates in a wide range of environmental conditions would be prevalent in the algal communities. Algal germinations, survival and development may be determined significantly by the growth strategies of both attached diatoms and young macroalgae on the substratum in nature.

CHAPTER 7

ANTIDIATOM ACTIVITY OF YOUNG ALGAL PLANTS

7.1. Introduction

Antibiotic activity is a common feature of antagonistic interactions. To date, its occurrence and behaviour in algae are still not well known. Earlier studies have shown that certain unicellular algae exuded substances toxic to other organisms (Pratt, 1940, 1948; Rice, 1954; Proctor, 1957; Fitzgerald, 1969) and there is a vast literature on red tide causing microbial algae (e.g. Gunter et al., 1948; Wilson and Ray, 1956; Gorham, 1964 etc). Proctor (1957) and Lefévre (1964) indicated the existence of auto- and heteroantagonisms between algae. Extracts of many marine macroalgae display a variety of antimicrobial activities (e.g. Mautner et al., 1953; Allen and Dawson, 1960; Burkholder et al., 1961; Almodovar, 1964; Hornsey and Hide, 1974; 1976a,b; Khaleafa et al., 1975; Ehresmann et al., 1977; Richards et al., 1978, Caccamese and Azzolina, 1979; Caccamese et al., 1980, 1981). Excretion of tannic substances from physodes or fucosanvesicles of living brown algae has been reported (Haug and Larsen, 1958). In culture studies, the yellow substances released by Fucus vesiculosus both inhibits the growth of unicellular algae and cause cell lysis (Craigie and McLachlan, 1964; McLachlan and Craigie, 1964). The tannins from Sargassum natans and Ralfsia verrucosa also affected growth and survival of many fouling epifauna and planktonic animals (Conover and Sieburth, 1964, 1966; Sieburth and Conover, 1965). Caccamese et al (1981) reported the lack of epibionts on the brown alga Zanardinia prototypus Nardo.

Antibiosis in the sporeling phase of marine algae has received much less attention. The growths of two red algal crusts, Porphyrodiscus simulans Batt. and Rhodophysema elegans (Crouan frat, et J.Ag) were inhibited on the sides adjacent to Ralfsia spongicocarpa Batt., a brown alga, in mixed cultures (Fletcher, 1975). Khfaji and Boney

(1979) described antidiatom activity of crustose discs of the red alga Chondrus crispus with the formation of inhibition zones. Antibacterial activity was described for extracts from this alga (Hornsey and Hide, 1974, 1976[a,b]). In nature, sporelings often grow in association with diatoms on rocky substrata. Any interactions involving antibiosis are therefore of ecological interest. In the previous chapters, antagonistic phenomena between marine algae and diatoms have been demonstrated in various growth features. The present study describes some antidiatom behaviour of Fucus germlings and Chondrus sporelings under different culture conditions.

7.2. Materials and Methods

7.2.1 Algal materials

Fucus vesiculosus, F. serratus and Chondrus crispus were collected from the Isle of Cumbrae (Fig.1) during July-September, 1981. Diatom-free germlings and sporelings were obtained from the fertile thalli according to the methods described in Chapter 4, and they were allowed to grow in plastic petri dishes (50mm diameter) containing sterile seawater. The diatom Nitzschia closterium (GB 16) was kept growing in both liquid and solid enriched media at 15 ± 1°C, 3000 lux and 14h illumination per day before experiments.

7.2.2 Experimental methods and conditions

The diatom-free sporelings and germlings in petri dishes were pre-cultured in sterile seawater under the above experimental conditions for two days. Subsequently they were refreshed with a further 10ml sterile seawater. Identical volumes of N. closterium suspensions (4-47 x 10^4 cells ml^{-1}) taken in the exponential phase

were added separately to the petri dishes in which either sporelings or germlings were grown. The experiments were carried out at different light intensities, salinities, temperatures and nutritional conditions (enriched and non-enriched seawater media) as indicated in the results. In the experiment with treated and non-treated diatom cultures, the diatoms were either pre-cultured with or without F. serratus germlings in sterile seawater for 8 days prior to an experiment.

7.2.3 Assessment of antidiatom activity of algal germlings and sporelings

The inhibition zones around germlings and sporelings were clearly seen under the microscope (Vickers Patholux Microscope). Care was taken to avoid disturbance of cultures during diatom cell counting. Diatom numbers in the inhibition zones were estimated by counting the cells in five squares of an eyepiece grid from algal germlings or crustose discs to outer layers of the inhibition zones. Seven directions with 35 squares were determined equally around each embryo and disc, and 10 germlings or discs were randomly chosen from each petri dish, and measurements were made in three separate cultures.

7.3 Results

7.3.1 The formation of inhibition zones around young algal plants

The inhibitory effects of algal sporelings on the diatom Nitzschia closterium were demonstrated in the clear zone formation. The inhibition zones were formed around the crustose discs of Chondrus crispus and the embryos but not rhizoids of Fucus germlings (Plate 3,4). Within the zones, the diatom cell numbers generally increased as the distance from discs or embryos to the outer layer of the zones increased (Fig.2). In Fucus, the antidiatom activity appeared as soon

Figure 2 Diatom cell densities in the inhibition zones around Chondrus crispus sporelings; A.B = determined at the 2nd, 4th days after treatment; NI = in non-inhibition zones; bars indicate mean ± S.D.

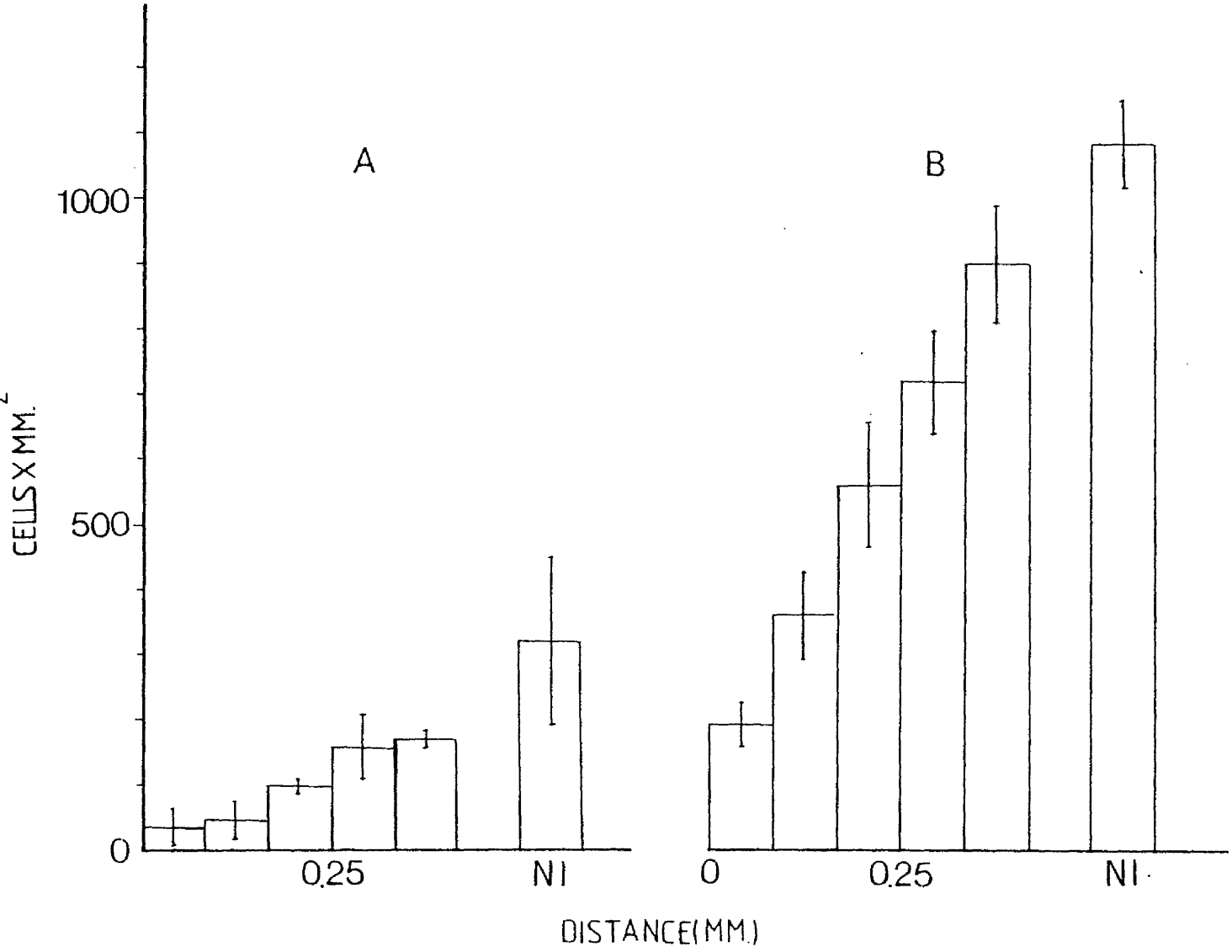
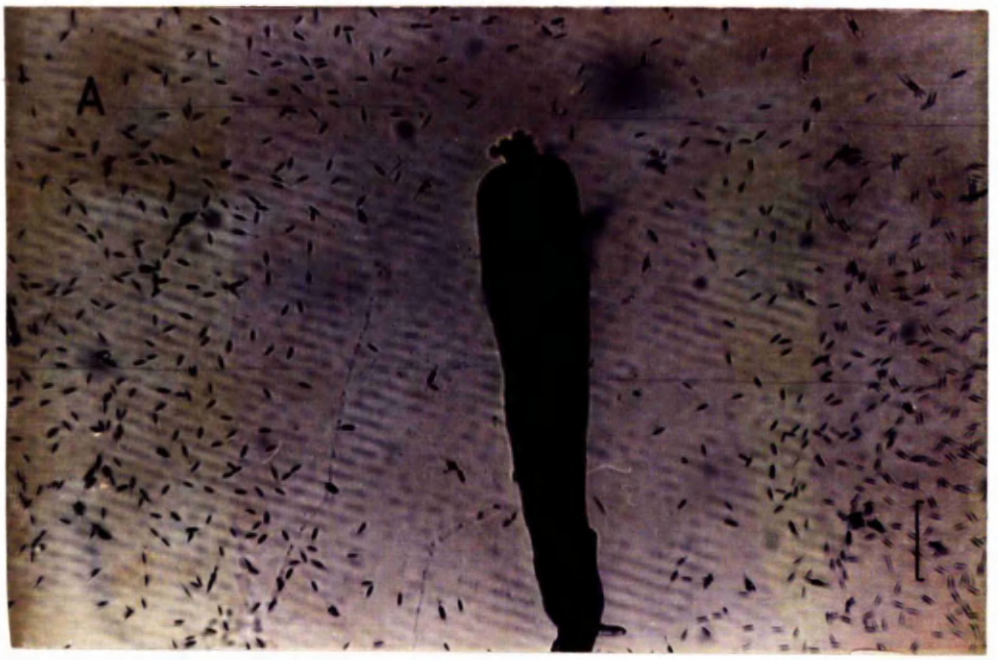

A
B
1000
500
0
CELLS X MM.-2
0.25
NI
0
0.25
NI
DISTANCE(MM.)

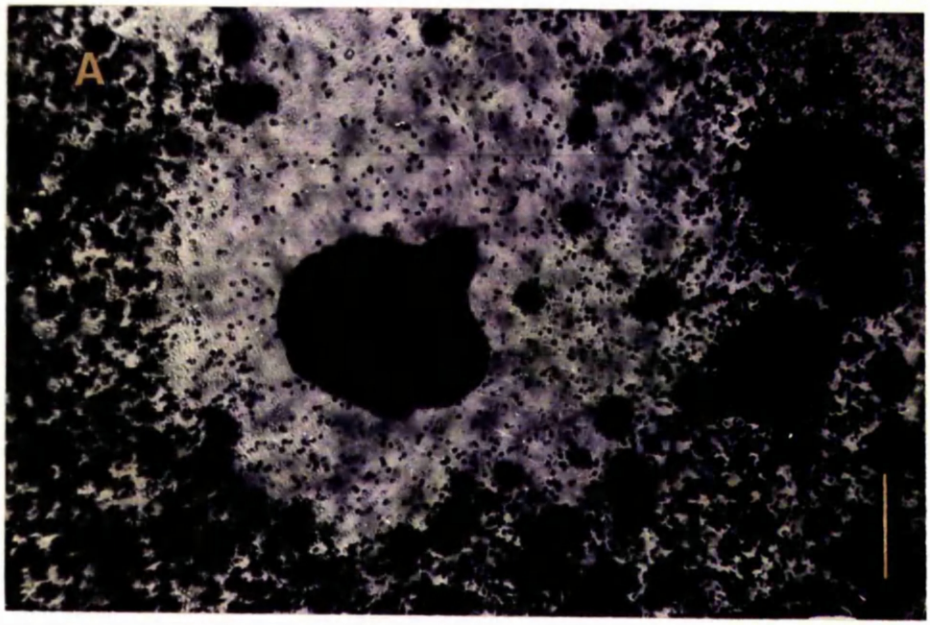
Plate 3 Photographs showing the formation of inhibition zones around Fucus serratus embryos (A, B) and the absence of inhibition zones around the dead germling (C) and rhizoids (B, D); scale = 100μm.

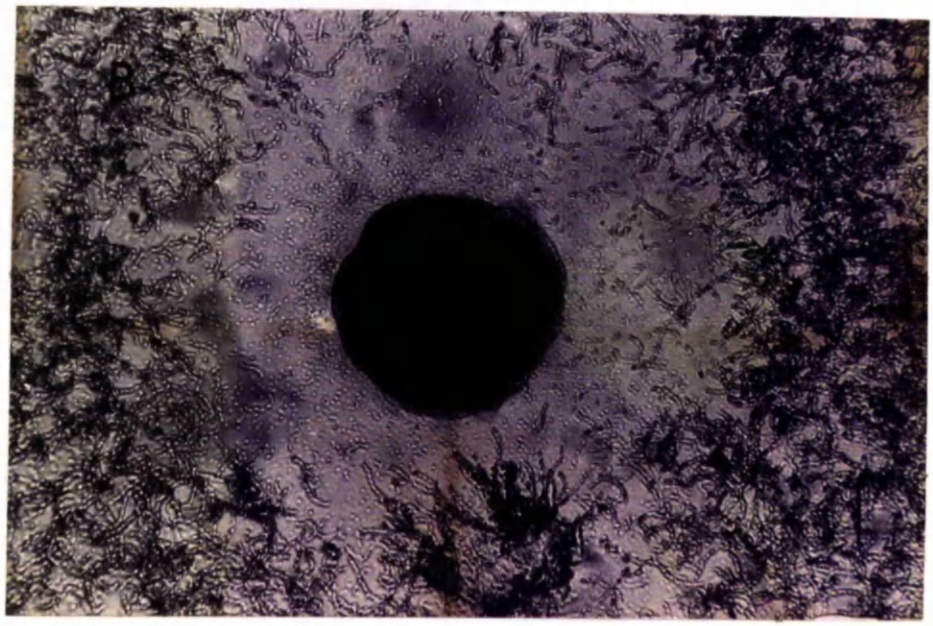

A


B


D

Plate 4 The inhibition zones of Chondrus crispus (A) and Gigartina stellata (B) crustose sporelings; scale = 100μm.


A


B

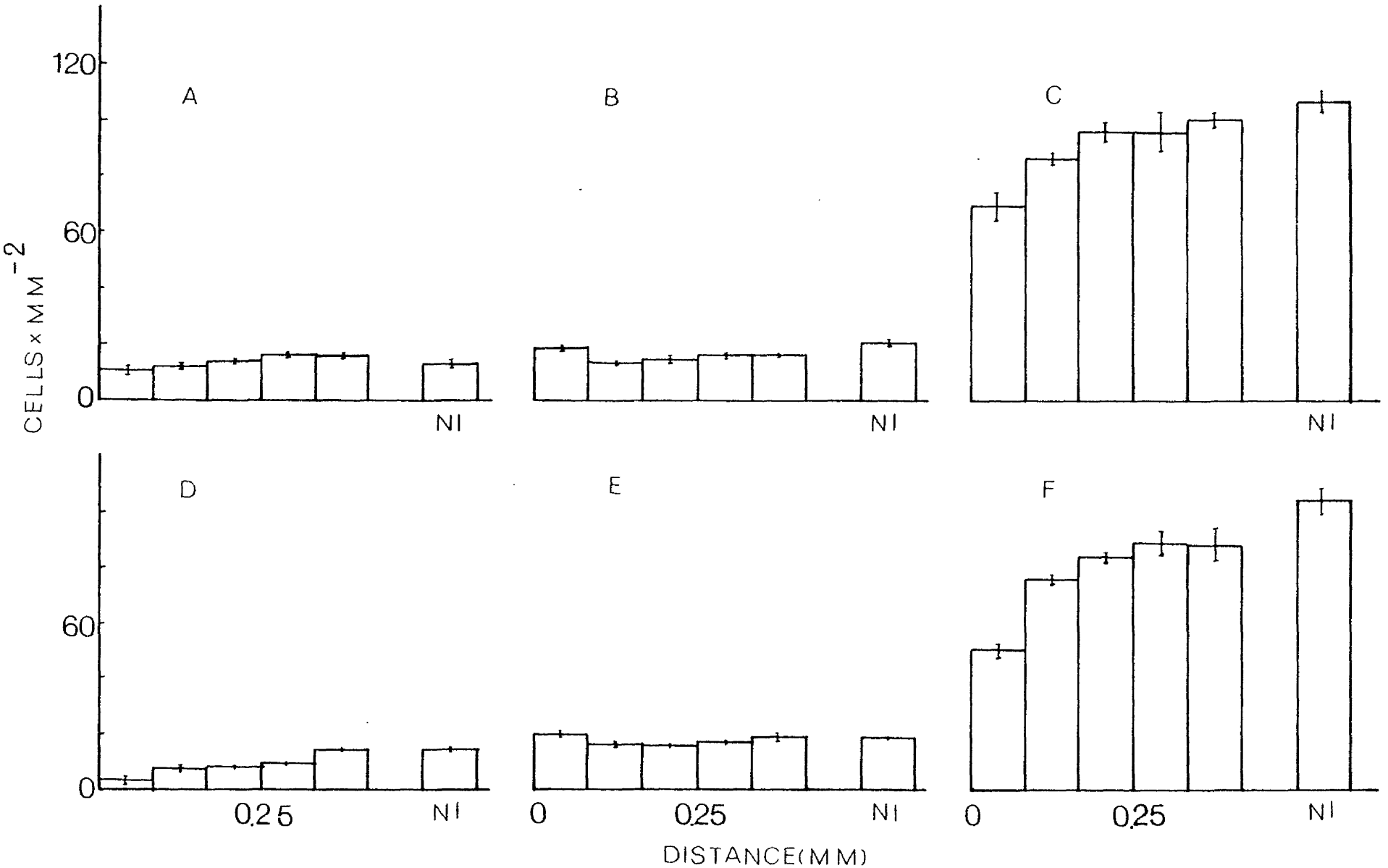
as the zygotes germinates, i.e., two days after germination in the present study. With Chondrus crispus, it did not appear until after several weeks of spore germination. Inhibition zones were observed with both large and small living sporelings and germlings, but not dead ones (Plate 3). Inhibition zones appeared in most of the fucoid germlings observed, whilst it was less common in Chondrus crispus sporelings (less than 30% of total sporelings examined, see data in Appendix 10). Fig.3 indicates that there was a slight difference of total diatom cell numbers in the clear zones of 2 and 6-day Fucus vesicuolosus germlings at the 2nd and 4th day after treatments ($P < 0.05$). In Chondrus the diatom cell density in the inhibition zones was slightly decreased by an increase of size of crustose sporelings at the 2nd day of treatment; however, low diatom densities were also found in some small crusts. The formation of erect fronds from the crusts did not induce antidiatom activity (Table 28). As shown in the results the algal

Table 28 The correlations between diatom cell numbers of Nitzschia closterium in the inhibition zones and the sizes and erect frond formation of Chondrus crusts (28-day old) in seawater cultures; Culture conditions: 15±1°C, 3500 lux and light/dark regime = 14/10h (r = correlation coefficient between diatom cell numbers and diameters of crusts).

Time period (day)	Diameter (mm) of of crust	% of sporelings with erect frond	Diatom density (cells/mm^2) in inhibition zone	r
2	0.121 - 0.350	60.00	57.14 - 97.10	-0.496 (P<0.01)
6	0.161 - 0.411	70.00	600.00 -4057.00	-0.023 (N.S.)

germlings or sporelings did not inhibit diatom growth and cell division, and the inhibition zones disappeared gradually when

Figure 3 Diatom cell densities in the inhibition zones around Fucus vesiculosus embryos; A,B,C = 2-day old germlings, D,E,F = 6-day old germlings; A,D., B.E., and C,F. = determined at the 2nd, 4th and 6th days after treatments; NI = in non-inhibition zones; bars indicate mean ± S.E.


120
60
0
CELLS x MM^{-2}
A
B
C
D
E
F
NI
0.25
0
DISTANCE(MM)

diatom growth and cell number increased rapidly after 6 days of treatment.

7.3.2 Effects of certain physico-chemical conditions on the formation of inhibition zones.

Culture conditions affected the formation of the inhibition zones. Inhibition zones were rarely formed with light intensities above 4000 lux. At lower light intensities, i.e. 500 and 1000 lux, more than 50% of total sporelings and germlings of Chondrus and Fucus serratus showed inhibition zones (Table 29).

Table 29 Percentages of total numbers of young algal plants with inhibition zones at different light intensities (Chondrus crispus - 42-day old, Fucus serratus - 19-day old; determined at the 7th day after inoculation of the diatom Nitzschia closterium, 15.58 x 10^4 cells/dish). Inhibition clear zone determined by visual inspection.

Light intensity (lux)	% (Mean ± range) Chondrus crispus	Fucus serratus
500	-	96.00 ± 2.00
1000	55.59 ± 13.17	84.00 ± 1.00
2000	24.90 ± 5.67	15.00 ± 1.00
3000	49.23 ± 10.77	3.50 ± 3.50
4000	16.66 ± 16.66	0
5000	0	0
7000	7.32 ± 6.97	0

Diatom cell number within zones increased in proportion to the light intensities studied. In dark conditions, no inhibition zones appeared, and the diatom cell number was greater around the germlings (Fig.4). Total diatom numbers in the inhibition zones of Fucus serratus embryos also different with the salinities used (Fig.5). The number

Figure 4 Diatom cell densities in the inhibition zones of Fucus serratus germlings; C = without pretreatment with fucoid germlings, T = with pretreatment, D = without pretreatment and in the dark; NI = in non-inhibition zones; bars indicate mean ± S.D.

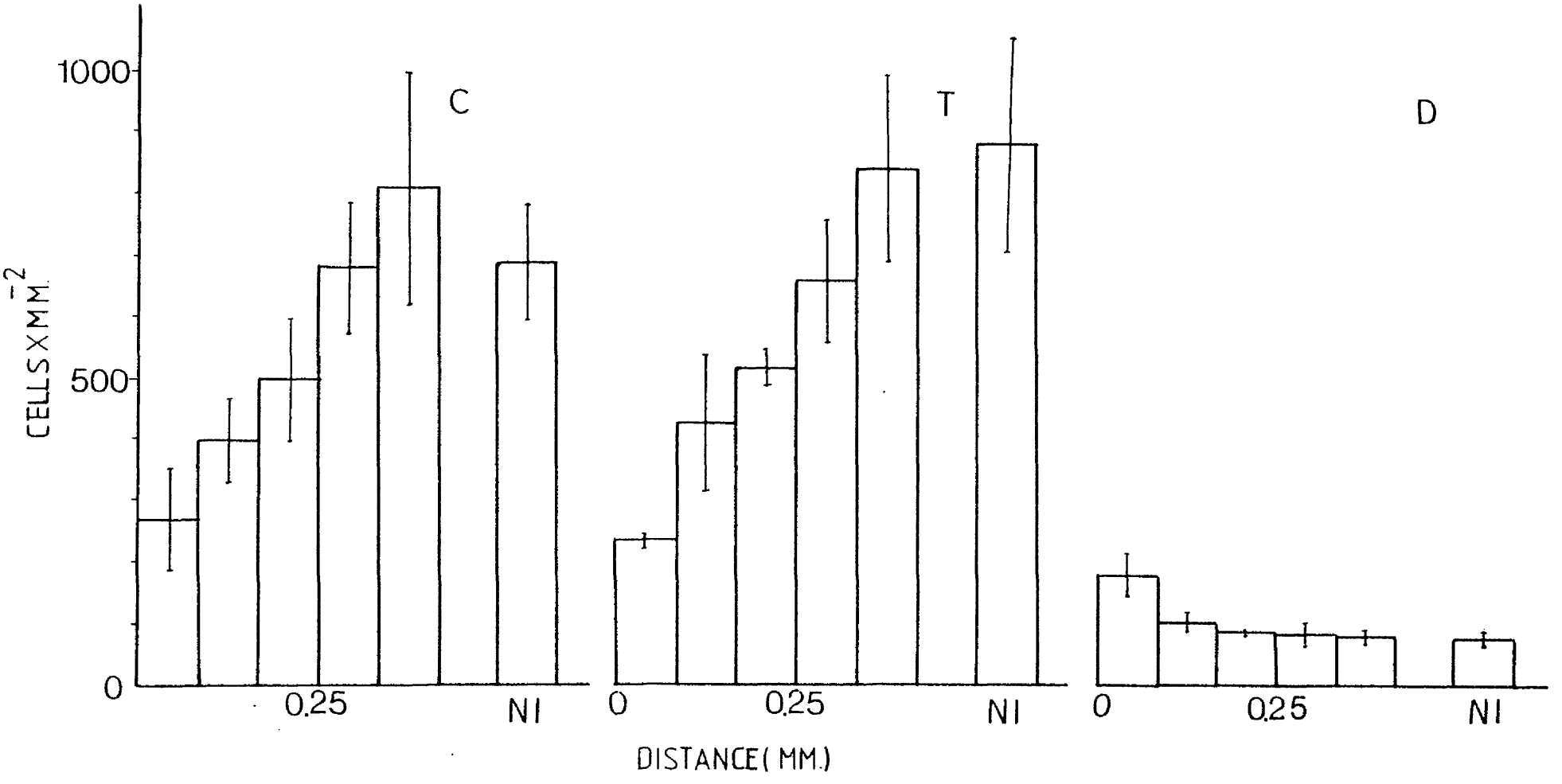
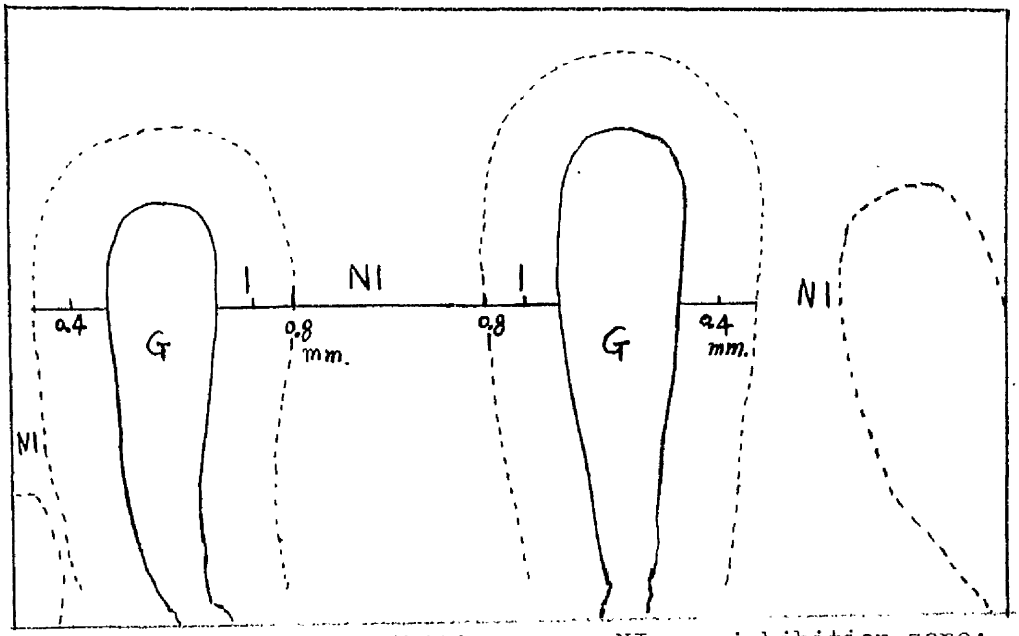

1000
500
0
CELLS X MM^{-2}
C
T
D
0
0.25
NI
0
0.25
NI
0
0.25
NI
DISTANCE (MM.)

Figure 5 Average diatom cell densities in the inhibition zones 0.42mm from Fucus serratus embryos (solid circles) and in non-inhibition zones (empty circles) at different salinities; age of germlings = 12 days;



G=Fucus germling; I=Inhibition zone; NI=non-inhibition zone;

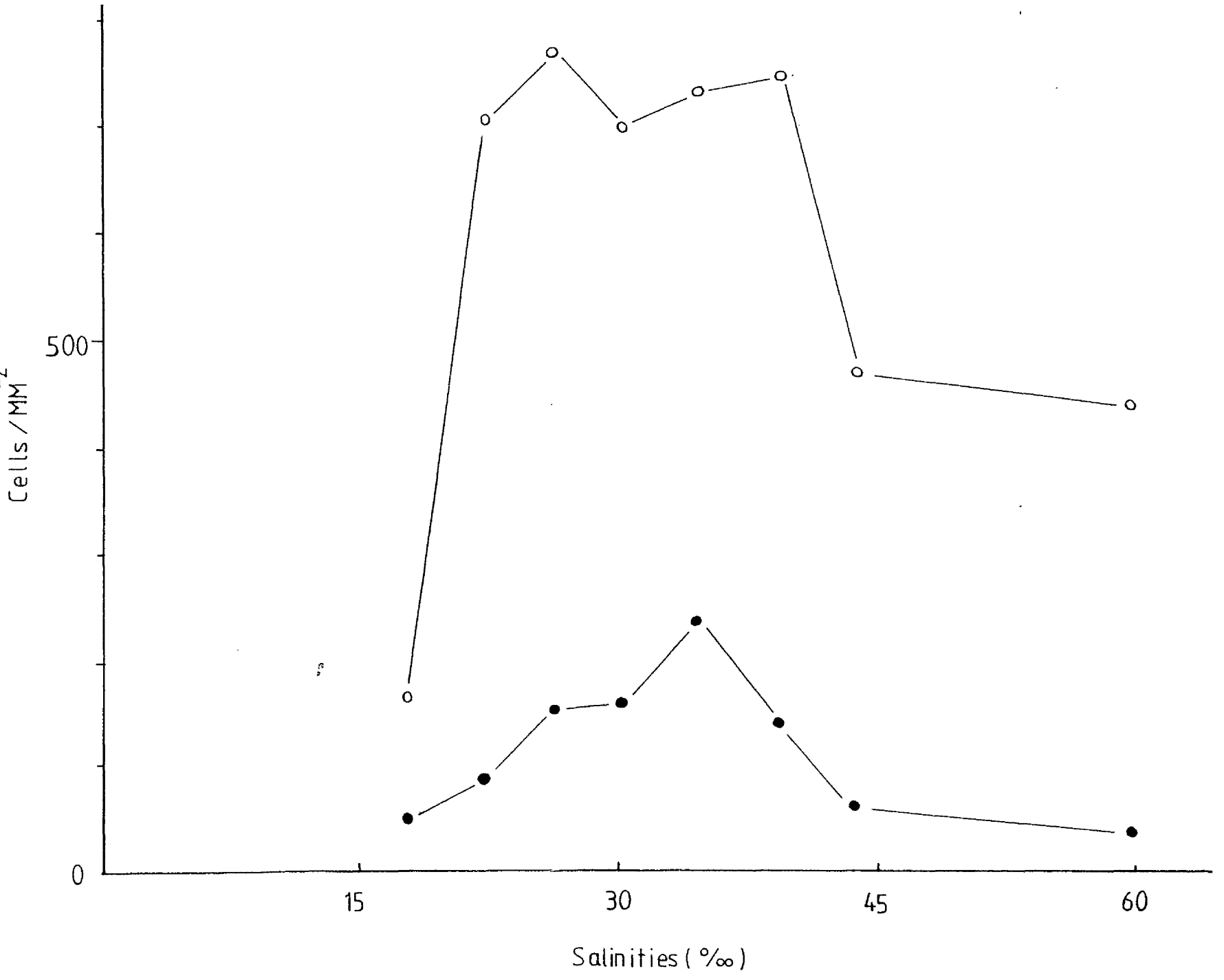
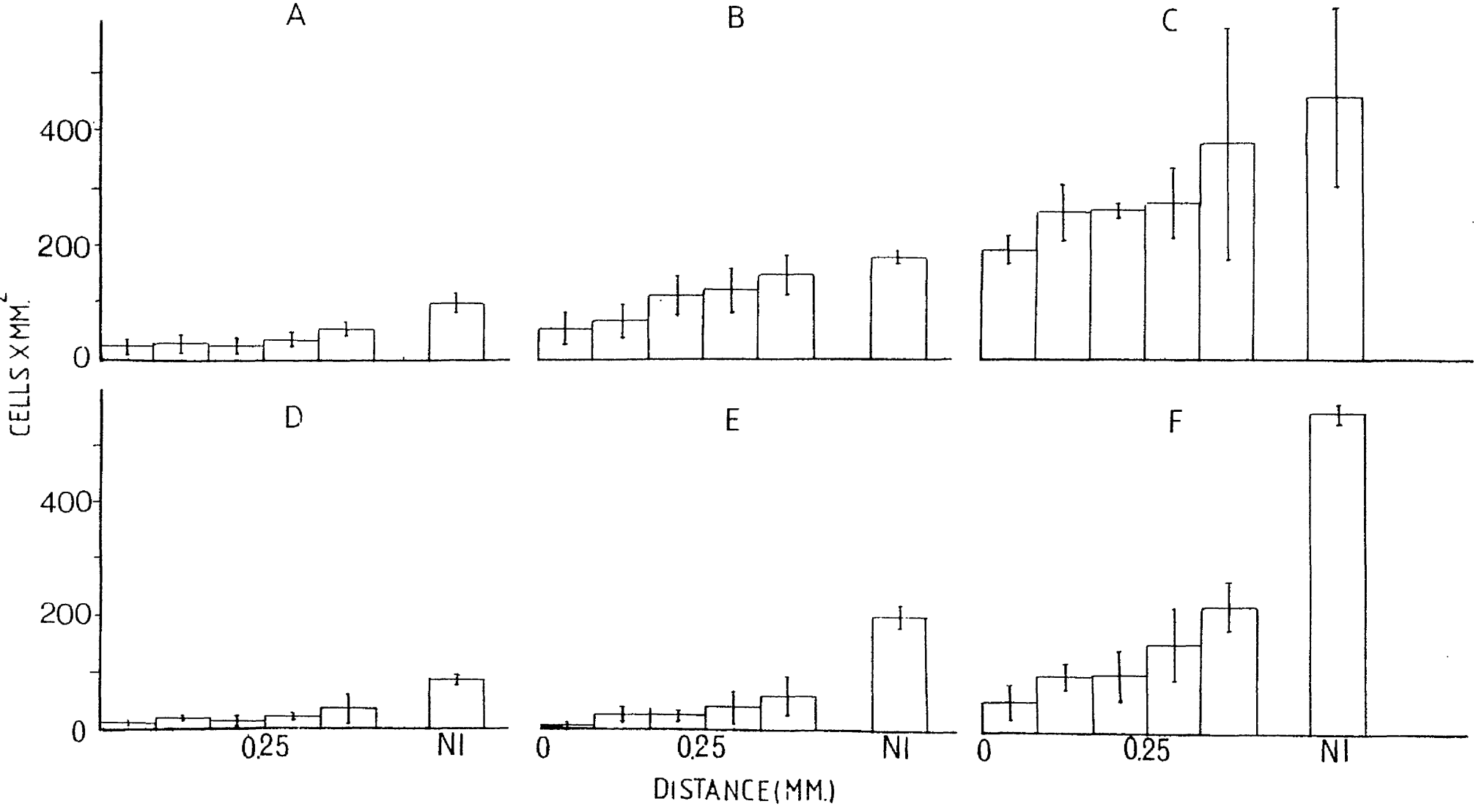

500
0
Cells / MM-2
15
30
45
60
Salinities (‰)

Figure 6 Diatom cell densities in the inhibition zones of Fucus serratus germlings in the enriched (A, B, C) and non-enriched (D, E, F) seawater media; A.D., B.E., and C.F = determined at the 2nd, 4th and 6th days after treatments; NI = in non-inhibition zones; age of germlings = 10 days; bars indicate mean ± S.D.


A
B
C
D
E
F
400
200
0
CELLS X MM.$^{-2}$
0.25
NI
DISTANCE (MM.)

of cells per mm^2 of inhibition area was increased towards normal salinities, i.e. 30.31 and 34.59‰, and decreased when salinities became lower or higher, i.e. 22.30 and 43.90‰ respectively. Cell density outside the inhibition zone (0.42mm from the germlings) was increased , i.e. 2.8 - 5.6 orders of that in the inhibition zone based on the cell number per mm^{-2}. Distribution of diatom cells in the inhibition zones of Fucus serratus was similar both at 10°C and 15°C with slightly fewer cells at 10°C than 15°C. Enrichment of seawater encouraged an increased growth of diatoms in the cultures so that the inhibitory effects of algal germlings in enriched cultures became less apparent than non-enriched ones (Fig. 6).

7.3.3 Effects of pretreatment with Fucus germlings in seawater

Pretreatment of the diatom N. closterium growing with Fucus serratus germlings for 8 days prior to an experiment had no effects on the subsequent response of the diatom to Fucus germlings growth (Fig. 4). Inhibition zones appeared equally in treated and non-treated cultures.

7.4 Discussion

The antidiatom activities of both Fucus germlings and Chondrus sporelings with Nitzschia closterium are clearly demonstrated in the results either by the formation of narrow clear zones or of radiating regions with progressively smaller numbers of diatoms towards the germlings and sporelings. Khfaji and Boney (1979) described the inhibition zones appearing around 3 month old crustose discs of Chondrus crispus forming complete barriers against diatom invasion. Sieburth and Conover (1965) found that the accumulation of polyphenols (tannins), which are inhibitory to fouling organisms, was greatest in the distal tips of Sargassum natans thalli. An increase of anti-

biotics was also found in meristematic or younger tissues of certain seaweeds (Ross, 1957; Hornsey and Hide, 1976), *eg.* the peripheral cells of Chondrus discs, which are meristematic. Extracellular substances are more likely to be excreted from the metabolically active meristematic cells and this is in agreement with the phenomena observed in the results. Thus inhibition zones are formed around the distal region of the fucoid embryos but not rhizoids. The inhibition zones can be seen more easily with the germlings growing towards the light during germination and development. No inhibition zone was observed around dead embryos or with those kept in the dark. Diatom numbers in inhibition zones were not correlated with the size of Chondrus crustose discs. Nevertheless, the metabolic activity of meristematic cells of individual sporelings of the same species or different algal sporelings was not identical as was revealed in the unequal diatom numbers in the inhibition zones formed under the same conditions.

McLachlan and Craigie (1964) indicated that the toxicity of yellow extra-cellular phenolic substances of F. vesiculosus appeared to be non-specific on caused cellular lysis of unicellular algae. As shown in the present results, the diatom cell numbers increased rapidly 6 days after inoculation. The inhibition zones, therefore, would seem to be directly attributable to the diatom cells being temporarily sensitive to certain extracellular substances of algal sporelings. The inhibition is gradually reduced as diatom cell numbers increase. The results in Chapter 4 have indicated that the growth rate of a great number of isolated littoral diatoms is greatly induced by the associated young algal plants particularly fucoid germlings. The sensitivity of unicellular algae to extracellular substances, in fact, varies considerably between species (McLachlan and Craigie, 1964; 1966. Mason and Gleason, 1981). It might be that diatom cells are less sensitive to extracellular

substances which significantly affect the growths of other unicellular algae. It is also possible that substances released from juvenile plants are relatively less active in toxicity than those from mature plants. Indeed, the phenolic compounds released from algal germlings and sporelings can be chelated by metal ions in seawater (Chapters 4, 5 and 6) (Ragan et al., 1980) and consequently, become less toxic to diatom cells. Therefore, in prolonged cultures, i.e. after 6 days as shown in fucoid germlings, the inhibitory effects of phenolic compounds to diatom cells reduce as the latter increase greatly in numbers. The similar inhibition zones which appeared in treated and non-treated diatom cultures suggest that any immunity or adaptation of diatom cells to toxic substances does not remain after cell division.

The substances involved in antibiotic activity in seaweeds have been studied (Gerber et al., 1958; Ehresmann et al, 1977; Caccamesse et al, 1980, 1981). Among the seaweeds investigated, extracts of Fucales demonstrate the strongest antibacterial activity (Ross, 1957; Caccamesse and Azzolina, 1979). This also has been shown in the present study with diatoms, where inhibition zones were formed earlier and for longer periods with fucoid germlings (up to 6 days in fucoid germlings and less than 4 days in Chondrus sporelings in most cases). In other words, the diatom, N. closterium appears to be comparably more sensitive to Fucus than to Chondrus inhibitory substances.

It is well known that environmental conditions can affect the antibiosis of certain marine algae (McLachlan and Craigie, 1964; Ross, 1957; Hornsey and Hide, 1976). The inhibitory effects of algal sporelings on diatoms varied greatly with the culture conditions examined. So far it is not yet clearly understood why algal germlings become more active in the inhibition of diatom growth at lower light intensities

and in certain nutritional conditions. However, two possibilities should be considered : an increase of exuded antibiotic substances from algal germlings, which increase the "phycosphere" (Bell and Mitchell, 1972) surrounding the algal sporelings to prevent diatom invasion in mixed cultures or a decrease of diatom growth and mobility. Schonbeck and Norton (1979) indicated that diluting the enriched medium can eliminate contaminating diatoms from cultures of F. spiralis germlings. Similar observations with epiphytes of aquatic weeds and a filamentous green algae in relation to available nitrogen compounds were also proposed by Fitzgerald (1969). The induction of diatom mobility by higher light intensities has been shown in various reports (Nultsch, 1956; Hopkins, 1963, 1965; Hopkins and Drum, 1966; Round and Eaton, 1966; Harper, 1969); this increases diatom invasion and diatom stress on young algal plants. Toxin production by Chlamydomonas reinhardi (Proctor, 1957) and Microcystis aeruginosa (Gorham, 1964) is also closely related to light condition. Nevertheless, the lack of inhibition zones in dark conditions implies that light is essential to the antibiotic activity of fucoid germlings either in the releasing or formation of substances toxic to diatoms. In addition, the pH of the medium in close proximity to the germlings might also play an important role in the toxicity of released substances (Proctor, 1957; McLachlan and Craigie, 1964; Kroes, 1970).

It was noticeable that on occasions, clear zones formed around the red alga, Gigartina stellata, in old cultures heavily contaminated by unclassified epiphytes other than diatoms (Plate 4). Antibiotic activity in G. stellata has not yet been reported though Gigartina is related to C. crispus in spore ontological development (Hornsey and Hide, 1974; Khfaji and Boney, 1979; Prince and Kingsbury, 1973).

In the previous chapters, all results showed that G. stellata is more sensitive to diatom inhibition than C. crispus. Therefore, the above findings together with the antibiosis in the brown alga Ralfsia spongiocarba Batt. (Fletcher, 1975) suggest that antibiosis is common in young algal plants of red and brown algae under certain environmental conditions and algal growth stages. Although the antidiatom activity of algal germlings and sporelings appears in rather short periods mostly less than 7 days in the present study, this may be sufficient to allow algal sporelings and germlings to become established before diatom invasion. This is quite important in relation to algal distribution in nature from an ecological point of view. A study of the antidiatom behaviour of juvenile plants undoubtedly adds to our understanding of antagonistic behaviour of co-existing species.

CHAPTER 8

EFFECTS OF DIATOM MUCILAGE ON THE GROWTH AND MORPHOLOGY OF ALGAL SPORELINGS AND GERMLINGS

8.1. INTRODUCTION

The attachment and germination of algal spores are greatly enhanced by the excretion of mucilaginous substances in intimate contact with the substratum (Moorjani and Jones, 1972; Quatrano and Crayton, 1973; Boney, 1975, 1981; Fletcher, 1976, 1981; Forbes and Hallam, 1979). The nature of the substratum plays an important role in bio-adhesion (Baier, 1970; Corpe, 1970; Norton and Fetter, 1981). Culture studies indicate that insufficient contact of spores with the substratum results in rhizoid-like sporelings or irregular cell masses in the red algae Gigartina stellata (Stackh.) Batt and Chondrus crispus L. (Chen and McLachlan, 1972; Chen et al., 1974; Prince and Kingsbury, 1973). The occurrence of discoid holdfasts in Polysiphonia is related to the surface nature of substratum (Fletcher, 1976). The shape of germinated zygotes of Bifurcaria bifurcata Ross (Hardy and Moss, 1979) and the attachment of the haptera of Laminaria digitata (Huds.) Lamour (Moss and Tovey, 1978) are affected by the surface character of substrata. The importance of a suitable substratum for the attaching zoospores of green algae was also proposed by Christie and Shaw, (1968). In nature, the substrata colonized by algal propagules are often ones previously occupied by micro-organisms, especially bacteria and diatoms, (Woods Hole Oceanographic Institution, 1952; Sieburth, 1975; 1979; Fletcher, 1974). These precursors on the substratum directly or indirectly affect the species which follow as well as their growth phenomena (Miller, 1946; Horbund and Freiberger, 1970). Diatoms usually dominate the surface of submerged substrata before or after bacterial attachment (Wood, 1950; Skerman, 1956; Corpe, 1970). Many diatom species found in the littoral zone

produce large amounts of mucilaginous substance (Hendey, 1951; 1964), which then form slimy films on the rock face. Laboratory studies have shown that the slime films of benthic diatoms are of significance in sediment stabilization, (Holland et al., 1974). Scheer (1945) found that the diatom community provides favourable conditions for the settlement of bryozoans. The diatom mucilage film together with the aggregated soil grains and organic debris undoubtedly provide fouling macro-organisms with both a nutrient source and a place for settlement. Little is known of the chemical nature of the diatom mucilaginous substance. Lewin (1958) and Lewin et al., (1958) showed that the mucilaginous substance in Amphipleura rutilans (Trent.) Hisle and the capsular material in Phaeodyctylum tricornutum Bohlin comprised predominantely different forms of mono- and polysaccharides. Since these mucilage-producing littoral diatoms are often early colonisers of cleared areas, it seemed worthwhile examining the effects of diatom slimes on the early sporeling and germling development of marine algae.

8.2. Materials and Methods

8.2.1 Diatom mucilage and release and attachment of reproductive cells of marine algae.

The naviculoid diatom, Navicula grevilleana Hendey (GB 25), was collected and isolated from a population growing on Plumaria elegans (Bonnem.) Schm. (Rhodophyta) (Appendix 4). In unialgal culture, this diatom produces a large quantity of mucilaginous substance, which gradually forms a thick slimy film on the bottom of a petri dish. Before an experiment, identical amounts of diatom suspension were cultured in 50mm diameter petri dishes (Sterilin) which contained 10ml enriched seawater medium at 3000 lux, 15 $\pm$ 1^{o}C and 14h illumination day^{-1}. The diatoms were kept growing in the cultures without change of medium for about

five weeks. The slime films formed on the bottom of the dishes were then air-dried at room temperature for six weeks to kill the cells. The viability of diatom cells in the slime film was tested before the experiment by culturing them in enriched seawater medium for at least three weeks. They were then examined under a microscope to ensure that there was no living cells in the cultures. During the experiments any culture with obvious diatom contamination was eliminated. The dried slime film in the dish was rinsed carefully with sterile distilled water and seawater (three times). In this way the effect of the diatom mucilage was separated from that of living diatom cells to simulate conditions in intertidal habitats, in which dried diatom slimes are left on the rock faces. Finally 10ml of enriched seawater medium was added to the dish, and the dishes were kept at $15\pm1^{o}C$ and 14/10 h light /dark regime.

Fertile thalli of eight species of marine benthic algae- Ulva lactuca, Pelvetia canaliculata, Fucus spiralis, F. vesiculosus, F.serratus, Ascophyllum nodosum, Chondrus crispus and Gigartina stellata were collected from the littoral zone (Fig.1). Methods for obtaining the release of carpospores, gametes, antherozoids and eggs were the same as described in 4.2.1. Identical volumes of the zygotes (Ulva, Fucus, and Ascophyllum) and carpospores (Chondrus and Gigartina) were added separately to 50mm diameter petri dishes with or without diatom mucilage described above, which contained 10 ml enriched medium.

8.2.2 Determinations of percentage germination and growth of zygotes and carpospores

Percentage germination for the brown algae was calculated from the number of germinated zygotes in the total number of zygotes observed in the whole dish. Unattached or loosely attached germlings were assessed

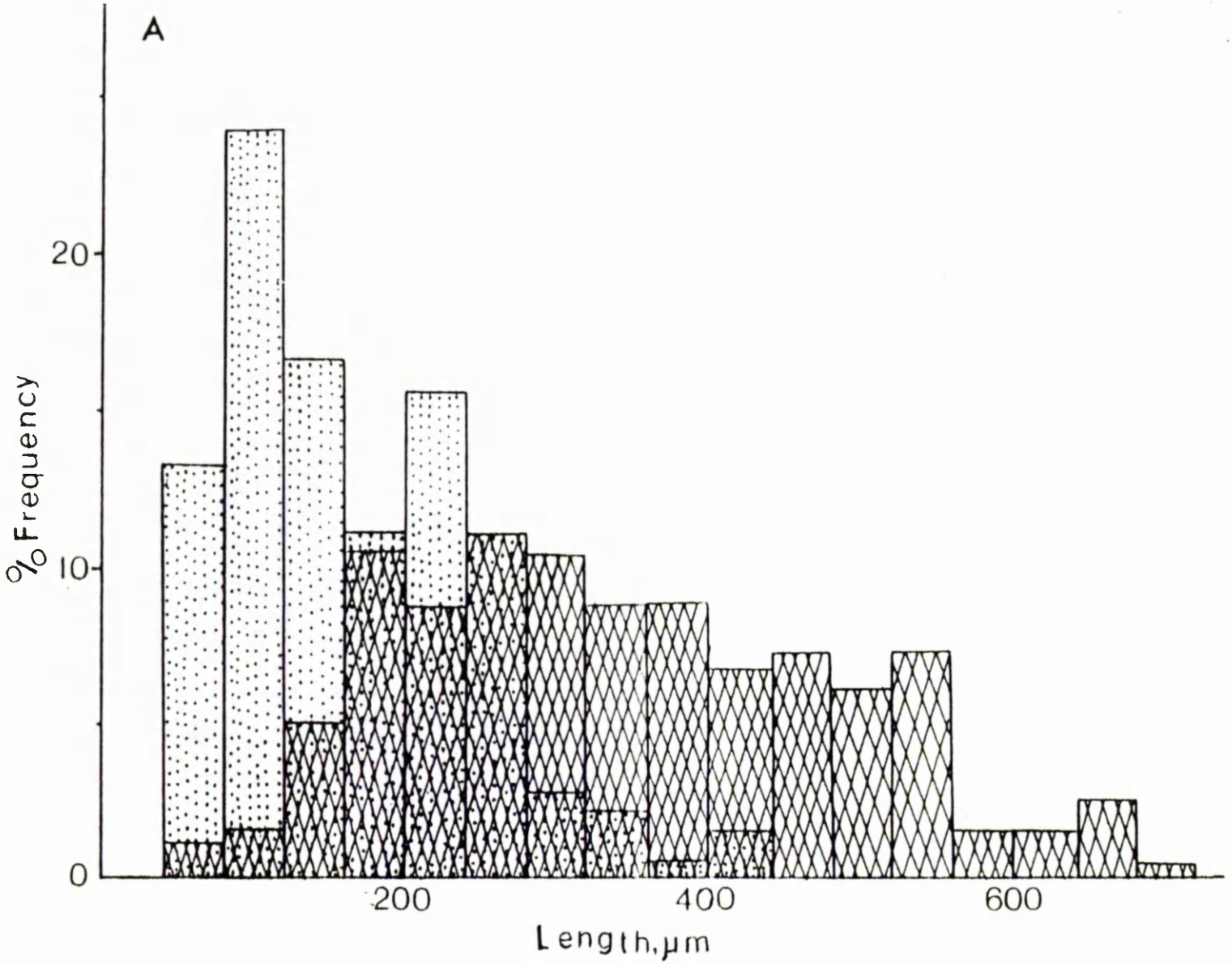
when germlings failed to attach or became attached with a small part of rhizoid on the bottom surface of dish; these were seen when the dish was moved from side to side. For sporelings of red algae, percentage germination was determined by counting the germinated spores in a dish. The number of spores added to the dishes were also counted on each occasion. Lengths and widths of oo embryos and the diameters of disc-form sporelings were also measured for the assessment of growth rates.

8.3. Results

The results indicated that both growth and development of the algal sporelings and germlings varied with the species in both treatments With Ulva lactuca, the primary rhizoid was long and unbranched on the slime film (Plate 5 E) and secondary rhizoids did not appear until about the tenth day after germination. By contrast, the primary rhizoid in control sporelings (without diatom slime) was comparatively short and many secondary rhizoids appeared in a few days (Plate 5B). Histogrammic summaries (Figs. 7A,B) showed that the germlings growing on the slime film generally had longer fronds than those on the clean surface and the fronds became branched earlier (see Plate 5 E). Transverse sections of germlings from both experimental treatments appeared identical.

With the fucoid algae, the percentage germination of zygotes on diatom slime was slightly higher than that on clean surfaces though not significantly so (Table 30). Generally, the number of unattached or loosely attached germlings of the four species was consistently higher in the control than in the slime treated cultures during the first three weeks in the present study, particularly with F. spiralis and A. nodosum (Table 30). The dimensions of fronds were comparatively larger on the

Figure 7 Size distribution of *Ulva lactuca* germlings growing on clean surfaces and on diatom mucilage; A = lengths, B = widths; clean surfaces = stippled, diatom mucilage = cross hatched, data based on 3 replicates and 30 germlings in each replicate .


A
20
10
0
% Frequency
200
400
600
Length, µm

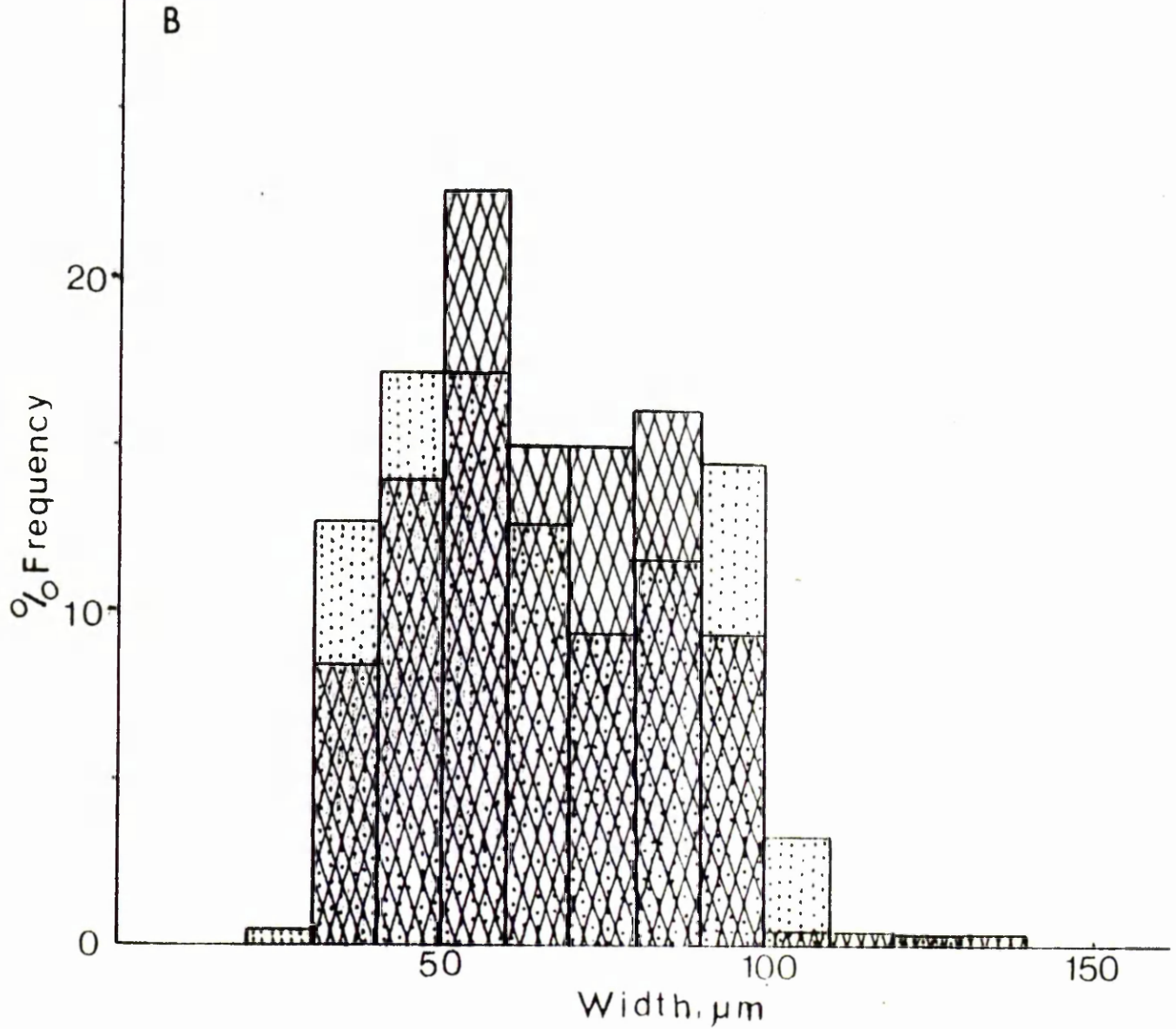
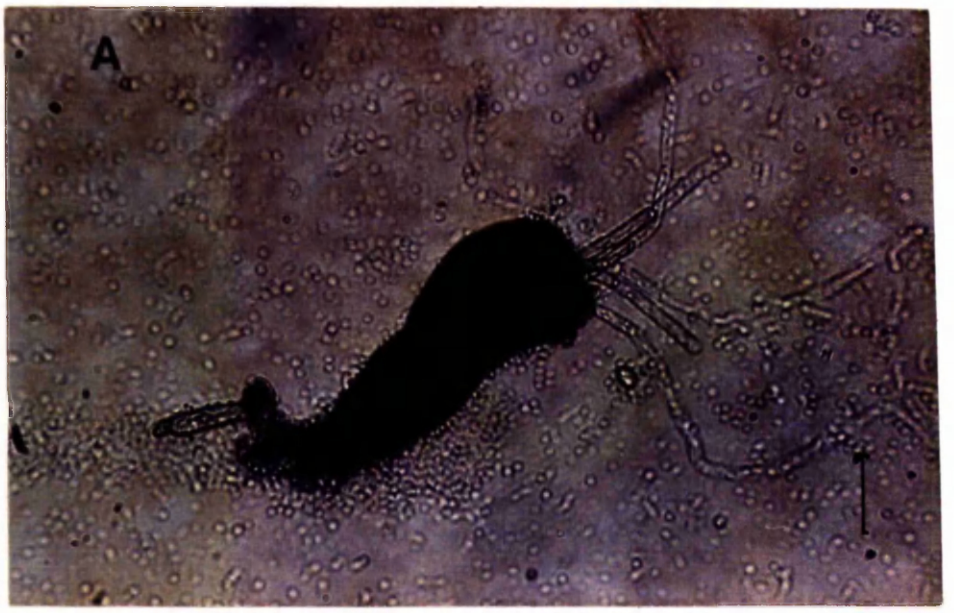
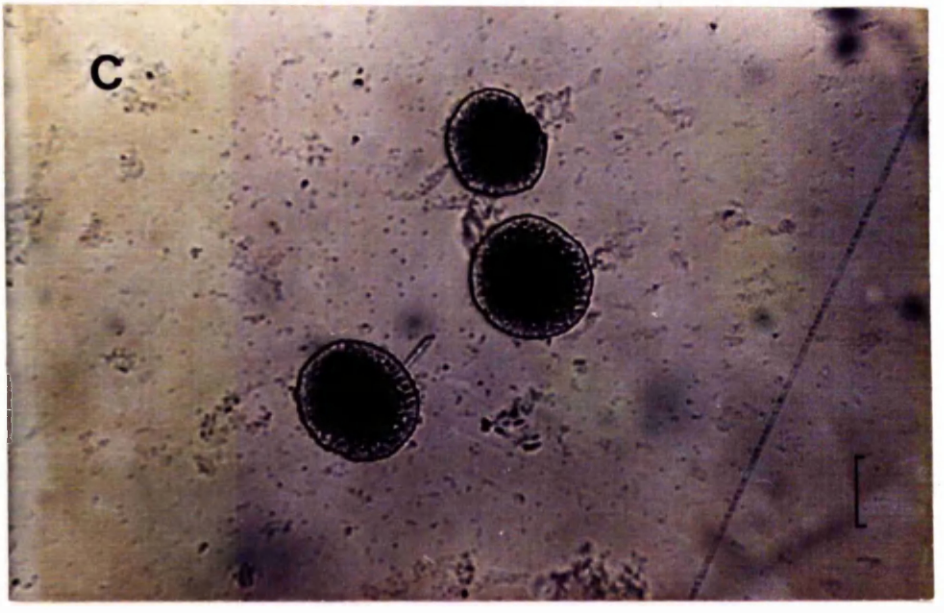

B
20
10
0
% Frequency
50
100
150
Width, µm

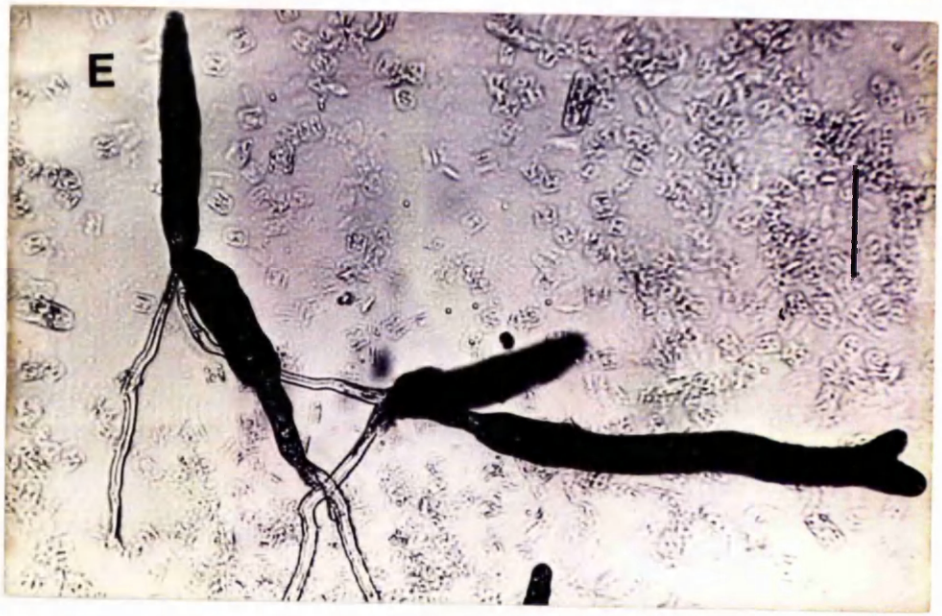
Plate 5 Ulva lactuca germlings after 6 days growth on clean surface (B and on diatom mucilage (E); Fucus spiralis germlings after 20 days gro on clean surface (A) and on diatom mucilage (D); Gigartina stellata sporelings after 23 days growth on clean surface (C) and on diatom mucilage (F); scale = 100 μm.


A


B


C


D


E

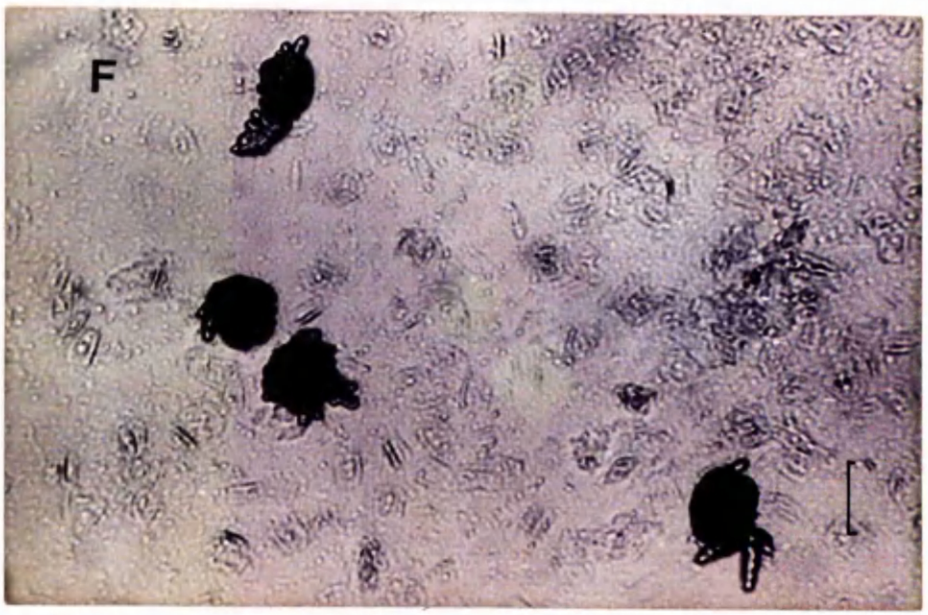

F

Table 30 Germination and growth of embryos of marine brown algae in cultures : NM = not measured, * determined at 20th day (lengths and widths were based on 3 replicates and 30 germlings in each replicate).

		after 6 days		after 20 days		
		% germination	% unhealthy or unattached embryos	% unattached or loosely attached embryos	size of embryos (μm) length (Mean ± S.E.)	width
Fucus spiralis	clean surface	82.04	19.06	38.00	310.84 ± 32.16	154.41 ± 3.82
	diatom mucilage	95.98	17.31	1.33	364.39 ± 37.82	171.37 ± 12.51
F. vesiculosus	clean surface	94.72	21.12	4.18	510.55 ± 15.37	203.61 ± 4.84
	diatom mucilage	92.72	20.30	1.00	598.57 ± 21.73	203.78 ± 8.71
F. serratus	clean surface	96.95	29.04	5.50	646.73 ± 62.17	172.19 ± 7.89
	diatom macilage	99.17	24.00	0.33	720.27 ± 61.22	163.06 ± 11.01
Ascophyllum nodosum	clean surface	91.98	41.18	30.60	281.67 ± 13.57	146.36 ± 2.46
	diatom mucilage	93.65	20.79	5.00	327.59 ± 4.33	156.88 ± 2.38
*Pelvetia canaliculata	clean surface	90.25	17.25	NM	germlings healthy - not measure (see text)	
	diatom mucilage	96.53	1.88	NM	" " " " "	

slime than on the clean surface (Table 30). Germlings of F.serratus and F.vesiculosus grew much faster than those of F.spiralis and A.nodosum under the same cultural conditions. The lengths of apical hairs varied greatly in the germlings even in the same culture, and were not persistently intact throughout the experiment. Rhizoid production in the brown algae was clearly induced by the diatom slime, particularly with F. spiralis, in which a fully developed rhizoidal system was found on the slime film whereas unhealthy and poorly-developed rhizoids appeared on the clean surface after three weeks (Plate 5.A,D). There was difficulty in measuring the length and width of P. canaliculata germlings since the fronds grew upright with tufts of apical hairs on the tip under the microscope. However, all the germlings in both treatments appeared healthy. Rhizoid production of P. canaliculata sporelings on the slime film was little different from that on clean surfaces. Possibly the large quantity of Pelvetia mesochiton greatly decreases the effects of external factors on zygote development.

With both Gigartina stellata and Chondrus crispus sporelings the early stages of development are disc-like. Whilst in C. crispus germination rates were generally slow in the first 6 days; the number of germinated spores on the slime were smaller than those on clean surfaces (Table 31). After 22 days, the average diameter of the discs

Table 31 Germination of red algal spores after 6 days on clean surfaces and on diatom mucilage (data based on 6 replicates)

	% Germination	
	clean surface	diatom mucilage
Chondrus crispus	10.36	6.17
Gigartina stellata	38.85	44.35

Table 32 Growth of carpospores of Chondrus crispus after 22 days on clean surfaces and on diatom mucilage (diameter of discs based on 3 replicates and 30 crustose discs).

	% disc-form sporelings	% rhizoid-form sporelings	% sporelings with hyaline hairs	% sporelings with erect fronds	diameter of discs (μm) (Mean ± S.E)
Clean surface	48.65	51.35	64.11	34.88	218.50 ± 12.75
diatom macilage	47.19	52.81	36.70	0	126.20 ± 16.58

of C. crispus sporelings on slime was approximately half that of sporelings on clean surfaces (Table 32). In addition, hyaline hairs and erect fronds were absent while they were observed in the control sporelings. In G. stellata, this inhibitory effect was more distinct in the cultures. Sporelings in the cultures with diatom slime were all abnormal in appearance (Plate 5C,F) whilst those on clean surfaces were disc-like. Most of the abnormal sporelings appeared as spherical masses of cells overlapping each other with one or several rhizoid-like protuberances. Whilst sporelings adhered firmly to the slime film, they showed no signs of erect branch formation. Rates of germination were higher than with Chondrus, and were a little enhanced on the slime layer.

8.4. Discussion

The effects of diatom mucilage on the growth of algal sporelings and germlings varied with the species observed. In general the effects on germination were less significant than those on developmental morphology. Whilst the average size of fucoid germlings growing on the mucilage was larger than that on clean surfaces, it was smaller with Chondrus crispus sporelings. The more striking features were seen in the effects of diatom mucilage on both rhizoid production and germling development in Ulva lactuca and the fucoid algae. It has been shown for the zygotes of fucoid algae (F. vesiculosus) that both RNA and protein synthesis are necessary pre-requisites in rhizoid formation (Quatrano, 1968). Adhesion of fucoid germlings appears to be dependent on production of the sulphated fucoidan outside the rhizoidal cells (Quatrano and Crayton, 1973; Hardy and Moss, 1978; Forbes and Hallam, 1979). A shortage of sulphated polysaccharides will result in either complete failure or loose attachment of zygote rhizoids. Plants of upper shore fucoid algae contain more fucoidan than the lower shore Fucus serratus (Evans et al.,

1973b). In the present work, increased rhizoid production was observed with germlings of F. spiralis when in contact with the diatom mucilage, and much more than with germlings of F. serratus. A higher proportion of loosely attached germlings was observed in control germlings of F. spiralis, which might be attributed to a shortage of fucoidan production under the conditions of experiments, or to slower growth rates when compared with germlings of F. serratus. However, on the diatom mucilage there was both enhanced rhizoid production and germling growth, which would seem to indicate the release of growth promoting substances by the mucilage layer. Similar results with Fucus germlings have been described when IAA and certain indole compounds are applied to cultures (duBuy and Olson, 1937; Davidson, 1950). With Pelvetia canaliculata germling rhizoid production on the mucilage was little different from that on clean surfaces. This may be a result of the greater importance of the mesochiton in the early stages of zygote germination (Moss, 1974), possibly serving a function similar to that of the diatom mucilage with the other fucoid algae examined.

The change in rhizoid morphogenesis seen with Ulva lactuca germlings on diatom mucilage, with the formation of a long and unbranched primary rhizoid and without secondary rhizoid formation, was strikingly different from the results obtained with the fucoid algae. Long, unicellular, unbranched rhizoids were found in germinated tetraspores of the red alga Polysiphonia on coverglass (Fletcher, 1976). This abnormal rhizoid growth resulted in a less efficient attachment to the substratum than was seen with the germlings with normal rhizoid development on cleaned surfaces, and would seemingly be disadvantageous in nature. The morphogenesis of green algal germlings has been shown to be variable under a variety of experimental conditions. Restoration of normal morphology in axenically grown Ulva germlings has been observed in the presence

of certain bacteria (Provasoli and Pintner, 1972,1980). Induction of rhizoid formation by green algae was induced by exogenous growth substances (Jacobs, 1951; Eaton et al., 1966). It would seem that growth promoting substances in the diatom slime were responsible for the change in the pattern of rhizoid development in Ulva.

The diatom mucilage had marked effects on both the growth and morphogenesis of sporelings of Gigartina stellata and Chondrus crispus. With both species, ontological developments of carpospores normally result in the formation of prostrate discoid cell growths, initially monostromatic, later becoming polystromatic in the centre with subsequent erect branch formation. Further anticlinal and peripheral cell divisions result in the increased thickness and expansion of the disc-like holdfast (Chen and McLachlan, 1972; Chen et al., 1974; Prince and Kingsbury, 1973). Gigartina sporelings grown on diatom mucilage produced an irregular cell mass. These were different in appearance from the rhizoid-form sporelings which are known to arise either as a result of insufficient contact with the substratum or after a long delay in germination (Chen et al., 1974). Erect branch formation with Gigartina was not obtained in the present experiments. Chondrus crispus sporelings showed both growth inhibition (with smaller disc formation) on the diatom mucilage, and the absence of erect branch formation. Evidently the diatom mucilage induces clear inhibitory effects on primary disc formation by these red algae with especially severe effects on Gigartina. Whether these effects are the result of the substance or substances in mucilage which induced growth abnormalities in the green and brown algae is not known.

The results indicate that dried diatom mucilages containing dead cells release either growth substances, or compounds which simulate

the effects of growth substances. Growth substances have been identified both in unicellular algae and the extracellular products released by them (Bentley 1958, 1960; Hellebust, 1965; Mowat, 1965; Fogg, 1966; Augier, 1976). Such substances have been recognised as controlling both growth and development, including mechanisms of cell elongation and division, cellular differentiation, morphogenesis and organogenesis (Augier, 1976). Slime or mucilage films of bacterial or diatom origins are known to be of significance for the attachment of other organisms (Gray, 1966; Floodgate, 1968; Horbund and Freiberger, 1970). Mucilage-secreting diatoms are common in eulittoral habitats. The present results show that the residual diatom mucilage, left after drying out and death of the constituent cells, can have marked effects on both the attachment mechanisms and subsequent morphogenesis of certain algal sporelings.

CHAPTER 9

EXCHANGE OF DISSOLVED ORGANIC MATTER BETWEEN CERTAIN LITTORAL DIATOMS AND SPORELINGS OF RED ALGAE

9.1 Introduction

Most algae are affected in various ways by organic substances in the environment (Fogg, 1970). Apart from man-made activities and animal release, there is a considerable amount of dissolved organic matter in seawater derived from littoral algae via excretion, autolysis and bacterial decomposition (Jones, 1962; Khailov and Burlakova, 1969; Sieburth, 1969; Sieburth and Jensen, 1969; Newell et al., 1980; Lucas et al., 1981). Some organic substances can be taken up by various algae and assimilated into intracellular components or respired inside the cell (Williams, 1970; Hellebust, 1974; White, 1974). The brown alga, Fucus vesiculosus, is capable of absorbing exogenous mannitol which is then converted into residual carbohydrates (Bidwell and Ghosh, 1962). Drew (1969) found that two out of the eleven brown algae investigated converted absorbed ^{14}C-glucose into mannitol. Uptake and assimilation of amino acids and vitamins in red and green algae are also well documented (Watanabe, 1937; Boalch, 1961; Tatewaki and Provasoli, 1964; Fries and Petterson, 1968; Crawford and Richardson, 1974). Heterotrophic uptake and utilization are more common in diatoms, particularly those living in the littoral zones (Lewin and Lewin, 1960; Cooksey, 1972; Lewin and Hellebust, 1976; Hellebust and Lewin, 1977). There is often a significantly enhanced growth of diatoms in the presence of organic supplements (Bunt, 1969; Carlucci and Bowes, 1970; Ragan et al., 1980). The relatively higher uptake rate of organic compounds in light (photoheterotrophy) is thought to be a temporary compensation for the inadequate photosynthesis under certain conditions (Admiraal and Peletier, 1979b). Heterotrophic growth on exogenous organic compounds in complete darkness is not uncommon in diatoms (Smiley and Darley, 1972;

Lewin and Hellebust, 1970). However, uptake and assimilation of organic matter are rather dependent on the metabolic system developed within the cells or their heterotrophic capacity (Oaks,1962a,b; Hellebust and Guillard, 1967; Cooksey, 1972; Lylis and Trainor, 1973; Bekheet and Syrett, 1977; Darley et al., 1981). Recently, Admiraal and Peletier (1979b) found that this heterotrophic capacity is most noticeable in mud-living littoral diatoms. The attached littoral marine algal assemblages may play a mixed-trophic role at lower levels of the detrital food web (Saks et al., 1976). The question remains whether a selectively heterotrophic capacity exists in the associations of certain epiphytic diatoms and host macro-algae in the littoral zone. Early suggestions of "suitability" (Aleem, 1950a)and "chemical dependence" (Edsbagge, 1966a)were not borne out by subsequent investigation.

The translocation of photosynthetic products in symbiosis is well known (Allen, 1971; Evans et al., 1973a; Goff, 1979). Jones (1967) used ^{15}N as a tracer and found that nitrogenous extracellular products of the littoral blue-green alga, Calothrix scopulorum, were transferred to the unicellular alga, Chlorella marina, and the seaweeds Porphyra umbilicalis and Fucus spiralis etc. It is possible that both marine diatoms and macroalgae can serve as dissolved organic matter pools in the littoral zones since organic substances which are immediately available for uptake and assimilation may be transferred to various types of algae in different forms. In the laboratory studies so far reported, one or several labelled compounds are normally used and treated separately with the algae investigated. Obviously, these compounds cannot represent all the dissolved organic compounds in natural seawater, although they often appear as the major components in algal extracellular substances.

In nature the littoral marine diatoms are early colonizers of new surfaces between the tide marks, and are abundant on rocky shores and epiphytes on larger algae on certain seashores. The diatoms compete for space with sporelings and germlings of marine algae and with larval stages of sessile invertebrates. Whilst there is an increasing evidence of various interactions between diatoms and sporelings of marine algae, evidence of exchange of dissolved organic matter (DOM) between the two seems not to be available. In the present study, ^{14}C-labelled DOM from red algal sporelings and from one littoral diatom have been used in experiments to study the possible transference of photoassimilated compounds between the two. The quantities of DOM released, whilst adequately measurable in terms of ^{14}C-labelling have been too small to enable characterization of the individual components by chemical analysis and are consequently expressed as total DOM.

9.2. Materials and Methods:

9.2.1 Isolation and culture of algal sporelings and littoral diatoms

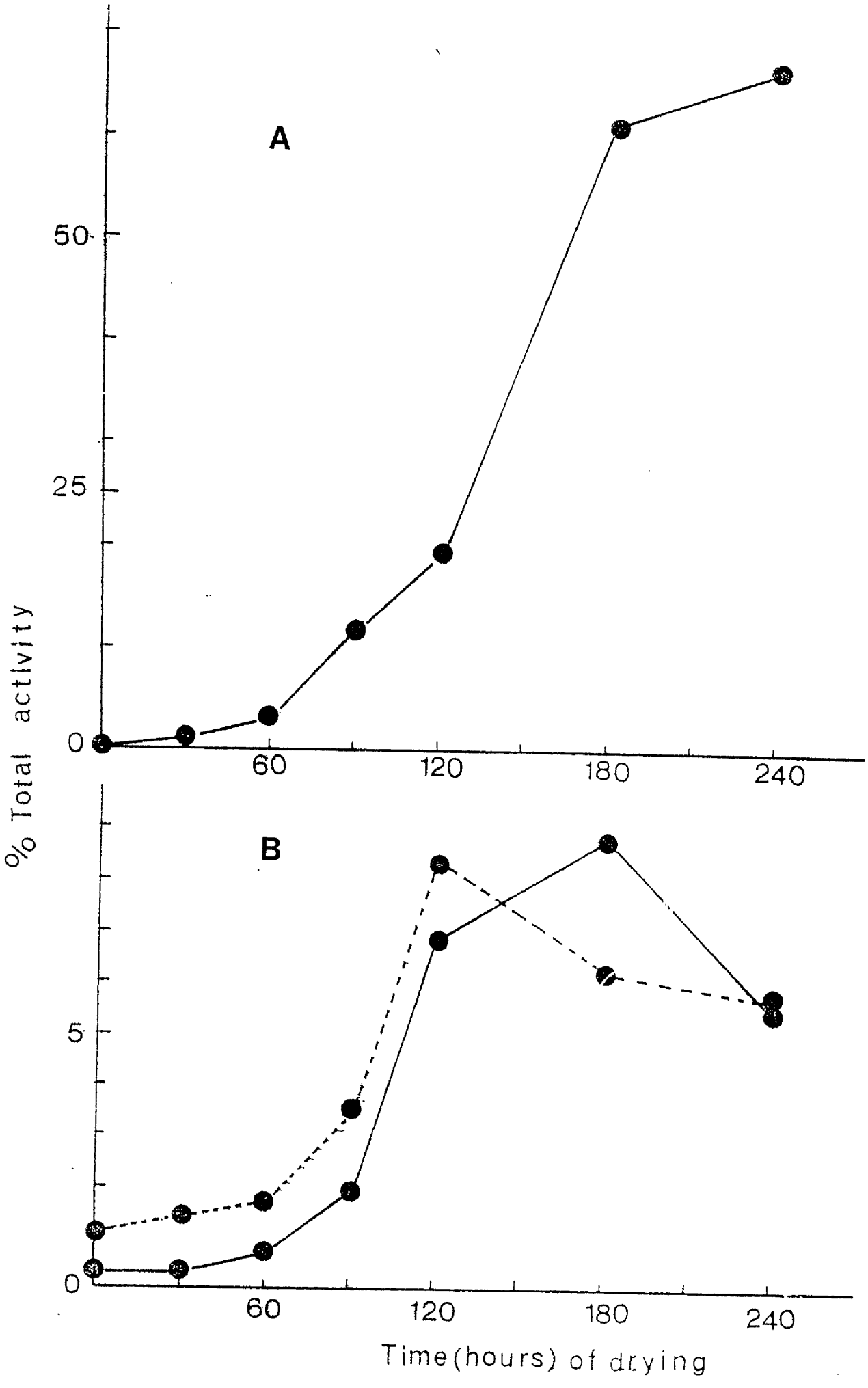
9.2.1.1 Algal sporelings

Fronds of red algae Gigartina stellata and Chondrus crispus were excised from the lower littoral zone at Ardmore Point, Dumbarton, Scotland in September, 1981. Methods for obtaining diatom-free sporelings growing in 50mm diameter petri dishes were the same as already described in Chapter 4.

9.2.1.2 Littoral diatoms

The unialgal cultures of pennate diatoms, Stauroneis constricta, Nitzschia closterium and Cocconeis stauroneiformis (Chapter 3) were used in the present study. The diatoms were kept growing in both liquid

Figure 8 Effects of air-drying periods on the release on ^{14}C-labelled organic compounds in the seawater; A = release in the first hour, B = release in the 2nd-4th hours after re-immersion of Chondrus crispus sporelings in non-labelled seawater.


A
50
25
0
60
120
180
240
% Total activity
B
5
0
60
120
180
240
Time (hours) of drying

and solid media at 15 ± 1°C, 3000 lux and with 14h of illumination per day. However, an axenic culture of Stauroneis constricta was also made for the experiment in 9.2.4.1 by repeatedly streaking diatom colonies on fresh enriched agar medium (Appendix 5). Sterility of S. constricta was tested with marine bacterial agar(Zobell, 1946), then the agar plates were incubated at 20 ± 2°C for ten days. Axenic cultures were not obtained with N. closterium and C. stauroneiformis.

9.2.2 Effects of air-drying on the release of organic compounds from living algae

During the experiments, it was found that the amounts of organic matter (labelled with ^{14}C) released from continuously immersed young plants of marine algae were often too small to be demonstrated in the present study (less than 5% of total photosynthetically fixed carbon was released). However, about 10-60% of fixed carbon was released from the living Chondrus sporelings into seawater within 1-4 hours when the plants were temporarily air-dried at room temperature for 2-3h prior to immersion. Therefore, air-drying treatment can facilitate the release of organic matter from living plants and it was used in the present study. The amounts of ^{14}C-compounds released based on the % of total radioactivity and weight and surface area of algal plants with the time course are shown in Figure 8 and Table 33.

9.2.3 Excretion of labelled dissolved organic matter from algal sporelings and diatoms

9.2.3.1 Algal sporelings

In the present study, 2-3 month old algal sporelings were dislodged carefully from the petri dishes with a spatula and washed in tap and seawater several times to remove detritus. The sporelings were then placed in a 100ml glass-stoppered flask which contained 50ml enriched

Table 33 Activities of ^{14}C-labelled compounds released from air-dried Chondrus crispus sporelings.

Dry period (min)	After 1h (light)		After 3h			
			light		dark	
	d.p.m. mg.dry wt.$^{-1}$	d.p.m. mm^{-2}	d.p.m. mg dry wt.$^{-1}$	d.p.m. mm^{-2}	d.p.m. mg dry wt^{-1}	d.p.m. mm^{-2}
0	22.64	0.67	21.46	1.14	75.63	3.36
30	121.36	6.53	32.61	1.76	128.44	5.62
60	269.05	12.47	63.13	2.29	127.12	6.88
90	837.46	46.86	137.17	7.67	324.71	15.02
120	1627.70	84.10	577.09	29.81	680.14	34.86
180	4756.26	230.72	691.03	33.44	568.54	77.59
240	7015.67	344.55	592.95	28.86	545.80	24.68

seawater medium (Appendix 5) and 3-4 μCi $NaH^{14}CO_3$ under conditions of 15 ± 1°C, 4000 lux and a light regime of 14h per day. During incubation, the culture medium was continuously stirred with a magnetic bar. After 2 days the sporelings were washed again in cold seawater to remove the superficial inorganic labelled carbon from the algal sporelings. The washed sporelings (0.23 - 0.81gm wet weight) were either immersed immediately in sterile seawater (non-dried) under the above conditions or air-dried on aluminium foil paper for two hours prior to immersion in 20ml sterile non-labelled seawater for excretion of organic matter (the air-drying enhanced greatly the release of dissolved organic matter from the sporelings, 9.2.2). Some washed G. stellata sporelings (0.98gm wet weight) were ground with a small quantity of non-labelled filtered seawater in a mortar. Then the extract was diluted with seawater to an appropriate volume and radioactivity. The supernatant of seawater extract as well as the dissolved organic matter (DOM) excreted from sporelings were separately passed through glass microfibre papers, Whatman GF/C, then HA Millipore membranes (0.45μm diameter pore size). Both sterilized filtrates were kept separately in a refrigerator for use the following day.

9.2.3.2 Diatom cells

Of the three isolated diatoms, Stauroneis constricta was used for excretion of cell organic matter in non-bacteria-free condition. The diatom cells were grown in 20ml enriched seawater medium in a 70ml glass-stoppered bottle under the conditions described for algal sporelings. After 10 days, the diatom population approached exponential phase and grew densely over the bottom of the bottle. The culture medium was then replaced by 10ml fresh enriched seawater with 0.5 μCi $NaH^{14}CO_3$ for 2 days. Care was taken during refreshment of the culture medium

by means of sterile pipettes to avoid disturbing the diatom layer on the bottom of the bottle. Afterwards, the labelled diatoms were washed with 50ml sterile ice-cold seawater 10-12 times until the radioactivity of washing seawater was reduced to the background level (on the basis of dpm; Fig.9). It was found that a few cells were suspended in seawater during washing so causing slightly increased radioactivity. This was unavoidable and was ignored. The washed diatom cells were resuspended in a small quantity of seawater (10ml) and then separated on a glass microfibre paper (Whatman GF/C) at a low pressure. Then the paper, together with the cells, were air-dried on the aluminium foil for two hours under the conditions described for algal sporelings (9.2.3). For the release of organic matter from the diatom cells, the air-dried diatoms were re-immersed in the sterilized seawater for two hours. Then the released DOM-seawater was sterilized by passing the seawater through a 0.45μm pore size membrane described above. The diatom-DOM filtrate was stored in a refrigerator before use, and the total diatom cell number which excreted organic matter was also determined under a microscope.

9.2.4 Uptake of sporeling DOM by diatoms

9.2.4.1 Uptake of DOM excreted by dried and non-dried algal sporelings

3ml exudate-seawater solution was added to 25ml sterile vials which contained 1ml diatom suspension (ca.1.5-1.7 x 10^4 cells ml^{-1}). The radioactivity of total 4ml culture was determined prior to experiment. The cultures were either kept in the darkness or exposed to light of 3000 lux for 14h followed by 10h darkness at a temperature of 15 ± $1^{o}C$. After 24h and 48h, 6 samples of 0.5ml diatom suspension were taken from each of the replicated cultures, and the diatom cells were fixed immediately with one drop of 2% neutralized formalin solution. Three samples

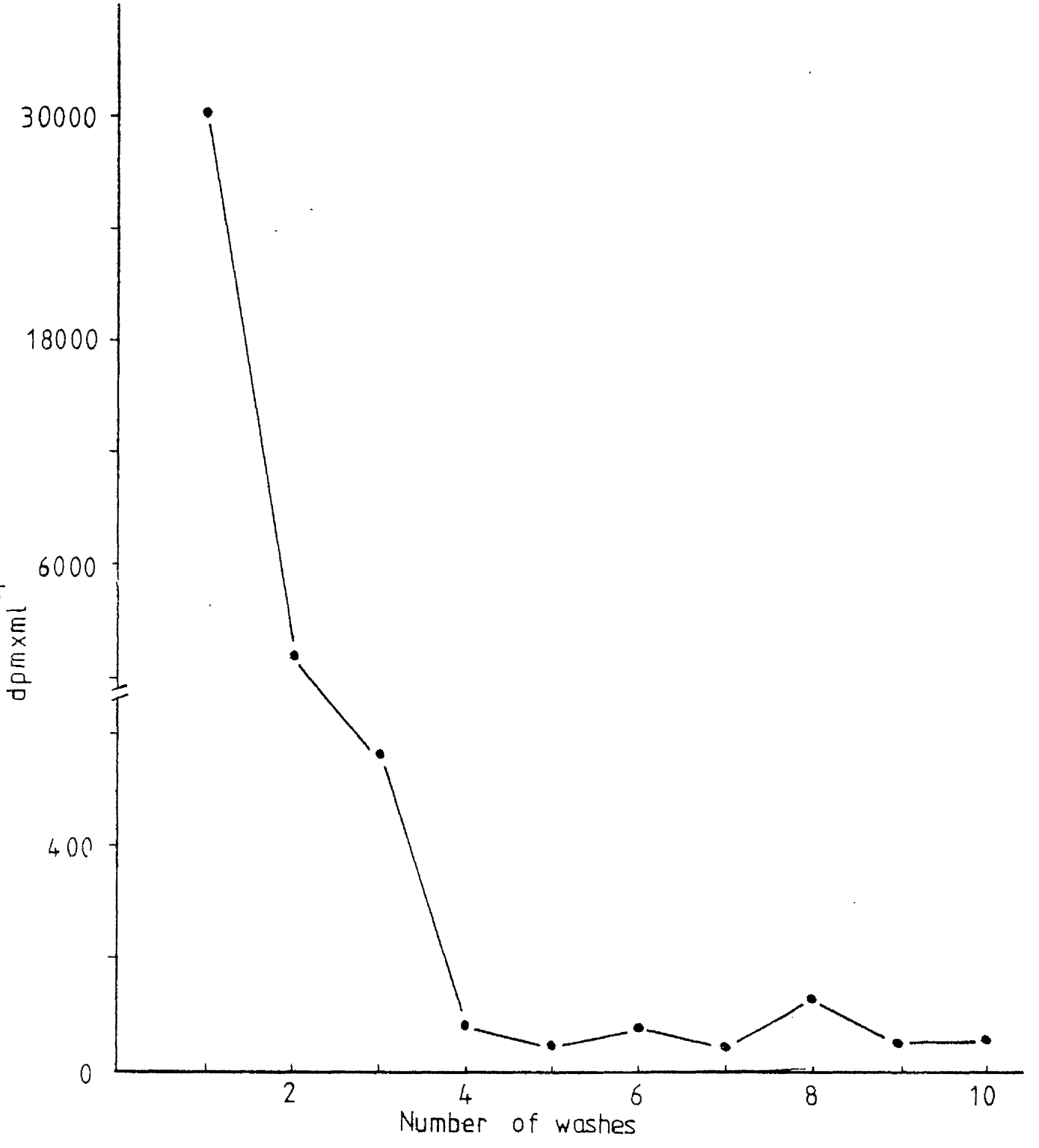


Fig. 9 The radioactivity (dpm ml^{-1}) in the wash water

were passed separately through glass microfibre papers in a filter apparatus at low pressure and the diatom cells on the filter papers were then washed by passing 10ml ice-cold non-labelled filtered seawater through the filter apparatus three times for each occasion. Finally, the filter papers along with diatom cells were placed in scintillation vials to which was added 10ml Packard Scintillant 299. The radioactivity of diatom cells was then determined in a Packard Liquid Scintillation Spectrometer of known counting efficiency. Another three samples of diatom suspension were used for total radioactivity determination without passing through filter papers. The remainder in the vials was used for counting diatom cell numbers.

In a short term experiment, the axenic culture of S. constricta growing exponentially in enriched seawater medium was transferred to sterile seawater and kept in darkness for 24h. Then the diatom cells were concentrated by centrifuging at $15^{o}C$ and 5000 rpm for 15 minutes. Afterwards, the diatom cells were re-suspended in fresh sterile seawater to a concentration about 4×10^{5} cells ml^{-1}. 1ml diatom suspension was mixed separately with 3ml Gigartina and Chondrus DOM filtrates in sterile vials and kept in light and dark conditions for over 6h. After 2, 4 and 6h, samples were collected for the determination of radioactivity as described above.

9.2.4.2 Uptake of seawater extract of Gigartina stellata

The seawater extract was added to a specially designed glass container (Fig.10). The central compartment A communicated with the three outer compartments B, C and D via three No. 2 sintered glass filters. (Pyrex, Borosilicate glass). The contents of A were stirred by means of a magnetic stirrer, and those of the three outer compartments were constantly aerated, so keeping the diatoms in suspension.

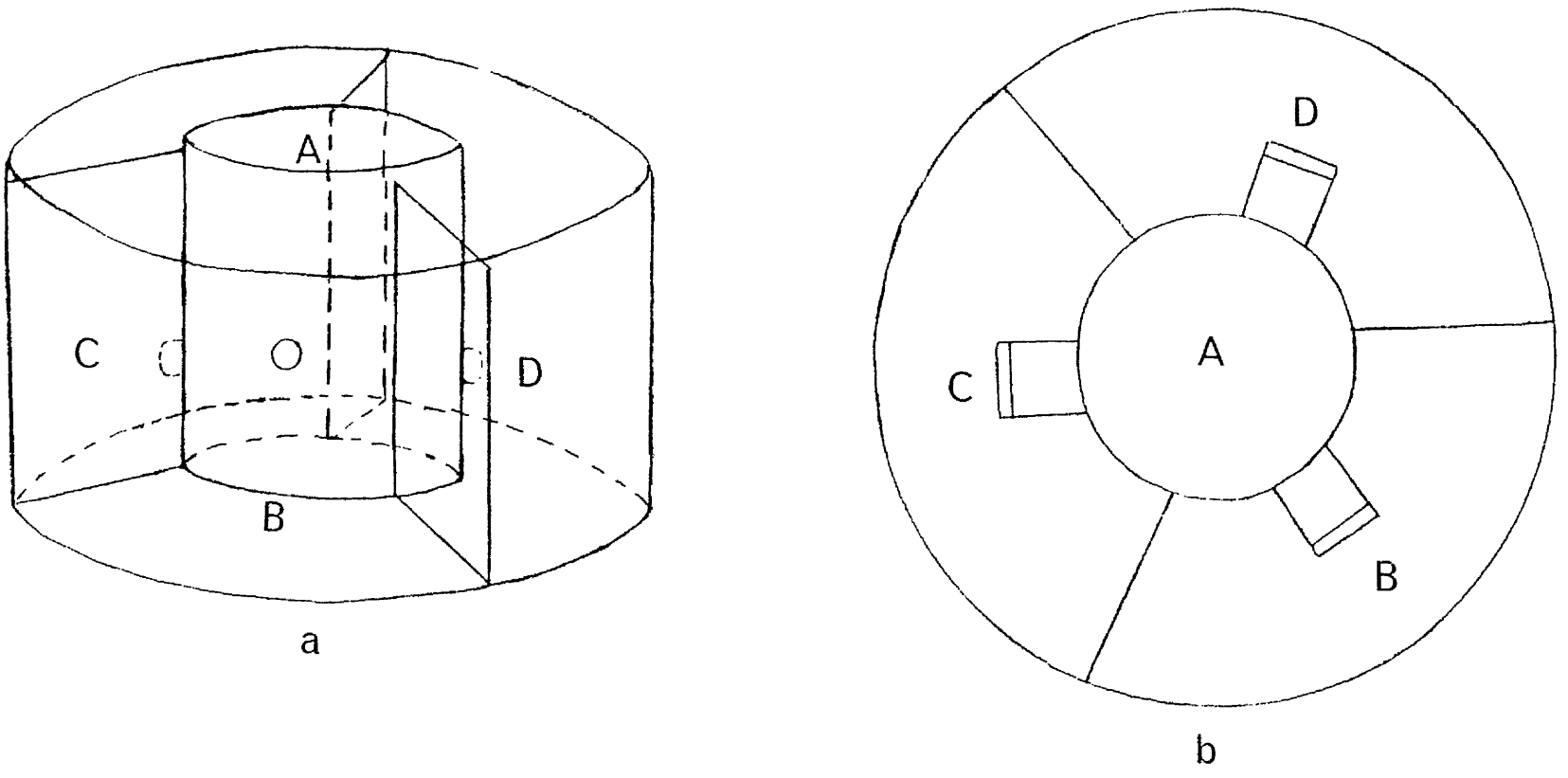


Figure 10 The compartmented-chamber device. The glass container, 90mm in diameter and 50mm in height was divided into three chambers with three pieces of glass slide connected to a small glass column of 40mm diameter and 45mm height. Three No.2 sintered glass filters are inserted into the column 15mm from the base, connecting the outer chambers B, C, D with inner column A. The glass lid of the petri dish has three holes allowing aeration tubes to be passed into the chambers (not included in the figures); a = side view, b = plan view.

Prior to the experiments the rates of diffusion of the contents of A were tested using the fluorescein solution. The passage of the dye into the three outer compartments proceeded at a steady state. Before the experiment, the four compartments were filled with non-labelled sterilized filtered seawater and kept in a growth cabinet under the experimental conditions, i.e. 15 ± 1 ^{o}C, 1500 lux and light/dark regime = 14/10h. 3-5ml suspensions containing three diatom species (Cocconeis stauroneiformis, Stauroneis constricta and Nitzschia closterium) were added separately to each of the outer compartments giving a final cell concentration of more than 1 x 10^{4}cells ml^{-1}. After 1h of preculture in seawater, 5ml seawater extract of Gigartina sporelings were added to the central compartment to give a final radioactivity strength of more than 9000 dpm ml^{-1}. The experiment was carried out over 6 days under the experimental conditions described above. Samples for radioactivity and cell number determination were taken after 2, 4 and 6 days. On each occasion, 7 samples of 1ml diatom suspension were taken from each diatom compartment, 3 for determination of cell radioactivity, 3 for assessment of total radioactivity, and 1 for cell number determination (fixed immediately after collection).

9.2.5 Uptake of diatom DOM by algal sporelings

The dislodged algal sporelings of G. stellata and C. crispus were washed in cold tap and seawater several times as described for the previous experiments. Aliquots of 0.08 and 0.15gm wet weight of washed sporelings of G. stellata and C. crispus respectively were separately placed in 25ml sterile vials containing 12ml diatom DOM seawater solution of a known radioactivity (approximately 5000 cpm ml^{-1}). Then the vials were either kept in darkness or exposed to light of an intensity of 3000 lux at 15 ± 1^{o}C. 3 replicates were set up for each condition of both

algal species. After 2, 4 and 6h, three aliquots of 0.5ml seawater solution were taken from each vial to measure the radioactivity remaining in the seawater. Then the amount of ^{14}C-compounds taken up by algal sporelings was calculated from the loss of radioactivity from seawater. After 6h, all vials were again exposed to light for 5h. This was followed by 10h darkness and finally 5h light according to the light-dark regime of the growth cabinet used. The sporelings were subsequently collected and washed in cold non-labelled filtered seawater 10-12 times to remove the organic ^{14}C possibly adsorbed on the algal surface. The washed sporelings were then oven-dried at 55-60^{o}C for 2 days to a constant dry weight. The surface area of algal sporelings treated was calculated from the correlation equation between wet weight and surface area of non-labelled sporelings of the same age and growth condition (Gigartina: $Y = 0.93+0.02X$, $r = 0.817$, $P < 0.05$; Chondrus: $Y = -1.16 + 0.13X$, $r = 0.930$, $P < 0.001$). Finally, the dried sporelings were combusted in an L.S. Sample Oxidizer (IN 41001) and the ^{14}C was trapped in a "cocktail" for later measurement of radioactivity in the Spectrometer.

9.3 Results

9.3.1 Uptake of sporeling DOM by diatoms

9.3.1.1 Seawater extract of Gigartina stellata sporelings

In the experiment with the compartmented chambers, all three isolated diatoms grew well in the seawater continuously supplied with G. stellata extract exuded from the central compartment through the sintered glass filters. However, their relative growth constants were not high, averaging from 0.05 day^{-1} in S. constricta to 0.22 day^{-1} in C. stauroneiformis respectively from day 2 to day 6. The ratio of cell surface area in S. constricta, N. closterium and C. stauroneiformis

Table 34 Uptake of Gigartina stellata seawater extract DOM by diatoms (data given as Mean ± S.E.)

Diatom species	Time period (h)	Total dpm ml^{-1}	% uptake	10^{-5} dpm/cell	n
Stauroneis constricta	48	347.58 ± 4.43	43.39	212.24 ± 6.86	3
	96	619.55 ± 64.48	28.88	198.86 ± 8.82	3
	144	1444.70 ± 57.84	48.90	793.86 ± 39.15	3
Nitzschia closterium	48	466.54 ±185.92	16.23	24.34 ± 4.00	3
	96	388.85 ± 4.53	28.53	28.68 ± 1.16	3
	144	935.09 ± 75.40	53.98	114.47 ± 3.70	3
Cocconeis stauroneiformis	48	651.27 ± 69.87	30.78	1336.80 ±254.93	3
	96	1042.50 ± 5.70	25.43	1104.75 ± 33.08	3
	144	1881.09 ±144.86	50.48	2316.39 ± 91.46	3

was approximately 7:3:2 and the volume was 12:3:1 respectively determined after 6 days of treatment; however, the surface area/volume ratio was 1:2:4 respectively. % uptake as well as the amount of organic-^{14}C taken up per unit of cell generally increased with the longer exposure period up to 144h (Table 34). After 6 days, the amount (based on the radioactivity, dpm ml^{-1}) taken up by diatom cells in each compartment was about 50% of total amount; whilst it increased 4, 5 and 2 orders of magnitude respectively for the radioactivities of S. constricta, N. closterium and C. stauroneiformis cells from day 2 to day 6. The ratio of cell radioactivity (dpm $cell^{-1}$) for these three diatoms determined after 48, 96 and 144h were 10:1:60, 7:1:38 and 7:1:20 respectively. Obviously, Cocconeis demonstrated the greatest capacity in the uptake and incorporation of radioactive carbon from Gigartina extract. On the basis of cell surface area (A)/volume (V), i.e. μm^{-1}, the uptake amounts of the diatoms investigated became 4:1:41 dpm μm^{-1} for Stauroneis, Nitzschia and Cocconeis respectively after 6 days. From the results (Table 34), there is a correlation between total radioactivity in the compartment and the uptake per unit of cell ($r = 0.78$, $P < 0.005$); thus accumulation of labelled carbon in diatom cells occurred with the time period. However, the comparatively low values of total radioactivity observed in the compartment of N. closterium were obviously due to the clogging of sintered glass filter by diatom cells which appeared densely at the outlet from the central compartment after 4 days of inoculation. This resulted in a slight contamination of this diatom in other compartments after 9-10 days. Nevertheless, diatom contamination did not appear at least in the present results with an exposure time of up to 6 days.

9.3.1.2 Exuded DOM by dried and non-dried algal sporelings

The results with substances from non-dried sporelings (Table 35)

Table 35 Uptake of Gigartina stellata DOM by diatoms after 24 and 48 h. of treatment (data given as mean ± S.E.; D = dark condition, L = light-dark regime of 14-10h).

		24h			48h	
		n	% uptake	10^{-5} dpm/cell	% uptake	10^{-5} dpm/cell
Stauroneis constricta	L	3	15.32	426.05 ± 56.27	7.39	47.54 ± 3.86
	D	3	13.95	—	5.53	42.07 ± 3.92
Nitzschia closterium	L	3	30.34	509.36 ± 28.46	32.22	111.24 ± 11.76
	D	3	5.66	—	8.04	39.19 ± 3.55

showed that the uptake rate for ^{14}C in N. closterium appeared to be similar to that of S. constricta after 24h (light) and 48h (dark). The particularly high activity in N. closterium in light conditions after 48h is suspect. After 2 days of exposure, the ratios of radioactivity per unit of diatom cell (dpm $cell^{-1}$) in both light and dark conditions were 1:1 for Stauroneis and 3:1 for Nitzschia respectively. However, cell radioactivities of both diatoms largely decreased (approximately 50-90%) with the increase of diatom cell number (approximately 50-100%) in the light condition.

With Chondrus-labelled substances, the uptake pattern for the three diatoms investigated was quite similar to that observed with G. stellata. The % uptake was generally larger in the first 24h; it was generally higher in the light than in the dark (Table 36). However, the increased threshold of radioactivity per unit of diatom cell was not proportional to the increase of % uptake, particularly after 48h of treatment. The results clearly demonstrated that the highest value of cell radioactivity appeared in C. stauroneiformis as shown in the previous results with G. stellata sporeling extract. The ratio of the averaged amount of ^{14}C taken up by S. constricta, N. closterium and C. stauroneiformis was approximately 2:2:3 respectively based on dpm/unit of diatom cell and 1:1:4 respectively based on dpm μm^{-1} diatom cell.

In the short term experiment over 6h, light and dark uptakes were even more significantly different when S. constricta was treated separately with the substances from air-dried Gigartina and Chondrus plants (Table 37). The uptake rate in both light and dark conditions gradually increased in the first 4h, then it decreased significantly in the next 2h. In general, the uptake rate was consistently higher in the light than in the dark. The results also showed that the amount of algal

Table 36 Uptake of Chondrus crispus DOM by diatoms after 24 and 48h of treatments (data given as mean ± S.E; L = light-dark regime of 14-10h, D = dark condition)

Diatoms		n	24 h		48 h	
			% uptake	10^{-5} dpm/cell	% uptake	10^{-5} dpm/cell
Stauroneis constricta	L	3	8.26	150.17 ± 11.05	35.45	486.81 ± 19.38
	D	3	4.64	95.10 ± 11.16	10.91	185.41 ± 14.78
Nitzschia closterium	L	3	8.07	166.68 ± 9.79	26.45	277.35 ± 18.94
	D	3	4.25	76.28 ± 3.00	10.14	277.32 ± 5.63
Cocconeis stauroneiformis	L	3	7.83	218.40 ± 22.81	15.73	308.82 ± 11.72
	D	3	6.87	186.49 ± 20.61	12.14	524.86 ± 17.63

Table 37 Uptake of algal sporeling DOM by the diatom Stauroneis constricta (data based on 2 replicates and given as mean ± range)

Algal sporelings	Time period (h)	Light		Dark	
		10^{-4} dpm/cell	10^{-10} mg(dry)sporelings/cell	10^{-4} dpm/cell	10^{-10} mg(dry)sporelings/cell
Gigartina stellata	2	8.64 ± 0.19	71.22 ± 2.46	8.14 ± 0.04	67.09 ± 0.33
	4	12.38 ± 2.01	102.00 ± 16.52	11.64 ± 1.29	95.94 ± 10.63
	6	9.65 ± 3.05	79.54 ± 24.65	7.37 ± 5.92	60.71 ± 5.57
Chondrus crispus	2	4.82 ± 0.13	94.35 ± 2.54	4.67 ± 0.06	91.32 ± 1.28
	4	8.42 ± 1.03	164.77 ± 20.21	6.40 ± 1.63	125.13 ± 31.76
	6	6.29 ± 0.09	123.12 ± 1.76	6.35 ± 0.69	124.30 ± 13.31

Table 38 Uptake of diatom DOM by sporelings (* = dpm/mg (dry), ** = dpm/cm^2 sporelings, n = replicate number, L = light condition, D = dark condition. Data given as net mean ± S.E.)

		Time period (h)						
		n	2 *	4 *	6 *	n	24 *	24 **
Gigartina stellata	L	3	32.10 ± 12.00	22.12 ± 16.60	15.42 ± 10.95	6	54.04 ± 5.39	1046.99 ± 83.17
	D	3	32.89 ± 16.11	39.27 ± 24.69	42.71 ± 10.09			
Chondrus crispus	L	3	46.27 ± 5.40	17.64 ± 10.32	-4.90 ± 1.74	6	83.93 ± 7.05	811.68 ± 64.99
	D	3	43.35 ± 5.40	25.81 ± 15.96	9.50 ± 4.98			

sporelings (based on mg dry weight) required to excrete the same quantity of labelled carbons taken up by unit of diatom cell was larger in Chondrus than in Gigartina by up to two orders of magnitude.

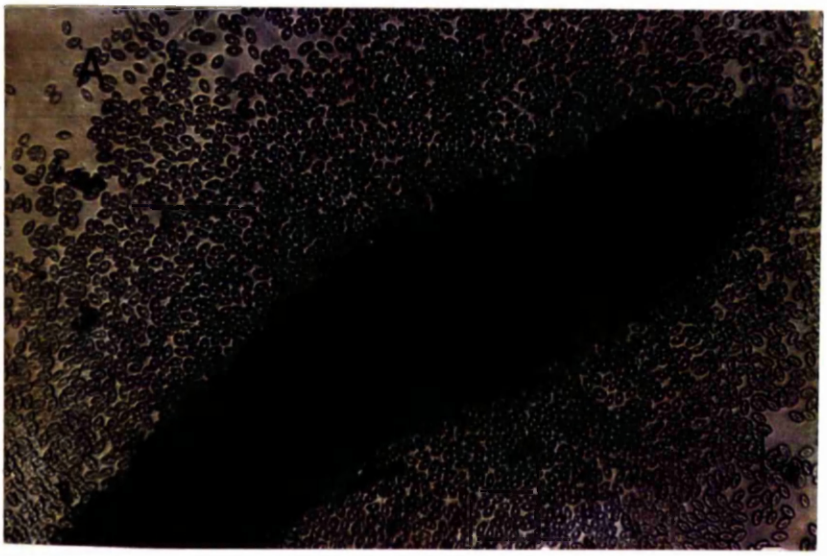
9.3.2 Uptake of diatom DOM by algal sporelings

The uptake of diatom-fixed organic matter by algal sporelings (judged from the loss of radioactivity in seawater) also occurred. Generally, the net loss of radioactivity of ^{14}C labelled DOM from the seawater was irregular with the time course up to 6h in the present study (Table 38). However, the uptake rate in both red algae generally decreased with an increase of exposure time, particularly in the light condition. It showed that the total amount of diatom-fixed-^{14}C taken up by algal sporelings was consistently higher in the dark than in the light, and was higher with G. stellata than C. crispus based upon weight and surface area. The ratio of total uptakes of Gigartina and Chondrus was 3:2 dpm mg^{-1} dry weight of plants, but it was 1:3 dpm cm^{-2} plants or diatom cells cm^{-2} surface area of plants. After 24h, the average radioactivity of the organic compounds remaining in G. stellata and C. crispus plants were 54.04 and 83.93 dpm gm^{-1} (dry weight) or 1046.99 and 811.68 dpm cm^{-2} algal plants respectively (Table 38).

9.4. Discussion

The transfer of ^{14}C labelled photosynthetic products (as dissolved organic matter) between algal sporelings and littoral diatoms has been clearly demonstrated in the present study. The uptake rate for labelled dissolved organic matter by diatoms varies greatly with species investigated, but is invariably higher in the light than in the dark. Undoubtedly light uptake is induced by certain metabolic processes within the diatom cells (Oaks, 1962a, 1962b; Miller et al., 1963; Hellebust, 1971; Cooksey, 1972; Coughlan, 1977; Hellebust and Lewin, 1977).

Plate 6 The diatom Cocconeis stauroneiformis growing around the Fucus serratus germling (A) and Chondrus crispus sporelings (B); scale = 100μm.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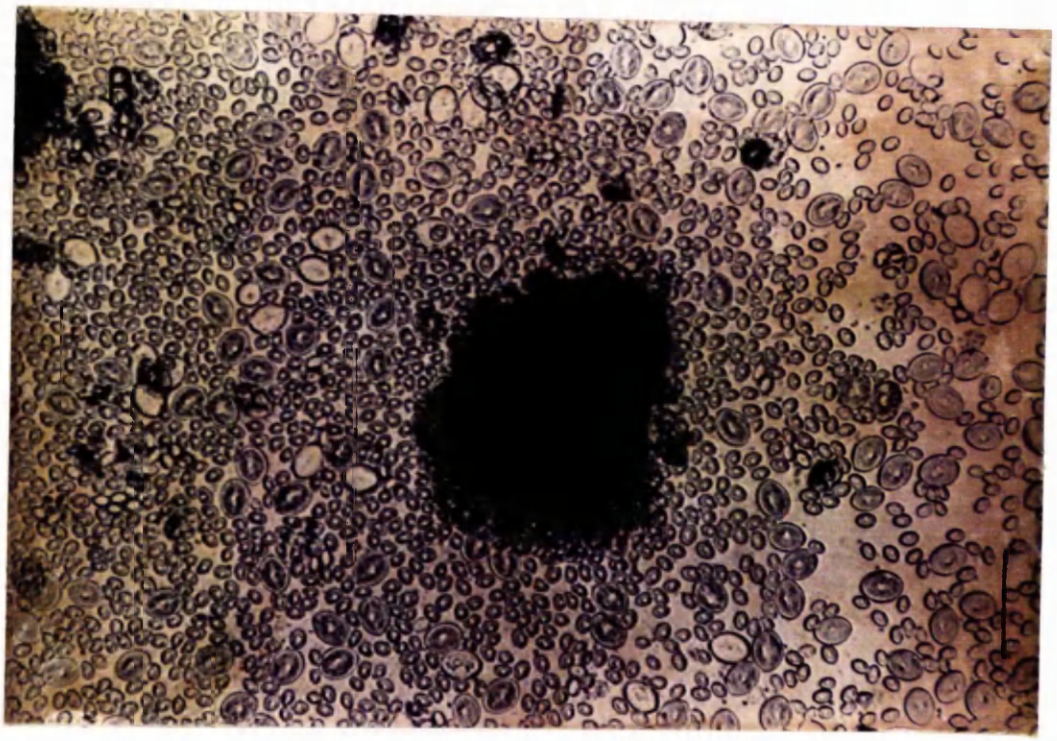

B

The ability of uptake for organic compounds in unicellular algae is quite selective and varies with species and compounds (Lewin and Lewin, 1960; Sloan and Strickland, 1966; Hellebust and Guillard, 1967). Thus the specific radioactivity per unit of diatom cell is not constant in the diatoms examined in the present work. In experiments both with Gigartina stellata and Chondrus crispus the cells of the diatom Cocconeis stauroneiformis had noticeably higher specific activity of ^{14}C-organic compounds than the other two diatoms, Stauroneis constricta and Nitzschia closterium, although it has the lowest cell division rate of the three. Munk and Riley, (1952), Bellinger (1977) and Admiraal and Peletier (1979b) indicated that cell uptake capability for organic matter is related to cell size in unicellular algae. C. stauroneiformis has a relatively larger cell area/volume ratio than S. constricta of 2-4 orders of magnitude. In addition, the greater affinity and incorporation ability of diatoms to the sporeling photosynthetic products might also be involved in such differences of uptake rates. In nature, Cocconeis frequently appears as a major epiphytic diatom on marine algae (Edsbagge, 1966b; DeFelice and Lynts, 1978; Chapter 2). In cultures, this diatom always appears in large amounts around fucoid germlings and red algal crusts (Plate 6). N. closterium has shown a limitation of incorporation ability to dissolved organic matter in the present study possibly either due to an unsufficient amount of compounds available for incorporation or greater increase of cell number, i.e. higher cell division rate in the cultures, though it has relatively higher area/volume ratio than S. constricta. Its uptake rate for sporeling substances is less than those of S. constricta and C. stauroneiformis based on dpm/unit of cell.

The radioactivity of fixed ^{14}C in the diatom cells investigated

is considerably high both in short and long term experiments; this suggests that ^{14}C labelled compounds released from red algal sporelings are by no means immediately utilised and incorporated into cell components. The organic compounds taken up by these three diatoms seem likely to be common to some extent but different in certain compounds. Presumably, the cell radioactivity reflects the efficiency of incorporation of photosynthetic products of algal sporelings into diatom intracellular substances; the higher the activity per unit of diatom cell, the higher efficiency of DOM of sporelings for incorporation. In the short term experiment over 6h the dissolved organic matter was collected and sterilized immediately after release from both red algae. The diatom S. constricta was grown axenically in non-enriched seawater in the dark for more than 24h prior to the experiment. Thus the diatom cells were starved of exogenous organic matter, and they took up authentic DOM (without it being decomposed by bacteria) rapidly as soon as they were exposed to the ^{14}C labelled compounds. The results recorded in Table 37 are in agreement with those of long term experiments of more than 24h. Clearly, the Gigartina compounds are generally superior to Chondrus compounds for diatom incorporation based on the weight of algal sporelings per unit of diatom cell. In other words, Gigartina probably contributes a comparatively broader spectrum of ^{14}C labelled organic compounds readily taken up by diatom cells and subsequently incorporated into metabolic processes, particularly in the light. It supports greater diatom biomass than Chondrus based on the cell number of diatoms per unit of weight of sporelings under the same conditions. The variety of chemical nature of sporeling DOM between these two red algae could be expected. Harrison (1978) claimed that Ulva fenestrata growth is promoted by Zosteria marina detritus but not Spartina altermflora since the former contains greater amounts of nitrogen and phosphorus. Whether

there is a linkage between the low uptake rate and antibiotic substances from Chondrus crispus (Hornsey and Hide, 1974, 1976; Khfaji and Boney, 1979) remains to be explained. Compared with seawater extract (or intracellular substances), the exudates (or extracellular substances) either from dried or non-dried algal sporelings seem to be less efficient for the diatom S. constricta. By contrast certain extracted substances may be utilised immediately following the uptake by diatom cells. Therefore, the intracellular substances of macroalgae are equally important as extracellular substances for diatom heterotrophy and this can also explain the endophytic behaviour of certain diatoms, i.e., Navicula endophytica and Cocconeis scutellum (Ha$|e, 1968; Taasen, 1972).

Heterotrophic uptake of diatom DOM by algal sporelings has also been demonstrated in the experiments. G. stellata has shown a greater uptake capacity for diatom DOM than C. crispus based on the surface area or weight. The amounts taken up were larger in the dark than in the light on each occasion. This might suggest that: (a) a passive diffusion plays a large part in the uptake mechanism. (b) organic compounds uptake is less efficient or not significant when photosynthesis occurs in optimum light conditions. The variable uptake rates or capacities of the two red algae reveal a possible difference of chemical natures (as described above) either in the cell walls or intracellular compounds to couple diatom substances. After a 24h light-dark regime, the appreciable amounts of labelled organic compounds remaining in the combusted algal plants confirms that uptake and assimilation of diatom DOM by marine algae did occur. Such heterotrophic behaviour has been well documented by many authors, e.g. carbohydrates and nitrogenous compounds (Watanabe, 1937; Crawford and Richardson, 1974) and vitamins (Tatewako and Provasoli, 1964; Fries

and Petterson , 1968). Nevertheless, the amount of organic compounds taken by algal sporelings is comparatively smaller than those by diatoms on the basis of biomass. This further suggests that uptake and incorporation of organic compounds in marine algae is probably restricted to metabolically actie cells in the plant body, i.e. the growth region.

In fact, algal uptake behaviour for DOM is a function of environmental factors including various organic substances and organisms which comprise a microniche in nature. Jones (1967) found that extracellular products (labelled with ^{15}N) of the blue-green algae Calothrix scopulorum are assimilated directly by not only bacteria and fungi but also various algae, including unicellular representatives, Fucus, Porphyra and other macroalgae, and the living cells remove more of the available nitrogen than dead cells. The bilateral transformation of photosynthates between littoral diatoms and algal sporelings shown in the present work confirms the ecological importance of certain algal dissolved organic matter in nature. Certainly, bacterial activity involved in the decomposition of organic matter in nature plays an additional important role (Harrison and Mann,1975; Sepers, 1977; Lucas et al., 1981; Koop et al., 1982). The difficulty of chemical identification of algal substances in the present study is understood when the small biomass of algal sporelings and diatoms is considered. In a recent paper, Robertson et al (1982) obtained dissolved organic matter from oven-dried plants. This is different from the air-dried method used in the present study; the latter may not result in serious cell injury with drought periods of 1-4h since most of the greening of branch tips return to normal colour in a few days after re-immersion in seawater. Indeed, many marine algae can resist desiccation during spring and neap tide periods (Baker, 1909,

1910; Biebl, 1952; Schonbeck and Norton, 1978). However, in view of the 10-60% of photosynthetic products of carbon released from air-dried sporelings (Fig. 9), the immersion and emersion due to the tidal regime could result in a great loss of algal organic matter into seawater in nature (Khailov and Burlakova, 1969; Sieburth, 1969). The present study demonstrates the organic linkage between littoral diatoms and young plants of marine algae and a possible role of both serving as dissolved organic matter "pools" in the littoral area, which can be either consumed by animals (Castenholz, 1961; Anderson and Stephens, 1969; Chia and Warwick, 1969, Efford and Tsumura, 1973; Ferguson, 1971; McIntire and Moore, 1977) or released directly to the surrounding water to affect the growth of other organisms, (Lefévre, 1964; Fogg, 1966) as shown in Chapters 4, 5, 6, 7 and 8 of this thesis.

CHAPTER 10

GENERAL DISCUSSION AND CONCLUSION

The periodical seasonal development of littoral diatoms in the study area on Cumbrae Island has been clearly demonstrated. The spring bloom of diatoms occurs when temperature and length of sunshine period increase from the minimum values in the winter. However, zonal distribution is much dependent on the local environments, i.e., tidal levels and phytal taxa. Phytal morphology may be an important factor but not necessarily so for the attachment of epiphytic diatoms. The similarity of epiphytic diatom assemblages collected from different macroalgal species increases during maximum development and decreases when there is a minimum development. The distribution of common epiphytic diatoms is mainly restricted to certain macroalgae in the lower littoral zone and tide pools. From the results, the diatoms-macroalgae associations are probably in a physio-chemical complex; both abiotic factors and the characters of associated diatoms and host plants are equally important in the association formation. There is a great difference of diatom flora on the non-living substrata from that on macroalgae; the former consists predominantly of mucous tube-lining diatoms, whilst the latter are mostly sessile taxa.

The interactions between littoral diatoms and young algal plants are demonstrated in the change of their growth rates. The presence of algal sporelings and germlings, in general, greatly enhances diatom growth; whilst the reverse is less apparent in the results. Diatoms inhibit the growth of macroalgae immediately after the attachment of spores and zygotes to the substratum.

Inhibitory effects of certain benthic diatoms on the growth rate

of young macroalgae appear regardless of the change of light intensities, temperatures, salinities and nutritional conditions. The common littoral diatoms, Nitzschia closterium and Stauroneis constricta isolated from nature demonstrate a great affinity to the attached crustose sporelings of Gigartina stellata on the substratum. This character creates unfavourable growth conditions for algal sporelings, such as mechanical damage of crustose surface cells by the attached mucilage excreted from diatom cells, reduced light intensity and increased diatom extracellular substances in the immediate vicinity of the algal crusts. However, when the above diatoms are associated with Cocconeis stauroneformis, a common epiphytic diatom in experiments with Gigartina sporelings then the inhibitory effects of diatoms on algal sporelings are as much dependent on the interactions between the diatoms as between diatoms and sporelings. The inhibitory effects of C. stauroneiformis on N. closterium and S. constricta are of ecological interest regarding the wide distributed habit of the species in nature. In the cultures with many macroalgal sporelings and germlings the effects of diatoms on the growth of young algal plants are relatively less obvious.

Extracellular substances of Chondrus sporelings and fucoid germlings have antibiotic effects on N. closterium. However, the antidiatom activity varies with the metabolic activities of individual young algal plants, light intensities, salinities and nutrient concentrations in the culture media. It gradually decreases as diatom cell number increases 6 days after inoculation. Antidiatom activity does not occur in the dark. Pretreatment of diatoms with Fucus extract had no effect on the subsequent response of diatoms to Fucus germling growths.

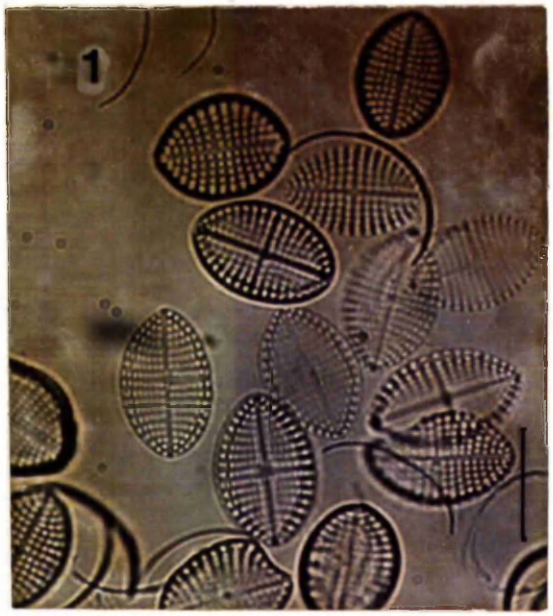
The diatom Navicula grevilleana secretes large amounts of mucilaginous substance which covers the surfaces of substrata and affects remarkedly the morphology and growth features of attached algal sporelings and germlings. Its effects appear with relatively larger sized and more developed rhizoids of fucoid germlings, unusually irregular shapes of Gigartina crusts, a long unbranched primary rhizoid of Ulva and smaller disc-form crusts of Chondrus sporelings. It is believed that the above features are caused by certain unidentified compounds with activities similar to plant growth substances in the mucilage. Thus the distributions of mucilage producing diatoms in littoral habitats could be of ecological significance in early settlement processes.

Transformation of photosynthetic products between littoral diatoms and algal sporelings has been demonstrated using radioisotope techniques. The amount of ^{14}C-labelled DOM (dissolved organic matter) of Gigartina and Chondrus sporelings taken up by littoral diatoms varies with the diatom species studied. Of the three diatoms studied, Cocconeis stauroneiformis has the greatest capability for uptake and assimilation of DOM, followed by Stauroneis constricta and then Nitzschia closterium the least. The uptake rate of ^{14}C-labelled DOM of Gigartina sporelings by diatoms is generally greater in the light than in the dark, and decreases with an increase of diatom cell number. The diatom S. constricta has different uptake capabilities for the assimilation of Gigartina and Chondrus DOM; the capability is greater for Gigartina DOM than Chondrus DOM. Whether this is due to the different nature of substances of both sporelings is not known. It is likely that seawater extracts of algal sporelings provide a great variety of DOM for diatom uptake and incorporation into cell components than the exudates from dried and non-dried thalli. Heterotrophic uptake of diatom DOM does take

place with algal sporelings, and it is greater in the dark than in the light. Evidence of such transformation of dissolved organic matter between diatoms and algal sporelings reflects again the great significance of extracellular substances released from various algae under different conditions in nature.

The results of the researches described in this thesis indicate clearly that the interactions between littoral diatoms and young algal plants are rather complicated, and involve various physico-chemical and biotic factors. No single factor could seem to predominate. However, algal extracellular substance ("ectocrines") and the mechanical attachment of diatoms on algal germlings and sporelings might be the most important factors in the interactions. Whether the interactions are beneficial or non-beneficial for either diatoms or young algal plants is entirely governed by the algal growth strategies. With the abundant population of diatoms which can occur at times in intertidal habitats, it could seem very likely that interactions of the types described will play significant roles in the early microbial phases of colonization.

Plate 7 Isolated diatoms; 1 = Cocconeis stauroneiformis (GB 9, 31), 2 = Stauroneis constricta (GB 3), 3 = Navicula grevilleana (GB 25, 30), 4 = Navicula sp. (GB 22, 23, 24), 5 = Nitzschia sp. (GB 19), 6 = Fragilaria sp. (GB 10); scale = 10 μm.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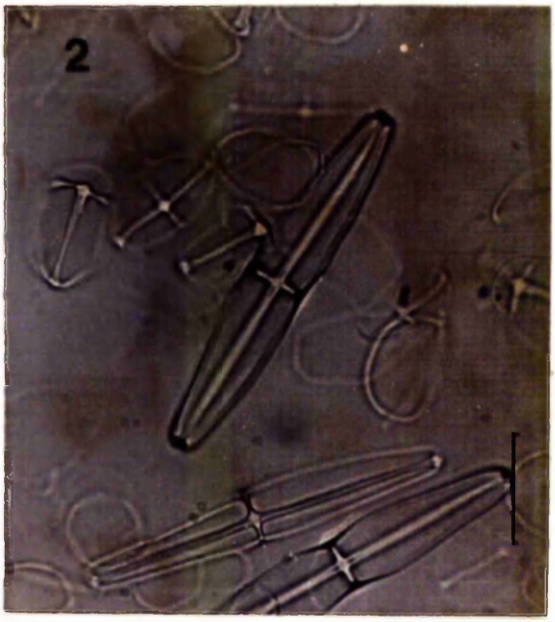
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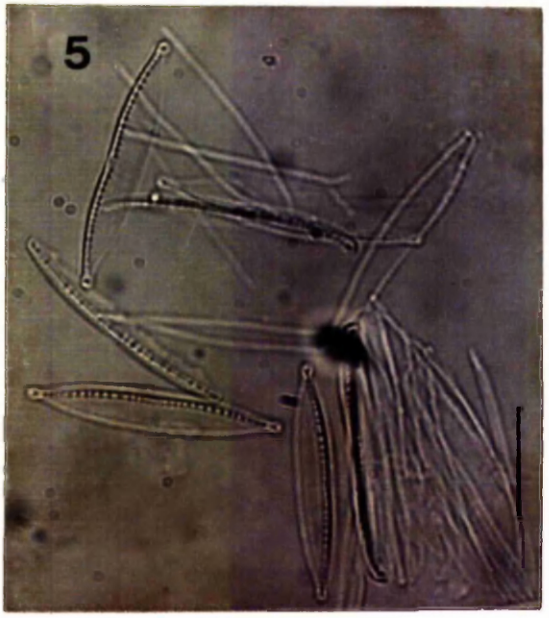
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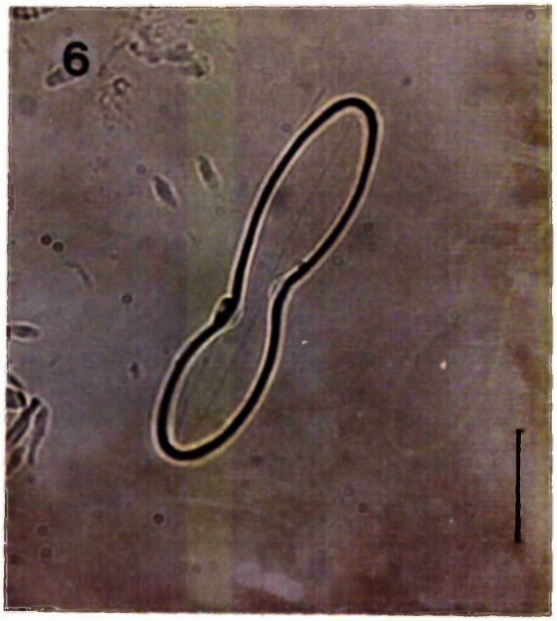

6

APPENDICES

Appendix 1 Dates of sample collections of macroalgae, seawater, barnacles and glass slides from the study area.

Date	Macroalgae	Seawater	Barnacles and slides
27 Mar. 1980	+	+	-
12 Apr. 1980	+	+	-
12 May, 1980	+	+	-
12 June 1980	+	+	-
12 July 1980	+	+	-
25 July 1980	-	-	-
8 Aug. 1980	+	+	-
7 Sept. 1980	-	+	-
3 Oct. 1980	-	+	-
31 Oct.1980	+	-	-
4 Nov. 1980	+	+	-
13 Dec.1980	+	-	-
12 Jan.1981	-	+	-
4 Feb. 1981	-	+	-
17 Mar.1981	-	-	+
28 Apr.1981	-	-	+
19 May 1981	-	-	+
15 June 1981	-	-	+
27 July 1981	-	-	+

Appendix 2 Numbers of genus and species identified from the collected samples (I = macroalgae, II = slides and barnacle debris, III = seawater).

	I	II	III		I	II	III
Achnanthes Bory	10	7	6	Grammatophora Ehrenberg	4	3	1
Actinocyclus Ehrenberg	1	1	1	Guinardia H. Peragallo	0	0	1
Amphipleura Kutzing	0	2	1	Hantzschia Grunow	1	2	1
Amphiprora Ehrenberg	0	1	2	Licmophora Agardh	8	4	3
Amphora Ehrenberg	10	8	6	Mastogloia Thwaites ex W.Smith	0	0	0
Asterionella Hassall ex W.Smith	0	0	1	Melosira Agardh	3	2	6
Auricula Castracane	0	0	1	Navicula Bory	22	12	13
Bacillaria Gmelin	2	1	1	Nitzschia Hassal	20	12	9
Bacteriastrum Shadbolt	0	0	1	Opephora Petit	3	1	2
Biddulphia Gray	1	1	0	Paralia Heiberg	1	1	1
Brebissonia Grunow	1	0	0	Pinnularia Ehrenberg	0	1	0
Caloneis Cleve	1	1	1	Pleurosigma W. Smith	1	1	0
Campylodiscus Ehrenberg	1	0	0	Podosira Ehrenberg	1	1	0
Chaetoceros Ehrenberg	0	1	1	Rhabdonema Kutzing	2	3	1
Cocconeis Ehrenberg	12	8	9	Rhizosolenia Brightwell	0	0	2
Coscinodiscus Ehrenberg	6	4	4	Rhoicosphenia Grunow	1	1	0
Cyclotella (Kutzing) de Brebisson	2	1	4	Skeletonema Greville	1	0	1
Cymbella Agardh	1	0	0	Stauroneis Ehrenberg	2	1	0
Denticula Kutzing	1	1	1	Stephanopyxis Ehrenberg	0	0	1
Diploneis Ehrenberg	3	2	11	Surirella Turpin	1	0	0
Ditylum L.W. Bailey	0	0	1	Synedra Ehrenberg	5	8	3
Fragilaria Lyngbye	1	1	1	Tabellaria Ehrenberg	1	0	0
Glyphodesmis Greville	1	0	0	Thalassiosira Cleve	3	2	6
Gomphonema Hustedt in Pascher	0	0	0	Trachyneis Cleve	1	0	0
				Tropidoneis Cleve	0	0	2
Total					135	95	106

Appendix 3 32 most common diatoms in epiphytic assemblages used for diatom community analyses.

Achnanthes brevipes Agardh	Melosira granulata (Ehr.) Ralfs
A. delicatula Kutzing	Navicula cancellata Donkin
A. groenlandica Cleve	N. clementis Grunow
A. parvula Kutzing	N. directa (W.Smith) Ralfs
Amphora ovalis Kutzing	N. endophytica Halse
A. proteus Gregory	N. forcipata Greville
Cocconeis placentula Ehrenberg	N. grevilleana Hendey
C. scutellum Ehrenberg	N. ramosissima (Ag.) Cleve
C. stauroneiformis (Van Heurck) Okuno	Nitzschia angularis W. Smith
Denticula tenuis Kutzing	N. lanceolata W. smith
Gomphonema sp. (1)	N. fonticola Grunow
Gomphonema sp. (2)	Rhabdonema adriaticum Kutzing
Grammatophora marina(Lyngb.)Kutzing	R. minutum Kutzing
G. oceanica Ehrenberg	Rhoicosphenia curvata(Kutz.)Grunow
Hantzschia amphioxys(Ehr.) Grunow	Synedra affinis Kutzing
Licmophora gracilis (Ehr.) Grunow	S. investiens W. Smith.

Appendix 4 The dates of collections and sources of isolated diatoms collected from study area and Keppel Pier, Millport, Isle of Cumbrae

Diatom species	Date	Source
Achnanthes linearis (GB 7)	Feb.80	Cladophora sp.
Achnanthes sp. (GB 29)	May 80	Ectocarpus sp.
Amphora acutiuscula (GB 6)	Feb.80	Enteromorpha intestinalis
A. perpusilla (GB 5)	Mar.80	Cladophora rupestris
Cocconeis stauroneiformis (GB 9)	Mar.80	Ascophyllum nodosum
C. stauroneiformis (GB 31)	Nov.80	Gigartina stellata
Fragilaria pinnata (GB 8)	Mar.80	Fucus spiralis
F. tabulata (GB 4)	May 80	Ectocarpus sp.
Fragilaria sp. (GB.10)	May 80	Rock surfaces
Guinardia flaccida (GB 26)	May 80	Cladophora sp.
Navicula directa (GB 21)	Mar.80	Slides suspended from Keppel Pier
N. grevilleana (GB 25)	May 80	Plumaria elegans
N. grevilleana (GB 30)	Jan.80	Rock surfaces
N. seminulum (GB 1)	Feb.80	G. stellata
N. seminulum (GB 2)	Feb.80	A. nodosum
Navicula sp. (GB 22)	Mar.80	Ectocarpus sp.
Navicula sp. (GB 23)	May 80	Rock surfaces
Navicula sp. (GB 24)	May 80	Polysiphonia lanosa
Nitzschia angularis (GB 15)	Jan.80	Rock surfaces
N. closterium (GB 16)	Mar.80	E. intestinalis
N. fonticola (GB 13)	Dec.79	G. stellata
N. fonticola (GB 18)	Mar.80	Rhizoclonium sp.
N. lanceolata (GB 19)	Apr.80	Slides suspended from Keppel Pier
Nitzschia sp. (GB 14)	May 80	Rock surfaces
Pleurosigma strigosum (GB 17)	May 80	Rock surfaces
Rhabdonema minutum (GB 28)	Mar.80	Slides suspended from Keppel Pier
Stauroneis constricta (GB 3)	Dec.79	Barnacles
Synedra affinis (GB 11)	May 80	P. elegans
S. affinis (GB 12)	May 80	Ectocarpus sp.
S. investiens (GB 20)	Mar.80	Slides suspended from Keppel Pier
Surirella ovata (GB 27)	Mar.80	Slides suspended from Keppel Pier

Appendix 5 The composition of enriched seawater medium (Boney and Burrow, 1966) (BSWM)

Solution A:

50ml $NaNO_3$ 0.4% (W/V) in distilled water and 2ml of each of the following:

$MnSO_3.4H_2O$	1.47 g/l	$NaMoO_4.2H_2O$	0.23 g/l
$CuSO_4.5H_2O$	0.0023 g/l	$LiCl_2.H_2O$	0.005g/l
$CoCl_2.6H_2O$	0.064 g/l		

Solution B:

$ZnSO_4.7H_2O$ 4.98 g/l

Solution C: Tetra sodium salt of DEDTA 2.6 g/l

$FeSO_4.7H_2O$ 0.12 g/l

Solution D: $Na_2HPO_4.12H_2O$ 15 g/l

To one litre pasteurized seawater add: 60ml solution A, 2ml solution B, 15ml solution C and 1.5ml solution D.

For agar medium, 14gm Bacto-agar (Difco) was added to 1 litre BSWM

Appendix 6 Composition of modified artificial seawater medium (ASWM) (modified from Harrison et al, 1980).

Solution 1: ($mmole\ l^{-1}$)

NaCl	362.66	KBr	7.24×10^{-1}
Na_2SO_4	24.99	H_3BO_3	3.71×10^{-1}
KCl	8.03	NaF	6.57×10^{-2}
$NaHCO_3$	2.06		

Solution 2: ($\mu mole\ l^{-1}$)

$MgCl_2.6H_2O$	47.17
$CaCl_2.2H_2O$	9.13
$SrCl_2.6H_2O$	8.20×10^{-2}

Vitamin complex: ($\mu g\ l^{-1}$)

Thiamine HCl	100.00
Vitamin B_{12}	2.00
Biotin	1.00

Nutrients and trace metals: ($\mu mole\ l^{-1}$)

$NaNO_3$	600.00	$ZnSO_4.7H_2O$	0.25
$NaH_2PO_4.2H_2O$	60.00	$CoSO_4.7H_2O$	5.69×10^{-2}
Na_2SiO_3	105.60	$CuSO_4.5H_2O$	0.04
Na_2EDTA	14.86	$Na_2MoO_4.2H_2O$	41.32
$FeCl_3.6H_2O$	6.00	$LiCl_2.H_2O$	0.16
$MnSO_4.4H_2O$	2.42	RbCl	2.30
		KIO_3	0.08

The pH value of the combined colutions was adjusted to 7.6-7.8 after the addition of Tris(hydroxymethyl)methylamine, 0.25g l^{-1}.

Appendix 7 The dates of collection of marine algae from Millport, Isle of Cumbrae. (Chapter 4).

Marine algae	dates
Chlorophyta	
Ulva lactuca	10 Aug. 1980
Phaeophyta	
Ascophyllum nodosum	14 Feb. 1981
Fucus vesiculosus	10 May. 1981
Fucus spiralis	24 July 1981
Rhodophyta	
Gigartina stellata	3 Oct. 1980
Chondrus crispus	23 Nov. 1980

Appendix 8 The ages of experimental algae, culture media and periods of experiments: *ASWM = artificial seawater medium, BSWM enriched seawater medium.

Seaweeds	Age (day)		Culture media*	Experiment period (days)
	sporelings & germlings	Diatoms		
Ulva lactuca	2	8	ASWM	10
Gigartina stellata	3	8	ASWM	17
Chondrus crispus	5	8	ASWM	15
Ascophyllum nodosum	14	8	1/10 BSWM	14
Fucus spiralis	14	6	1/10 BSWM	15
Fucus vesiculosus	12	7	1/10 BSWM	15

Appendix 9 Initial and final cell concentrations (10^5 cells. ml^{-1}) of diatoms Stauroneis constricta(*) and Nitzschia closterium(**) in the cultures of some experiments (referred to Table numbers); UD = undetermined.

Table number	Initial cell concentration	Final cell concentration							
		Medium							
		B	B/2	B/5	B/10				
15(*)	0.0060	20.97	21.48	16.77	13.86				
		Light intensity (lux)							
		1000	2000	3000	4000	5000			
17(*)	0.0060	33.44	33.41	33.23	48.32	27.85			
		Temperature (^{o}C)							
		15			10				
20(*)	0.0065	19.15			UD				
20(**)	0.0067	108.60			UD				
		Salinity (‰)							
		49.60	42.50	36.03	33.05	28.20	24.11	21.78	16.84
21(*)	0.0065	66.76	73.80	81.92	83.70	90.41	85.20	65.20	90.70
		49.18	44.32	39.35	35.10	30.85	26.20	21.63	16.30
22(*)	0.0066	45.00	49.86	70.56	51.28	61.60	65.00	66.28	3.91

Appendix 10 Inhibition zone (clear zone) formation of the 3-month *Chondrus crispus* sporelings in the cultures with diatoms (the length and width of erect fronds of sporelings ranged from 147.27 to 1767.24μm and 112.91 to 392.72μm respectively; * : + = slightly clear, ++ = clear, +++ = very clear)

Diatom species	Number of sporelings with clear zone	Number of sporelings Total	%	clear zone * assessment
Achnanthes linearis (GB 7)	1	61	1.64	++
Amphora acutiuscula (GB 6)	58	172	33.72	+++
Fragilaria pinnata (GB 8)	3	41	7.32	+++
F. tabulata (GB 4)	11	36	30.56	++
Navicula fonticola (GB 13)	1	21	4.76	+++
N. fonticola (GB 18)	9	29	31.03	+++
N. seminulum (GB 1)	7	16	43.75	+
Nitzschia sp. (GB 14)	8	24	33.33	+
Mean			23.26	

Appendix 11 Preparation of algal materials for SEM

Gigartina stellata sporelings growing with diatoms on glass cover slips (No. 0) were fixed and dehydrated using the following procedures (fixation method was recommended by Dr. A.H. Mandourah, whilst the dehydration method was described in Hayat, 1978).

Fixation:

5% glutaraldehyde in 0.1M Na-cacodylate and 0.25M sucrose for overnight.

washed in 0.1M cacodylate and 0.25M sucrose for 30 min.

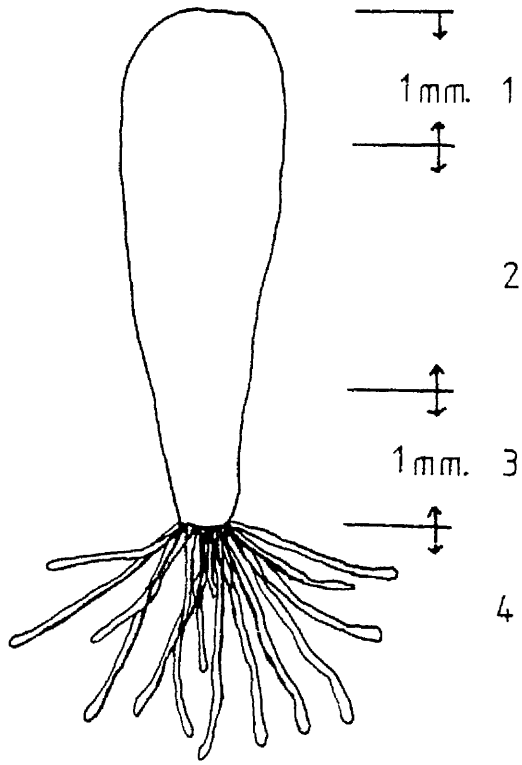
washed in 0.1M cacodylate and 0.125M sucrose for 30 min.

Post-fixed in 1% OSO_4 in 0.1M cacodylate for 2.5 hr.

Dehydration:

25%	acetone	5-10 min.
50%	"	"
70%	"	"
90%	"	"
95%	"	"
100%	"	"
100%	"	"

The dehbdrated algal materials were dried in a critical point drier and then fixed on stubs and gold shadowed.



Appendix 12 Distribution of radioactivity in 2.5-month old Fucus vesiculosus germlings two days after treatment with $NaH^{14}CO_3$ (method for combustion of algal plants and radioactivity determination is described in 9.2.5).

Portion of germlings	Dry wt. (mg)	Radioactivity (dpm)	dpm/mg
1	2.8	2618.18	935.06
2	3.3	1866.41	565.57
3	2.4	1251.18	521.32
4	1.1	319.61	290.55

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