MEIOFAUNAL NEMATODES AND THE DECOMPOSITION OF KELP

(One volume)

Michael Patrick Benwell

A thesis submitted for the degree of Doctor of Philosophy in the University of Glasgow, following research conducted at the University Marine Biological Station, Millport.

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TABLE OF CONTENTS

DECLARATION

I hereby certify that the work embodied in this thesis, for the degree of Doctor of Philosophy, is a result of my own work, which has not previously been submitted for any degree.

.....

M.P. BENWELL

I certify that this study has been performed under my supervision.

.....

DR. P.G. MOORE

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I would like to thank my supervisor, Dr. P.G. Moore, for his encouragement and advice throughout the project.

Particular thanks are also due to Mr. P.J. Lonsdale for his assistance with the underwater work. The following have also acted as diving partners: Messrs. A.P. Bedford, G.A. Fisher, P.J. Morgan, R.D.M. Nash, I.J. Robbins and M. Service.

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SUMMARY

Several aspects of the involvement of free-living nematodes in the decomposition of the kelp Laminaria saccharina have been investigated.

The seasonal pattern of growth and decay of the L. saccharina population at a site on the Isle of Cumbrae was followed. Brightfield, fluorescence and scanning electron microscopy were used to study the distribution of all epiphytes from bacterial size upwards on fronds of different year-groups over a period of one year. The areas of decomposing tissue at the frond tip had a distinctive microbial community: bacteria of several morphological types, yeasts, diatoms, flagellates and ciliates. Most epiphytes on the intact frond surface (diatoms, rod-shaped bacteria) were most abundant on the older, apical part of the frond. In the spring and early summer a network of filaments of an ectocarpoid alga developed at the frond tip, and around these filaments accumulated a mucilage matrix containing bacteria, diatoms, flagellates, ciliates, sediment grains, faecal pellets etc. The distribution of hydroids, bryozoans etc. is described.

The distribution and abundance of nematodes were studied over the same period. Four species dominated the fauna: Monhystera disjuncta, M. refringens, Chromadora nudicapitata and Theristus acer. Nematodes were virtually absent from the meristem and mid-frond regions. The first three of the above species are of similar body size, were present throughout the year, both on the tip surface and in the decomposing tissue, and bred continuously. An attempt is made to relate their seasonal changes in abundance to those of particular epiphytes, considering also data from gut contents analyses. T. acer is a larger nematode. As the ectocarpoid filaments grew in spring, progressively larger T. acer colonized the frond. One cycle of T. acer breeding took place, and then the filaments died, the material trapped around them dispersed and T. acer

disappeared from the frond. Gut contents analyses suggested that T. acer fed on mucilage and bacteria.

M. disjuncta appeared to be the nematode most closely associated with decomposing tissue. This species was cultured and its feeding on bacteria isolated from the frond was investigated using ^{32}P as a label. There was significant uptake of label from only one of the four bacterial strains tested. Comparison of the gut retention time determined by microscopic observation, with the time-course of uptake of label showed that assimilation was occurring. Uptake of label from solution was insignificant.

L. saccharina plants were allowed to decompose below the photic zone, and changes in the nematodes and epiphytes were followed. The rate and process of decomposition were essentially the same as those of distal tissue loss in living plants. A preliminary attempt at respirometric measurement of decomposition rates in the laboratory is described.

CHAPTER 1

INTRODUCTION

Fringing vegetation is a feature of most aquatic ecosystems. Around the edges of lakes and rivers grow submerged and emergent angiosperms: water lilies, reed beds etc. Around the coast, saltmarshes, mangrove swamps and seagrass meadows are found on soft bottoms, while macroalgae form communities on rocky substrata. In both salt and fresh water there are also microphytes, simple or colonial microalgae, forming mats on the bottom or living interstitially or attached to macrophytes. The primary productivity of this fringing vegetation is usually high (see reviews by Mann, 1972b; Teal, 1980), comparable with that of productive agricultural crops and perhaps 1-50 times as high as phytoplankton productivity in the same habitat. The proportion of this productivity which is contributed by the microphytes varies from less than 1% to more than 50% (review by Teal, 1980). A high proportion of the microphyte productivity may be consumed by herbivores such as insect larvae (Allanson, 1973), gastropods (Castenholz, 1961) or crustaceans (Jansson, 1969). In contrast, several studies have found that <10% of the production of the macrophytes of the fringing vegetation is grazed (Teal, 1962; Miller and Mann, 1973); the remaining 90% must enter decomposer food webs.

Free-living nematodes are abundant in both freshwater and marine environments, living in the sediment and on macrophytes. The very large numbers in which they occur (10^5 m^{-2} would be common in a marine sediment)

and their rapid growth and reproduction suggest that they are of considerable ecological importance (Gerlach, 1971). Despite this, little is known about the ecology of these animals, probably because their small size and their difficult taxonomy have deterred many ecologists.

This thesis describes a study of some of the ways in which free-living nematodes are involved in the decomposition of the kelp Laminaria saccharina (L.) Lamour.

Kelps of the order Laminariales (e.g. Macrocystis, Ecklonia, Laminaria) are the dominant macrophytes on sublittoral rock in the cold and cold temperate seas in both the N and S hemispheres. Of these algae, the genus Laminaria is essentially northern in distribution, although a few species are found in the S hemisphere (see maps in Mann, 1972b). The obvious large Laminaria plant is the sporophyte, which produces haploid zoospores from sori on the older parts of the frond. These zoospores develop into microscopic, benthic, filamentous, male and female gametophytes, which in turn produce gametes which fuse to give the zygotes which develop into the next sporophyte generation. A Laminaria sporophyte consists of a frond, a stipe and a holdfast. New frond and stipe tissue are produced by a meristem at the frond-stipe junction. The various fates of frond tissue are shown schematically in Fig. 1.1., see also Johnston (1971). Some tissue is consumed directly by herbivores such as gastropods and sea urchins, but, as mentioned above, this probably amounts to less than 10% of net production in a steady-state kelp bed (Miller and Mann, 1973). The remainder of the net production enters decomposer food chains in a variety of ways. Some dissolved organic matter (DOM) is released by healthy algal tissue. Early attempts to quantify this release in a range of brown algae produced conflicting results; Moebus and Johnson (1974), for example, found much lower rates of release than did Sieburth (1969) and Khailov & Burlakova (1969).

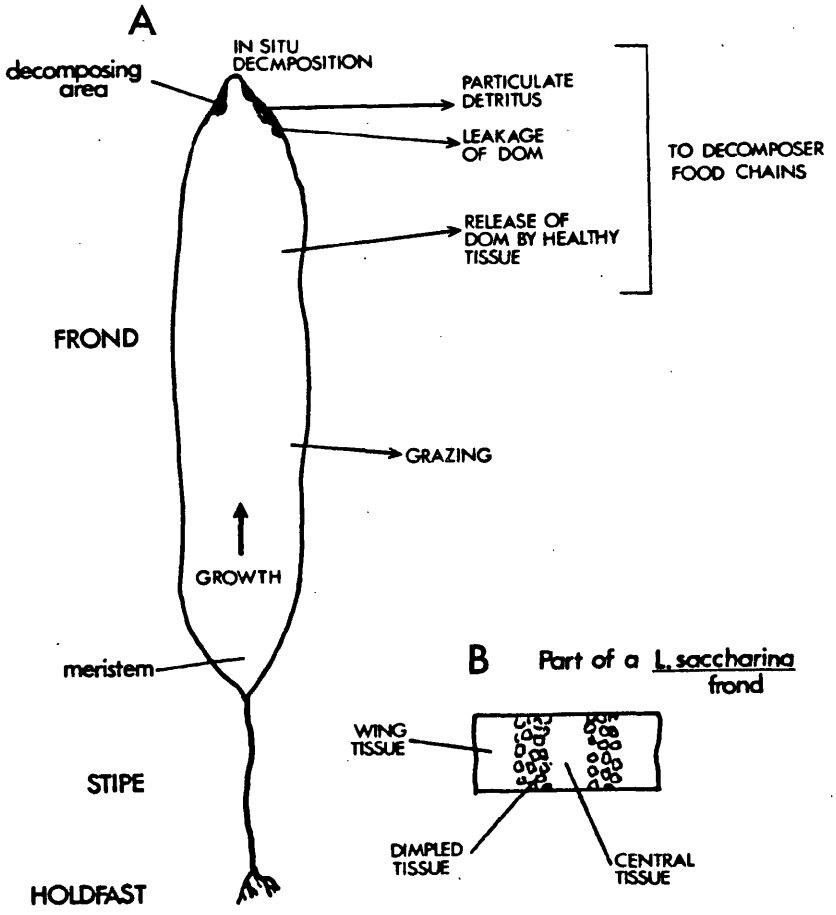


FIG. 1.1. (a) Diagram of a Laminaria saccharina frond showing pattern of growth and processes of decomposition.

(b) Part of a L. saccharina frond showing position of central, dimpled and wing tissue (see Chapters 2 and 3).

Two more recent studies, on Laminaria spp., have given results closer to those of the last two of these studies: Hatcher et al (1977) attributed to release of DOM the 35% of the net production of L. longicruris ce la Pyl. which was otherwise unaccounted for; Johnston et al (1977) found that 20% of the net production of L. saccharina plants was released as DOM from healthy tissue. Small areas of decomposing tissue are always present around the tip of a Laminaria frond. These contribute to decomposition in three ways. Firstly, some Laminaria tissue decomposes in situ in these areas. Secondly, there is leakage of DOM from the decomposing cells; Johnston et al (1977) estimated that 16% of the net production of L. saccharina was lost in this way. Thirdly, the decomposing areas continuously produce particulate detritus. In addition to the small detrital particles produced directly, the decomposing areas can weaken the frond so that large sections are torn off by wave action. Johnston et al (1977) found that 64% of net L. saccharina production was lost in particulate form. Whole Laminaria plants, particularly young plants and those on unstable substrata can be removed by wave action in the autumn and winter. A large amount of particulate detritus is thus produced by Laminaria plants in a variety of ways.

The DOM released by kelps may decompose by several pathways. Some will be taken up by planktonic bacteria (for a discussion of these bacteria see Sieburth, 1979). Some may form organic aggregates by mechanisms such as those described by Riley (1970). Both bacteria and aggregates may be consumed by filter-feeding zooplankton. DOM of algal origin may thus be decomposed in the water column or may settle out in faecal pellets, carcases etc. and enter detritus food webs. Fine algal detritus particles may similarly be decomposed in the water column or may settle out directly or indirectly as faecal pellets etc. Sedimentation of detrital particles originating from kelps was described by Webster et al (1975). Larger particles are more likely to arrive on the bottom. Drift

kelp is regularly trawled from depths of 40-50m in the Clyde near Millport, and observations while diving have shown that it can accumulate in localized dense beds, though the occurrence of these is rather unpredictable. Cowper (1978) recorded similar accumulations of drift algae from seagrass beds on the Texas coast. Beds of drift algae provide a habitat for a distinctive community of animals, and the role of these animals in the decomposition of the algae has been studied at Millport by Mr. A.P. Bedford (Ph.D. thesis in preparation). Some large pieces of Laminaria end up on the strandline, where they are colonized by another community of animals, of largely terrestrial origin (insect larvae, oligochaetes, rhabditid nematodes etc.), (Backlund, 1945).

Various aspects of the decomposition of marine macrophytes are reviewed by Fenchel (1972), Mann (1972b, 1976).

In such a complex process there are clearly many points at which nematodes are likely to be involved. Much of the energy input into coastal sediments comes from macrophytes, and these sediments usually have a diverse nematode fauna the biology of which is very poorly understood. Gerlach (1978) discusses the way in which meiofauna may stimulate bacterial productivity and hence speed up decomposition. Decomposition in the strandline involves a variety of nematodes: Inglis & Coles (1961) describe strandline rhabditids; the one sample taken in this present study contained rhabditids, monhysterids and oncholaimids. Neither decomposition in the sediment nor decomposition on the strandline were considered in this study.

Chapters 2, 3 and 4 describe a seasonal study of the distribution of nematodes and of all epiphytes from bacterial size upwards on living Laminaria saccharina. An understanding of the ecology of nematodes on living fronds is necessary if any changes which take place when tissue becomes detached and decomposes are to be recognized and interpreted. Such a study should also reveal whether nematodes are involved in in situ decomposition at the frond tip and the associated leakage of DOM and

production of particulate detritus. This is the most detailed study which has yet been made of the relationship between nematodes and epiphytes on a marine alga; several previous workers have suggested that such a study would be useful (e.g. Ott, 1967; Warwick, 1977). There has only been one previous seasonal study of an algal nematode fauna (Warwick, 1977).

Chapter 5 describes experiments to investigate feeding by the nematode Monhystera disjuncta, which appeared to be the species most closely associated with the areas of decomposing tissue, on bacteria isolated from L. saccharina fronds. Such aspects as feeding rates, gut retention times and selective feeding are considered.

Chapter 6 describes a field experiment on the decomposition of L. saccharina plants attached below the photic zone to a line running along the bottom. The rate of tissue loss was measured, and changes in the epiphytes and nematodes were monitored. The second part of this chapter concerns a preliminary attempt to measure decomposition rates in the laboratory using a respirometric technique.

CHAPTER 2

THE FIELD STUDY : THE STUDY AREA ; THE BIOLOGY OF LAMINARIA SACCHARINA POPULATION ; SAMPLING STRATEGY

2.1. THE STUDY AREA

2.1.1. The Clyde Sea area - general physical features (Fig. 2.1.).

Bathymetry; surface sediments; solid geology. The shallow drowned estuary of the Clyde enters the area at the NE corner and runs into a system of steep-walled, glacially deepened trenches separated by shallow sills. South of Arran is a relatively shallow, relatively level plateau which forms a sill over which water must pass to exchange between the deeper areas to the north and south. The bathymetry is discussed in Barnes and Goodley (1971) and Deegan et al (1973), both of which include bathymetric charts. The distribution of surface sediments is largely controlled by bathymetry. Deegan et al (1973) recognized three major facies: the Coarse Littoral Facies, the Transitional Facies, and the Deep Silty Clay Facies, and discussed in detail their character and distribution. The Highland Boundary Fault runs through the area along a course which is in part uncertain. Dalradian rocks outcrop generally to the north of the fault, while to the south are mainly Old Red Sandstone, and Carboniferous lavas, mudstones and sandstones. Tertiary sills, vents, dykes and larger intrusions are numerous.

Water movements. Hydrographic studies include those by Barnes and Goodley (1961), Johnston et al (1974) and Dooley (1979). The freshwater input, principally from the Rivers Clyde and Leven, is small relative to the volume of the Firth. The sill in the south separates the Firth

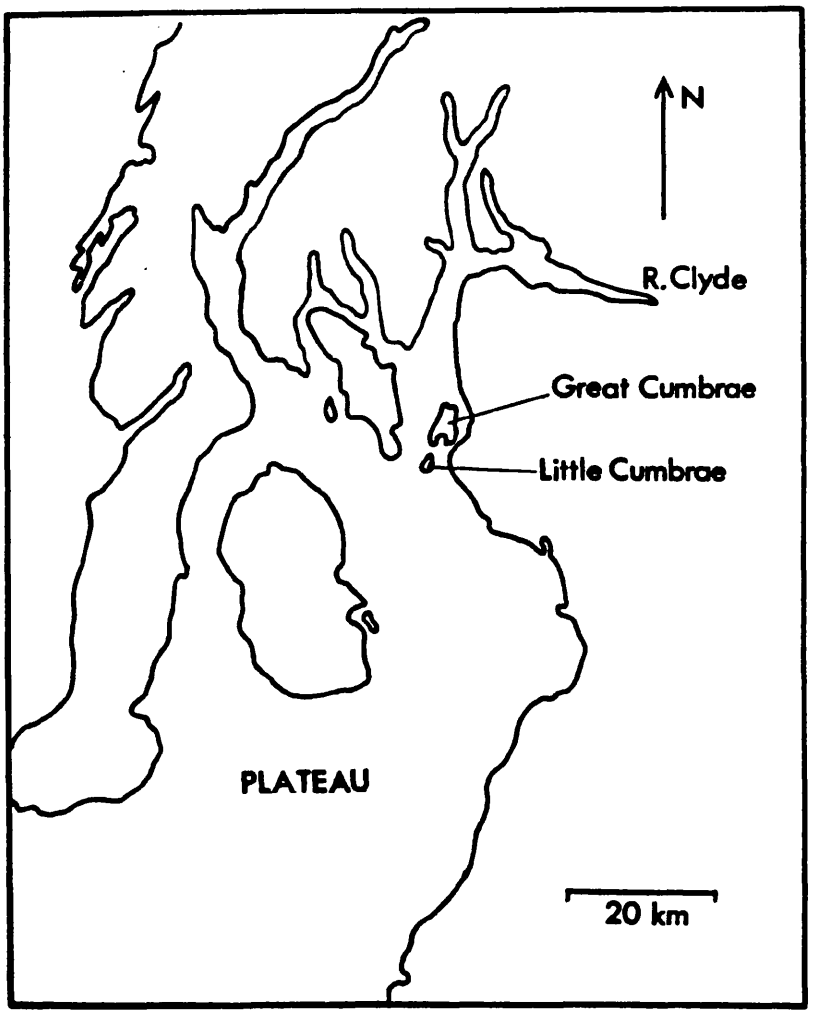


FIG. 2.1. Map of the Clyde Sea Area

from the very strong tidal streams of the North Channel, and tidal streams within the Firth are generally low, though rather faster in the narrower, shallower sections in the north. The resulting low levels of turbulence and mixing give rise to a patchy and variable water structure with large vertical and horizontal density gradients. This density distribution interacts with wind-driven flows to give a complex and variable pattern of water movements.

Salinity; temperature. Barnes and Goodley (1961) summarized surface salinity data for Keppel Pier, Millport for the years 1949-1953. The grand mean salinity was 32.10‰, the months with the lowest and highest overall means being January and June, with 31.26‰ and 32.98‰ respectively. The effect of rainfall on surface salinity was greatest in the summer months. Johnston et al (1974) found that in April 1970, salinity at 3m depth ranged from 31.5‰ at the mouth of the R. Clyde to 33.74‰ near the Mull of Kintyre, with 33.2 - 33.5‰ over much of the Firth. Values in the same range were found by Dooley (1979).

Barnes (1959) summarized surface sea temperatures at Millport for the years 1949-1958. The month with the lowest ten-year mean was March, with 6.76°C, the month with the highest, August, with 13.39°C. Data for 1959-1979 are summarized by Moore (in press); some temperature-depth profiles are given by Dooley (1979).

Nutrient levels. Johnston et al (1974) recorded nitrate levels of 5-11 μg-at NO_3-N l^{-1} over much of the Firth in March-April 1970. Levels were higher (12-16 μg-at NO_3-N l^{-1}) in the waters of salinity 33‰ in the upper Firth, and much higher (12-65 μg-at NO_3-N l^{-1}) in the Irvine Bay area where industrial effluents are discharged. Even the lowest of these levels is somewhat higher than is usually found in surface waters.

2.1.2. The study site

The Wishing Well (grid ref. 183556, OS 1:50000 First Series sheet 63) is on the East coast of Great Cumbrae Island, 1.5 km north of the Marine Station (Fig. 2.2.). A small stream runs down the shore. A profile of the beach is shown in Fig. 2.3. A gently sloping rocky platform runs from +3.3m to -0.8m (heights above Chart Datum). The bedrock is Old Red Sandstone and on the platform this is overlain with stones which on the Wentworth scale (see Buchanan and Kain, 1971) fall into the categories: pebbles, cobbles and boulders. There are boulders of up to 70x60x40cm at all levels on the platform, and larger ones of up to 150x130x120cm on the upper part. Most of these stones are Old Red Sandstone but a few are basalt, probably from the nearby dykes. The platform ends with a steep rock and boulder slope dropping from -0.8 to -2.7m in a horizontal distance of 3m. Below this is a long sandy slope which continues down to -28m, the lower limit of observations made by diving. There are a few boulders lying on the sand immediately below the steep rocky slope. The maximum depth of this part of the Largs Channel (a glacially deepened trough, see above) is 50m.

At the south side of the beach a rocky outcrop, the Butter Lump, rises from the lower part of the platform. It is just covered at HWS and is connected to the rest of the beach at LW. Its outer side is steep and has no boulders, and inside it is a small patch of muddy gravelly sand with scattered boulders. While the whole coastline of Great Cumbrae, except for Farland Point (Fig. 2.2.), is fairly sheltered from wave action, the Butter Lump provides additional shelter for the littoral and shallow sublittoral at the Wishing Well.

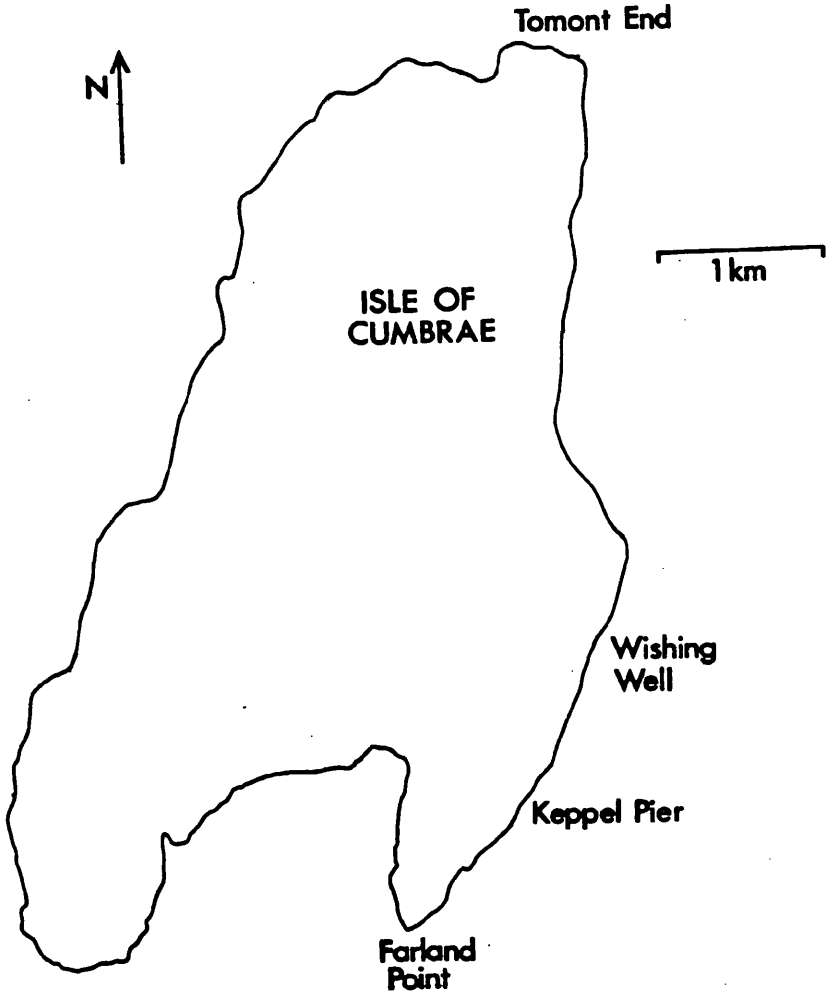
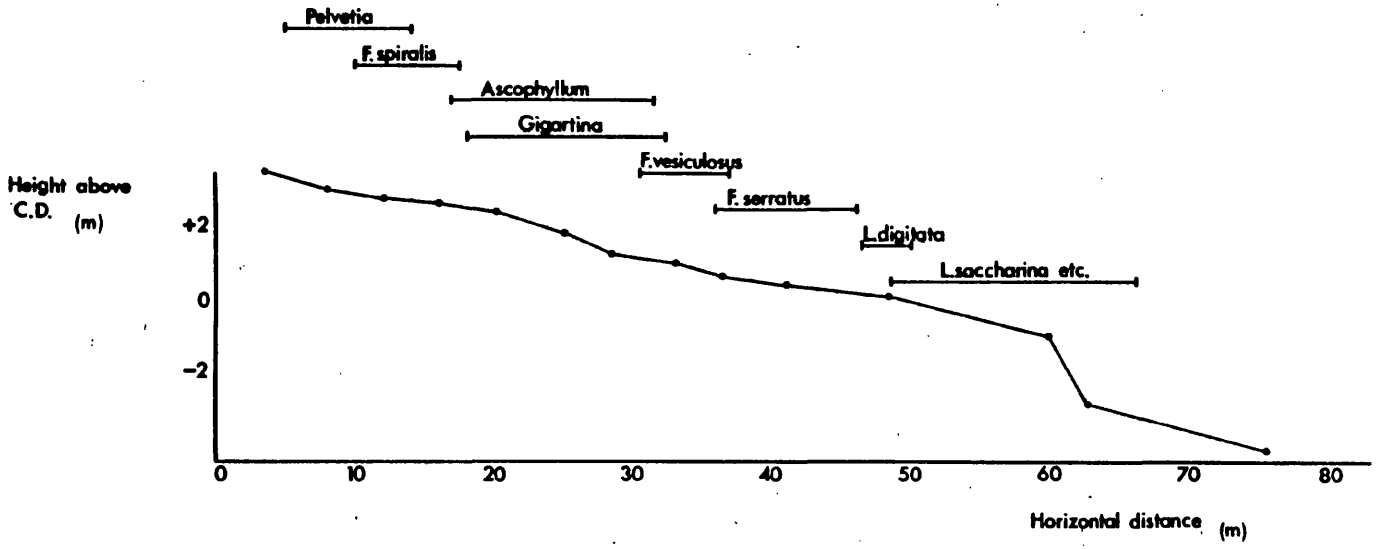


FIG. 2.2. Map of Great Cumbrae, showing the study site (the Wishing Well) and other sites mentioned in the text.

FIG. 2.3. Profile of the beach at the Wishing Well, showing position of the main algal zones.
C.D = CHART DATUM.


Pelvetia
F. spiralis
Ascophyllum
Gigartina
F. vesiculosus
F. serratus
L. digitata
L. saccharina etc.
Height above C.D. (m)
+2
0
−2
0
10
20
30
40
50
60
70
80
Horizontal distance (m)

Zonation of the algae. The zonation of the littoral algae down the centre of the beach is typical of that seen on boulder shores on Great Cumbrae. The dominant species are Pelvetia canaliculata (L.) Dene. et Thur., Fucus spiralis L., Ascophyllum nodosum (L.) Le Jol., Gigartina stellata (Stackh. in With.) Batt., F. vesiculosus L. and F. serratus L., and the positions of their zones are indicated in Fig. 2.3. The algae are more or less confined to the bedrock and the larger boulders, and the overlap of zones shown in Fig. 2.3. occurs because the boulders provide a considerable range in height at one level on the beach. Green algae, mainly Enteromorpha spp., grow in small amounts where the stream flows over the upper shore.

The kelp zone was the site of this study. At +0.4m there is a narrow belt of Laminaria digitata (Huds.) Lamour. From here to the bottom of the boulder slope (-2.7m) the zone is dominated by L. saccharina, with small amounts of Desmarestia aculeata (L.) Lamour., Chorda filum (L.) Stackh. and Saccorhiza polyschides (Lightf.) Batt. Various smaller algae (mainly Rhodophyceae) grow beneath the laminarian canopy. A few L. saccharina plants grow on the boulders at the top of the sandy slope. Further down the slope is a zone dominated by the brittle star Ophiocomina nigra (Abildgaard) and the burrowing anemone Cerianthus lloydi Gosse.

A different pattern of zonation is seen on the outer side of the Butter Lump which reflects its slightly greater exposure to wave action. In particular, the mid shore has a broad zone with a mosaic of green algae (species of Enteromorpha and Ulva), the red algae Gigartina stellata and Porphyra sp., the mussel Mytilus edulis L., and the barnacle Balanus balanoides (L.). Between this mosaic and the L. digitata belt is a zone with Palmaria palmata (L.) O. Kuntze (= Rhodymenia palmata) and a little F. serratus.

2.2. BIOLOGY OF THE L. SACCHARINA POPULATION

2.2.1. Age structure. The population was first surveyed in October 1978. The plants from several $1m^2$ quadrats were harvested and brought back to the laboratory for measuring and ageing. Plants were aged by counting the dark zones visible in longitudinal sections of the holdfast and the basal part of the stipe (Parke, 1948). The shallower part of the L. saccharina zone from +0.1 to -0.3m, was populated largely by 0-class (1978) sporophytes, while most of the plants from -0.4m to -0.8m were 1-class (1977) plants. (Table 2.1.).

	SHALLOW ZONE			DEEP ZONE		
Quadrat	A	B	C	D	E	F
1977 plants	0	1	4	7	2	9
1978 plants	18	11	15	2	0	2

TABLE 2.1. Numbers of L. saccharina plants of different year-groups in $1m^2$ quadrats from the shallower and deeper parts of the L. saccharina zone in October 1978.

There was some overlap, or an irregular boundary between the two zones. The difference between the zones persisted throughout the study period. The 1977 plants did not survive the 1978/1979 winter, so that in the spring and summer of 1979 the dominant plants in the deeper zone were 1979 plants. Many of the 1978 plants in the shallower zone did survive the 1978/1979 winter, and this year-group again dominated the zone in 1979. Many of the 1979 plants, but apparently none of the 1978 plants, survived the 1979/1980 winter. L. saccharina at the Wishing Well thus appeared to have a 2 year maximum lifespan, and plants of three year-groups, 1977, 1978 and 1979, were involved in the study.

2.2.2. Growth of plants of the different year-groups

1977 plants. In October 1978 these were attached to the bedrock or to large boulders (those with bases > 50x50cm) rather than to small stones. The fronds were mostly 20-50cm long and 16-20cm wide, thus falling outside the length-width curve of 1978 plants in this month. By January 1979 most of these plants had been reduced to bare stipes; a few retained remnants of frond up to 12cm long. These 1977 plants did not regenerate their fronds in the spring of 1979 and the stipes slowly rotted, finally disappearing in the autumn of 1979, some having served as a substratum for the settlement of 1979 plants.

1978 plants. In October 1978 these were attached mainly to boulders, but some to smaller stones. The plants ranged in length from 9cm to 140cm and in width from 2 to 20cm. By January 1979 all the smaller plants and many of the larger had been lost and the remaining plants had been reduced in size, the longest found being 90cm long and 15cm wide. Most of these plants had lost the wing and dimpled tissue from the apical part of the frond, giving a narrow strap-like tip (see Fig. 3.1.). The plants started to grow rapidly in February 1979, the new tissue being much broader than the winter frond which remained attached to it. In March 1979 the new tissue was 50-60cm long and 20-25cm wide, and the total frond length was 100-130cm. By May 1979 the winter fronds were being lost from the tips and the fronds were 120-150cm long and ~30cm wide. By July the fronds were up to 200cm long and 40cm wide. In September the fronds were still long but showed considerable damage, with large portions missing from the edges in several places. Over the next three months the damage increased and the fronds tore through. By January 1980 most of the 1978 plants had been reduced to bare stipes, and none had fronds longer than 15cm. These plants showed no frond regeneration in 1980.

1979 plants. New sporophytes began to appear in late March 1979. These were 1-2cm long when first noticed and seemed to be attached indiscriminately to boulders, pebbles and the stipes of older plants. More sporophytes continued to appear until September. Growth in the spring was rapid, the earliest sporophytes reaching 40cm long and 7-8cm wide in May and up to 100cm long and 20cm wide by July. Growth then slowed and by September the fronds showed some damage which increased through the winter. During the winter most of the smaller, later-developed plants were lost, as were the plants which had developed on pebbles or on the stipes of other plants. In January 1980 most of the remaining plants were only 40-80cm long, and some as little as 5cm.

2.2.3. Growth rates

The way in which a Laminaria plant grows was described in Chapter 1. Some information on the rates of basal growth in L. saccharina at the Wishing Well can be derived from the observations on frond lengths presented above. 1979 plants which appeared in March had reached 50-60cm by May and 100cm by July. Little apical decomposing tissue was present in these first few months of growth (see section 3.3.) which suggests that distal tissue loss was minimal. The rate of basal growth was therefore 0.8-1.0cm day^{-1} over this period. The growth rate of 1978 plants in the spring of 1979 could be observed directly by following the junction of new and old fronds, which was marked by an abrupt change in frond width. The rate was 1.0-1.5cm day^{-1}, rather higher than in the 1979 plants.

The punched hole method introduced by Parke (1948) and subsequently used in a number of other studies (e.g. Mann, 1972a; Johnston et al 1977; Chapman and Craigie, 1978) can reveal the rates of basal growth and distal tissue loss at all times of year. A hole is punched in the frond above the meristem, at a known distance from the frond base, and at intervals thereafter the total frond length and the new distance of the hole from

the frond base are measured. When the hole approaches the frond tip a new hole is punched above the meristem.

If: l_o cm = frond length at time zero

l_t cm = frond length at time t days

X_o cm = distance of hole from base at time zero

X_t cm = distance of hole from base at time t days

G cm day^{-1} = mean daily rate of basal growth from time zero to time t days

D cm day^{-1} = mean daily rate of distal loss from time zero to time t days

$$\text{then } G = \frac{X_t - X_o}{t}$$

$$\text{and } D = \frac{(l_o - l_t)}{t} + G$$

Parke (1948) found that all production of new tissue occurred within 10cm of the frond base. In the present study, holes were punched 15cm above the base to ensure that the meristem would not be damaged. A 15cm length of 1cm diameter brass piping with one end sharpened to form a cutting edge was used to make the holes, and punched plants were marked by tying around the stipe a large (25x15cm) label cut from a sheet of flexible white plastic. Eight 1978 plants in the shallow zone were punched in May 1979. Some of these were later damaged or lost and further plants were punched to replace them. Observations on the plants were rather sporadic but the results are consistent within themselves and with the rates estimated above (Table 2.2.).

Growth rates in the period May-July did not vary much from plant to plant and ranged from 1.0 to 1.8cm day^{-1}. Distal loss in the same period was 0.2-1.9cm day^{-1}. From July onwards growth rates were lower, 0.5-0.7cm day^{-1} in July-August and 0.1-0.4cm day^{-1} in August-December. In the

PLANT	INITIAL DATE	FINAL DATE	l_o	l_t	G	D
A	27.4	12.6	110	136	1.4	0.8
	12.6	23.7	136	168	1.0	0.2
	23.7	4.9	168	153	0.7	1.1
B	27.4	12.6	220	205	1.6	1.9
C	27.4	12.6	105	137	1.7	0.9
	12.6	23.7	137	172	1.1	0.3
D	27.4	12.6	150	142	1.1	1.3
E	27.4	12.6	125	181	2.0	0.7
F	27.4	23.7	220	231	0.8	0.7
	23.7	4.9	231	197	0.5	1.3
	4.9	23.10	197	81	0.3	2.2
G	23.7	4.9	183	155	0.6	1.2
	4.9	23.10	155	94	0.2	1.4
	4.9	23.10	174	60	0.3	2.5
	4.9	23.10	132	62	0.4	1.7

TABLE 2.2. Results of punched hole measurements of basal growth and distal tissue loss. All dates refer to 1979.

autumn distal tissue loss was rapid, and the rate of loss varied considerably among the plants, presumably because large pieces of tissue were lost at irregular intervals when the fronds tore through.

2.2.4. Population density

No attempt was made to measure accurately the density of the L. saccharina population. It is evident from the results presented above that the density was seasonally variable and that the spatial distribution of the plants was not random. Estimates of summer and winter density can however be made from occasional quadrat samples taken for other purposes. Results from these are given in Table 2.3.

Late summer density was ∿50 plants m^{-2}. Most of these were small 0-class plants which would not survive the following winter. In the winter a boulder of area 0.25 m^2 carried about 5 plants. Boulders stable enough to allow winter survival of plants covered ∿30% of the area; winter population density was thus ∿6 plants m^{-2}.

2.3. DISCUSSION

The Wishing Well is topographically typical of the SE coast of Cumbrae, and of much of the N part of the Firth of Clyde, in that there is a narrow rocky platform above a steep sandy slope running down into deep water. The zonation of the littoral algae on the main part of the beach is typical of fairly sheltered sites within this generally sheltered region; the stream appears to have a significant effect only on the upper shore.

A kelp zone dominated by L. saccharina below a narrow fringe of L. digitata is also typical of the Inner Firth. Round much of Britain L. saccharina is outcompeted in this zone by L. hyperborea (Gunn.) Fosl., which forms a dense perennial forest (Kain, 1975). The usual outcome of competition seems to be reversed in two situations: on unstable substrata,

MONTH	ZONE	QUADRAT SIZE	NO. OF PLANTS	REMARKS
October 1978	shallow	$1m^2$	18	
			12	
			19	
	deep	$1m^2$	9	
			2	
			11	
January 1979	shallow	$(\frac{1}{2}m)^2$	5	
			12	
			10	
			3	
	deep	$(\frac{1}{2}m)^2$	1	only plants with some
			2	frond left are included
March 1979	shallow	$(\frac{1}{2}m)^2$	8	taken over boulder
			5	none between
			9	" " "
	deep	$(\frac{1}{2}m)^2$	0	only plants with frond
			0	left included
			1	" " "
May 1979	shallow	$(\frac{1}{2}m)^2$	14	boulder and pebbles
			6	pebbles
			23	boulder and pebbles
	deep	$(\frac{1}{2}m)^2$	25	all young plants
			7	" " "
July 1979	shallow	$(\frac{1}{2}m)^2$	14	pebbles. young plants
			5	boulder
January 1980	shallow	$(\frac{1}{2}m)^2$	4	boulders
			0	boulders
	deep	$(\frac{1}{2}m)^2$	9	
			7	

TABLE 2.3. Quadrat counts of L. saccharina plants.

and in more sheltered sites (Kain, 1962). L. saccharina is successful on unstable substrata probably because its flexible stipe produces less leverage on the substratum than the rigid stipe of L. hyperborea. Around much of Cumbrae even the bedrock carries L. saccharina, and so shelter is probably the important factor. This is confirmed by the occurrence of L. hyperborea in the most exposed situations in the area, i.e. in the upper part of the kelp zone at sites such as Farland Point, Great Cumbrae and Gull Point, Little Cumbrae (Kain, 1962 and personal observations). There is no obvious explanation for the success of L. saccharina in sheltered conditions; L. hyperborea can grow even in very sheltered situations, where it may assume the non-digitate form L. hyperborea f. cucullata (Svendsen and Kain, 1971), but its growth in such habitats is slow. In the absence of vigorous water movements, the rate of uptake of dissolved nutrients might limit growth. It could be that uptake is more efficient in L. saccharina than in L. hyperborea, perhaps because the dimples in a L. saccharina frond give rise to turbulent flow over the surface. Nitrate levels in the Firth of Clyde might be too high to limit growth, but the concentrations of other nutrients could be limiting.

The depth to which L. saccharina extends at the Wishing Well is limited by substratum availability. On a rope which was laid out along the bottom perpendicular to the shore from -0.8m to -28m, sporophytes developed down to -7m, which is about 2m below the lowest boulders lying on the sandy slope. This figure of -7m agrees with that given by Clokie and Boney (1979), who found that in the Clyde L. saccharina reached 40% of the maximum depth to which algae grew, and that in the Largs Channel this latter depth, given by the lower limit of Conchocoelis (Clokie and Boney, 1980) was 16-20m below C.D. The sea urchin Echinus esculentus L., though present at the Wishing Well (particularly on the steep rocky slope), was not found in large numbers, and sea urchin grazing does not appear to control the lower limit of the L. saccharina

population (cf. Jones and Kain, 1967; Kain, 1977).

At the Wishing Well, sporophytes were produced from May to September. Of these, the only ones to survive the following winter were a proportion of those which developed early in the season on stable substrata, i.e. bedrock and large boulders. Summer sporophytes and those on unstable substrata were lost in the autumn and early winter. Most of the plants which survived their first winter persisted through the following summer, but in their second winter they suffered heavy damage and there was no regeneration in the third spring. The maximum lifespan was thus two years. This agrees well with the results of Parke (1948) who studied populations in a variety of habitats in Devon and Argyll. She found that in shallow sublittoral habitats sporophyte production ceased in the winter, and spring sporophytes dominated most of her populations as a result of their better survival. Except in very sheltered habitats, depopulation was rapid even among spring sporophytes, with 94-97% lost in the first year, and more than 99% by the end of the second. The species in the Simplices section of the genus Laminaria are in general shorter-lived than those in the Digitatae. Most have a maximum lifespan of two years, whilst L. digitata and L. hyperborea, for example, can live for up to five and eighteen years respectively (literature reviewed by Kain, 1979).

The growth rates of L. saccharina at the Wishing Well agree well with those measured by Parke (1948) and Johnston et al (1977). Parke (1948) studied both littoral and sublittoral population; growth rates in the sublittoral were higher, and closer to those measured in this study. As at the Wishing Well, growth was fastest in the second year of life. In the population studied by Johnston et al (1977) (in Loch Creran, Argyll) a secondary peak of distal tissue loss, which was attributed to "poor water conditions", occurred in June-July. This might correspond to the increase in tissue decomposition observed at the Wishing Well in August-September 1979 (see section 3.3.). All Laminaria species which

have been studied show similar seasonal patterns of growth, though some, such as L. hyperborea, have a more marked reduction of growth in the slow-growth period than does L. saccharina. Information on photosynthesis and respiration rates, the accumulation and depletion of reserves of laminaran, mannitol and nitrogen, translocation, the factors limiting growth at different times of year, etc. can be found in Black (1950), Kain (1976), Luning (1969, 1971, 1979), Luning et al (1973), Johnston et al (1977), Hatcher et al (1977), Chapman and Craigie (1977, 1978) and Mann (1972a, 1979). Most of this literature is reviewed by Kain (1979).

The largest L. saccharina plants at the Wishing Well were those on the boulders at the top of the sandy slope. The same is found at other sites on Great Cumbrae, and a possible explanation for this lies in the effects of water movements. The plants growing in shallow water will be subject to greater wave action than those in deeper water. Wave action will tend to increase growth rates by promoting nutrient exchange, but at the same time will tend to decrease them by accelerating distal tissue loss and hence decreasing the amount of photosynthetic tissue. Parke (1948) found that rates of basal growth were lower at more exposed sites, indicating that at her sites the second of these effects outweighed the first. This may also be the case at the Wishing Well; however, whilst diving it was noticed that the tidal stream was stronger below the steep rocky slope than on the platform. Tidal streams will promote nutrient exchange without having the same damaging effect as wave action, and may also hold the fronds in the optimum attitude for photosynthesis. The largest L. saccharina plants are found in the approaches to tidal rapids (Lewis, 1964).

2.4. SAMPLING STRATEGY FOR THE EPIPHYTE AND NEMATODE STUDIES

(a) Choice of year-groups

Since at any one time the L. saccharina population consists of two year-groups, each dominant in one part of the kelp zone, the opportunity was taken to sample separately each year-group from within its zone. This meant that samples were taken as follows, Table 2.4.

	JANUARY 1979	MARCH 1979	MAY 1979	JULY 1979	SEPTEMBER 1979	NOVEMBER 1979	JANUARY 1980
SHALLOW	1978	1978	1978	1978	1978	1978	1978
DEEP	1977	1977	1979	1979	1979	1979	1979

TABLE 2.4. Year-groups of L. saccharina used in the sampling programme in different months.

Plants growing on the boulders at the top of the sandy slope were not included. These formed only a small part of the total L. saccharina biomass, and they grew larger than the other plants (see section 2.3.) and were therefore atypical.

(b) Selection of plants within a year-group

To obtain a representative sample by carrying out a random sampling programme for each year-group would have meant examining many replicate plants, which would have been both time-consuming and unacceptably damaging to the site. So plants were chosen which appeared to be typical of their year-group. In the summer and early autumn when 0-class plants of a variety of sizes and ages were present, plants typical of the largest were taken, for two reasons: (i) it was these early spring sporophytes which would persist through the following winter, (ii) these larger plants

made up most of the biomass even when they were outnumbered by small plants. (Fig. 2.4. shows the distribution of "frond area" among different frond length classes of 1978 plants from three $1m^2$ quadrats taken from the shallow zone in October 1978. The "frond area" of a frond is the product of its length and its maximum width, and for a group of fronds, "frond area" should be roughly proportional to biomass). In each sample, two plants from each year-group were used for nematode studies, two for the brightfield examination of epiphytes, and one for fluorescence microscopy.

(c) Frequency of sampling

Shortage of time prevented sampling more frequently than once every two months. This is a major defect of the sampling programme. While differences within plants and between replicate plants were usually small (see Chapters 3 and 4), differences between bimonthly samples were often considerable, and more frequent sampling would have been a great advantage. Time involved in sample processing and the programme of experimental work unfortunately precluded any more intensive field effort.

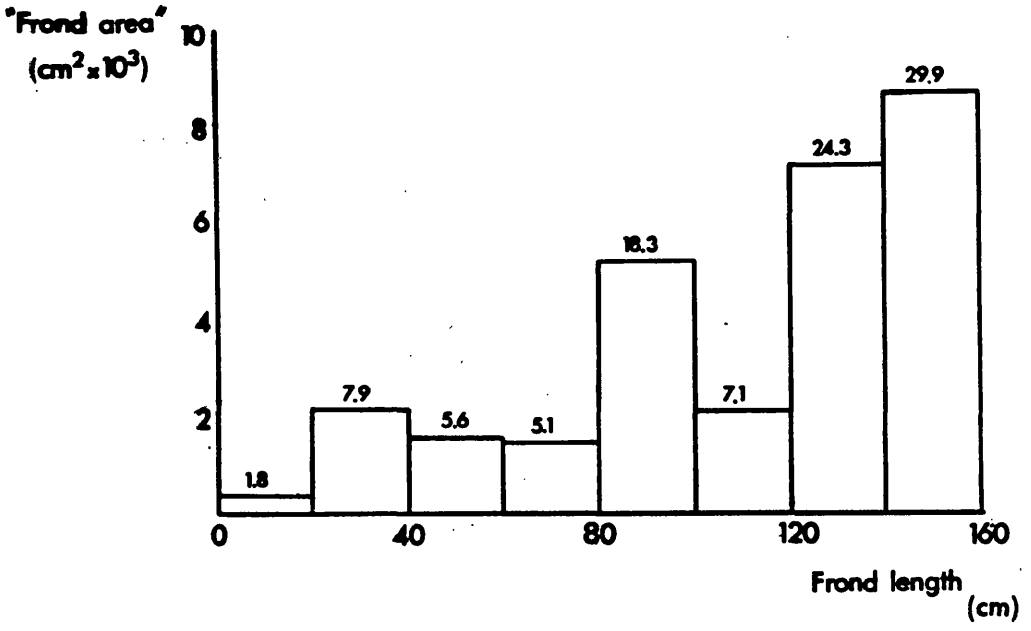


FIG. 2.4. Distribution of "frond area" (frond length x maximum frond width) for different frond length classes in the L. saccharina population, shallow zone, October 1978, based on plants from three $1m^2$ quadrats. Figure above each block is % of population "frond area" made up by that length class.

CHAPTER 3

EPIPHYTES ON LAMINARIA SACCHARINA FRONDS

3.1. METHODS

3.1.1. Collection of plants

All plants were collected underwater, usually by SCUBA diving, occasionally by snorkelling. Plants were detached either by pulling the holdfast from the substratum or by cutting through the stipe. They were then enclosed in a large (35x50cm) polythene bag, the mouth of which was closed with a repeatedly twisted elastic band. During these operations, disturbance to the plants which might have dislodged loosely attached epiphytes was minimized. In the laboratory the plants were placed in baths of filtered seawater (0.3µm Gamma-12 on-line filter, Whatman Ltd., connected to the laboratory seawater system).

3.1.2. Naked eye observations and brightfield microscopy

These were carried out on the same fronds. Portions ∿4x4cm were cut from appropriate parts of the frond with scissors, placed in Petri dishes of filtered seawater and examined under the stereomicroscope. Smaller pieces, ∿0.5x0.5cm, were mounted on microscope slides and examined under the compound microscope. Transmitted light was used and, provided it was bright enough, proved satisfactory for even the thickest pieces of frond. After microscopical examination was completed the frond was laid out on a bench and examined with the naked eye. The length of the frond and the width at 10cm intervals along the length were recorded.

3.1.3. Fluorescence microscopy

Pieces ∿0.5x0.5cm were cut from the frond with scissors. Two strips of a synthetic putty ("Glasticon", Kelseal Ltd.,) were placed on a flat microscope slide at a suitable distance apart to support a square coverslip. A piece of frond was laid between the strips and a drop of acridine orange solution (a 1:2500 solution of solid acridine orange freshly made up in 0.4µm Millipore filtered seawater) was put onto it. A coverslip was then laid across the "Glasticon" strips and firmly pressed down until it just made contact with the Laminaria surface. The mount was left for 2 minutes then examined under an incident-light fluorescence system consisting of a Leitz Orthoplan microscope, a Ploemopak fluorescence illuminator, and Placo NP1 planachromat objectives of magnifications x10, x40 and x100. Counts of bacteria were made under the oil-immersion objective using a squared eyepiece graticule; a special non-fluorescent immersion oil was not found to be necessary.

3.1.4. Scanning electron microscopy

One 1978 plant was collected from the shallow site on 23/6/79. Twelve pieces ∿1 x 1cm were cut from the frond with scissors. These included pieces of decomposing tissue from the frond tip and pieces with intact surfaces from the central, dimpled and wing regions of the tip, mid-frond and meristem. The pieces were fixed for 2 days in phosphate-buffered 4% glutaraldehyde, pH 7.4-7.6, then dehydrated in a series of alcohols. After 1 day in absolute alcohol they were transferred to a 50:50 mixture of absolute alcohol and amyl acetate for 1 hour, then to amyl acetate for 1 hour before critical point drying using liquid CO_2 as the transition fluid. On removal from the drier the pieces were immediately mounted on aluminium specimen stubs using Silver Dag (Agar Aids) as an adhesive, coated with Au/Pd and examined in a Cambridge Stereoscan 600 scanning electron microscope.

3.1.5. Discussion of methods

Naked-eye and stereomicroscope observations gave information on the distribution of decomposing tissue and of the larger epiphytes such as hydroids, bryozoans and macro-algae. The brightfield compound microscope provided most of the observations on diatoms, protozoans, small algal filaments and material collecting around algal filaments, and some of those on Leucothrix. Fluorescence microscopy was used in the seasonal study of bacteria and also gave some information about protozoans and diatoms. The SEM, with its greater resolving power and its ability to show three-dimensional structures, provided additional details about many epiphytes, particularly bacteria.

In the fluorescence method, the acridine orange binds to nucleic acids and the resulting complex fluoresces bright green. Dead bacteria can take up large quantities of stain and appear orange, while chlorophyll shows blood-red autofluorescence. Live bacteria thus appear green, but the Laminaria cells also fluoresce, and this background fluorescence can make seeing the bacteria difficult, particularly in mounts of meristem tissue. In most cases it was possible to see the bacteria by careful focussing and by ensuring that the coverslip was pressed against the Laminaria surface. Whenever there was doubt about whether all the bacteria on a mount were visible, the mount was discarded. About one in five meristem mounts were discarded for this reason. Errors may have occurred if areas with high background fluorescence have more or less bacteria than do other areas, or if wrong decisions were made about when to reject mounts. This must be borne in mind when interpreting the results, and comparison with results from the SEM provides a useful check. Burton (1976) used an acridine orange epifluorescence method similar to that described above to study bacteria on the surface of the green alga Ulva, but with the red alga Ceramium he resorted to fluorescence examination of filtered homogenates. With Fucus serratus neither method was successful, the

direct observation method failing because of background fluorescence which Burton attributed to the presence of polyphenols in the algal tissue. Mazure and Field (1980) found that dense aggregations of bacteria in the surface mucilage made acridine orange counts impossible on Laminaria pallida (Grev.)J.Ag. and Ecklonia maxima (Osbeck) Papenf.

One problem with the SEM procedure was that transferring the specimens through fixative, alcohols and amyl acetate led to the loss of loosely attached epiphytes. Some of the material originally trapped among brown algal filaments was lost in this way. Another problem was that mucilage which did remain on the specimens partly concealed bacteria attached to the frond surface. To get the most meaningful results from an SEM study of this type it would be preferable to use several methods of preparing the specimens and then to compare the results.

3.2. DAMAGE TO THE FRONDS, DISTRIBUTION OF DECOMPOSING TISSUE

3.2.1. Results

Scale drawings showing the shapes of plants used for naked-eye and brightfield observations are given in Figs. 3.1. and 3.2. These drawings were constructed from the measurements mentioned in section 3.1.1. In this and following sections distances given in cm, as in "decomposing tissue was present from 130 to 150cm", refer to distances from the frond base in these plants.

January 1979. 1978 plant. In the strap-like tip of this plant only the thicker central tissue remained. Decomposing areas were found at the edge of the frond, back to 15cm. In the mid-frond region there were extensive areas on the central tissue where the meristoderm had been removed. 1977 plant. The decomposing areas were more extensive than in the 1978 plant, occurring around the tip and edge of the frond.

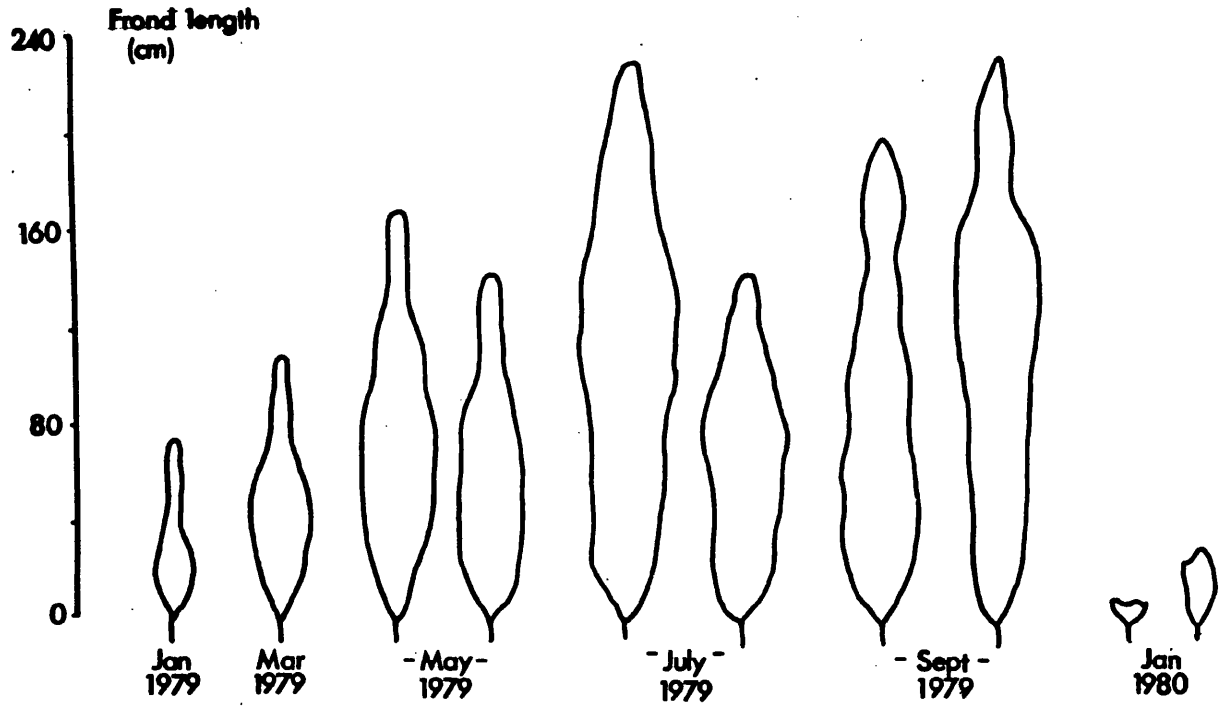
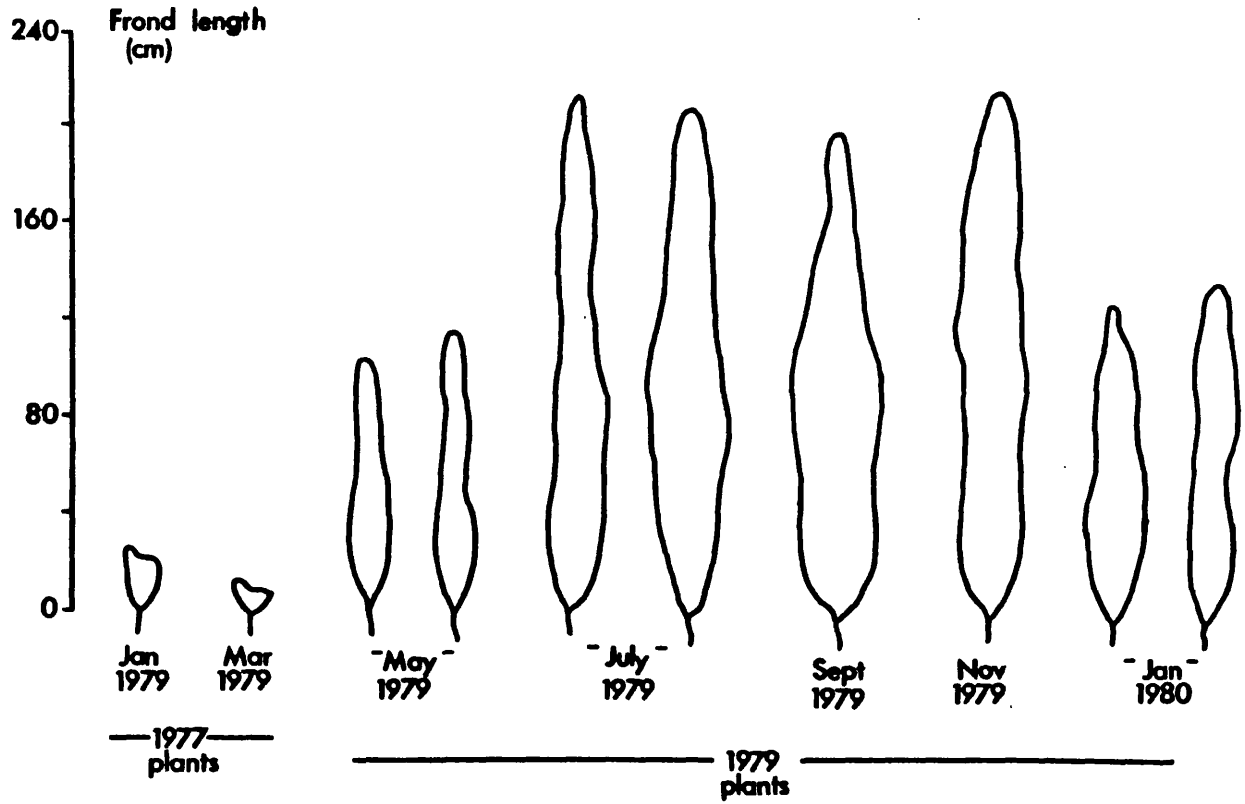

Frond length
(cm)
240
160
80
0
Jan
1979
Mar
1979
May
1979
July
1979
Sept
1979
Jan
1980

FIG. 3.1. Scale drawings of 1978 plants used for naked-eye and brightfield observations.

FIG. 3.2. Scale drawings of 1977 and 1979 plants used for naked-eye and brightfield observations.


Frond length (cm)
240
160
80
0
Jan 1979
Mar 1979
May 1979
July 1979
Sept 1979
Nov 1979
Jan 1980
1977 plants
1979 plants

May 1979. 1978 plant (i). Above 150cm the wings and dimpled tissue had been lost and ∿30% of the central tissue had lost its meristoderm. At 120 and 110cm in the central tissue were two large areas without meristoderm. The wing tissue had large ragged holes up to 1cm across from 130 to 150cm and smaller holes below this, damage being heavier above 60-70cm. 1978 plant (ii) was similar though rather less damaged. 1979 plants. In both plants the differentiation of wings, dimples and centre was just appearing in the meristem region. Plant (ii) had a little damage in the wing tissue. In neither plant did the decomposing tissue around the tip extend back for more than a few cells from the frond edge.

July 1979. 1978 plant (ii). The wings and dimpled tissue had been lost above 100cm. There was extensive decomposing tissue around the frond tip and smaller areas around the frond edge back to 100cm. There were a few places where the meristoderm of the central tissue had been grazed away, and from 70 to 100cm the wing tissue showed much damage. 1978 plant (i) was very similar. 1979 plant (i). Decomposing tissue was restricted to the extreme tip and was much less extensive than on the 1978 plants. The edges showed damage back to 90cm. 1979 plant (ii). Decomposing tissue was again restricted to above 90cm.

September 1979. 1978 plant (i). Above 100cm the whole frond showed extensive tissue decay. Most of the wing tissue had been lost. The frond edge had a whitish appearance; microscopic examination showed that this was where the meristoderm has been lost. From 100cm back to 10cm there were many holes in the wing tissue. From 60 to 100cm there were several areas where the central tissue had lost its meristoderm and in some cases these areas had become holes passing right through the frond surrounded by zones of decomposing tissue. 1978 plant (ii). Again damage and decay were extensive. The frond had almost torn off at 130cm and large pieces had been lost from the edge as far back as 80cm.

1979 plant. The wings and dimpled tissue were missing above 65cm, and the central tissue had areas without meristoderm as far back as 40cm. The wings were damaged as far back as 10cm.

November 1979. 1979 plant (i). Only the central tissue remained above 90cm, and from 40 to 90cm had holes and areas without meristoderm. The wings were more heavily damaged, with holes up to 3x1cm in size in the 40-90cm region. 1979 plant (ii). Again the decomposing areas at the tip were not large, extending back only for 1 or 2mm from the frond edge.

January 1980. 1978 plant (i). Virtually no wing tissue remained. The decomposing areas were very small but several large holes passed through the frond. 1978 plant (ii). Decomposing areas were again very small. 1979 plant (i). Wing and dimpled tissue were missing above 40cm and the central tissue had many holes and a generally tattered appearance. Below 40cm there was less damage.

3.2.2. Discussion

The following points emerge from these results:

(a) Fronds showed damage (pits, holes etc.) at all times of year.

(b) Damage was heavier on the older parts of the fronds.

(c) Damage was heavier on the wings than on the central tissue.

(d) Damaged areas were colonized by decomposer organisms (see section 3.10).

(e) In the upper part of all fronds at least some of the wing tissue had been lost and there was always a zone of decomposing tissue along the frond tip edge.

(f) There was a notable increase in the extent of decomposition in August and September 1979.

The processes which seemed to be involved in decomposition and tissue loss are summarized in Fig. 3.3.

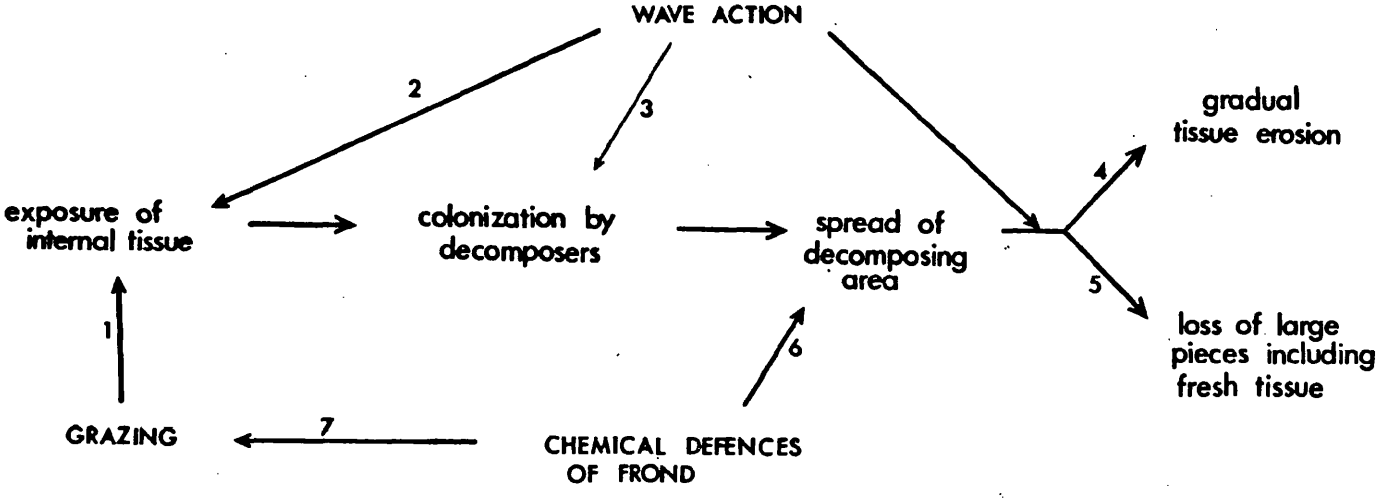

WAVE ACTION
2
3
gradual
tissue erosion
4
exposure of
internal tissue
colonization by
decomposers
spread of
decomposing
area
5
loss of large
pieces including
fresh tissue
1
6
GRAZING
7
CHEMICAL DEFENCES
OF FROND

FIG. 3.3. Processes involved in decomposition and tissue loss.

(1) See section 3.3.

(2) Wave action may initiate new points of damage or may extend existing damage.

(3) A possible effect. Removal of decomposing tissue with its associated decomposer organisms may reduce the efficiency of colonization of newly exposed internal tissue.

(4) Gradual erosion of decomposing tissue continues all year, mainly from the edges of the older parts of the frond.

(5) This occurs where decomposing areas initiated away from the frond edge spread, creating a point of weakness at which wave action then tears the frond through. Losses of this type are particularly common in the autumn and early winter.

(6) Polyphenols and other substances inhibitory to microbial growth are found in brown algae, but may not be present in the internal cells (see section 3.13.).

(7) The frond may contain substances distasteful to grazers (see section 3.13.).

As would be expected, damage was heavier on the older, distal parts of the frond. Damage was also more noticeable on the wings than on the central tissue. Whether the wings are subjected to more stress, for example through being flexed by waves, or whether because of their relative thinness or some other feature of their structure they are more susceptible to damage, is not certain. In the upper part of the frond the wings and dimpled tissue were progressively lost, giving the frond its tapered tip. It is not known why L. saccharina has a frond with central, dimpled and wing regions; one suggestion is that the dimples promote nutrient exchange by breaking up the laminar flow of water over the frond surface.

Norton (1978) found that when Saccorhiza polyschides sporophytes were transplanted into Lough Ine, a region of higher water temperatures than that from which they were taken, distal decay was abnormally rapid. He discussed the possibility that this was the result of a disease which attacks S. polyschides at high water temperatures. The increase in the extent of decomposition in L. saccharina fronds at the Wishing Well occurred at the time of maximum water temperature, and while the symptoms were not as drastic as those observed by Norton, it is possible that a temperature-induced change in the composition or activity of the microbial community of the decomposing tissue was responsible. If the activities of decomposer organisms are controlled by inhibitory substances (a moot point, see section 3.13.) a sudden decrease in the levels of these substances could have caused the increase in decomposition. Some studies have found that levels of inhibitory substances in various algae are lower during the slow-growth season, which in L. saccharina starts in July (see section 2.2.), but other studies have not confirmed this (see section 3.13.).

3.3. GRAZERS

3.3.1. Observations

Several gastropods known to be consumers of algal tissue were found on the fronds. Gibbula cineraria (L.) was present throughout the year at a density of 2-6 individuals frond^{-1}. A small number of adult Lacuna vincta (Montagu) were present in January 1979 and in the March and May samples their ring-shaped egg masses were abundant. In July no eggs were found but there were up to 70 newly hatched juveniles on each frond. Numbers then declined until January 1980. Littorina littorea (L.) and Patina pellucida (L.) were only occasionally seen. The sea urchin Echinus esculentus L. was present in small numbers on the rocky platform at the Wishing Well.

Some observations were made in the laboratory of the type of damage caused by the feeding activities of Lacuna and Gibbula. Lacuna makes roughly circular holes, ∿2mm in diameter, which can pass right through a frond in the wing regions. Gibbula makes larger, irregularly oval, scoop-like pits in the frond surface.

3.3.2. Discussion

Despite the laboratory observations mentioned above it was often difficult to recognize, on plants collected from the field, which areas of damage were the result of grazing and which of other factors, principally wave action. Wave action and decomposition quickly altered the shape of damaged areas, making it impossible to assess the relative importance of grazing and wave action in causing the initial damage. Mann (1972b) makes the generalization that only about 10% of kelp production is directly consumed by herbivores, and this would seem to be true of the L. saccharina population at the Wishing Well. The observations on Lacuna are consistent with the biology of this species on the coast of NE England as described by Smith (1973).

3.4. BACTERIA ON INTACT FROND SURFACES

3.4.1. Results

Fluorescence microscopy - A few filamentous bacteria were seen on the frond tip surface at all times of year, these are probably flexibacteria (see section 3.4.2.). By far the greater part of the bacterial flora appeared as green dots (Plate 1) which the SEM later showed were not cocci but rods viewed end-on (see below). The two-dimensional nature of the frond surface meant that the bacteria could be counted using the squared eyepiece graticule. In each bimonthly sample one plant of each year-group was taken and counts made from the tip and meristem regions, and in some cases also from the mid-frond. From each region five mounts were made, the pieces mounted being taken at random from the region concerned. From each mount the bacteria in ten squares taken at random were counted. A preliminary count in October 1978 showed no apparent differences in bacterial density between the wings, dimpled and central parts at one level on the frond.

The main features of the results can be seen from Fig. 3.4. In this figure each point represents the mean of the ten counts from one mount, converted from bacteria/square (1 square = 82.6 μm^2) to bacteria/100μm^2. In all plants examined the frond tip had more bacteria than the meristem. Most of the meristem means fell within the range 0.5-15 bacteria/100μm^2, most of the tip means within the range 3-25 bacteria/100μm^2. Although there was some indication that numbers were lower during the rapid growth season (January to June) than during the slow growth season, the range of seasonal variation was less than one order of magnitude.

Scanning electron microscopy - Bacteria on intact frond surfaces can be seen on several of the SEM plates. Plates 2 and 3 are of the frond surface within a "stand" of the diatom Licmophora (see section 3.5.). The bacteria present are mainly rods 1.5μm long, attached end-on to the

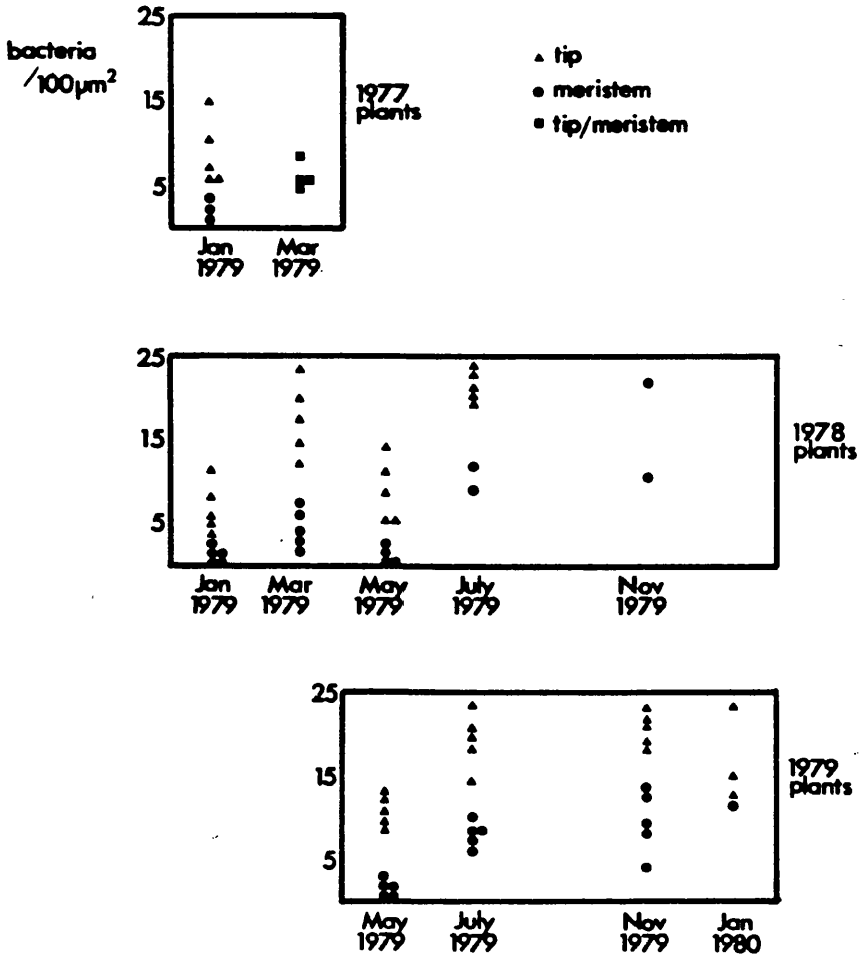
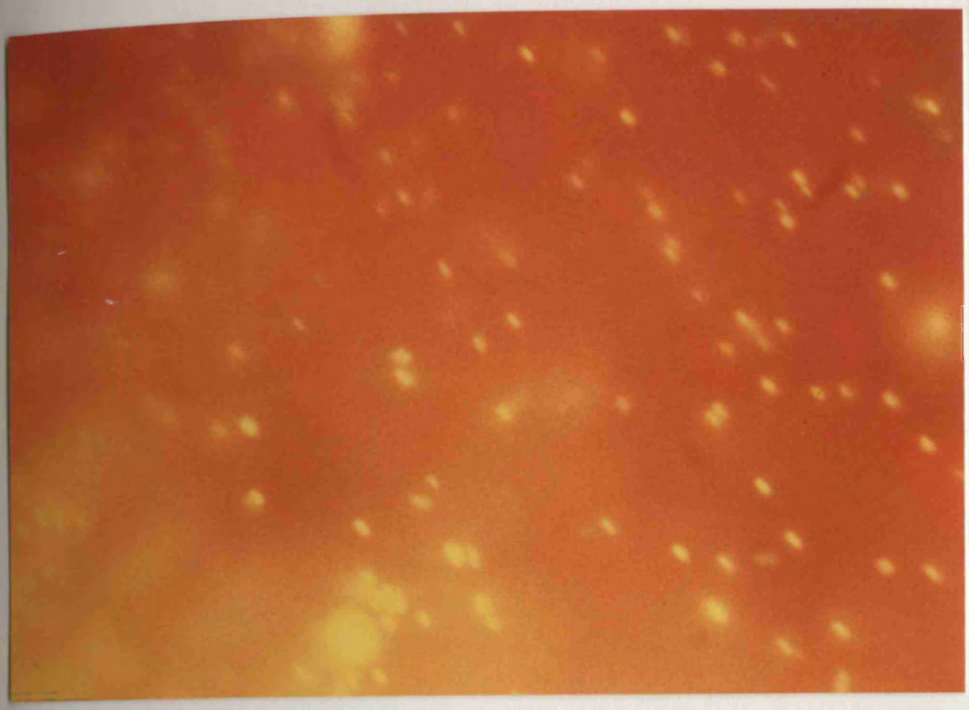


FIG. 3.4. Results of fluorescence counts of bacteria on intact frond surfaces. (1977 plants in March 1979 were very short and no distinction was made between tip and meristem.

PLATE 1 Fluorescence micrograph showing rod-shaped bacteria on frond tip surface. 1978 plant, April 1979. x1500.

PLATE 2 SEM of frond tip surface, 1978 plant, June 1979. Rod-shaped bacteria attached end-on to surface. Scale bar = 4μm.



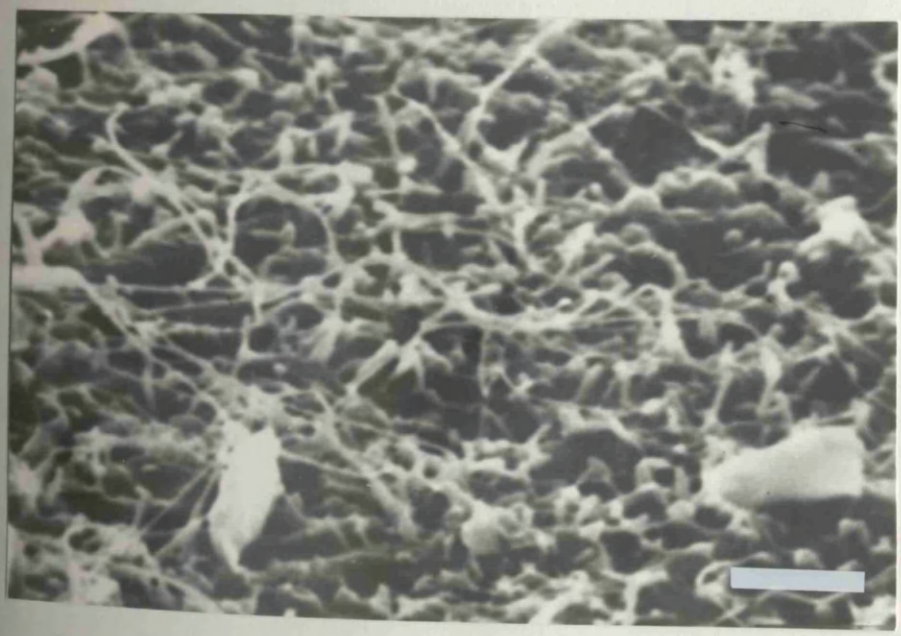
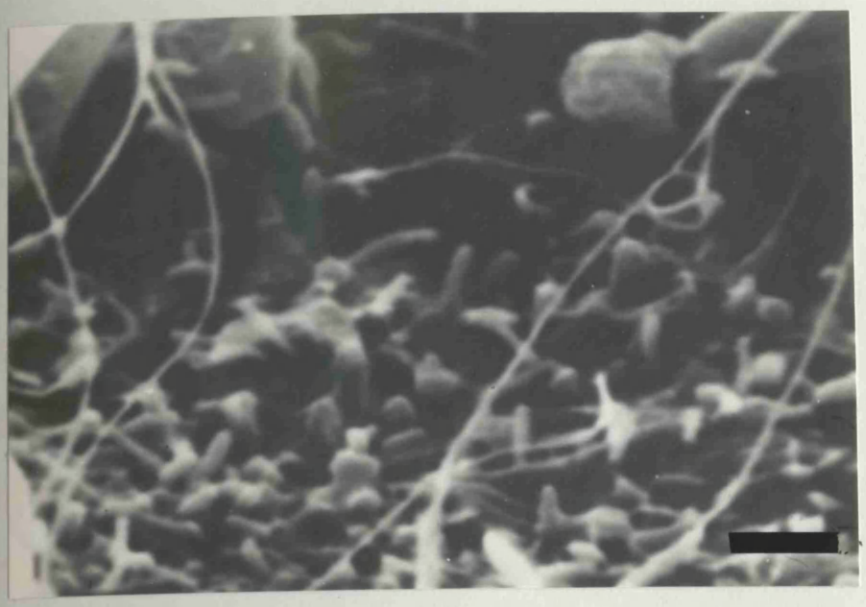


PLATE 3 SEM of frond tip surface, 1978 plant, June 1979. Rod-shaped bacteria attached end-on to surface, higher magnification. Scale bar = 2μm.

PLATE 4 SEM of mid-frond surface, 1978 plant, June 1979. Low magnification view. Scale bar = 20μm.



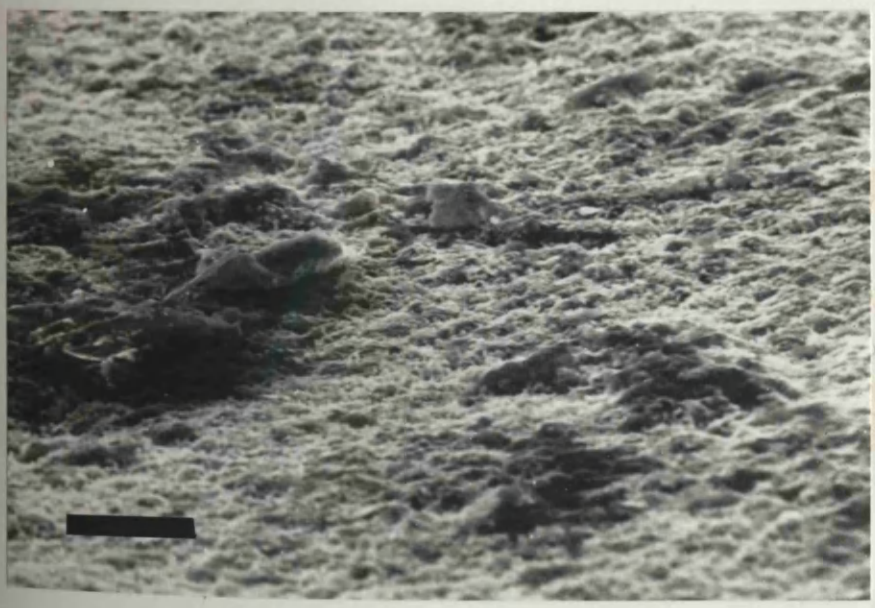


PLATE 5 SEM of mid-frond surface, 1978 plant, June 1979. Rod-shaped bacteria, largely concealed by mucilage. Scale bar = 4μm.

PLATE 6 SEM of meristem surface, 1978 plant, June 1979. Low magnification view. Scale bar = 10μm.

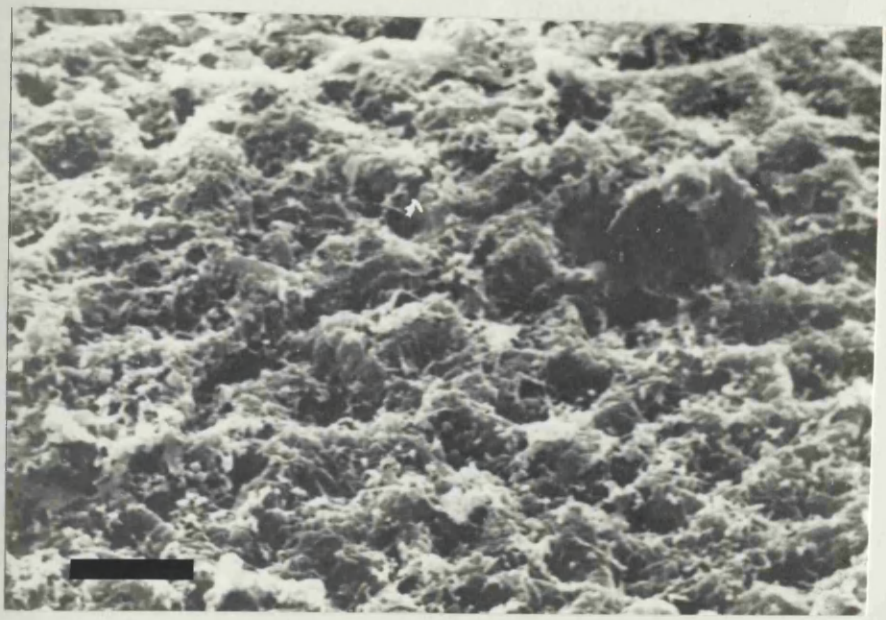
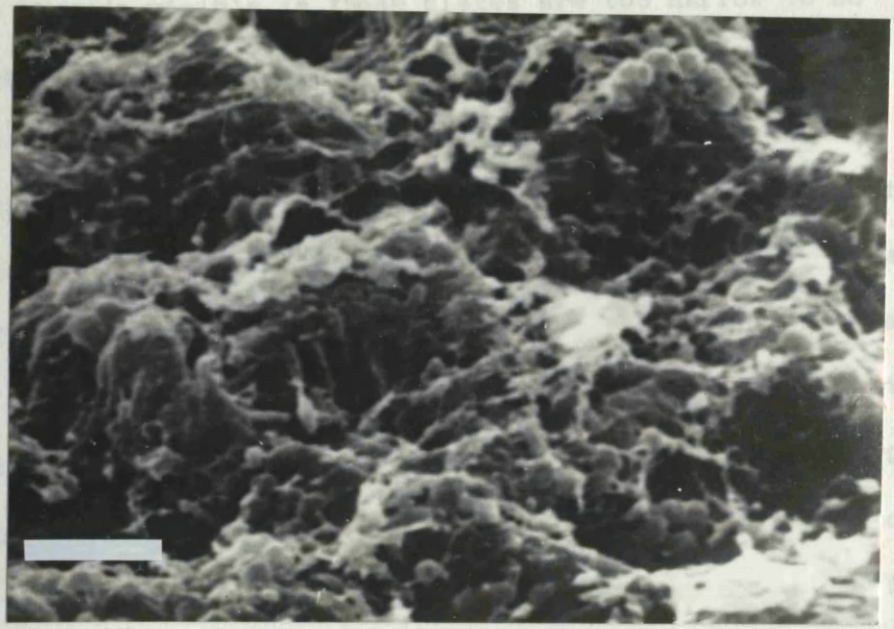
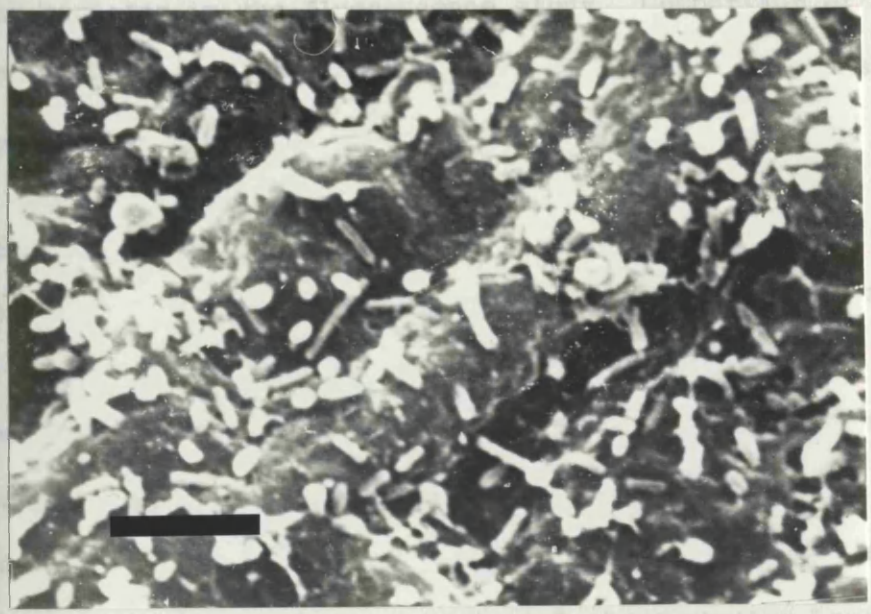


PLATE 7 SEM of meristem surface 1978 plant, June 1979. Rod-shaped bacteria largely concealed by mucilage. Scale bar = 4µm.

PLATE 8 SEM of meristem surface, 1978 plant, June 1979. View more directly down onto surface, rod-shaped bacteria at density of about 20/100um^2, Scale bar = 4µm.





surface. The threads visible in these plates are too narrow to be filamentous bacteria and are probably dried mucilage. Plates 4 and 5 are of the mid-frond region. Plate 4 gives a low magnification view of the surface (compare with views at the same magnification of the frond tip surface, Plate 10, and the meristem surface, Plate 6). Plate 5 is at a higher magnification, and rod-shaped bacteria are visible but are largely concealed by mucilage (see comments in 4.2.5.). Plates 6-8 are of the meristem region. Plate 6 is a low magnification view, Plate 7 a higher magnification view. Again bacteria are present but are largely concealed by mucilage. Plate 8 is taken looking more directly down onto the frond surface in an area where most of the mucilage has been lost and the bacteria are more easily visible. The density of bacteria can be estimated from this plate as about 20/100μm^2, which is slightly higher than any of the fluorescence means, but is within the range of the individual counts found for the meristem in the fluorescence study. Comparison of Plate 8 with Plate 7 and other SEM pictures not included in this thesis shows that this density is typical for the meristem surface.

3.4.2.. Discussion

(a) Counts - The results of previous studies of the numbers of bacteria on brown algae are summarized in Table 3.1. From these limited data some generalizations can be made. It appears that more bacteria are present during the slow growth period of an alga than during its rapid growth period, and also that on laminarians there are more bacteria on the frond tip than on the meristem. It is not clear whether Laycock (1974) and Maizure and Field (1980) included decomposing tissue in their tip samples; this tissue is always present at the frond tip in L. saccharina (see section 3.2.) and has many more bacteria than the intact frond surfaces. In the present study, counts from the tip were taken exclusively from intact surfaces and were still higher than those from the meristem.

SOURCE	ALGA	PART OF FROND	NO. cm^{-2} IF NO STUDY OF SEASONAL VARIATION	MONTH OF MAXIMUM COUNT	MAX. NO. cm^{-2}	MONTH OF MINIMUM COUNT	MIN. NO. cm^{-2}	METHOD
Chan and McManus (1969)	Ascophyllum nodosum	whole	-	June (1)	$-10^7/g$ (2)	July (1)	$10^4/g$ (2)	plate count
Laycock (1974)	Laminaria longicruris	decomposing tips	-	February	10^5	September	$2x10^3$	plate count (3)
		mid-frond	-	September	$2x10^3$	March	10^2	
		meristem	-	October	10^3	April	10	
Burton (1976)	Fucus serratus	whole plant	-	May	$7x10^3$	November	$5x10^2$	plate count
	Laminaria digitata	whole plant	$3-8x10^3$	-	-	-	-	
Cundell et al (1977)	Ascophyllum nodosum	3rd year growth	$1.1x10^8$	-	-	-	-	SEM
Mazure and Field (1980)	Laminaria pallida and Ecklonia maxima	tips	-	± constant all year	10^7	± constant all year	10^7	plate count
		meristem	-	March (4)	10^6	August (4)	10^3	

1 - Study only ran from May to Sept. 2 - N.B. bacteria g^{-1} not bacteria cm^{-2}.
3 - Plates incubated at ambient sea temp. in each month, not all at one temp.
4 - In S. Africa, southern hemisphere.

TABLE 3.1. Summary of results of previous counts of bacteria on the surfaces of brown algae.

Table 3.1. also shows that results from direct counts are usually one or two orders of magnitude higher than those from plate counts. Confirmation of this was provided by Burton (1976) who found that on the algae Ceramium and Ulva, plate counts detected only 4-30% of the bacteria observed using epifluorescence techniques. In the present study, direct observation showed smaller seasonal differences and smaller differences between different frond regions than have been found in previous studies using plate counts. The plate count method was adapted for use on marine algae by Chan and McManus (1967) and subsequent studies have used basically the same technique. Sources of error in plate counts are discussed by Jensen (1968). Those which could lead to an underestimate of bacterial numbers include (i) persistence in the homogenate of groups of bacteria attached to substratum particles or to each other. (ii) damage to bacteria during attempts to break up such groups. (iii) slow or zero growth of certain bacteria on the plating media used, so that the colonies are not seen or are overgrown by other colonies. It is also usual to find that the number of colonies is not inversely proportional to the dilution, an effect normally attributed to adhesion of bacteria to pipettes or to mutual inhibition of bacteria on the plates. Burton (1976) found an effect of this type. He also found that if the homogenate was spun down and the pellet resuspended in sterile saline before dilution, the count increased, by a factor of x45 for Fucus serratus. This suggests that a serious problem with plate counts on algae may be the presence in the tissues of inhibitory substances which are brought into contact with the bacteria during homogenization. Since the concentration of such inhibitory substances may vary both seasonally and between different parts of the frond (see section 3.13.), this effect could explain why larger seasonal and regional differences are found by plate counting than by direct observation.

Fluorescence microscopy will distinguish live from dead bacteria, but it is possible that some of the bacteria detected are in an inactive state. Ramsay (1974) found that on Elodea leaves 17-45% of the bacteria seen using a direct observation method incorporated ^{3}H-gluocose in amounts detectable by autoradiography.

(b) The identity of frond surface bacteria - Sieburth et al (1974), Sieburth (1975) and Cundell et al (1977) have used the SEM to study microbial communities on algal surfaces. Regular members of these communities included a variety of rods, (including curved or ring-shaped Flectobacillus-like cells) cocci, unicellular bacterial filaments (flexibacteria), multicellular bacterial filaments such as Leucothrix, yeasts of various types, and diatoms. The microbial communities of both the frond surface and the decomposing tissue of L. saccharina at the Wishing Well are composed of organisms of these types. The decomposing tissue community will be considered in section 3.10 and frond surface diatoms in sections 3.5. and 3.7. Consisting almost exclusively of rods and flexibacteria, the bacterial flora of intact L. saccharina surfaces is simpler than those of many of the algae examined in the studies listed above, being comparable to that of Monostroma (Sieburth 1975) or the basal parts of Ascophyllum (Cundell et al 1977). This simplicity may be connected with the relatively short lifespan of any piece of Laminaria tissue or with antimicrobial activity of the alga (see section 3.13.).

Several studies have involved the isolation and characterisation of bacteria from marine algae: Chan and McManus (1969), Laycock (1974), Burton (1976), Kong and Chan (1979), and the present study (see Chapter 5). Most of the isolates have proved to be Gram-negative rods and have been assigned to such genera as Vibrio, Pseudomonas, Xanthomonas, Altermonas and Flavobacter. The taxonomy of the pseudomonad genera and of "Flavobacter" is still unsettled and is reviewed by Sieburth (1979). The rods which make up the bulk of the bacteria on L. saccharina surfaces are presumably of

this general type, which is ubiquitous on particulate matter in the sea. Flexibacteria (e.g. Flexibacter and Cytophaga) are also important in the decomposition of organic matter in the sea, but are often inhibited on the rich organic media used to cultivate the dominant Gram-negative rods. Their taxonomy and culture are discussed by Lewin (1969) and Sieburth (1979).

3.5. DIATOMS ATTACHED TO INTACT FROND SURFACES

3.5.1. Results

The small diatom Licmophora sp. was found on all the plants examined, at all times of year. On many plants it was the only diatom actually attached to the intact Laminaria surface. As shown in Fig. 3.5., it stands up from the frond surface attached by its smaller end (see also SEM pictures, Plates 9-11). The distribution of this diatom was similar on all the plants.

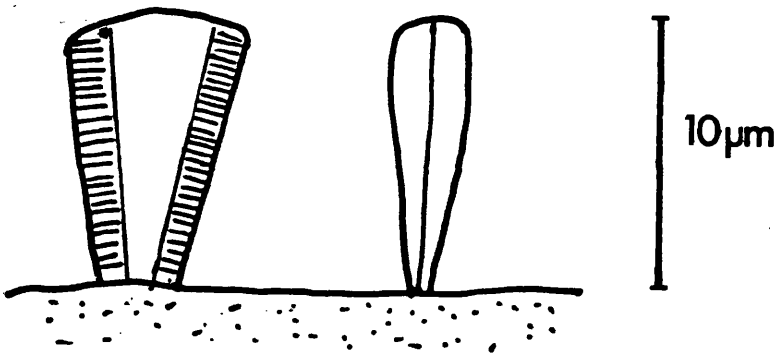
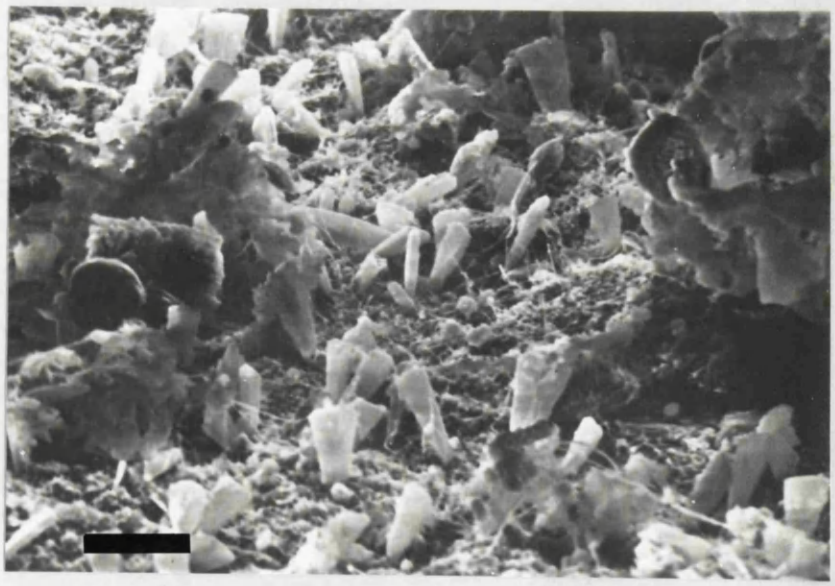


FIGURE 3.5. Licmophora sp.

PLATE 9 SEM of frond-tip surface, 1978 plant, June 1979. Stand of Licmophora, low magnification. Scale bar = 40µm.

PLATE 10 SEM of frond-tip surface, 1978 plant, June 1979. Stand of Licmophora, higher magnification; mucilage threads also visible. Scale bar = 20µm.



It was most abundant at the frond tip; here there were some scattered individuals but most of the diatoms were found in "stands" of up to 0.2mm in diameter and of rather irregular shape, within which they were distributed apparently at random at densities in the range 75-250 cells mm^{-2}. These stands usually occupied about 20% of the surface area at the extreme frond tip. Lower down the frond the density of stands, the size of stands and the density of diatoms within stands all progressively decreased, until only a few scattered individuals, and finally none at all, remained. It was generally at 30-50cm above the frond base that the Licmophora finally disappeared, though on one or two plants, notably the remnants of 1978 plants in January 1980, a few cells were seen almost at the frond base. It might be expected that the lower limit of the Licmophora would be further from the frond base in the rapid growth season, but no evidence of this was found, possibly because not enough plants were examined. There were fewer Licmophora on the very young 1979 plants in the May 1979 sample than on any other plants, only very small stands in the tip region. On some plants a small number of a Rhoicosphenia species were found growing together with the Licmophora, attached to the frond in the same upright position.

3.5.2. Discussion

The diatom flora of L. saccharina fronds at the Wishing Well consisted of three separate microhabitat associations:

(i) those attached directly to the frond surface (considered in this section).

(ii) those associated with other epiphytes on the frond surface - filamentous brown algae, hydroids and bryozoa (see sections 3.7., 3.8. and 3.9. respectively).

(iii) those living on decomposing tissue and in pits and cracks in the surface (see section 3.10).

Aleem (1950) and Round (1971) both observed that large mucilaginous algae such as Laminaria had relatively few epiphytic diatoms compared with filamentous algae such as Cladophora or Ectocarpus. Round (1971) also stated that some large algae have an epiphytic flora consisting almost solely of one species, often a Licmophora or an Achnanthes. These remarks certainly apply to the flora of intact L. saccharina surfaces, which seem unattractive to most diatoms, possibly because of the physical properties of the frond or the presence of antimicrobial substances (see section 3.13.). The Licmophora has presumably evolved a method of overcoming this; an alternative explanation, that Licmophora itself excludes other diatoms from the frond surface, seems unlikely because of the low percentage cover which it achieves compared, for example, with that reached by Cocconeis spp. on Cladophora (see pictures in Sieburth et al (1974)). Why Licmophora does not reach a higher density, and why it occurs in stands, are not apparent; perhaps the cell division rate of this species is low in the frond-surface habitat. Although it seems to be specialized for life on the frond, and might possibly be partially nutritionally dependent on dissolved organic matter released by the Laminaria, there is no evidence for a semi-parasitic or pathogenic way of life as shown by the Cocconeis species which penetrate the epidermis of hosts ranging from algae to whales (see discussion in Sieburth, 1979).

3.6. MACROSCOPIC ALGAE ON THE FROND SURFACE

3.6.1. Results

Table 3.2. shows the distribution of macro-algal (and fungal) epiphytes on two 1978 and one 1979 L. saccharina plants in April 1979. On the 1978 plants more epiphyte species were found on the stipes than on the fronds. The ectocarpoid filaments which were the only epiphytes on the frond were not found in the meristem region, but there was no obvious difference in species number between the older and younger parts of the stipe.

	STIPE					FROND				
1978 plant (i)	-50	-40	-30	-20	-10	+10	+20	+30	+40	+50
Ectocarpoid filaments	+	+	+	+	+	-	-	+	+	
Spongomorpha sp.	-	-	-	+	+	-	-	-	-	-
Urospora sp.	-	-	-	+	-	-	-	-	-	-
Rhodymenia palmata	+	+	+	+	+	-	-	-	-	-
Porphyra sp.	-	-	+	-	-	-	-	-	-	-
Fungus	+	-	-	-	-	-	-	-	-	-

1978 plant (ii)	-30	-20	-10	+10	+20	+30	+40	+50	+60	+70	+80
Ectocarpoid filaments	+	+	+	-	-	-	-	+	+	+	+
Rhodymenia palmata	-	+	+	-	-	-	-	-	-	-	-

1977 plant	-50	-40	-30	-20	-10	+10	+20
Ectocarpoid filaments	+	+	+	+	+	+	+
Punctaria sp.	+	-	-	-	-	-	-
L. saccharina (sporelings)	-	+	-	+	+	-	-
Urospora sp.	+	-	-	-	-	-	-
Monostroma sp.	+	-	-	+	-	-	+
Spongomorpha sp.	+	+	+	-	-	-	-
Ulva lactuca	+	-	-	-	-	-	-
Rhodymenia palmata	+	+	+	+	+	-	+
Polysiphonia urceolata	-	-	-	+	-	-	-
Fungus	-	-	+	-	-	-	-

Distance from frond stipe junction (cm)

TABLE 3.2. Distribution of macroalgal epiphytes on three *L. saccharina* plants in April 1979.

The 1977 plant carried more species than did the 1978 plants; again there were more species on the stipe, but three species in addition to the ectocarpoid filaments were found on the frond. Ectocarpoid filaments were found even in the meristem region.

The ectocarpoid filaments on the frond were studied in more detail in the bimonthly sampling programme. Identification of algae in this group is difficult, but it appeared that there was one species which was present throughout the year. These filaments emerged in clumps from "craters" in the Laminaria surface (see Fig. 3.6. and Plate 12), which suggests that this was an endo-epiphyte of the type described by Fritsch (1945) in which the basal filaments ramify within the host and the erect

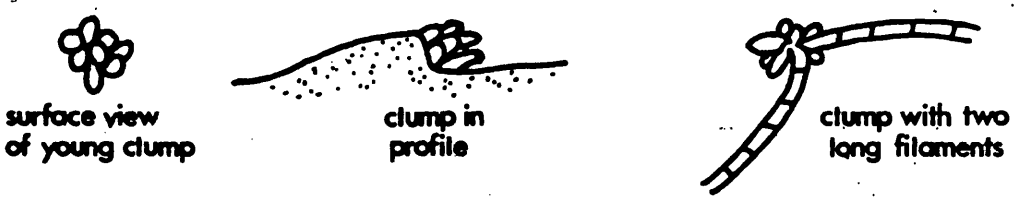
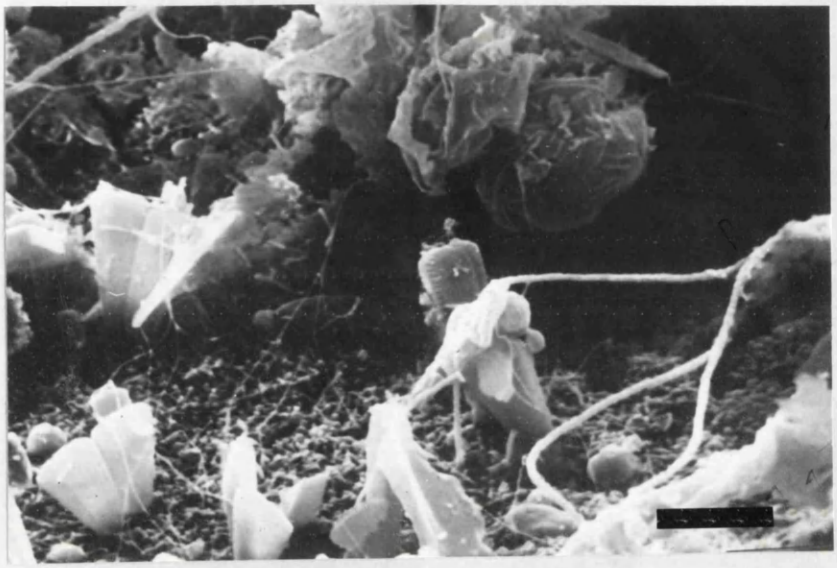


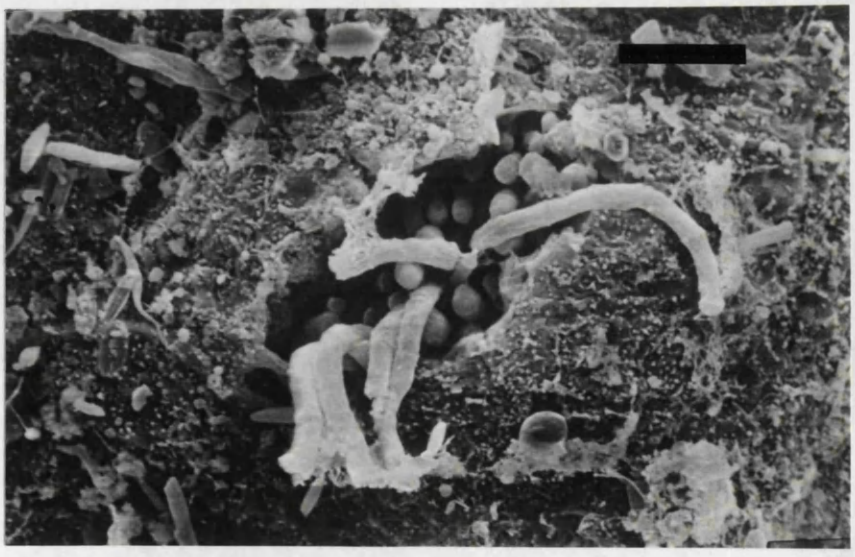
FIGURE 3.6. Emergent filaments of the endo-epiphytic ectocarpoid

filaments emerge through its surface. Ectocarpoid filaments of one or more other, apparently non-endophytic, species were present on the fronds from February to June.

PLATE 11 SEM of frond tip surface, 1978 plant June 1979. Within stand of Licmophora, showing also multicellular bacterial filaments, mucilage threads, rod-shaped bacteria on frond surface. Scale bar = 10μm

PLATE 12 SEM of frond tip surface, 1978 plant, June 1979. Young clump of emergent filaments of the epi-endophytic ectocarpoid alga. Scale bar = 20μm.





Seasonal pattern of growth and distribution of ectocarpoid filaments - 1978 plants

In January 1979 clumps of the emergent filaments of the endo-epiphyte (hereafter referred to simply as "clumps") were present on the frond tips at a density of 3-6mm^{-2}. Most clumps had 3-6 filaments, but some had up to 20. The size of the cells in the filaments was ∿10x12μm and most of the filaments were only 2 or 3 cells long, though a few clumps had 1 or 2 filaments up to 90μm long. Lower down the frond the density of clumps progressively decreased and none were seen below 30cm. On the 1978 plant (i) in the May 1979 sample young clumps were found as far back as 90cm, and at the frond tip, clump density was 4-6mm^{-2}. Most of the filaments in the frond tip clumps were only a few cells long but others, perhaps 30%, were longer, up to 7 or 8mm. The cells in the apical parts of these filaments were ∿6x25μm in size, longer and narrower than those at the filament base. A small number of groups of filaments up to 1cm long belonging to non-endophytic ectocarpoid species were present on the frond tip. In June 1979 both the epi-endophytic and the other filaments reached their maximum lengths, ∿1cm and ∿2.5cm respectively. In July 1979 the distribution of filaments was much as in May 1979, but all the non-endophytic filaments, and all the larger and almost all the shorter filaments in the clumps, appeared to be dead; the cells were empty, only the walls remaining. Spores were probably produced before the filaments died, this was not observed in 1979 but micrographs taken in 1980 show sporangia (Plate 13). In September 1979 clumps were present on the frond tip of 1978 plant (i) at a density of ∿1mm^{-2} and most of the filaments seen were dead. The density of clumps again decreased down the frond and none were seen below 70cm. In November 1979 and January 1980 young clumps again appeared, the density reaching ∿2mm^{-2} on the frond tip in January. 1979 plants. When first examined in May 1979 clumps were present on the frond tip at a density of 1-2mm^{-2} and on neither plant were any seen below 50cm. Growth and death of these filaments, and the appearance and disappearance of non-endophytic filaments, was much as described for 1978 plants.

3.6.2. Discussion

Ballantine (1979) studied the macro-algal epiphytes on a variety of algae off Puerto Rico. He found that some host species carried more epiphyte species than did others and that these "good" hosts also had heavier epiphyte loads (g epiphyte/g host). He also found more epiphyte species and individuals on the older (basal in the case of his host species) parts of the host frond. On the grounds of its epiphyte species number L. saccharina would fall into Ballantine's "poor host" category, though epiphyte diversities in general might be expected to be lower in Scotland than in Puerto Rico. The low diversity and the zonation of epiphytes on the frond are discussed in section 3.13.

Norton et al (1977) noted that the stipes of L. hyperborea carried a heavier and more diverse epiphyte flora than the fronds, and the same was true of L. saccharina at the Wishing Well. Two factors which may explain this are the shorter life of any section of frond tissue, and the harder, less flexible nature of the stipe. Many of the epiphytes on L. saccharina stipes were smaller, presumably shorter lived, species than those recorded from the stipes of L. hyperborea by Norton et al (1977). This is probably related to the shorter lifespan of L. saccharina and possibly also to the greater smoothness and flexibility of its stipe.

It is not known to what extent epi-endophytic ectocarpoid algae are nutritionally dependent on their hosts. Russell (1964) found that the endophytic filaments of Laminariocolax tomentosoides (Farl.) Kylin, which grows mainly on Laminaria spp., penetrated the host cells, and that an extensive endophytic system probably belonging to this species was present in Laminaria digitata during July and August when no epiphytic filaments were visible. The endophytic system can receive little light and Russell (1964) found that the chloroplasts of endophytic L. tomentosoides filaments were "rather reduced". On the other hand Russell (1964) and Kylin (1937, quoted in Russell 1964) successfully grew L. tomentosoides in culture without any

organic supplement, so the association with the host is not obligatory.

When the ectocarpoid filaments on L. saccharina are longest, in April, May and June, other epiphytes collect around them (see below).

3.7. MATERIAL ACCUMULATING AROUND ECTOCARPOID FILAMENTS

3.7.1. Results

In late spring and early summer a prominent feature of the frond surface, particularly towards the tip, was what appeared to the naked eye as a flocculent light brown material which was loosely attached to the frond, being easily washed off by violent water movements. Under the light microscope it could be seen that this material consisted of a mucilage matrix in which were found sediment grains, faecal pellets, flagellates, ciliates and a great variety of diatoms. None of these was attached to the frond; all lay free in the matrix or were attached to the ectocarpoid filaments. It seemed to be these filaments, assisted by filamentous diatoms, which retained the material on the frond. Material also collected in the bottom of dimples in the dimpled region of the frond.

Plates 13-18 are light micrographs of the mucilaginous material. Plate 13 shows an ectocarpoid filament with sporangia, Plate 14 a faecal pellet, probably from a harpacticoid copepod. Plates 15-18 show diatoms within the matrix, species of Melosira, Rhabdonema and Pleurosigma in Plates 14-17 respectively, and of Synedra and Cocconeis in Plate 18. A colonial Rhabdonema can be seen in Plate 20, and other diatoms present included Grammatophora and solitary and tube-forming species of Navicula. Plates 19-21 are SEM pictures taken to show how material collected around ectocarpoid and diatom filaments. Much of the material has been washed off during fixation and dehydration (see section 3.1.5.) but enough remains to illustrate the point. In all three plants mucilaginous material overlies stands of the diatom Licmophora (see section 3.5.). Plates 19 and 20 look directly down onto the surface, while Plate 21 looks down obliquely to show how the material stands up from the surface.

PLATE 13 Light micrograph of frond tip surface, 1978 plant, May 1979. Ectocarpoid filament with sporangia. Trapped mucilage. x150.

PLATE 14 Light micrograph of frond tip surface, 1978 plant, May 1979. Faecal pellet in mucilage matrix trapped among algal filaments. x600.

PLATE 15 Light micrograph of frond tip surface, 1978 plant, May 1979. Diatom *Melosira* in mucilage matrix. x600.

PLATE 16 Light micrograph of frond tip surface, 1978 plant, May 1979. Diatom *Rhabdonema* in mucilage matrix. x600.

PLATE 17 Light micrograph of frond tip surface, 1978 plant, May 1979. Diatom *Pleurosigma* in mucilage matrix. x600.

PLATE 18 Light micrograph of frond tip surface, 1978 plant, May 1979. Diatoms *Synedra* and *Cocconeis* in mucilage matrix. x600.

PLATE 19 SEM of frond tip surface, 1978 plant, June 1979. Mucilage material collected around algal filaments. Scale bar = 100μm.

PLATE 20 SEM of frond tip surface, 1978 plant, June 1979. Mucilage material collected around filament of a colonial diatom, (*Rhabdonema* sp.). Scale bar = 100μm.

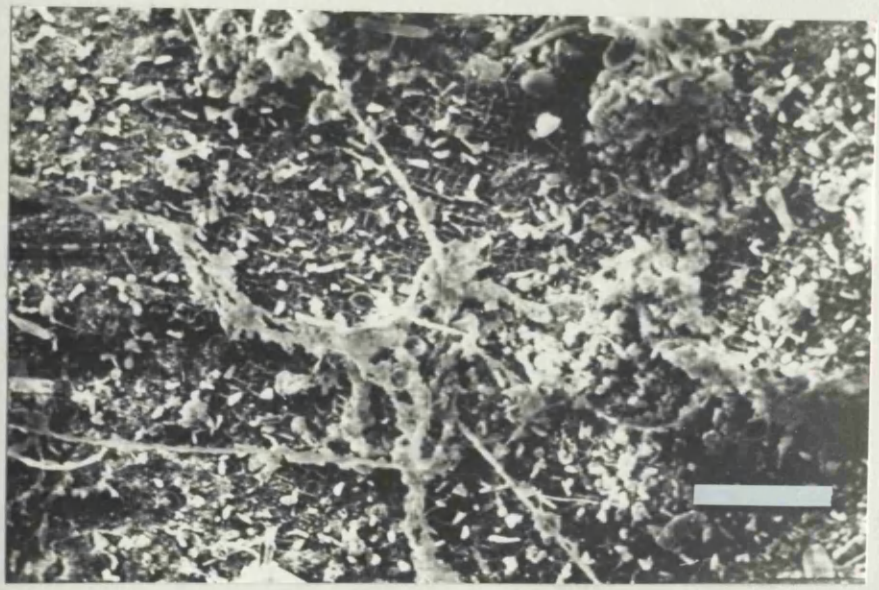


PLATE 21 SEM of frond tip surface, 1978 plant, June 1979. Oblique view to show how mucilage material stands up from frond surface. Scale bar = 100µm.

Many of the ciliates contained diatoms which they had ingested. Fluorescence microscopy showed that bacteria were numerous in the matrix; they were mainly short rods, with a few flexibacteria. The flagellates were presumably feeding on these bacteria.

Seasonal aspects - 1978 plants - In January 1979 a small number of diatoms of most of the genera mentioned above were collected around the bases of clumps of ectocarpoid filaments. In March the filaments were longer and the number of diatoms greater. By May the material described above had developed, in places on the frond tip in such quantities that the diatom cover exceeded 100%. The amount present decreased progressively down the frond and none was seen below 60cm on plant (i) and 50cm on plant (ii). In the region 60-100cm on both plants the material was noticeably more abundant in the dimples of the dimpled region of the frond than on the flat portions, and contained fewer diatoms than the material at the frond tip, some of the smaller particles consisting almost entirely of mucilage with a few sediment grains. In July most of the material had disappeared leaving only a small amount around the dead ectocarpoid filaments, and only very small amounts were seen in September or November 1979. 1979 plants - these followed the same pattern but the amount of material present was never as great as on the 1978 plants.

3.7.2. Discussion

The development of this material was correlated in time with the growth of the ectocarpoid filaments. The mucilage matrix probably came from the Laminaria and became trapped in the filaments. There seems to be no information on the production of mucilage by brown algae and in particular on its seasonal variation, but it seems likely that at least some is produced all year and in the absence of epiphytic filaments is lost into the sea. A filament-mucilage complex would be expected to trap sediment grains suspended by wave action. The appearance and disappearance of the diatoms coincided with those of the rest of the material, and it seems

likely that in the absence of the filaments, unattached diatoms were unable to remain on the frond surface. Aleem (1950) found that littoral diatoms showed in general two seasonal peaks of abundance, one in the spring and a lesser one in the autumn. Studies by Castenholz (1961) and Janason (1969) showed that in some cases at least the summer minimum is the result of grazing. This pattern of abundance was not shown by the diatoms in the mucilage matrix, but potential grazers of diatoms (ciliates, turbellarians, harpacticoid copepods) were present and could have played a subsidiary part in reducing diatom numbers within the overall pattern set by the growth and death of the ectocarpoid filaments.

Aleem (1950) observed that during calm weather in late summer the fronds of Laminaria in sheltered pools at St. Andrews, Fife, were covered with a slimy growth of diatoms, mainly species of Achnanthes, Licmophora, Synedra, Amphora, Grammatophora, Cocconeis and Campyloneis. This could have been material similar to that described above, either unattached and remaining on the frond in the absence of water movement, or trapped in filaments of some type not found on L. saccharina at the Wishing Well at this time of year.

3.8. HYDROIDS

3.8.1. Distribution and abundance

Only one species of hydroid, Obelia geniculata (L.), was recorded from the fronds, never in very large quantities. On 1978 plants, for example, a few stems were present on the frond tips in January 1979 and March 1979. In May and July 1979 rather more was present on all parts of the frond except the meristem, indicating that settlement occurred at this time of year. Obelia was present on the tip and mid-frond in September 1979 and on the tip in November 1979, but was absent in January 1980.

3.8.2. Organisms associated with hydroids

In July 1979 Obelia stems served as a substratum for the attachment of various diatoms (including Licmophora, Cocconeis and Rhabdonema) a few green and red algal sporelings, and a large number of filaments 2-3μm in diameter which were probably blue-green algae. Most of these were never seen attached to Laminaria surfaces or were much less abundant there.

3.8.3. Discussion

At Plymouth O. geniculata carried gonophores from March to September, and Obelia medusae were found in the plankton mainly from spring to autumn (Marine Biological Association, 1957). A long period in spring and summer during which settlement can occur is consistent with the observations described above. Because Obelia was never abundant on the fronds, hydroid stems were not quantitatively important as a substratum for epiphytes, but the diversity of organisms attaching to them contrasted sharply with the lack of diversity on intact L. saccharina surfaces.

3.9. BRYOZOANS

Membranipora membranacea (L.) was the only bryozoan found on the fronds except for two colonies of Electra pilosa (L.) seen in the May 1979 sample.

3.9.1. Distribution and growth - 1978 plants

In January 1979 there were a few colonies >1cm in diameter above the 30cm level on 1978 plant (i), while in March and May 1979 no bryozoans were seen. The distribution of colonies on the July plant (i) is shown in Fig. 3.7., the distribution on plant (ii) was similar. Settlement of bryozoans had occurred preferentially on the lower mid-frond and the range of size shown by the new colonies (<1mm-0.6cm) indicated that it had continued for several weeks. Although the size of colonies increased up the frond there was some overlap, with small and large colonies at the

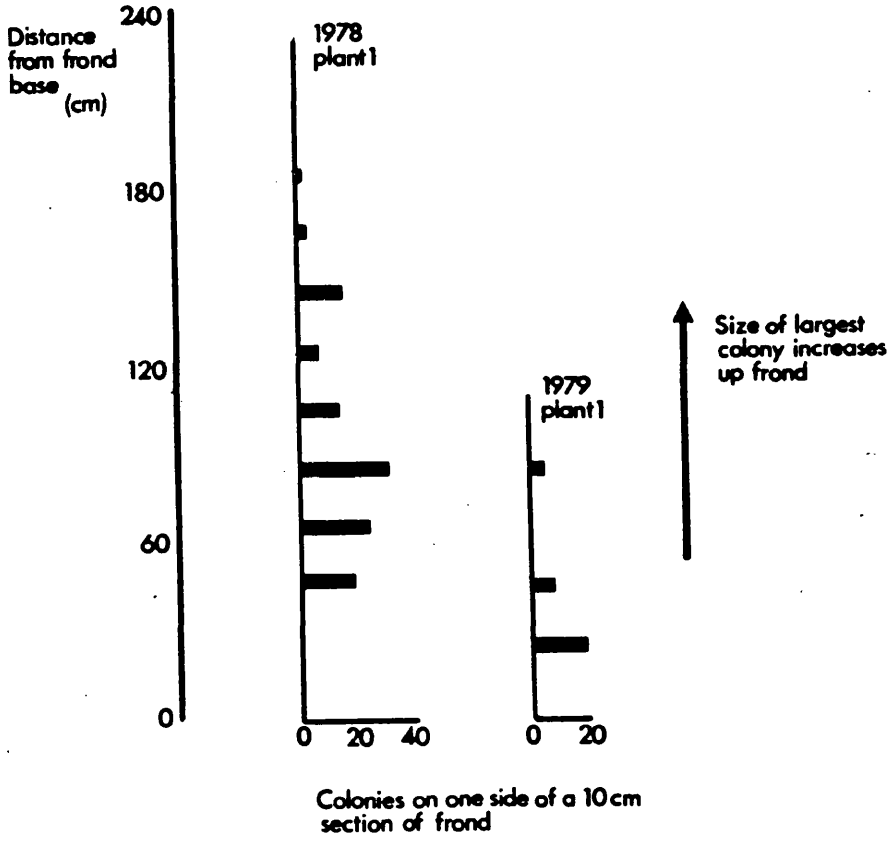


FIG. 3.7. Distribution of Membranipora colonies on 1978 and 1979 plants, July 1979. Only colonies on one surface of the frond are included; similar numbers were present on the other surface.

same level on the frond, which indicated that the selection of the level on the frond at which to settle was not precise. On neither frond was there any apparent difference between the number of colonies on the wings, dimpled and central tissue, or between the two surfaces of the frond. Membranipora reached its peak of abundance in September 1979. Table 3.3. shows the distribution of colonies on 1978 plant (i). The presence of colonies < 1cm in diameter showed that limited settlement had occurred in July or August. The confluence of colonies on the upper part of the frond resulted from the growth of colonies which had settled in May or June. In November 1979 the frond tip of the 1978 plant (i) had a Membranipora cover of ∿60% and no colonies were present below 40cm. No colonies were seen on the 1978 plants in January 1980. 1979 plants. The seasonal pattern was very similar to that on 1978 plants. The first colonies appeared in May 1979, and the distribution of colonies in July is shown in Fig. 3.7. In January 1980 a few colonies > 1cm in diameter remained on the upper two thirds of the fronds.

3.9.2. Organisms associated with bryozoan colonies

In January, September and November 1979 and in January 1980 some of the Membranipora colonies included many dead zooids. The empty zooecia were often almost completely filled with diatoms of several types, principally motile Navicula speciès, together with occasional red and green algal sporelings. Up to 200 diatoms were seen in one zooecium, and in November 1979 there were certainly more diatoms in Membranipora zooecia than on the rest of the frond put together. Ciliates, many of them with diatoms in their food vacuoles, were also numerous. The surfaces of live zooids were colonised by a small number of diatoms.

3.9.3. Discussion

The cyphonautes larvae of Membranipora were present in the plankton off Plymouth throughout the year but chiefly in autumn (Marine Biological

130cm - tip	few small colonies
120-130cm	colonies ∿ 2cm diameter; confluent; 75% cover
80-90cm	colonies ∿ 2cm diameter; confluent; 40% cover
40-50cm	3 colonies ∿ 1cm in diameter
20-30cm	no colonies
0-10cm	no colonies

TABLE 3.3. Distribution of Membranipora colonies on 1978 plant (i) in September 1979.

Association, 1957). Nair (1962) found that in Norway settlement occurred from April onwards. In the present study settlement took place from May until September.

It has been demonstrated that certain bryozoan species show

(i) preferential settlement of larvae on the younger parts of substrate organisms.

(ii) preferential orientation of ancestrulae, and

(iii) preferential direction of colony growth. (Ryland, 1959; Ryland and Stebbing, 1971; Stebbing, 1972). Membranipora growing on Laminaria spp. shows random orientation of ancestrulae (cf. basal facing orientation when growing on Fucus serratus) but colony growth is directed basally (Ryland and Stebbing, 1971). Preferential settlement of larvae on the younger parts of Laminaria fronds has been demonstrated in Scrupocellaria reptans (L.) (Ryland and Stebbing, 1971; Stebbing, 1972) but apparently has not previously been observed in Membranipora.

Orientation of Membranipora ancestrulae on Fucus is probably a response to the direction of water flow; directional colony growth may similarly be a rheopositive response, or may depend on the detection of an age-dependent polarity in the substrate organism (Ryland and Stebbing, 1971). Stebbing (1972) used choice-chamber experiments to show that Scrupocellaria larvae can distinguish younger from older Laminaria digitata surfaces. The demonstration in the present study that the bacterial flora of intact L. saccharina surfaces is morphologically similar at all levels on the frond makes the suggestion of Stebbing (1972), that the age-dependent polarity which bryozoans can detect in their hosts is dependent on the presence or absence of particular micro-organisms, seem unlikely to be correct. There is no evidence to support a hypothesis involving the recognition of particular micro-organisms rather than one depending on detection of differences in the concentration or chemical nature of substances contained in or released by different parts of the frond.

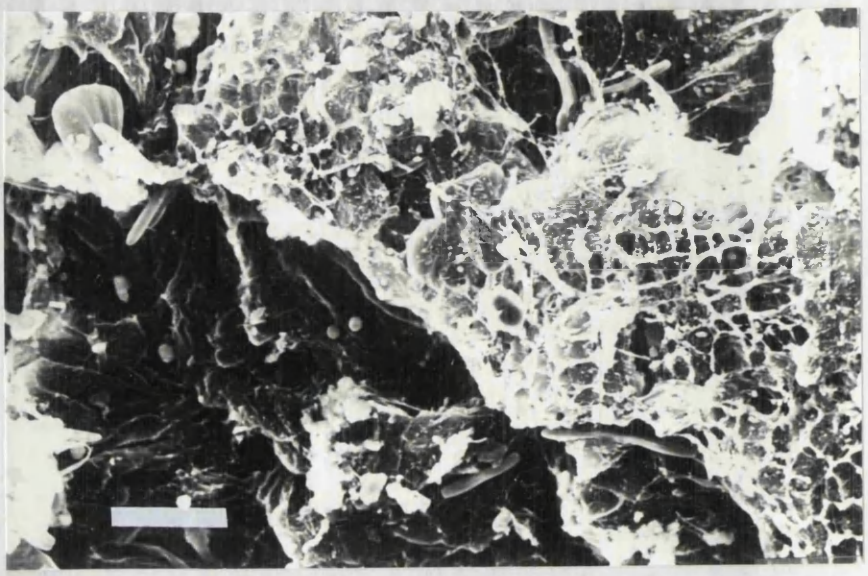
3.10. THE COMMUNITY ON DECOMPOSING TISSUE

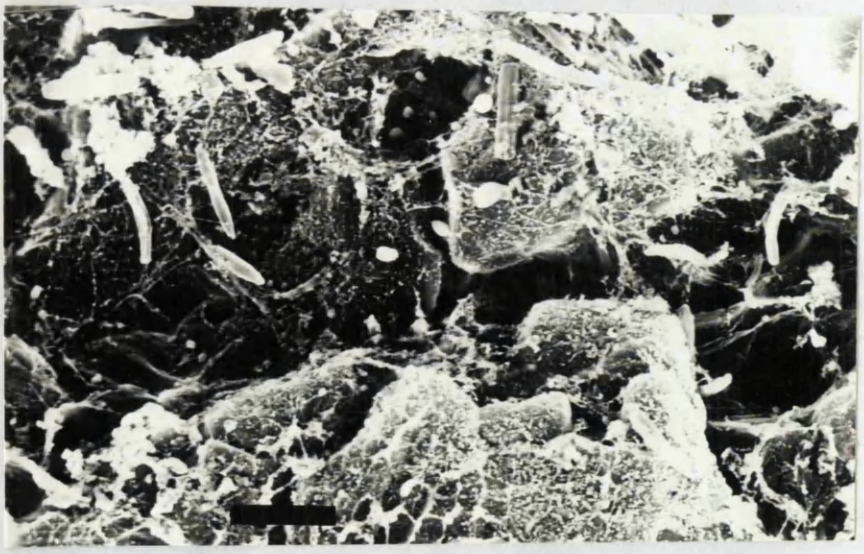
The distribution of decomposing tissue on the fronds and the processes involved in its formation and extension were discussed in section 3.2.

3.10.1. SEM observations

Plates 22-24 show low magnification views of areas of decomposing tissue from the frond tip. In some areas the cell walls of the meristoderm remain, while in others this tissue has been lost completely, exposing the larger cells of the underlying cortex. Plate 25 shows an area typical of the small pits and cracks common in the tip surface. Pennate diatoms are found in these decomposing regions, some attached to the walls of meristoderm and cortex cells, others, presumably motile forms, unattached. The attached forms include Rhoicosphenia (Plate 22) and Cocconeis (Plate 24), the unattached are predominantly species of Navicula. Bacteria can be seen in these three plates as dots on all the Laminaria surfaces and as fine filaments; they are shown in more detail in later plates.

Plates 26-30 are higher magnification views of decomposing regions. Plates 26-28 show that the dominant morphological type of bacterium over extensive areas of Laminaria cell surface is a curved rod about 2x0.3μm in size. These bacteria probably belong to Flectobacillus, although no ring forms were seen (see discussion, 3.10.2.). It appears that they can be attached to the substrate in almost any orientation. Straight rods of several sizes are also common. Plate 30 shows an area with many short rods, ∿1.0x0.3μm in size, again attached to Laminaria cell walls in a variety of positions. Longer rods can be seen in Plate 29, where some of them are apparently dividing, and in Plates 26 and 28. Bacterial filaments are present. The obvious filament in Plate 26 is about 0.5μm wide and 40μm long, and seems to be unicellular, since no cross walls are visible. The filament at the right of Plate 28 and those at the top of Plate 31 are similar. These are probably Flexibacter or a related genus. The filaments at the top left of Plate 27 are of similar diameter but are





PLATES 22 and 23 SEMs of decomposing tissue, frond tip, 1978 plant, June 1979. Low magnification views to show structure of frond-edge decomposing areas. Walls of meristoderm cells remain in some areas; cortical cells are exposed. Numerous attached and unattached diatoms; bacteria on all surfaces. Scale bars = 40µm.

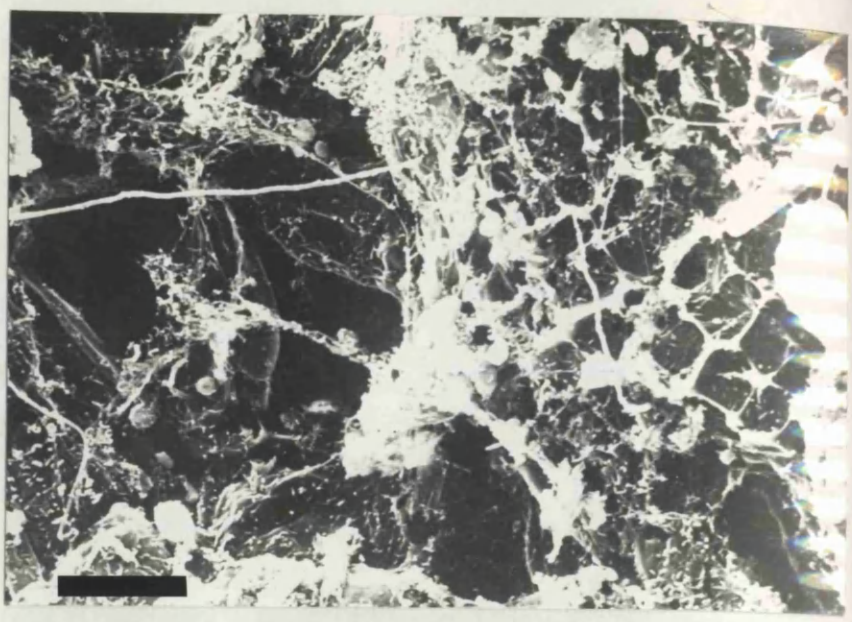


PLATE 24 SEM of decomposing area, frond tip, 1978 plant, June 1979. Low magnification view to show structure of decomposing area. Diatom Cocconeis. Scale bar = 20 µm

PLATE 25 SEM of frond tip, 1978 plant, June 1979. Small pit in surface, colonized by diatoms. Scale bar = 100µm.

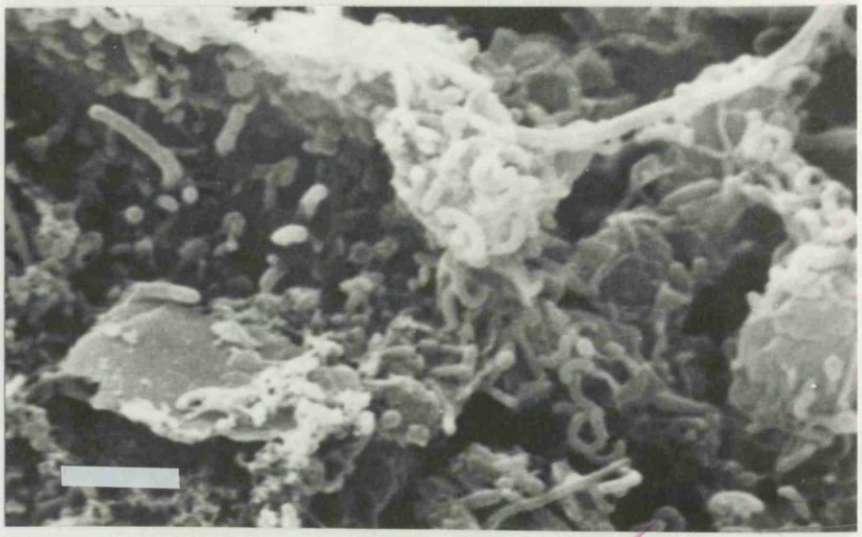
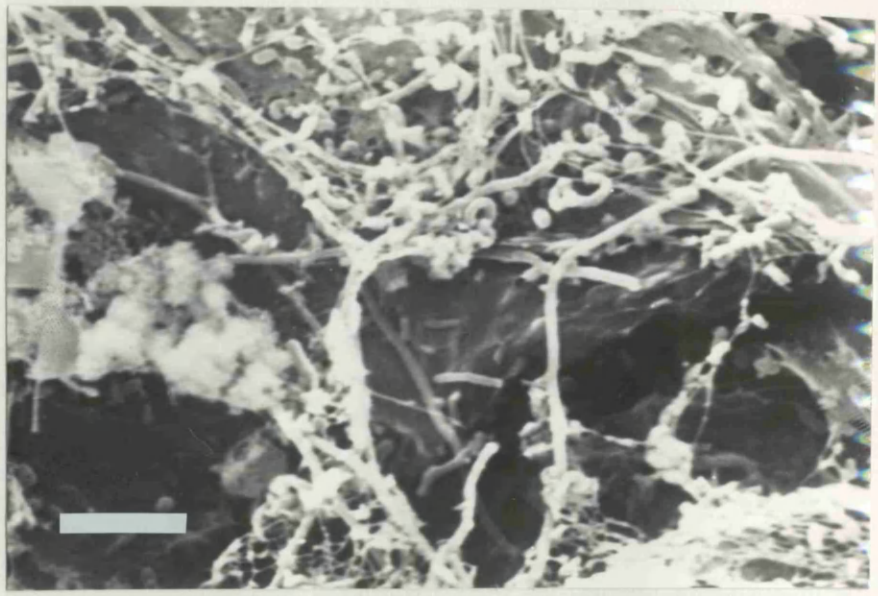


PLATE 26 SEM of decomposing tissue, frond tip, 1978 plant, June 1979. Curved rods and long unicellular bacterial filament. Scale bar = 4μm

PLATE 27 SEM of decomposing tissue, frond tip, 1978 plant, June 1979. Dense cover of curved bacteria (Flectobacillus), multicellular bacterial filaments at top left. Scale bar = 4μm.



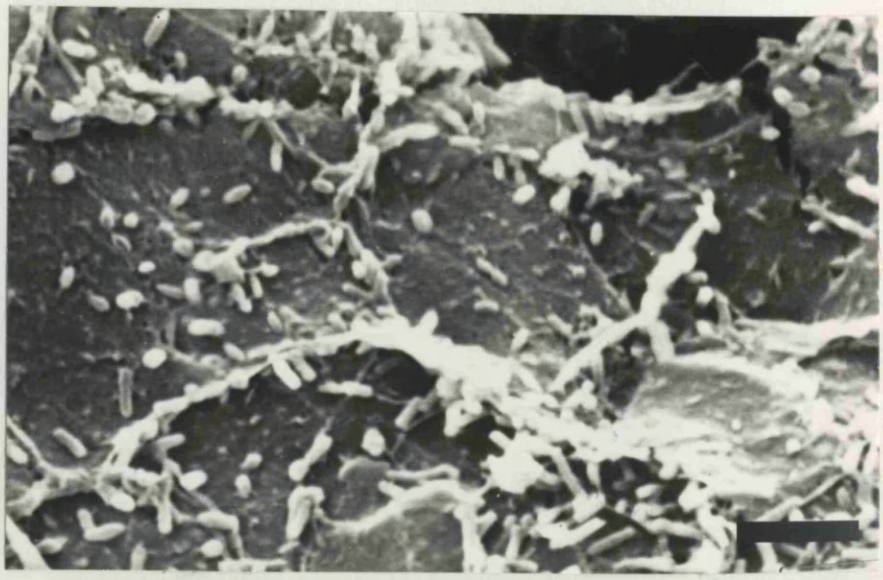
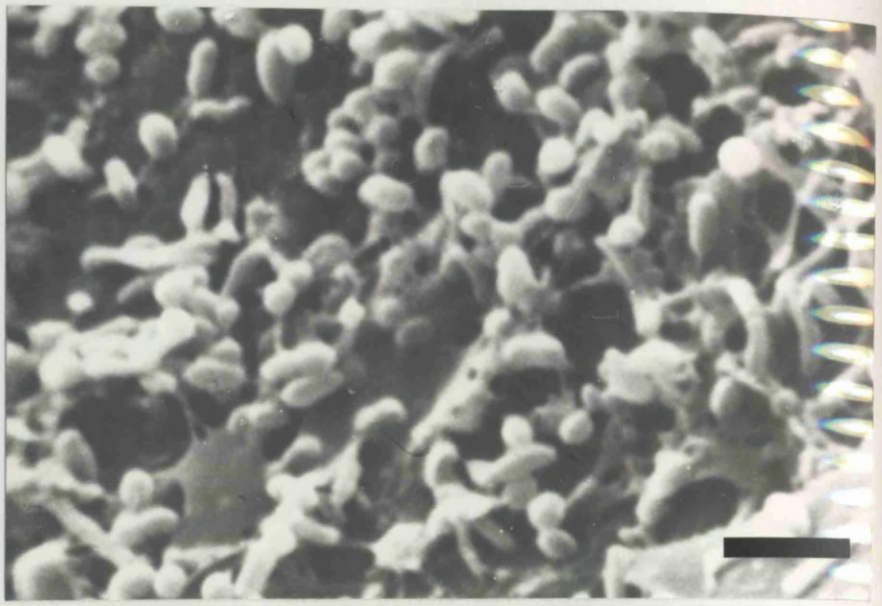


PLATE 28 SEM of decomposing tissue, frond tip, 1978 plant, June 1979. Curved bacteria (Flectobacillus), unicellular filamentous bacteria (flexibacteria), and straight rod-shaped bacteria. Scale bar = 4μm.

PLATE 29 SEM of decomposing tissue, frond tip, 1978 plant, June 1979. Bacteria mainly longer straight rods. Scale bar = 4μm.



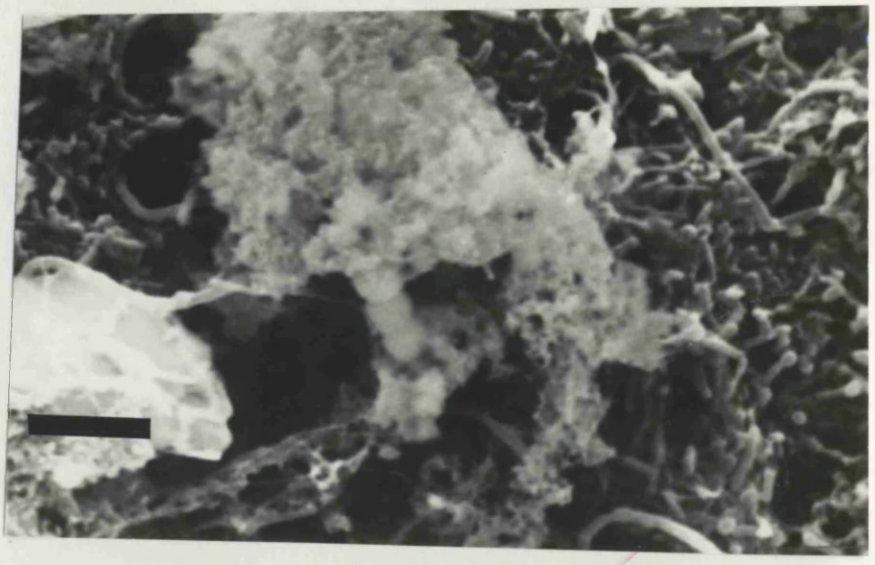
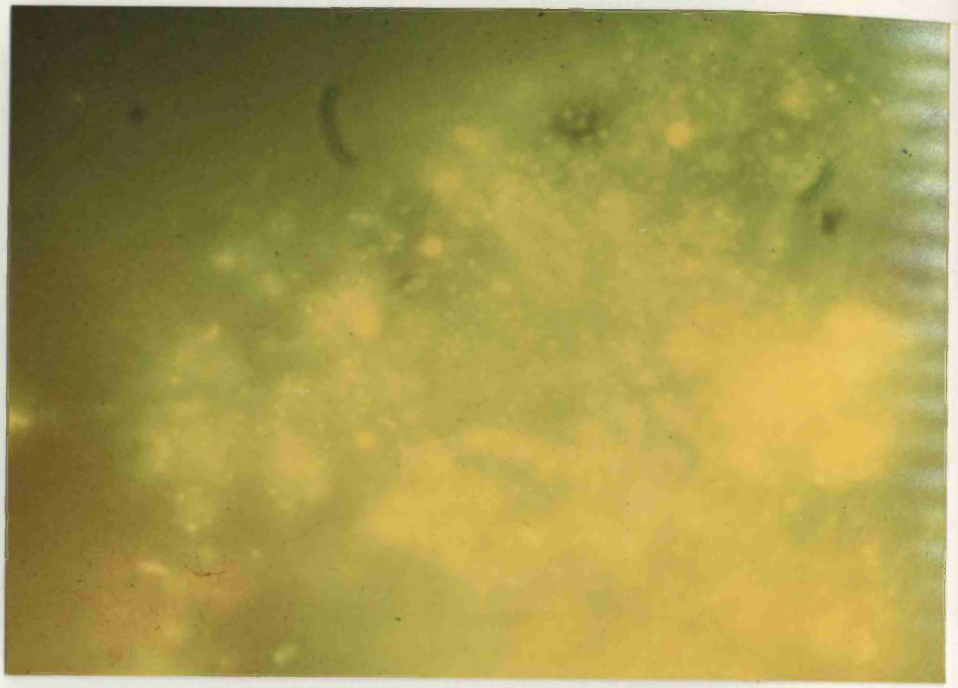


PLATE 30 SEM of decomposing tissue, frond tip, 1978 plant, June 1979. Dense cover of short rod-shaped bacteria. Scale bar = 2μm.

PLATE 31 SEM of decomposing tissue, frond tip, 1978 plant, June 1979. Colony of a cryptococcoid yeast, surrounded by mucilage. Flexibacteria also present. Scale bar = 4μm.



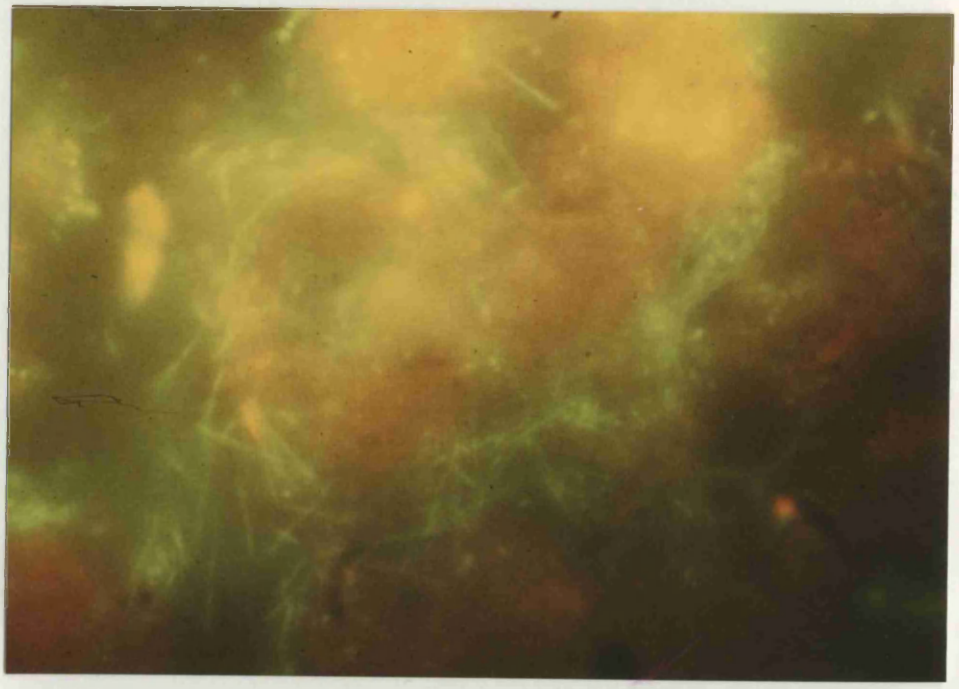


PLATE 32 Fluorescence micrograph of decomposing tissue, frond tip, 1978 plant, May 1979. Bacteria are predominantly rods. x 1500

PLATE 33 Fluorescence micrograph of decomposing tissue, frond tip, 1978 plant, May 1979. Rod-shaped and filamentous bacteria. x 1500

multicellular; filaments of this type up to 20μm long were seen. Their size indicates that they are procaryotic, but their identity is uncertain. The filaments seen extending over the frond surface in Plate 11 may be older and hence larger filaments of the same general type. Plate 31 shows a cryptococcoid yeast colony. A large amount of mucilage collects around these colonies but the spherical yeast cells can clearly be seen. The cell size appears to vary with the colony size; the cells in Plate 31 are about 1.5μm in diameter, while in larger colonies cells of up to 4.5μm were seen. No definite spatial pattern of the different types of micro-organisms in the decomposing areas could be distinguished. Some small areas such as that in Plate 30 have predominantly one type of bacterium, but in most areas several types are interspersed. The percentage cover of micro-organisms on the Laminaria cell surfaces is high, 5-70%, compared with a maximum of 20% on intact frond surfaces.

3.10.2. Fluorescence and brightfield observations

Plates 32 and 33 are fluorescence micrographs of decomposing tissue from the tip of a 1978 plant in April 1979. They confirm that bacteria are much more abundant on decomposing tissue than on intact frond surfaces (see Plate 1) and that a greater variety of morphological types is present. Plate 32 shows predominantly rods, Plate 33 has both rods and filaments. Most of the bacteria seen to be attached to Laminaria cell surfaces. Both long and short rods are present; a few of the longer rods, usually seen in small colonies, have an orange-fluorescing capsule around the green-fluorescing cells, others of the longer rods are curved and are presumably Flectobacillus. The filamentous bacteria often stand up from the surface to some extent.

Diatoms similar to those seen with the SEM were present in all samples. In addition the decomposing tissue of both 1978 plants examined in January 1980 had a large population of an apochlorotic Nitzchia species, apparently

the same as that previously observed on plants decomposing below the euphotic zone (see Chapter 6). Fluorescence observations confirmed that the cells contained no chlorophyll. Flagellates and ciliates were constant members of the community on decomposing tissue. Pigmented flagellates, which may have been algal zoospores, were occasionally seen, but most of the flagellates were colourless. Various colourless forms were seen, some with almost spherical, and some with elongate, cells. Their distribution was not uniform; some decomposing areas had only a few flagellates while some had very many, and where large numbers were present they were usually predominantly of one type, as though a sudden bloom had occurred on a particular decomposing area. The commonest ciliates belonged to the genera Holosticha and Euplotes.

The composition of the microbial community did not show marked seasonal changes. Bacteria of the various types, flagellates and ciliates were present in all samples in similar amounts. The only obvious seasonal difference was that the multicellular filamentous bacterium Leucothrix was more abundant in September 1979 and November 1979 than in any other sample, indicating an autumn-early winter peak of abundance.

3.10.3. Discussion

As mentioned in section 3.4.2., the bacteria of the decomposing tissue are of types observed on algal surfaces in previous studies. The only previous direct observations of bacteria on decomposing algal tissue were those of Sieburth (1975), who showed three SEM pictures. These were of a filamentous alga with a dense cover of the diatom Cocconeis; one point of similarity to the present study is that curved Flectobacillus-like cells were present.

Rods - Rods on intact frond surfaces were discussed in section 3.4. Those on decomposing tissue are presumably exposed to higher levels of dissolved nutrients and possibly to lower levels of inhibitory substances

(see section 3.13.). They might therefore be expected to have different biochemical properties, e.g. different reaction kinetics of the enzymes used in the uptake of dissolved organic matter, but no studies have been made.

Flectobacillus etc. - These are curved rods with rather variable morphology. On L. saccharina only horseshoe shapes were seen but other studies have also recorded rings and spirals. Sieburth (1975) found these bacteria on various algae and on decaying mangrove leaves. Their biology is reviewed by Raj (1977b) and Sieburth (1979).

Leucothrix - These pluricellular bacterial filaments have often been recorded from marine algae and also from fish eggs and crustacean larvae. The literature on Leucothrix is reviewed by Raj (1977a) and Sieburth (1979).

Flexibacteria - These are discussed in section 3.4.

Yeasts - Seshadri and Sieburth (1975) found more yeasts on red and green algae than on brown algae which included Laminaria digitata and L. longicruris, and on all the algae more yeasts in warm water periods than in cold. Colourless yeasts of the genus Candida were the most abundant, with small numbers of Rhodotorula present in the spring. Budding cells and pseudomycelia were observed with the SEM. In the present study only small, presumably young, colonies were seen; the reason for this is not apparent, possibly larger colonies would have been found in a more extensive SEM examination. For a review of the biology of marine yeasts see Sieburth (1979).

Flagellates and Ciliates - These are found on most detrital particles in the sea (Fenchel, 1970; Fenchel and Harrison, 1976) and so would be expected on decomposing Laminaria tissue. The flagellates observed by Fenchel (1970) included Bodo, Monas, Choanoflagellates and colourless euglenoids. Non-pigmented marine flagellates appear to feed on bacteria rather than using dissolved organic matter (Haas and Webb, 1979).

Holosticha and Euplotes, the commonest ciliates on the decomposing L. saccharina tissue, are also bacterial feeders (Fenchel, 1968b) and were among those which colonised Thalassia detritus (Fenchel, 1970).

Diatoms - On decomposing tissue, diatoms probably find shelter from wave action and surfaces less unfavourable than those of the intact meristoderm on which to settle. Levels of dissolved organic matter are presumably higher and the apochlorotic diatoms seen must have been wholly dependent on this as their carbon source. Pringsheim (1956) observed Nitzchia putrida on Fucus decomposing in laboratory tanks. Lewin and Lewin (1967) cultured three colourless Nitzchia species isolated from littoral habitats, showing that lactate or succinate could serve as the sole organic carbon source and that there was a requirement for exogenous sources of thiamine and cobalamine. They also found that under heterotrophic conditions all three apochlorotic strains grew considerably faster than normally pigmented strains. It seems likely that apochlorotic forms originated as mutants of pigmented strains and then evolved more efficient mechanisms for the uptake of dissolved organic matter as a result of competition with other heterotrophic organisms such as bacteria. Some pigmented diatoms are facultative heterotrophs (Lewin and Lewin, 1960), and it is possible that some of the pigmented forms on the decomposing tissue adopt this mode of nutrition.

3.11. MISCELLANEOUS OTHER EPIPHYTES

Spirorbid polychaetes were present throughout the year on all parts of the frond except the meristem. Vorticellid ciliates were present on the intact tip surfaces of both the 1978 plants in the May 1979 sample. In September 1979 small numbers of the dinoflagellate Ceratium were present on the decomposing tissue of all the plants. These had probably settled out from the plankton, where a bloom of this dinoflagellate was observed at this time.

3.12. SUMMARY

Differences in type, distribution or abundance of epiphytes on replicate plants were usually small; the most obvious being in the number of species of macro-algae on the stipes (see Table 3.2.).

Differences between the epiphytes on the central, dimpled and wing tissue at one level on a frond were also small. More of the mucilage material described in section 3.7. accumulated in the bottom of dimples than elsewhere on the fronds.

Similarly, the two surfaces of a frond differed little in their epiphytes. If a frond was consistently held in one position, the upper surface might be expected to develop a denser growth of photosynthetic epiphytes than the lower. The absence of such an effect agrees with field observations that the fronds grew tangled together and that they were often turned over by wave action.

There were only minor differences between plants of different year-groups. These were most obvious when one of the year-groups in a sample was either very young (1979 plants in May 1979) or very old (1977 (plants in January 1979; 1978 plants in January 1980). Very young plants had lower numbers of Licmophora and emergent ectocarpoid filaments. In very old plants the fronds were often colonised by macro-algal epiphytes otherwise seen only on the stipes.

Differences along the length of a frond were obvious. Most of the frond surface epiphytes (Licmophora,ectocarpoid filaments, rod-shaped bacteria) showed a gradient of abundance, with the highest numbers at the frond tip. This would be expected because the greater age of the distal tissue means that epiphytes have had longer to develop; there may also be an age-related gradient in the concentration of inhibitory substances within the Laminaria tissue (see section 3.13.). Only bryozoans and hydroids did not show this pattern of distribution on the frond surface; in the case of bryozoans,preferential settlement on the younger parts of the frond was demonstrated.

There were major differences between the epiphytes seen in different bimonthly samples. The seasonal patterns of abundance of the various epiphytes are summarized in Fig. 3.8.

3.13. GENERAL DISCUSSION: L. SACCHARINA SURFACES AS A SUBSTRATUM FOR EPIPHYTES

The epiphytes living on intact L. saccharina surfaces are not abundant and belong to only a few species. Organisms which colonize the fronds provide a much more favourable surface for settlement than does the frond itself. This could be because the frond contains inhibitory substances. In higher plants, polyphenols are known to act as a defence against diseases. Polyphenols are present in most brown algae, contained in special intracellular vesicles called physodes (review by Ragan, 1976). Polyphenol content may be high, often 10-14% of dry matter (Ragan and Jensen, 1977, and literature reviewed therein), and may vary seasonally (Ragan and Jensen, 1978). Other classes of compound which are present in some algae and which are known to be involved in host defence in higher plants include terpenes and allyl sulphides (Sieburth, 1968).

There is some evidence that extracts and exudates of macro-algae can have an inhibitory effect on a variety of organisms. Roos (1957) and Hornsey & Hide (1974) tested extracts of a range of algae against a few non-marine bacteria. Inhibitory activity was found in 22 out of 25 and in 54 out of 151 species respectively. Some species showed no seasonal variation in activity while others had maxima and minima at various times of year. Roos (1957) looked for differences in activity between different parts of the plant in three species including L. saccharina, but found none. Roos (1957) found that L. saccharina extracts were active against Staphylococcus aureus and Bacillus subtilis but not against Escherichia coli, and that activity was highest in late summer and autumn. Hornsey and Hide (1970) found activity against S. aureus, B. subtilis, E. coli, Proteus morganii and Streptococcus pyogenes in spring and winter but no activity in summer or autumn.

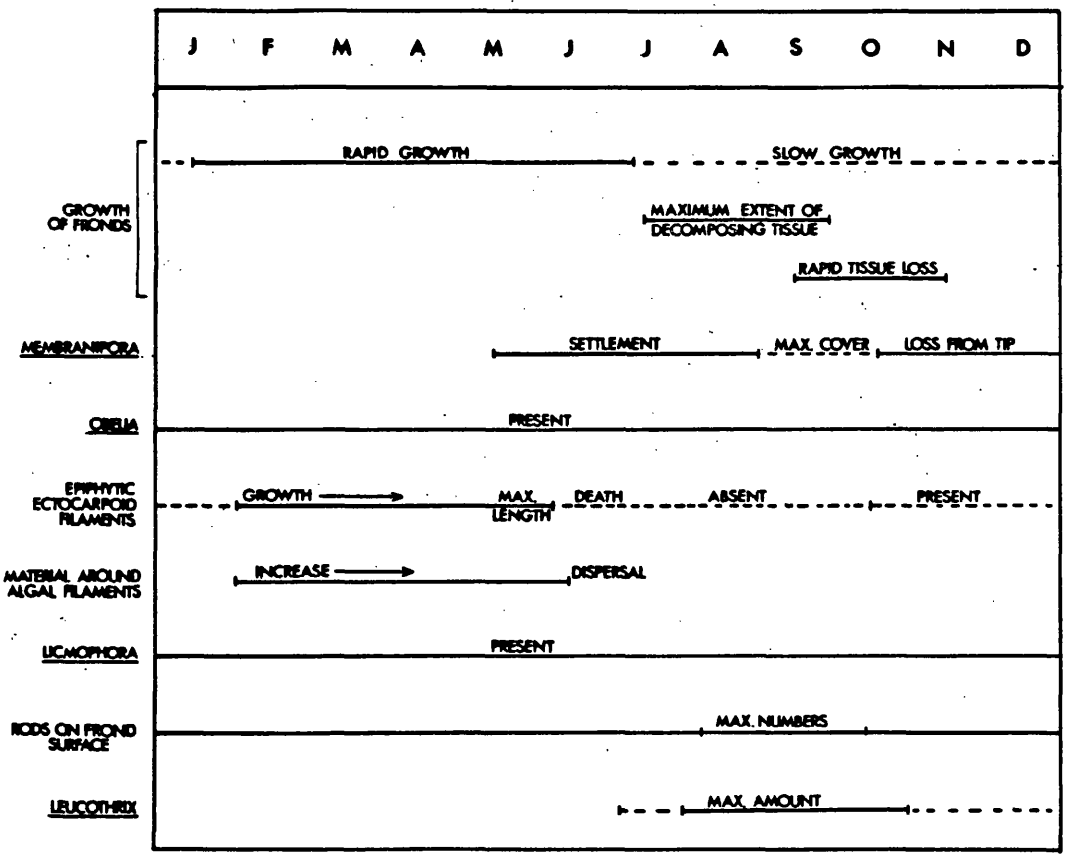


FIG. 3.8. Summary of main events on a L. saccharina frond in the course of a year.

When marine bacteria have been used as test organisms, rather different results have been obtained. Burton (1976) prepared extracts and exudates from three species of algae and isolated a range of bacteria from each species. He then tested each extract and exudate against each bacterium and in no case did he observe inhibition. Ballantine (Ph.D. thesis, 1977, quoted in Ballantine, 1979) tested extracts from several algae, both "good" and "poor" hosts (Ballantine, 1979) against "sensitive marine bacteria" and again found no inhibition. Conover and Sieburth (1964) found that activity against a Vibrio isolated from strandline Sargassum was not present in extracts of heavily fouled Sargassum plants but was present in extracts of the growing tips of healthy plants. Sieburth and Conover (1965) showed that the inhibitory substance was a tannin, and that it was also toxic to hydroids, and at higher concentrations to nematodes and turbellarians. Langlois (1975) found that exudates of Cladophora and Polysiphonia promoted, and extracts of Fucus, Ascophyllum and Scytosiphon inhibited, settlement and growth of telotrochs of Vorticella marina.

Most of the studies mentioned above have used extracts from algae, and give no information about whether any inhibitory substances which may be present in the algal tissues are released in sufficient quantities to affect epiphytes. Sieburth and Jensen (1969) found that three brown algae exuded precursors which in the alkaline medium of seawater formed polyphenols toxic to plaice larvae. Complexes formed by the oxidation of algal polyphenols and their combination with carbohydrate and protein material of algal and other origin make up a considerable part of marine "Gelbstoff" (Sieburth and Jensen, 1968, 1969).

It is still difficult to assess the significance of inhibitory compounds in the control of epiphyte growth on algae. The ecological significance of some of the results discussed above can be questioned, particularly where extracts have been used. Other findings, particularly on seasonal variations in activity, are contradictory. Matters which

would repay further investigation include:

(i) The concentration and chemical nature of inhibitory substances, both in the water and in algal-surface mucilage, to which epiphytes are actually exposed.

(ii) The sensitivity of different organisms to these substances. Specialized epiphytes may have evolved resistance, so with bacteria, for example, a range of strains isolated from a variety of marine habitats should be tested.

(iii) Seasonal patterns of inhibitory activity and polyphenol content. Some of the results obtained so far suggest that inhibitory activity may be lowest in spring and summer, the seasons of greatest potential epiphyte growth.

It must be remembered that the polyphenols found in algae may have functions other than acting as antifouling chemicals. They may be waste products of algal metabolism, or a defence against grazers rather than against epiphytes. Leighton (1966) suggested that specific distaste factors might explain the food preferences of algivorous invertebrates. There are also other factors which could be responsible for the different degrees of epiphyte growth on different surfaces; these include differences in roughness, and the presence or absence of mucilage coats.

Wherever the L. saccharina surface is damaged the exposed cortex or medulla tissue is colonised by a large number of organisms of the types described in section 3.10. This community is presumably dependent on high levels of dissolved organic matter leaking from Laminaria cells but its abundance and diversity suggest that whatever factor limits the growth of epiphytes on the intact frond surface is not acting here. Berthold (1882, quoted in Ragan, 1976) found that physodes were absent from the internal cells of brown algae, but Ragan (1976) questions this observation.

CHAPTER 4

NEMATODES ON L. SACCHARINA FRONDS

4.1. METHODS

4.1.1. Collection of samples

Selection of plants was discussed in section 3.4. In each bimonthly sample two plants of each of the relevant year-groups were used in the nematode study. All samples were taken by SCUBA diving or snorkelling. It had been observed in a preliminary study that the microbial community of decomposing tissue was very different from that of the intact frond surface, so it was decided to take separate nematode samples from each of these microhabitats. The frond surface samples were taken with samplers (Fig. 4.1.) constructed from rigid grey plastic piping ("Durapipe"), of 4.2cm internal diameter. Two sections of pipe, one with one end sharpened to form a cutting edge, were glued together using "Rapid Araldite", with a diaphragm cut from a toy balloon sandwiched between them. A 41mm rubber bung completed the sampler. To take a sample, the body of the sampler was held on one side of a L. saccharina frond and the bung on the other. The two were aligned and the bung pushed home. This cut a disc of standard area (13.6cm^2) from the frond and enclosed it in a roughly standard volume of seawater, ready for transport to the laboratory. Without an elastic diaphragm, the bung could not of course be pushed home. From each plant, three discs were taken from each of the regions: tip, mid-frond and meristem, unless the plants were for some reason very small (see section 2.2.), in which case the number of replicates had to be reduced. Decomposing tissue samples were taken by cutting the small

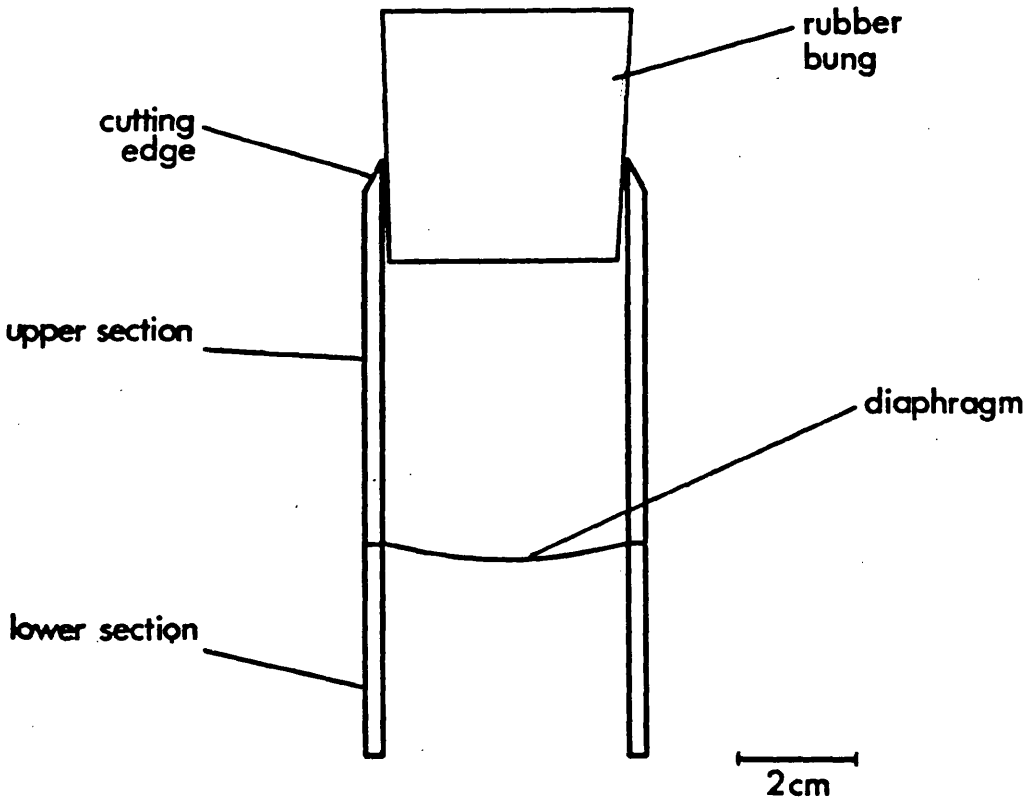


FIG. 4.1. Disc sampler.

decomposing areas from the frond with scissors or a scalpel and collecting them in polythene bags which were then closed with repeatedly twisted elastic bands. No attempt was made to make these samples quantitative.

4.1.2. Treatment of samples in the laboratory

On arrival at the laboratory all nematode samples were transferred to a refrigerator, and were then sorted within 6 hours of collection. A preliminary study had shown that a much larger count was obtained by sorting samples fresh than by sorting them preserved. This was because large amounts of mucilage were present; nematodes within this were often detected only by their movements. The nematodes were transferred to 4% formalin in seawater, then after 1-2 days to a mixture of 9 parts 50% ethanol to 1 part glycerol. This was left for the alcohol and water to evaporate, the final stages of dehydration being carried out in a desiccator, and the nematodes were then mounted in anhydrous glycerol.

Each individual was identified to species, measured, assigned to one of the "breeding categories" (see below), and its gut contents examined. Body length was measured by using a squared eyepiece graticule and squared paper to produce a scale drawing which was then measured with a map measurer. The four "breeding categories" were: juvenile, male, immature female, and mature female (ie. with fully developed eggs in the ovary).

4.1.3. Statistical treatment of data

The comparison of the relative abundances of different species in different samples involved the analysis of contingency tables, and the G-test (log-likelihood ratio, see Sokal and Rohlf, 1969) was used for this. It is considered advisable when using this test to keep to the same rule of thumb as for X^2 analysis, namely that no expected frequency should be less than 1.0, and that not more than 20% of the expected frequencies should be less than 5.0. In some cases these conditions were not met, but the usual remedy of combining rows or columns would have

defeated the purpose of the analysis. The G-test was therefore used even in these cases, and care was taken in interpreting the results; in particular a 1%, rather than a 5%, significance level was used throughout. In practice, most of the results were either highly significant ($p < 0.005$) or obviously non-significant, so it is not considered that major errors in interpretation were made. It was felt that in this case where only four species were involved, the use of a non-parametric test (e.g. Kendall's coefficient of concordance) would have meant an unnecessary waste of information.

4.2. RESULTS

4.2.1. Number of species present

Four species, Monhystera disjuncta Bastian, M. refringens Bresslau and Stekhoven, Chromadora nudicapitata Bastian and Theristus acer Bastian, dominated the nematode fauna. Other species made up only 1.1% of the total of 986 nematodes in the samples, and in no bimonthly sample did they form more than 7.1% of the nematodes found on either the frond surface or the decomposing tissue of plants of any one year-group.

4.2.2. Large-scale distribution of nematodes on the fronds

Nematodes were almost entirely confined to the tips of the fronds. They were only rarely found in the mid-frond and meristem samples, and then only in small numbers (Table 4.1.). All four species showed this pattern of distribution.

4.2.3. Frond surface samples: differences between replicate discs and between replicate plants

(a) Number of nematodes

Table 4.2. shows the number of nematodes found on each tip disc taken during the sampling programme. The number of replicate discs from any plant never exceeded three and this is too few to permit

	1977 PLANTS	1978 PLANTS	1979 PLANTS
Tip discs	20.3	48.7	70.0
Mid frond discs	0.0	4.5	1.0
Meristem discs	0.0	0.3	0.0

TABLE 4.1. Maximum sample mean values of number of nematodes/disc from different frond regions of plants of different year-groups.

SAMPLE	YEAR GROUP	PLANT A			PLANT B			PLANT C
*January 1979	1977	0	3	2	4	0	3	-
	1978	0	0	0	0	0	0	-
March 1979	1977	42	-	-	5	-	-	14
	1978	18	10	6	5	7	4	-
May 1979	1978	58	32	6	-	-	-	-
	1979	70	-	-	-	-	-	-
July 1979	1978	2	2	1	3	2	4	-
	1979	0	2	0	-	-	-	-
September 1979	1978	12	18	-	-	-	-	-
	1979	11	4	6	-	-	-	-
November 1979	1978	7	5	4	-	-	-	-
	1979	2	2	5	17	7	5	-
January 1980	1978	0	0	0	5	7	3	-
	1979	0	0	0	0	0	0	-

TABLE 4.2. Actual numbers of nematodes found on tip discs.

statistical testing to determine whether the distribution of nematodes was random at this scale within the tip region. No patchiness is obvious from an inspection of Table 4.2. Similar considerations apply to differences between replicate plants, though here there is a suggestion of differences among the three 1977 plants in the March 1979 sample.

(b) Relative abundance of the four species

Table 4.3. shows the results of G-tests for differences in the species compositions of replicate discs. In no case was the difference significant at the 1% level. Data from replicate discs were then pooled and further G-tests performed to test differences between replicate plants; the results are shown in Table 4.4. In only one case was a significant difference found, between the two 1978 plants in the March 1979 sample. Subdivision of the contingency table and further G-testing indicated that this difference was the result of a lower proportion of M. disjuncta in the 1978 plant b sample.

4.2.4. Frond surface samples: differences between plants of different year-groups

(a) Number of nematodes

Again the small number of replicates precludes statistical testing. In January 1979, nematodes were found on the surfaces of 1977 plants but not on those of 1978 plants. Similarly in January 1980, nematodes were found on the surfaces of 1978 plants but not on those of 1979 plants. There were no other obvious differences between plants of different year-groups.

(b) Relative abundance of the species

Table 4.5. shows the results of G-tests for differences in species composition between plants of different year-groups within the same bimonthly samples. Data from replicate discs and from replicate plants have been pooled except where significant differences have previously been shown (see above). In only two cases were differences significant at the

MONTH	PLANT	NO. OF DISCS	RESULT OF TEST
January 1979	1977 (a)	3	-
	1977 (b)	3	-
	1978	No surface nematodes	
March 1979	1977		
	1978 (a)	3	-
	1978 (b)	3	-
May 1979	1978	4 (inc. 1 mid-frond)	-
	1979	3	-
July 1979	1978 (a)	3	-
	1978 (b)	3	-
	1979	Only 1 species on surface	
September 1979	1978	3 (inc. 1 mid-frond)	-
	1979	3	-
November 1979	1978	3	-
	1979 (a)	3	-
	1979 (b)	3	-
January 1980	1978 (a)	Only 1 species on surface	
	1978 (b)	" " " " " "	
	1979 (a)	" " " " " "	
	1979 (b)	" " " " " "	

\- = Not significant at 1% level
\+ = Significant at 1% level

TABLE 4.3. Results of G-tests for differences in relative species abundances between replicate discs.

MONTH	PLANTS	RESULT
January 1979	1977 (a) and 1977 (b) 1978 plants - no surface nematodes	-
March 1979	1977 (a), (b), (c) 1978 (a) and 1978 (b)	- +
May 1979	Only one 1978 plant Only one 1979 plant	
July 1979	1978 (a) and 1978 (b) Only one 1979 plant	-
September 1979	Only one 1978 plant Only one 1979 plant	
November 1979	Only one 1978 plant 1979 (a) and 1979 (b)	-
January 1980	Only 1 species on 1978 surfaces Only 1 species on 1979 surfaces	

\+ = Significant at 1% level
\- = Not significant at 1% level

TABLE 4.4. **Results of G-tests for differences in relative species abundance between replicate plants (frond surface nematodes).**

MONTH	PLANTS	RESULT
January 1979	1978 plants have no surface nematodes	
March 1979	1977 plants (pooled) and 1978 (a) 1977 plants (pooled) and 1978 (b)	- +
May 1979	1978 and 1979 plants (1 of each)	-
July 1979	1978 plants (pooled) and 1979 plant (only)	-
September 1979	1978 and 1979 plants (1 of each)	-
November 1979	1978 (only) and 1979 (pooled)	+
January 1980	Only one species on surfaces	

\+ = Significant at 1% level
\- = Not significant at 1% level

TABLE 4.5. **Results of G-tests for differences in relative species abundance between plants of different year-groups (frond surface nematodes).**

1% level. In March 1979 there was no significant difference between 1978 plant a and the 1977 plants, but 1978 plant b was significantly different from the 1977 plants, having a lower proportion of M. disjuncta. In November 1979 there was a significant difference between the 1978 and 1979 plants, the result of the absence from the 1978 plants of C. nudicapitata and M. refringens, which between them made up 74% of the nematodes on the 1979 plant discs.

4.2.5. Seasonal variation of the surface nematode fauna

Fig. 4.2. shows the variation in the mean number of nematodes/tip disc, both overall and for each species separately, for 1977, 1978 and 1979 plants. The overall number of nematodes showed minima in January 1979, July 1979 and January 1980. There was a main peak in May 1979 and a smaller peak in September 1979. T. acer was present on the frond surface on only two of the bimonthly samples. Small numbers were present in March 1979 and large numbers in May 1979. This species was responsible for the May 1979 peak in overall numbers. C. nudicapitata was present in small numbers for most of the year, with minima in January 1979 and January 1980 and a maximum in September and November 1979. M. refringens was absent in July 1979 and January 1980, and was otherwise present in small numbers with a peak of abundance in September 1979. M. disjuncta was absent in July 1979 and was most abundant in January and March 1979 and again in January 1980.

Fig. 4.3. shows the results of G-tests for differences in species composition between the surface faunas of different bimonthly samples taken in pairs. Within each bimonthly sample data from replicate discs, replicate plants and plants of different year-groups have been pooled where they have previously been shown not to be significantly different at the 1% level (see above). Where heterogeneity has been shown, data have been treated separately. From Fig. 4.3. it can be seen that most

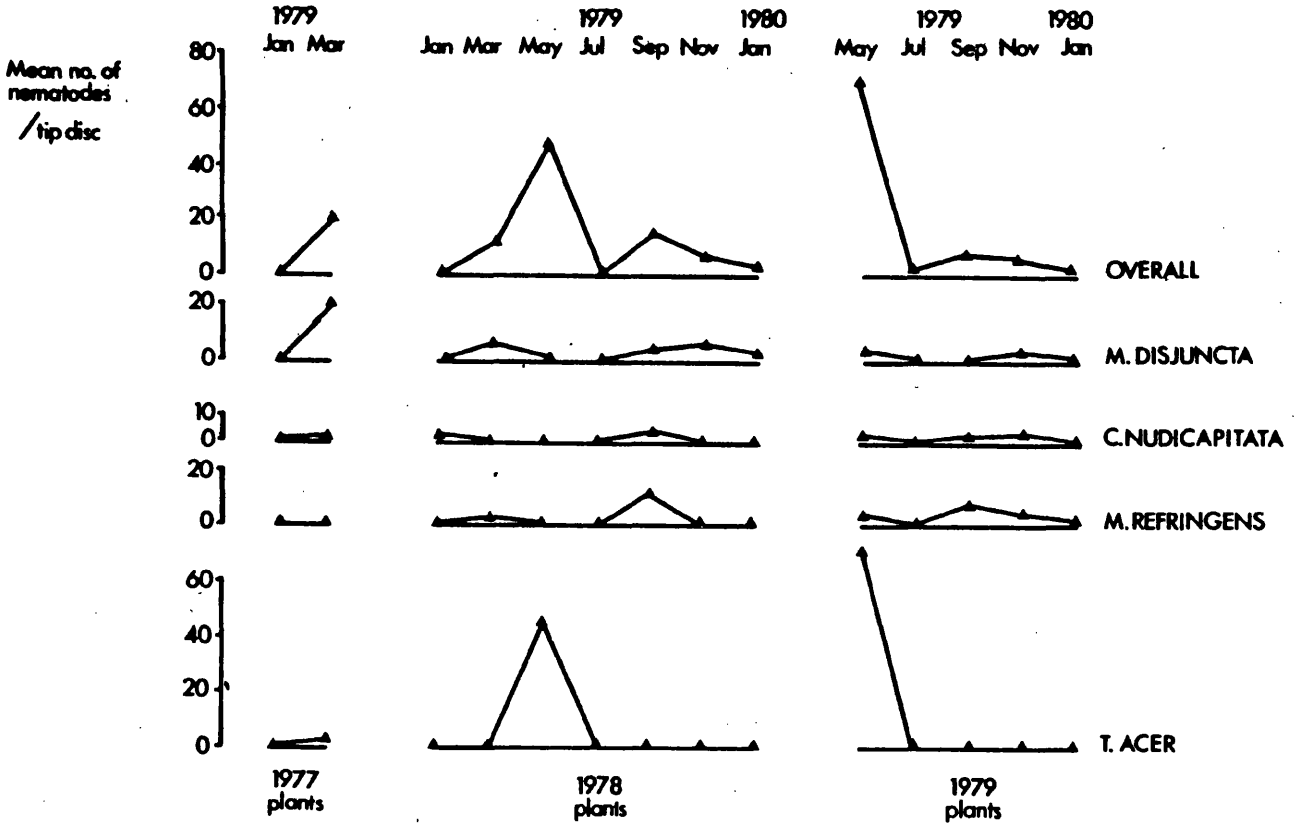

Mean no. of nematodes / tip disc
1979
Jan Mar
1979
Jan Mar May Jul Sep Nov
1980
Jan
1979
May Jul Sep Nov
1980
Jan
80
60
40
20
0
OVERALL
20
0
M. DISJUNCTA
10
0
C. NUDICAPITATA
20
0
M. REFRINGENS
60
40
20
0
T. ACER
1977 plants
1978 plants
1979 plants

FIG. 4.2. Seasonal variation in the mean number of nematodes/tip disc, overall and for each species separately, for 1977, 1978 and 1979 plants.

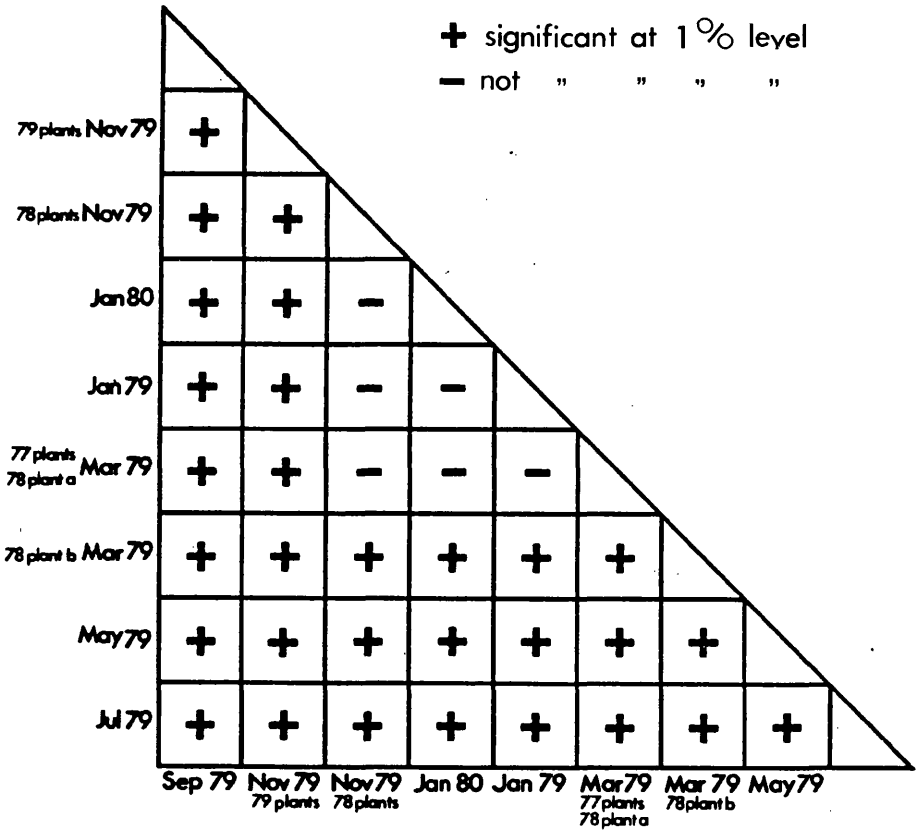


FIG. 4.3. Results of G-tests for differences in species composition between the frond surface faunas in different bimonthly samples taken in pairs.

bimonthly samples were significantly different in species composition from most other bimonthly samples. Differences within a group consisting of the January 1979 and January 1980 samples, one March 1979 and one November 1979 sample, were not significant. These samples can be considered to represent a winter fauna dominated by M. disjuncta.

4.2.6. Decomposing tissue: differences between replicate plants

Decomposing tissue samples were not taken from a known area or weight of tissue, so comparisons can only be made of the relative abundance of the different nematode species. Table 4.6. shows the results of G-tests for differences in species composition of the decomposing tissue samples from replicate plants. Because of the limited extent of decomposing tissue on very young plants (see section 3.2.), no decomposing tissue samples were obtained from 1979 plants in May or July 1979. The difference found between 1979 plant a and 1979 plant b in November 1979 is the result of the higher proportion of C. nudicapitata in the former. The difference between the 1978 plants in January 1980 is the result of the lower proportion of M. disjuncta in the 1978 plant a sample.

4.2.7. Decomposing tissue: differences between plants of different year-groups

Where no significant differences had been found between replicate plants, data from these were pooled before analysis of the differences between plants of different year-groups. The results of these further G-tests are shown in Table 4.7. In November, the 1978 plant sample was not significantly different from the 1979 plant a sample, but was significantly different from the 1979 plant b sample, having a higher proportion of C. nudicapitata. In January 1980, the 1978 plant sample was significantly different from the 1979 plant sample, the former having a lower proportion of M. disjuncta.

4.2.8. Decomposing tissue: differences between bimonthly samples

Fig. 4.4. shows the results of these G-tests. Within each bimonthly

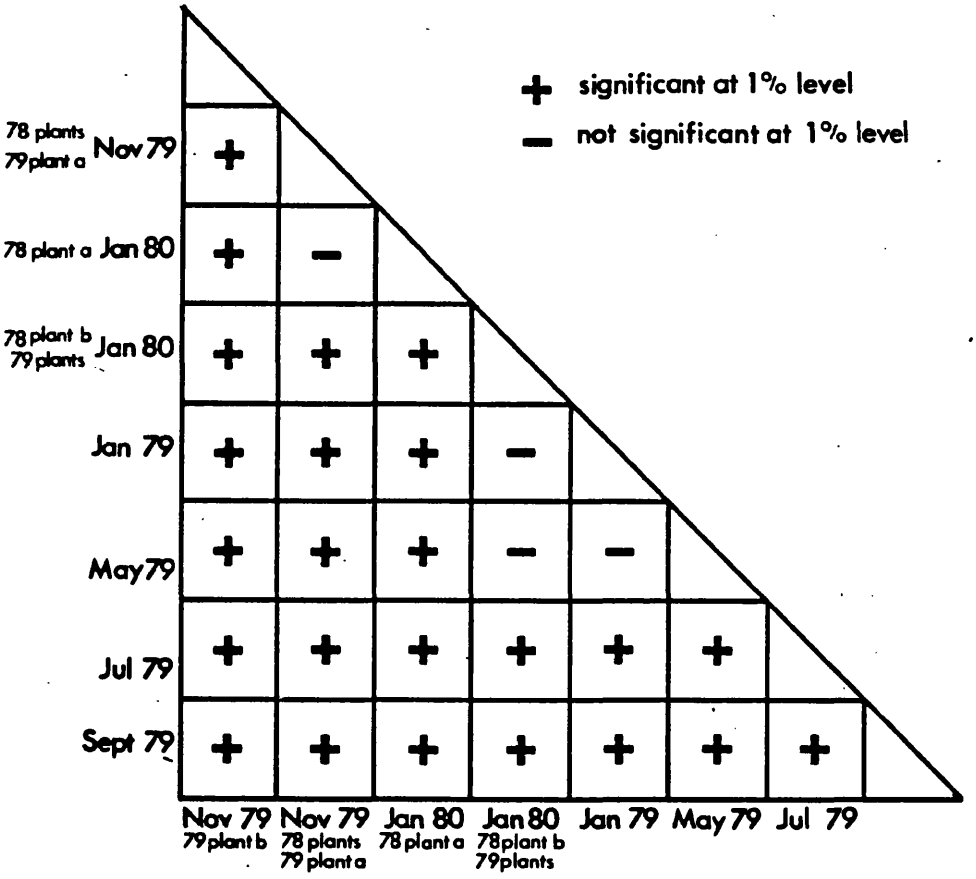


FIG. 4.4. Results of G-tests for differences in species composition between the decomposing tissue faunas in different bimonthly samples taken in pairs.

MONTH	PLANTS	RESULT
January 1979	Only one 1977 plant 1978 plant (a) and 1977 plant (b)	 -
March 1979	3 1977 plants - no separation of 1978 plants - no decomposing sample	
May 1979	Only one 1978 plant 1979 plants - no decomposing sample	
July 1979	1978 pl(i), 1978 pl(ii) & additional tissue 1979 plants - no decomposing sample	-
September 1979	Only one 1978 plants Only one 1979 plant	
November 1979	Only one 1978 plant 1979 pl(a) and 1979 pl(b)	 +
January 1980	1978 pl(a) and 1978 pl(b) 1979 pl(a) and 1979 pl(b)	+ -

TABLE 4.6. **Results of G-tests for differences in relative species abundance between decomposing tissue samples from replicate plants.**

MONTH	PLANTS	RESULT
January 1979	1977 (only) and 1978 (pooled)	-
March 1979	No separation of decomposing tissue in 1977 plants	
May 1979	No decomposing sample from 1979 plants	
July 1979	No decomposing sample from 1979 plants	
September 1979	No decomposing sample from 1979 plants	
November 1979	1978 and 1979 (a) 1978 and 1979 (b)	- +
January 1980	1978 (a) and 1979 (pooled) 1978 (b) and 1979 (pooled)	+ -

TABLE 4.7. **Results of G-tests for differences in relative species abundance between decomposing tissue samples from plants of different year-groups.**

sample, data from replicate plants and from plants of different year-groups have been pooled unless they have previously been shown to be different at the 1% level (see above). Most bimonthly samples were significantly different in species composition from most other bimonthly samples. Differences were not significant within a group consisting of the January 1979 and May 1979 samples and one January 1980 sample, nor between one November 1979 sample and the other January 1979 sample.

4.2.9. Comparison of surface and decomposing tissue samples

Figs. 4.5. and 4.6. show the mean relative proportions of the four common species and "other species" in the surface and decomposing tissue samples in each bimonthly sample for 1978 plants and 1977 and 1979 plants respectively. Table 4.8. shows the results of G-test comparisons of the relative proportions of the different species in tip disc and decomposing tissue samples within the same bimonthly samples. Data from replicate discs, replicate plants and plants of different year-groups have been pooled where data have previously been shown not to be significantly different at the 1% level. There is no obvious seasonal pattern in the occurrence of differences. Further G-testing on subdivided contingency tables gives the following information on which species are responsible for the observed significant differences. In May 1979 the decomposing tissue had a higher proportion of M. disjuncta. In November 1979 the decomposing tissue from the 1978 plants and the 1979 plant a had a higher proportion of C. nudicapitata than the 1978 plant surface sample, while the decomposing tissue from 1979 plant b had a higher proportion of C. nudicapitata than the 1979 plant surface sample. In January 1980 the decomposing tissue from 1978 plant b had a lower proportion of M. disjuncta than did the surface sample.

4.2.10. Breeding structure of the populations

For each species, length frequency histograms for each of the

FIG. 4.5. Proportions of the different species in surface and decomposing tissue samples, 1978 plants. Numbers show number of nematodes in each sample.

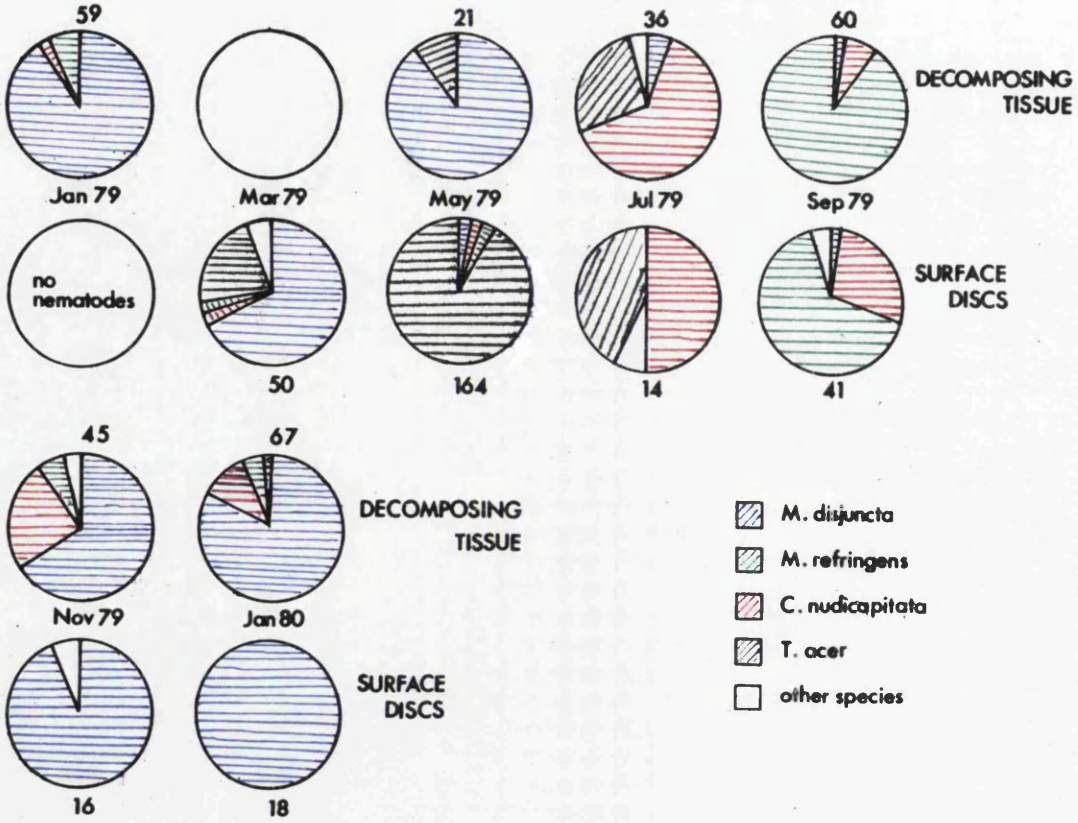
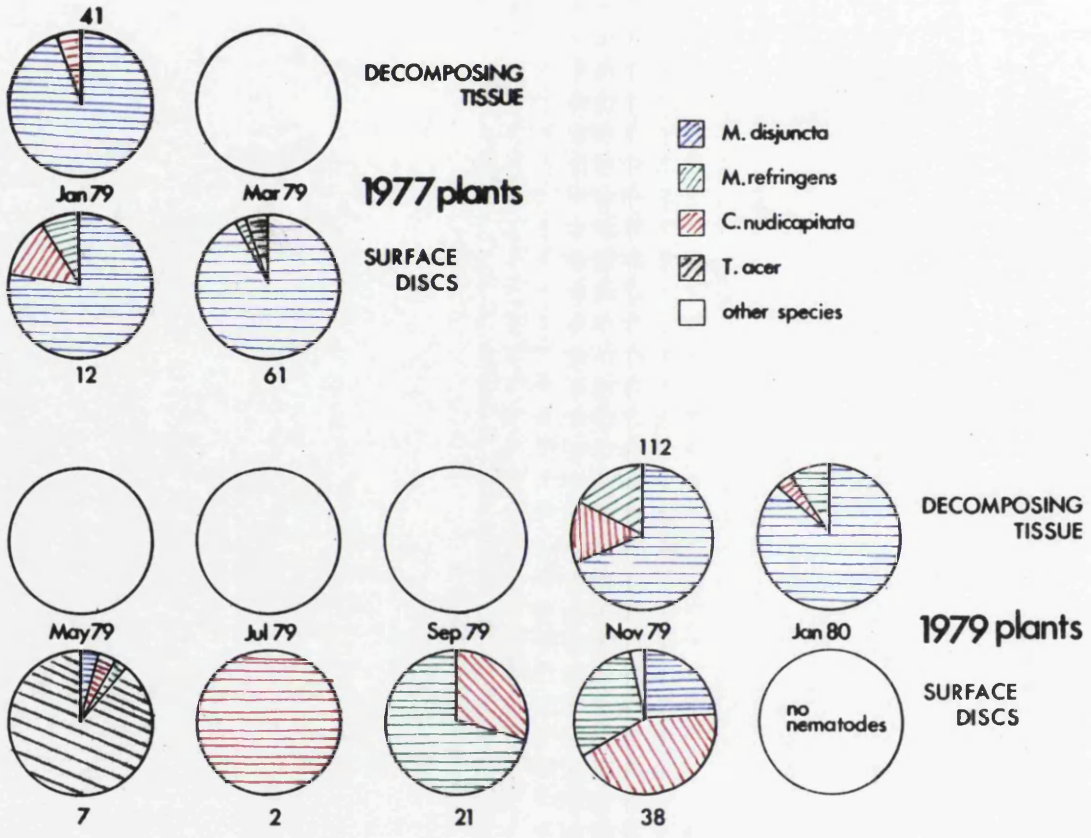

59
21
36
60
DECOMPOSING TISSUE
Jan 79
Mar 79
May 79
Jul 79
Sep 79
no nematodes
50
164
14
41
SURFACE DISCS
45
67
DECOMPOSING TISSUE
Nov 79
Jan 80
SURFACE DISCS
16
18
M. disjuncta
M. refringens
C. nudicapitata
T. acer
other species

FIG. 4.6. Proportions of the different species in surface and decomposing tissue samples, 1977 and 1979 plants. Numbers show number of nematodes in each sample.


41
Jan 79
Mar 79
DECOMPOSING TISSUE
1977 plants
SURFACE DISCS
12
61
M. disjuncta
M. refringens
C. nudicapitata
T. acer
other species
112
May 79
Jul 79
Sep 79
Nov 79
Jan 80
DECOMPOSING TISSUE
1979 plants
SURFACE DISCS
no nematodes
7
2
21
38

MONTH	DISCS	DECOMPOSING	RESULT
January 1979	Pooled	Pooled	-
March 1979	No comparison possible		
May 1979	Pooled	1978 (only plant)	+
July 1979	Pooled	Pooled	-
September 1979	Pooled	Pooled	-
November 1979	1979	1978 and 1979	+
	1979	1979 b	+
	1978	1978 and 1979 a	+
	1978	1979 b	-
January 1980		1978 b and 1979	-
		1978 a	+

TABLE 4.8. Results of G-tests for differences in relative species abundance between surface and decomposing tissue samples in the same bimonthly sample.

breeding categories (see section 4.1.) were constructed for each bimonthly sample. There were no apparent differences in location between the histograms for one species in the different bimonthly samples, so these were combined to give overall length-frequency histograms for the breeding categories in each species, shown in Figs. 4.7. - 4.10.

In M. disjuncta the two sexes became recognizable at a length of 400-600μm. Females became mature at a length of 600-750μm. The maximum length reached by females was about 1000μm, by males about 900μm.

In M. refringens the two sexes again became recognizable at a length of 400-600μm. Females became mature at 550-750μm. The maximum length reached by both males and females is about 800μm.

In C. nudicapitata males became recognizable at a length of 350-500μm, females at about 500μm. This implies that most of the larger "juveniles" were female. Females became mature at 600-800μm. The maximum length was 900-950μm in both sexes.

In T. acer the sexes became recognizable at 1200-1400μm in length. Females became mature at 1500-1900μm. The maximum length reached by females was 2800μm, but most adults of both sexes did not exceed 2300μm.

Seasonal variation in the breeding structure of the populations can then be shown in two ways; from the overall length-frequency histograms for each species (Figs. 4.11. - 4.14) and from seasonal variation in the relative proportion of the breeding categories in each species (Table 4.9.). For M. disjuncta, M. refringens and C. nudicapitata the histograms do not vary much in location or in skewness from month to month, indicating that the breeding structure of the populations was more or less constant throughout the sampling period. This is borne out by the lack of variation in the proportions of the breeding categories: G-tests for variation in the proportion of juveniles showed a significant

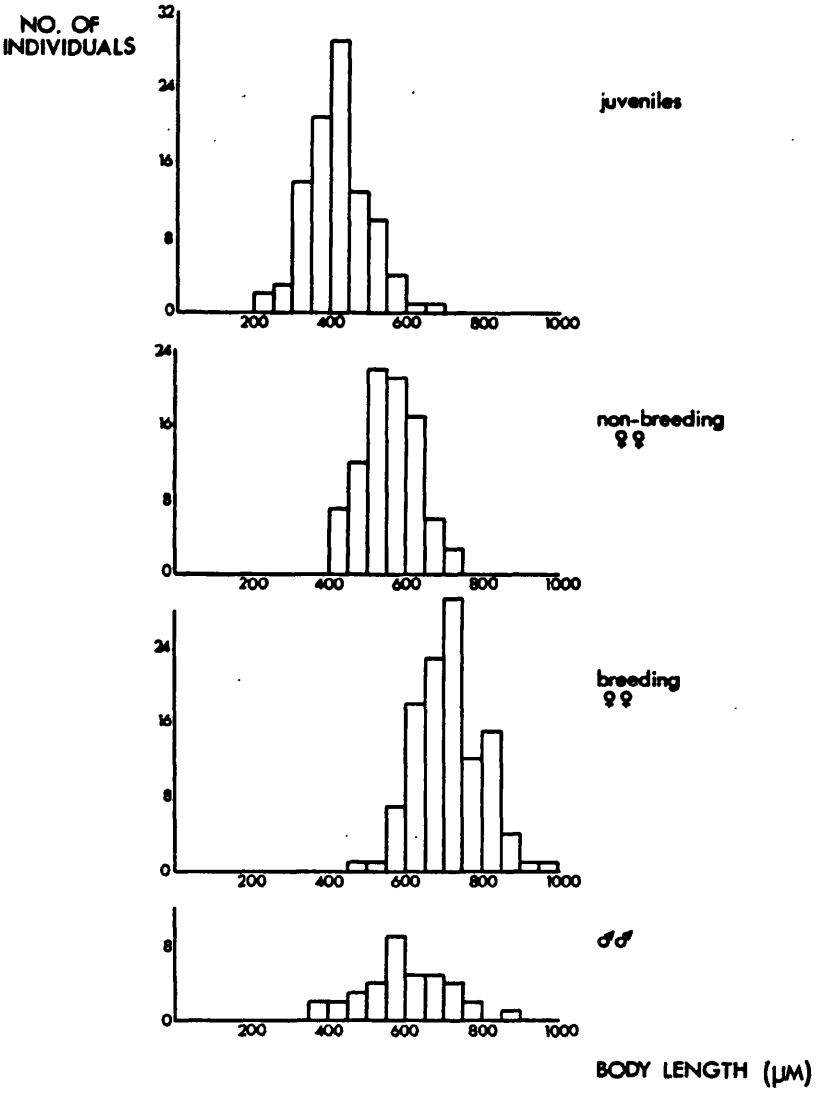


FIG. 4.7. Length-frequency histograms for the four breeding categories in Monhystera disjuncta.

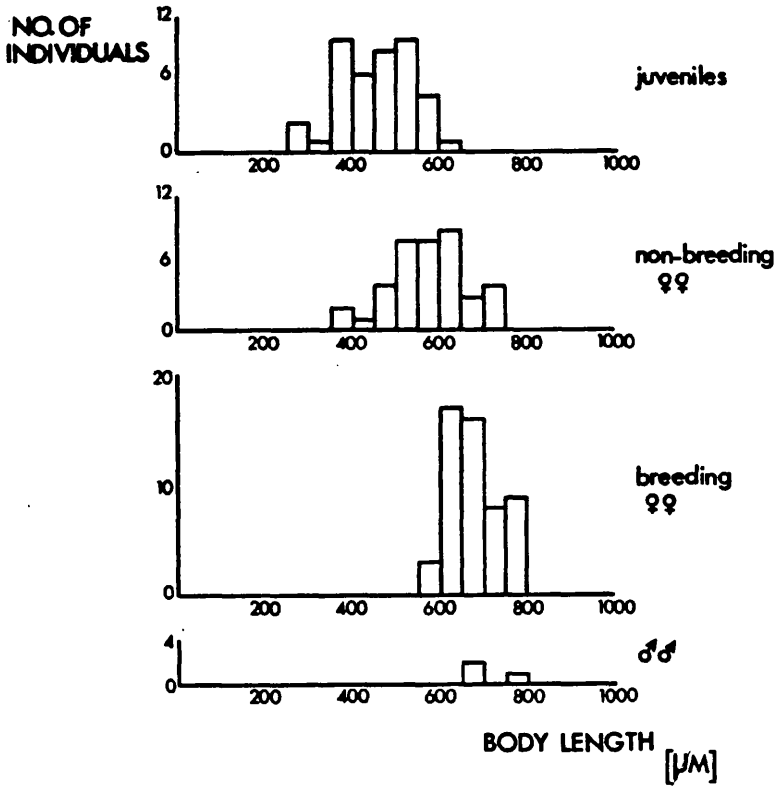


FIG. 4.8. Length-frequency histograms for the four breeding categories in Monhystera refringens.

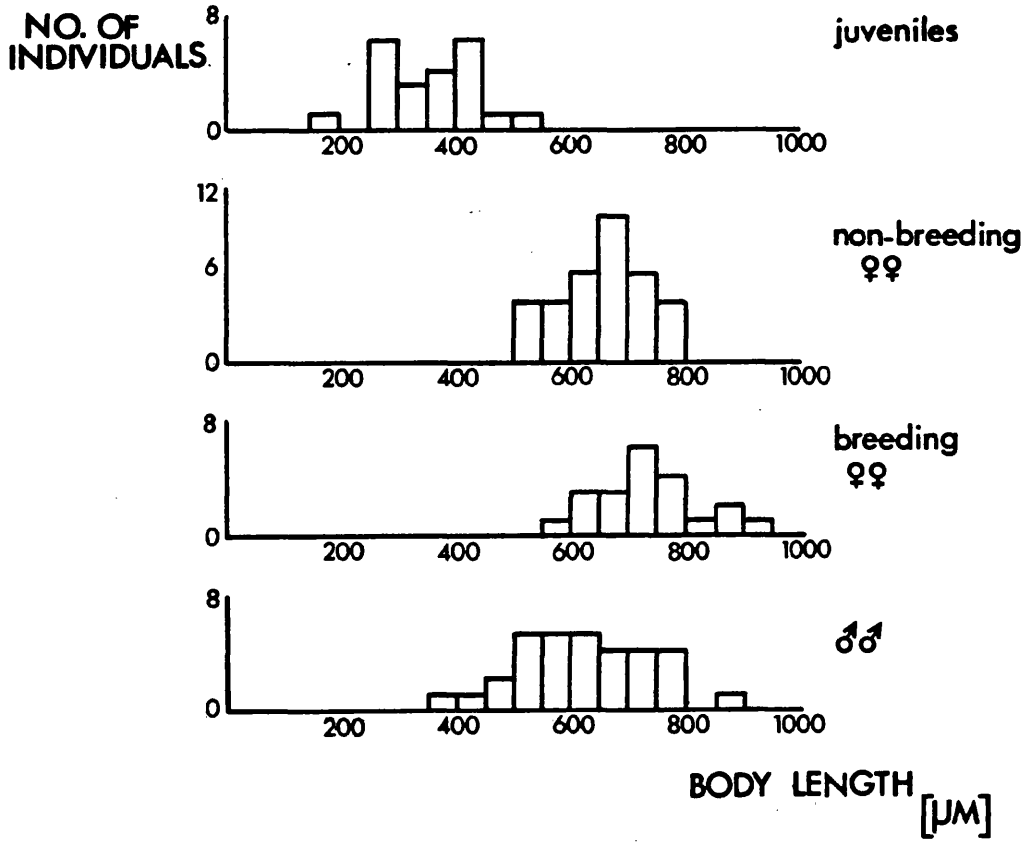


FIG. 4.9. Length-frequency histograms for the four breeding categories in Chromadora nudicapitata.

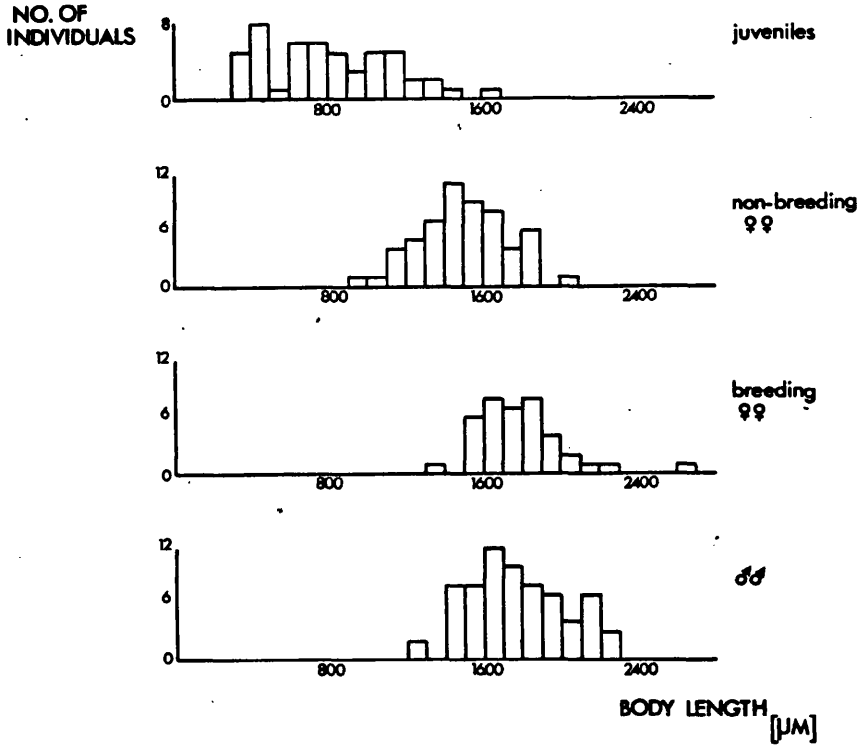


FIG. 4.10. Length-frequency histograms for the four breeding categories in Theristus acer.

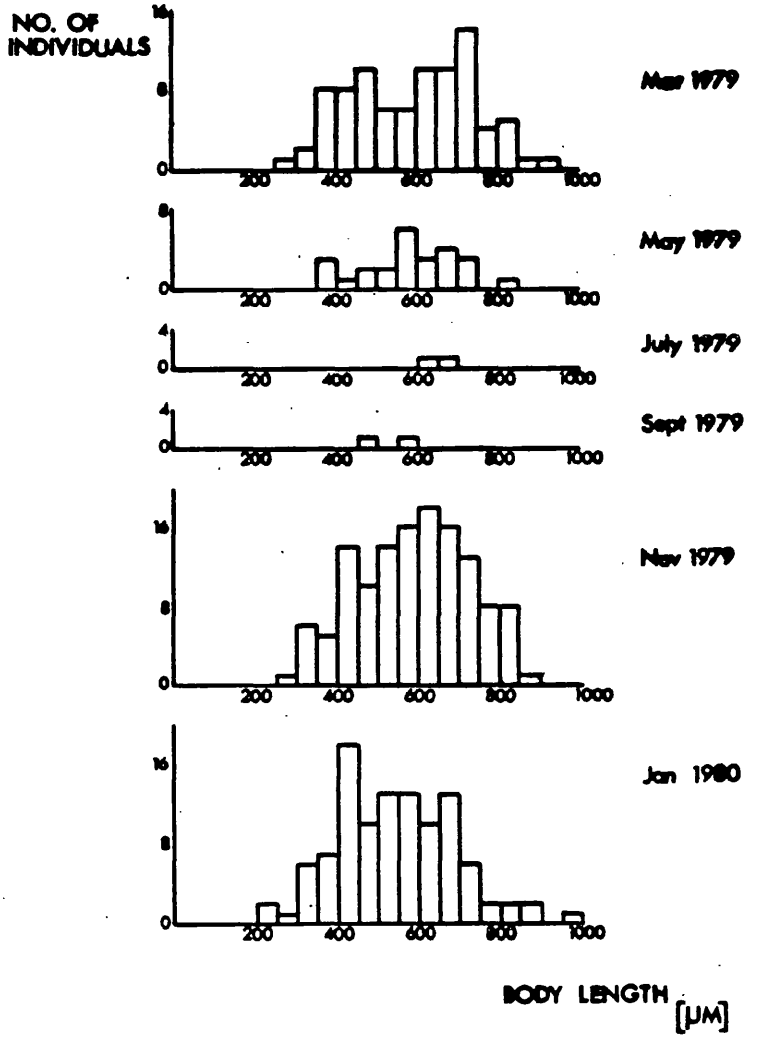


FIG. 4.11. Length-frequency histograms for Monhystera disjuncta in the different bimonthly samples.

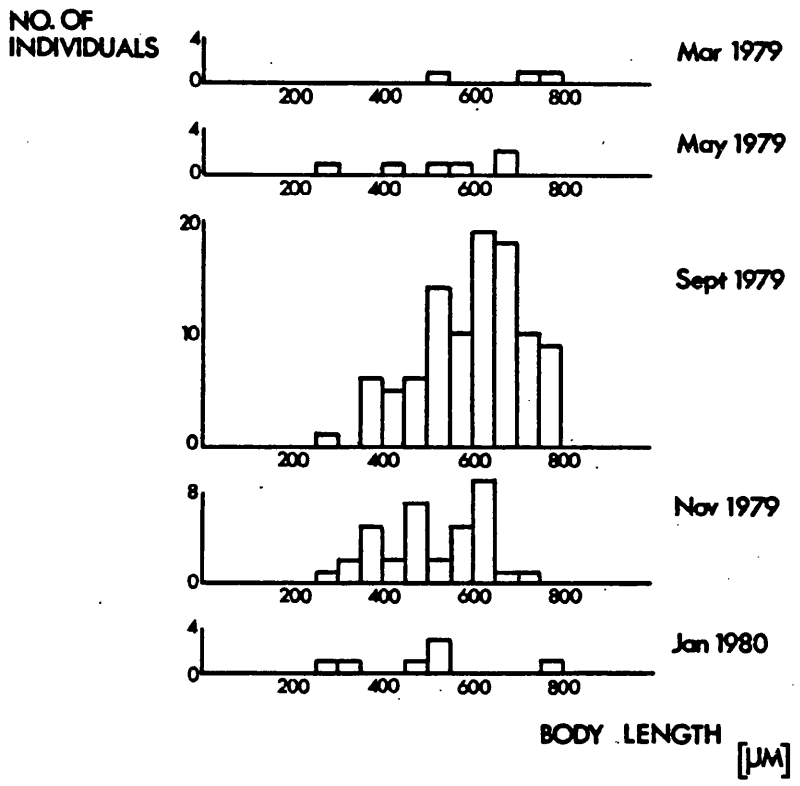


FIG. 4.12. Length-frequency histograms for Monhystera refringens in the different bimonthly samples.

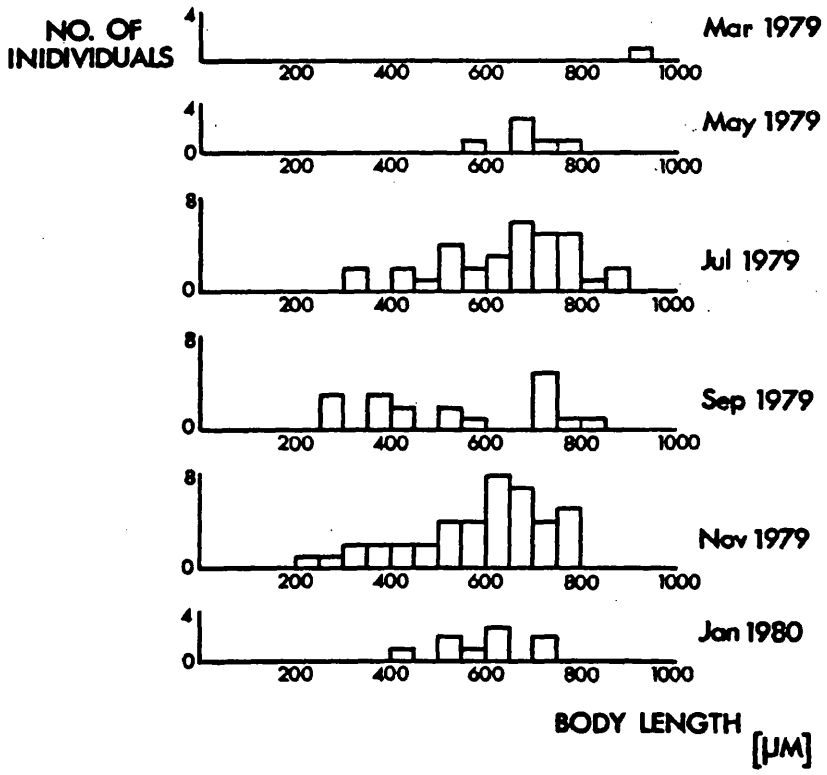


FIG. 4.13. Length-frequency histograms for Chromadora nudicapitata in the different bimonthly samples.

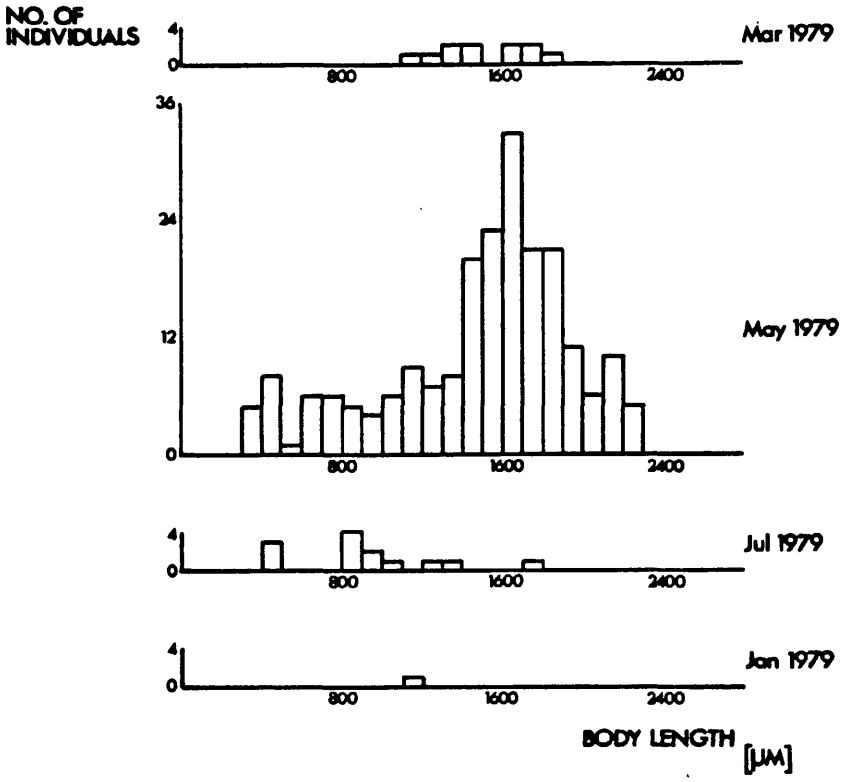


FIG. 4.14. Length-frequency histograms for Theristus acer in the different bimonthly samples.

SPECIES	MONTH	JUV.	NON BR. ♀	BR. ♀	♂
M. disjuncta	March 1979	23	23	32	8
	May 1979	1	11	11	3
	July 1979	0	1	1	0
	September 1979	0	1	0	1
	November 1979	27	36	54	13
	January 1980	40	34	20	12
M. refringens	May 1979	1	3	2	0
	September 1979	32	20	45	1
	November 1979	9	16	8	1
	January 1980	2	4	1	0
C. nudicapitata	March 1979	0	0	1	0
	May 1979	0	2	4	0
	July 1979	5	12	4	12
	September 1979	7	1	5	7
	November 1979	9	16	6	12
	January 1980	1	3	2	3
Theristus	March 1979	0	11	0	0
	May 1979	49	56	39	69
	July 1979	7	6	0	0
	January 1980	1	0	0	0

TABLE 4.9. Numbers of individuals in each breeding category (juveniles, immature females, breeding females, males) in different bimonthly samples.

difference only in the case of M. disjuncta which had a lower proportion of juveniles in the May 1979 sample than in the other samples. T. acer showed a different seasonal pattern. In March 1979 only immature females were present; immature and mature females, males and juveniles were found in May 1979, and in July 1979 only juveniles and small immature females remained.

Sex ratios, given by: $\frac{\text{No. of immature } ♀ + \text{no. of mature } ♀}{\text{No. of } ♂}$ were calculated from the data in Table 4.9. for each species for each bimonthly sample. G-tests showed that seasonal variation in sex ratio was not significant at the 1% level for M. disjuncta, M. refringens or C. nudicapitata, so data from the different bimonthly samples were pooled and overall sex ratios calculated for each species. The G-test was then used to test these overall ratios for deviation from a 1:1 ratio. The results are shown in Table 4.10. In C. nudicapitata the deviation from 1:1 is not significant at the 1% level; in the two Monhystera species it is highly significant.

SPECIES	SEX RATIO	PROBABILITY (G-TEST)
M. disjuncta	6.1	< 0.005
M. refringens	49.5	< 0.005
C. nudicapitata	1	$0.025 > p > 0.010$
T. acer	1.4	$p \simeq 0.025$

TABLE 4.10. Sex ratios for the four species and results of tests for deviation from a 1:1 ratio.

It can clearly be seen from Table 4.9. that T. acer does show seasonal variation in sex ratio. The ratio shown in Table 5.10. for this species is derived from the May 1979 sample only.

For each species, separate length-frequency histograms were constructed for each disc and decomposing tissue sample within each bimonthly sample. In many cases the numbers involved were very small, but there were no obvious differences in location or skewness between the histograms within one bimonthly sample. This suggests that at this scale there is little patchiness in size structure of a nematode population.

4.2.11. Gut contents analyses

(a) M. disjuncta

The gut of this species never contained diatoms or any other plant material. Occasionally small sediment particles were present, more often oil droplets and what might have been bacteria.

(b) M. refringens

The gut of this species often contained material similar to that described above for M. disjuncta. In addition, diatoms and diatom frustules, usually intact, and pieces of other plant material, were often present. Taken over all the samples:

8.1%	of the M. refringens	had 1 diatom in the gut
2.7%	"	2 diatoms in the gut
6.7%	"	3 or more diatoms in the gut
2.6%	"	intact but empty diatom frustules
2.6%	"	diatom fragments
4.0%	"	amorphous green matter
3.0%	"	other objects
70.5%	"	none of these

These percentages add up to more than 100% because some individuals had for example, diatoms and "other objects" in the gut.

The diatoms found in the gut were all from the smaller end of the size range of diatoms found on the frond (see sections 3.5., 3.7., 3.9. and 3.10.). They were mostly Navicula-like diatoms of about 25x5µm in size.

One Nitzchia was seen, and the largest of the Navicula-like diatoms was ∿35x5μm. The maximum number of diatoms seen in the gut of one individual was nine. The category "other objects" includes apparently colourless filaments about 4μm in diameter of which lengths of up to 30μm were seen; these resembled Leucothrix filaments (see section 3.10.). Also in this category were flattened spherical objects ∿5μm in diameter, which might have been colourless flagellates. The smallest M. refringens to have any of these recognizable objects in the gut was 490μm in length. About half the objects seen were in the posterior half of the gut, often just in front of the anus; about half were in the anterior half of the gut. Many of the objects appeared to be undergoing digestion, the cells often being ruptured and the chlorophyll degrading. It is of course possible that these objects were partly degraded before ingestion. On a few occasions apparently undamaged diatoms were seen in the posterior part of the gut. There was no apparent seasonal variation in the type of gut contents or in the proportion of individuals with recognizable objects in the gut.

(c) C. nudicapitata

Taken over all the samples, 41.3% of the C. nudicapitata had green matter in the gut. Much of this material was amorphous, but some appeared to be the remains of chloroplasts and of algal filaments. One intact diatom frustule was seen, as well as several colourless spherical objects which might have been flagellates. Green matter was seen in all parts of the gut, and the smallest individual in which it was noticed was 370μm long.

(d) T. acer

The guts of most individuals of this species in the May 1979 sample contained oil droplets, objects which appeared to be rod-shaped bacteria 1-1.5μm long, and unrecognizable amorphous material. 2.6% of individuals had sediment particles in the gut, and 3.2% had recognizable flagellates,

the flagella being visible in many cases. Small diatoms were seen in two individuals, and amorphous green-brown material in a few others. All these objects were present in both the anterior and posterior parts of the guts.

4.3. DISCUSSION

4.3.1. Previous records of the four abundant species

References are only given for records of particular relevance to this study. Other information is derived from the records listed by Gerlach and Riemann (1973, 1974).

Monhystera disjuncta

Much of the literature on this species is reviewed by Chitwood and Murphy (1964). It is widely distributed, having been recorded from the N. Sea, Baltic and Atlantic, and from Chile, the Falkland Islandsand Campbell Island. It also occupies a wide range of habitats: sandy tide pools, mud at 300-400m depth in fjords, decaying algae, littoral algae, Zostera fronds, between the pereiopods of amphipods and on the egg capsules of crabs. Some of the records from algal habitats are of particular relevance to the present study. Otto (1936) found M. disjuncta on decaying algae. Chitwood & Murphy (1964) describe how when blades of kelps, particularly Laminaria sinclairii, were left to rot in tanks a slime developed on the sides of the tanks and the remnants of the blades, and in this a monospecific bloom of M. disjuncta occurred. Wieser (1951) studied the nematode fauna of eight species of algae growing at Plymouth and found M. disjuncta only on Fucus serratus which was overgrown with the filamentous brown alga Elachista fucicola or with the hydroid Dynamena pumila and the bryozoan Electra pilosa.

Monhystera refringens

This species has been recorded only from marine algae. Bresslau and Stekhoven (1940) discovered it in the filamentous algae Cladostephus (Phaeophyceae) and Cystoclonium (Rhodophyceae) at Helgoland. Wieser (1951), in the study mentioned above, found M. refringens on the red alga Ceramium sp. and on Fucus serratus overgrown with Elachista, its vertical range being from Chart Datum to 4m above. Wieser (1959a) and Warwick (1977) recorded it from littoral algae, Wieser from Chile and Warwick from fine filamentous algae in the Scilly Isles.

Chromadora nudicapitata

Like M. disjuncta this species appears to be almost cosmopolitan, with records from the N. Sea, Atlantic, Mediterranean, Black Sea, Japan, Chile, the Falkland Islands and Campbell Island. Most records are from algae or from coarse sands in either the littoral or the shallow sublittoral. Wieser (1951) found C. nudicapitata on all the algae he examined, and it was also present in Chile on all algal growthforms except in the holdfasts of the large brown algae (Wieser, 1959a). Both in Chile and in the Mediterranean, Wieser (1959a, 1959b) found that in exposed situations C. nudicapitata was replaced by a species he identified as Chromadorina laeta [Inglis (1962) considers this a species inquirenda]. At Plymouth "C. laeta" was absent and C. nudicapitata extended its distribution into apparently more exposed situations (Wieser, 1951). Ott (1967) recorded C. nudicapitata from all parts of Cystoseira spicata plants in the Adriatic and found that on C. abrotanifolia it was more abundant at a sheltered, than at an exposed, site.

C. nudicapitata was present on most of the fine filamentous weeds from the Scilly Isles studied by Warwick (1977).

Theristus acer

This species also has a wide distribution: N. Sea, Baltic, Atlantic and Mediterranean, the Bay of Bengal, the Falkland Islands and Campbell Island. There are records from both sediment and algal habitats. The sediments include muds and sands from the supralittoral to 30m depth, but a high proportion of the records are from "mixed habitats", e.g.: "under stones amongst Spirorbis tubes" (Southern, 1914, as Monohystera acris), "shell sand with young Mytilus and algae" and "Much mud with some sand, tubes of annelids and young Mytilus" (both Stekhoven, 1935). Some records are from brackish water. The algal records came from a variety of algal growth forms. Bresslau and Stekhoven (1940) found this species on the filamentous algae Rhodocorton (Rhodophyceae), Cladostephus (Phaeophyceae) and Cladophora (Chlorophyceae) at Helgoland. Wieser (1951) recorded it from Gelidium, Fucus, Ceramium and Gigartina at Plymouth, Gerlach (1965) from Fucus and filamentous brown algae in Spitzbergen, and it was present on most of the fine filamentous algae, from the Scilly Isles, studied by Warwick (1977).

These four species are thus well known as inhabitants of marine algae and have been recorded from a wide range of algal growth forms. In trying to identify the ecological requirements of M. disjuncta, C. nudicapitata and T. acer living on L. saccharina fronds it must be borne in mind that these requirements can also be met in certain sediments, and in the case of M. disjuncta also by life in association with crustaceans. C. nudicapitata appears to have a preference for sheltered situations; the Wishing Well is relatively sheltered (see 2.12.) but no nematode samples were taken from more exposed sites in the region.

4.3.2. The niches of the four common species

In this study the most obvious feature of the spatial distribution of nematodes on the fronds was that they were almost completely confined to the frond tip. Within the tip region, differences in absolute numbers

of nematodes and in the relative proportions of the four species between replicate discs, between replicate plants and between plants of different year groups were small and, where they could be tested, usually not significant at the 1% level. Differences between bimonthly samples were much greater, and generally were significant at the 1% level, i.e. the fauna showed considerable seasonal variation.

There is a major difference between the ecology of T. acer and that of the other three species. T. acer was present on the frond for only part of the year, it was found predominantly in the surface samples, and the breeding structure of its population was not constant; it is also much larger than the other species. All these features can be related to the dependence of T. acer on the ectocarpoid filaments and their associated material. The other three species were present more or less throughout the year and were found in both the surface samples and the decomposing tissue samples. Each of the three had its own seasonal peak of abundance but none ever equalled the numbers achieved by T. acer at its peak. All three species are of much the same size and they appear to breed continuously. It is more difficult to correlate their spatial and temporal distribution with those of particular epiphytes. These three species can be considered to be the true, permanent fauna of the fronds, as distinct from T. acer which is an inhabitant of the epiphytic ectocarpoid filaments.

T. acer first appeared on the fronds in the March 1979 sample when a small number of immature females 1100-1900µm in length were found. The ectocarpoid filaments had started their rapid growth in February (see section 3.6.) and by March filaments up to 1.5mm long were present. With, on average, two filaments per clump having grown rapidly, and a clump density of $\sim 5mm^{-2}$, these filaments produced a tangled network standing up from the frond surface to a height of $\sim$0.5mm. By May 1979 the longest filaments were 7-8mm long, giving a network standing up to

a height of 3-4mm, within which the material described in 4.8. had accumulated. Many more T. acer were present, the mean density in the disc samples being $3.6cm^{-2}$, and these included mature individuals of both sexes, up to 2300μm long. The appearance of males indicated that there had been further colonization as well as growth of the individuals present in March. Breeding had commenced and juveniles 350-1600μm long were found. By July 1979 the ectocarpoid filaments had died and the material around them was dispersing. Only a small number of juvenile and immature female T. acer in the size range 400-1400μm were present in the sample, some of them in the decomposing tissue.

The occurrence on the frond of T. acer thus coincided exactly with the period when the ectocarpoid filaments grew rapidly, and this strongly suggests a causal relationship. It seems likely that the network of filaments provided a habitat within which nematodes the size of T. acer could move. When the filaments were still in the early stages of growth only small T. acer could colonize the frond, but as the filaments grew, larger, mature individuals appeared. Wieser (1951, 1959a) found that the nematode faunas of the foliaceous algae Nitophyllum punctatum and Porphyra laciniata consisted almost entirely of small species less than 1500μm in length, and suggested that this was because on these algae nematodes are dependent on epiphytes for the possibility of locomotion, and that the epiphytes present (which he did not describe in detail) were too small to allow the locomotion of any but the smallest nematodes. The other three species abundant on L. saccharina fronds fall into this size category. Apart from the ectocarpoid filaments the only epiphytes which stood up from the frond surface to any extent were the stems of the hydroid Obelia geniculata (see section 3.8.), and these were never present at a high enough density to form a network in the way that the ectocarpoids did. The situation on L. saccharina fronds thus appears to be the reverse of that found by Wieser (1951) on Fucus serratus, where a growth of the

hydroid Dynamena pumila and the bryozoan Electra pilosa (the growth forms of which are not unlike those of O. geniculata and Membranipora membranacea which were found together on the L. saccharina frond in the late summer and autumn) produced a nematode fauna with a higher proportion of larger species than did a growth of the filamentous epiphytic alga Elachista fucicola (which in growth form is not unlike the ectocarpoid filaments).

The colonization of the fronds by T. acer implies that there was a source population in some other habitat. T. acer was again abundant on the fronds in May 1980, which suggests that these migrations are a regular feature of the biology of this species. The location of the source population is not known. Warwick (1977) found that in the Scilly Isles T. acer was present on the littoral algae Asparagopsis armata and Falkenbergia rufolanosa throughout the year, with in some years a peak of abundance in the period May-July. Such a peak in the population density on other algae might lead to emigration which coincided with the availability of the ectocarpoid filament habitat on L. saccharina fronds. On the other hand, the peak observed by Warwick could have been the result of emigration from yet another habitat, possibly a sediment. Samples taken in May 1979 from the algae Polysiphonia urceolata and Ectocarpus sp. growing on boulders in the kelp zone at the Wishing Well did not contain T. acer.

It appeared that only one cycle of breeding took place on the Laminaria fronds. This agrees with the generation times measured by Gerlach and Schrage (1971) for the closely related T. pertenuis Bresslau and Stekhoven. Cultures derived from individuals collected with Laminaria holdfasts from Helgoland were maintained on seawater agar and showed a generation time of 71 days at 7°C and 47 days at 9-12°C, (these are the closest of their experimental temperatures to those encountered at the Wishing Well during the period in which T. acer was present on the fronds).

Further deductions about the niche of T. acer can be made from the results of the gut contents analysis, taken together with the structure

of the buccal cavity. Living among the ectocarpoid filaments, T. acer is presented with the complex material described in section 3.7., a mucilage matrix containing bacteria, flagellates, a variety of diatoms, sediment grains, faecal pellets and ciliates. T. acer has an unarmed buccal cavity, ∿ 6µm wide x 2.5µm deep in a juvenile of body length 800µm, 10µm wide x 4µm deep in an adult 1800µm long. The gut rarely contained recognizable plant material, so it does not seem that diatoms or the ectocarpoid filaments themselves were an important food. Flagellates and what appeared to be rod-shaped bacteria were regularly seen, but were often in an apparently undigested state even in the posterior gut. T. acer might have been selectively ingesting these organisms and digesting them. Alternatively the mucilage matrix could have been ingested unselectively together with all particles in it small enough to pass into the gut. In this case either the mucilage itself, or the particles contained in it, or both, could have been digested.

M. disjuncta was the most abundant nematode on the frond in both winters, i.e. in the samples taken in January and March 1979 and again in November 1979 and January 1980. In the disc samples, numbers were higher in March and November 1979 than in either January 1979 or January 1980. There was no large difference in the relative abundance of this species between the surface and the decomposing tissue samples except in May 1979, when it was still dominant in the decomposing tissue, but formed only a small proportion of the nematodes on the frond surface samples. If, however, the growth of the ectocarpoid filaments had not allowed T. acer to colonize the frond, M. disjuncta would have been the most abundant nematode on the surface as well as in the decomposing tissue. No epiphytes reached their seasonal peak of abundance during this winter period, but clues to the niche of this species come from the gut contents and the structure of the buccal cavity. The buccal cavity is unarmed and small, about 2µm wide x 4µm deep in an adult of body length 750µm, and as would be

expected, no large objects were ever seen in the gut. On the frond surface the only potential food would be perpendicularly attached rod-shaped bacteria and occasional flexibacteria, together with mucilage derived from the Laminaria (see section 3.4.). The two previous records of this species from decomposing algae (see above) raise the possibility that it is in the areas of decomposing tissue that M. disjuncta feeds. Potential foods in these areas were: rod-shaped bacteria of various types at higher densities than on the frond surface, filamentous bacteria such as Flexibacter and Leucothrix, and the decomposing Laminaria tissue itself, or at least dissolved organic matter and cell contents leaking from it (see section 3.10.).

M. refringens reached its maximum number in the September 1979 sample, where it was the most abundant nematode in both the surface and the decomposing tissue samples. It was in this sample that the decomposing tissue reached its maximum extent, reaching back for a considerable distance from the frond tips (see section 3.2.). Membranipora colonies also reached their maximum abundance in September 1979, but the maximum Membranipora cover was well behind the frond tip (see section 3.9.). M. refringens was most abundant at the frond tip, which suggests that it was associated with the decomposing tissue rather than with Membranipora. Even an association with the oldest bryozoan colonies which are found nearest the frond tip and in which dead zooids, with their distinctive community of diatoms and ciliates, were most abundant would not explain the observed distribution of M. refringens. M. refringens has a buccal cavity which is larger than that of M. disjuncta, 5µm wide x 3µm deep in an adult of body length 700µm, and is cup-shaped rather than elongate, and larger particles were seen in the gut of this species. While diatoms of the type seen in the gut were particularly abundant in dead bryozoan zooids, they were also present on the decomposing tissue (see section 3.10.), and in view of the distribution of M. refringens it seems that the nematodes

were obtaining them from this microhabitat. The Leucothrix-like filaments and the flagellates which were seen in the gut could also have been obtained in this microhabitat. It seems unlikely that a nematode could feed on such organisms from the decomposing tissue without also ingesting large numbers of rod-shaped bacteria and considerable amounts of Laminaria cell contents and organic matter derived from the Laminaria tissue. The Licmophora sp. which was abundant on the intact frond surface at all times of year was never seen in the gut of M. refringens. Leucothrix, small diatoms and flagellates were present in smaller quantities in the decomposing tissue throughout the year.

C. nudicapitata reached its maximum relative abundance in July 1979 and its maximum absolute abundance on the frond surface in September and November 1979. On the frond tip in July dead ectocarpoid filaments were present and small amounts of the mucilage material remained around their bases. The situation in September was described above, in the section on M. refringens. Like most chromadorids, C. nudicapitata has a buccal cavity with three small teeth and a set of cuticular pads (see, for example Deutsch, 1978). Previous observations have suggested that these are used to break open or pierce algal cells from which the contents are then sucked (see discussion below). The absence of cell walls and diatom frustules from the gut despite the regular occurrence of plant material provides further evidence that this is the method of feeding, and also makes it difficult to identify precisely which foods were being taken. One obvious potential food is diatoms, both those associated with decomposing tissue and bryozoan zooids (see sections 3.10. and 3.9.) and the Licmophora which grew on the intact frond surfaces. One intact Navicula frustule was found in the gut. Other possible sources of the plant material seen in the gut were epiphytic ectocarpoid filaments, which were present at least in an early stage of growth for most of the year (see section 3.6.), and the Laminaria tissue itself. It seems unlikely that, even with its

teeth, C. nudicapitata could feed directly on an intact Laminaria surface, but food might be obtained from the broken surfaces of decomposing regions. The few flagellates apparently present in the gut are most likely to have come from decomposing regions.

The three smaller species on the L. saccharina fronds appeared to breed continuously. Most of the studies of generation times in small monhysterids and chromadorids have been carried out at higher temperatures than are relevant to the present study. For example, Tietjen (1967) found a generation time of 24-35 days for Monhystera filicaudata growing at 20-25°C, and Tietjen and Lee (1973) a generation time of 22 days for Chromadora macrolaimoides at 25°C. One study at a relevant temperature showed M. disjuncta living on seawater agar to have a generation time of 17 days at 9-12°C and 22 days at 7°C (Gerlach and Schrage, 1971). The sex ratio in C. nudicapitata (like that in T. acer) was close to 1:1; this suggests that reproduction was bisexual and amphimictic. In the two Monhystera species, on the other hand, females greatly outnumbered males. Various types of hermaphroditism and parthenogenesis are known among free-living nematodes (see discussion in Nicholas, 1975), and it seems at least possible that in these Monhystera species reproduction is hermaphroditic, usually automictic, with occasional amphimixis. Such a breeding pattern might conceivably lead to a higher reproductive rate.

Most members of the family Monhysteridae (sensu Lorenzen, 1978) have either no teeth or very small teeth in the buccal cavity and it seems that the food must therefore be ingested whole. This was confirmed by Deutsch (1978) who compared the ingestion of foods in Diplolaimella sp. (Monhysteridae) with that in Chromadorina germanica Butschli (Chromadoridae), a species with teeth similar to those of Chromadora nudicapitata. One would then expect that the maximum size of food particles ingested by a monhysterid would be limited by the size of the buccal cavity or oesophagus. The observations on the two Monhystera spp. support this hypothesis; M. refringens has the larger buccal cavity, and larger objects were seen

in its gut. T. acer, on the other hand, has a much larger buccal cavity than does M. refringens and yet nothing larger than a flagellate was ever seen in the gut, so the relationship between buccal cavity size and maximum food size in this family is not simple. The foods seen in the guts of these three species are similar to those on which monhysterids have previously been observed to feed. Tietjen (1967) observed M. filicaudata Allgen feeding in the laboratory on "detritus", bacteria and dinoflagellates. Jennings and Deutsch (1975) demonstrated the presence in the gut of M. denticulata of the enzyme β-glucuronidase, which can attack important components of bacterial and algal cell walls; this enzyme was absent from the gut of C. germanica. Deutsch (1978) found that Diplolaimella sp. would ingest bacteria and small chlorophytes.

There is similar evidence that chromadoroids feed mainly in the way mentioned above in the section on C. nudicapitata. The combination of pads and teeth seems well adapted for this mode of feeding (Deutsch, 1978), although an alternative function, that of sorting mucus threads, was suggested for the similar mouthparts of sediment-living chromadoroids by Riemann and Schrage (1978). Deutsch (1978) found that C. germanica did not ingest the cell walls of the diatoms and large chlorophytes on which it fed in the laboratory. Tietjen and Lee (1973) observed that when Chromadora macrolaimoides Steiner fed on large diatoms the cell walls were not found in the gut but, that diatoms of length 10-20μm were frequently ingested whole. The Licmophora sp. which was common on the fronds was in this size range but only one intact diatom frustule (a Navicula) was seen in a C. nudicapitata gut. Jennings and Deutsch (1975) found no β-glucuronidase in the gut of C. germanica, though this does not necessarily mean that another enzyme with similar properties was not present.

Wieser (1953) divided the free-living marine nematodes into four groups (selective deposit feeders, unselective deposit feeders, epistrate feeders and predators/omnivores) on the basis of buccal cavity morphology which he compared with the limited information then available on the gut

contents and feeding habits of nematodes of different taxonomic groups. This was valuable as a preliminary study, but the use of only four groups, even if the feeding habits assigned to these are basically correct, is bound to ignore a lot of variation and specialization within each group. There has been a tendency in subsequent ecological studies merely to assign species to Wieser's categories, producing rather unhelpful generalizations such as "unselective deposit feeders are more abundant in finer sediments". More detailed observations of microhabitats and of gut contents, further studies of gut ultrastructure and digestive physiology (e.g. Deutsch, 1978) and further experimental studies (e.g. Tietjen and Lee 1973, 1977, and see also Chapter 5) are needed.

With the possible exception of C. nudicapitata which might have ingested only cell contents, none of the nematodes found on the L. saccharina fronds could have had anything approaching a monospecific diet. The scale of food patchiness is very small (see discussion below), most diatoms have bacteria on their surfaces, most epiphytes are surrounded by Laminaria mucilage, and dissolved organic matter is presumably present in the decomposing areas. This means that studies in which a nematode is cultured monoxenically with each of a series of food organisms, and growth or reproductive rates achieved on each food organism compared, may have little ecological relevance (see e.g. Lee et al 1976; Tietjen et al 1970).

Another source of confusion is the way in which Wieser (1959a, 1959b) and, following him, Ott (1967) use the term "sediment" to mean "all loose organic and inorganic matter deposited on algae" (Ott 1967). This use can easily obscure major differences such as those between the material found in Laminaria holdfasts, which does contain a large proportion of inorganic particles, and the material accumulated around ectocarpoid filaments in this study, which consisted mainly of mucilage, diatoms and bacteria. It would seem preferable to reserve the term "sediment" for material on the sea bed and to give a careful description at the microscopic level of

any material associated with algae.

The discussion above showed that consideration of the spatial and temporal distribution of the different species on the frond can provide some indication of the niche of each species. Most of the frond-surface epiphytes reached their maximum abundance at the frond tip, and the decomposing tissue was also concentrated in this region. It would therefore be expected that if nematode density was for any reason correlated with the density of any of these epiphytes, it would also reach a maximum at the frond tip. The reason for the complete absence of nematodes from the rest of the frond, despite the presence of epiphytes even if in lower numbers, is however not clear. Possibly a minimum density of epiphytes is necessary for nematode locomotion; possibly inhibitory substances deter nematodes from living on the younger parts of the frond. It may be that nematodes are physically capable of living on more of the frond but that they have evolved a behavioural mechanism to keep them at the frond tip where food is most abundant. This could be a response to chemical factors, similar to that which results in selection of particular species of algae by certain harpacticoid copepods (Hicks, 1977). If the population density on the tip had reached a higher level, some individuals might have moved onto the younger parts of the frond.

Within the tip region of one plant there was little variation between replicate discs in either the absolute number of nematodes or in the relative abundance of the different species. The discs were relatively large (4.2cm diameter) and the question arises whether patchiness in the distribution of epiphytes gives rise to patchiness in the distribution of nematodes at a smaller scale. Lee *et al* (1977) showed that presented with large patches of particular microorganisms, either in the laboratory or in the field, meiofaunal animals would preferentially colonize patches of certain species. Meyers and Hopper (1966) found that mycelial mats of two marine fungi placed within a seagrass bed attracted large numbers of gravid female *Metoncholaimus scissus* Wieser and Hopper. Meyers *et al* (1970)

also found that the distribution of this species within the sediments of the seagrass bed was correlated with the presence of mats of the diatom Pleurosigma baltica. In both these cases the patches of the microorganisms were large. On both the intact surfaces and the decomposing tissue of a L. saccharina frond, some epiphytes are distributed in patches. A "stand" of the diatom Licmophora, for example, is about 0.2mm in diameter, (see section 3.5.), while the diameter of a young clump of emergent ectocarpoid filaments is about 50μm (see section 3.6.). This compares with a body size of ∿1mm for the three smaller nematode species, and a maximum rate of movement which is probably of the same order of those recorded by Gray and Lissman (1964) for a Rhabditis sp. gliding between starch grains and for other species swimming, i.e. 200-500μm s^{-1}. A ciliate of body length 70μm might move at a speed of ∿200μm s^{-1}, and a flagellate of body length 10μm at ∿20-50μm s^{-1} (Alexander, 1979; personal observations). It might well be that the scale of epiphyte patchiness would be large enough to produce a heterogeneous spatial distribution of, for example, flagellates but too small to do so for nematodes. The body size of the three smaller nematode species may allow them to move all over the frond tip, both in the decomposing areas, where they burrow through the Laminaria tissue, and on the surfaces, where epiphytes permit locomotion. Each species could then consume its own particular foods wherever it encountered them. Microscopical observations of nematodes moving around on a piece of frond in the laboratory might provide useful information on these behavioural aspects.

Similar consideration of scale apply to the temporal aspects of distribution. No species consistently formed a higher proportion of the nematodes in the decomposing tissue than on the frond surface. In contrast to this, the decomposing tissue does have distinct flagellate and ciliate faunas. No section of Laminaria tissue will remain in the decomposing state for more than a few days before it is decomposed completely or lost from the frond (see section 2.2.). Zooflagellates of the type observed

in this tissue might have a generation time of 20-25h (estimated from data in Fenchel and Harrison, 1976); ciliates such as Euplotes can reproduce asexually every 30h at 10°C (Fenchel, 1968a). The populations of these protozoa probably achieve a reproductive rate high enough to offset losses caused by the loss of decomposing tissue from the frond. Their asexual reproduction also means that all stages in the life cycle are motile and could have evolved behavioural mechanisms for remaining on the frond. In M. disjuncta, Gerlach and Schrage (1971) found that with a generation time of 22 days at 7°C, development of the eggs took 5 days. It is unlikely that such an animal could become a specialized inhabitant of decomposing tissue because (i) even if reproduction is usually hermaphroditic or parthenogenetic (see discussion above), the generation time would be too long to produce a reproductive rate high enough to offset inevitable losses, and (ii) there is a long non-motile stage in which no behavioural adaptations for remaining on the frond could evolve.

Another matter which involves considerations of temporal scale is the form of the response shown by a nematode population to changes in the abundance of an epiphyte on which it is dependent. No change in nematode numbers will be produced if other factors keep the population below the level at which any limiting effect of epiphyte abundance would begin to act. If the population density was high enough, epiphyte abundance could limit the population size in a density-dependent fashion, so that an increase in the abundance of the epiphyte would raise, and a decrease lower, the carrying capacity of the habitat for that particular nematode species. The nematode population could respond to such a change by a change in the rate of immigration or emigration, reproduction or mortality. If the nematode was dependent on the epiphyte for food, an increased number of nematodes could then cause a decrease in the numbers of the epiphyte, and in the absence of other factors controlling epiphyte abundance some form of predator-prey oscillations might develop. Other factors controlling the

epiphyte population, particularly physical environmental factors, are however likely to be present. The change in nematode numbers which is observed will thus depend on the nature of the dependence of the nematode on the epiphyte, and if feeding is involved, on the extent to which the epiphyte population is reduced by grazing, also on the duration of the "bloom" of the epiphyte, which will at least in part be determined by the physical environment, and finally on the time-scale of the response of the nematode population. This last will depend on whether the means of response is immigration/emigration or reproduction/mortality, and, if reproduction is involved, on the reproductive rate.

T. acer was clearly dependent on the ectocarpoid filaments for the possibility of locomotion. It is uncertain whether, if it had been able to remain on the fronds in the absence of the filaments, this species would have found food at other times of year; the mucilage and rod-shaped bacteria on the frond surface might have been suitable. The period during which elongated ectocarpoid filaments were present lasted for about four months. The initial response of the nematode population was of necessity by immigration; and taken overall, immigration was the major means of response, continuing for two months before juveniles were produced.

In the cases of the other three species there was no such obvious correlation with a particular epiphyte, and the seasonal fluctuations in numbers were much smaller than for T. acer. As suggested above, it seems likely that many of the epiphytes found on the frond are of such a size as to permit locomotion of the three smaller species. It therefore seems unlikely that fluctuations in the abundance of any one epiphyte will significantly alter the carrying capacity of the frond for any of these species through an effect on their locomotion. Any limiting effect of the abundance of a particular epiphyte on the abundance of one of these species is likely to occur because the nematode feeds on the epiphyte, and the mechanism of limitation is likely to be depression of the reproductive

rate (limitation through an effect on locomotion would be more likely to act by forcing the emigration of surplus individuals). The nematode's response to an increase in the abundance of the epiphyte is thus likely to be principally an acceleration of reproduction. There is no evidence for immigration by any of these species, though it cannot be ruled out, particularly in the case of C. nudicapitata, which is known to live on a wide variety of algae. The duration of blooms of those epiphytes which do show seasonal fluctuations [Leucothrix, the amount of decomposing tissue (which can be considered as an epiphyte for the purposes of this discussion), Membranipora] is of the order of two months. There have been no experimental studies of the response times of nematode populations to changes in their food supply, but it seems likely that an increase in the food supply would reduce the time taken to reach maturity, or increase the rate of egg production by mature individuals. In this case a response might be noticeable within perhaps one month. If an increase in the abundance of an epiphyte was seen in one bimonthly sample, any increase in the abundance of a nematode dependent on it would therefore appear in the same bimonthly sample, or in the following one. It was thus reasonable to attempt to identify relationships between particular nematode and epiphyte species by comparing abundances within the same bimonthly sample. None of the epiphytes on which the smaller nematodes were dependent showed such marked seasonal fluctuations in abundance or size as did the ectocarpoid filaments, and this could partly explain why the fluctuations in the numbers of these nematodes were smaller than those of T. acer. Also, the duration of blooms of these epiphytes was only $\sim$2 months, and because breeding was the principal means of response, the nematode populations may never have reached the higher carrying capacity at the height of an epiphyte bloom.

An alternative explanation for the seasonal fluctuations in the numbers of these three species could be sought in their responses to environmental factors such as water temperature and day length. The effects

of these could easily be tested provided that the species could be cultured. Even if an explanation in terms of these factors was found, the question would remain as to why the different species had evolved different responses to them.

In general, sediment nematode faunas (see, for example, Tietjen 1969; Warwick and Buchanan, 1970) are more diverse than algal nematode faunas (see Wieser, 1951, 1959a; Warwick 1977), and among algal faunas, those from habitats where sediment (see comments on the use of this term) accumulates, such as kelp holdfasts (Moore, 1971), are perhaps more diverse than those from other algal habitats. Algal faunas also tend to show greater seasonal fluctuations in absolute numbers and in relative species abundance than do sediment faunas, as can be seen by comparing the present study and that by Warwick (1977) with those by Warwick and Buchanan (1971), Tietjen (1969) and Skoolmun and Gerlach (1971). Algal nematodes live in a habitat with seasonally variable physical structure and are dependent on a seasonally variable food supply. The physical structure of a sediment habitat is much more stable, and sediment nematodes are part of a detritus food web in which the slow turnover of organic matter to a large extent damps down the effect of seasonal fluctuations in its input. The greater seasonal stability of sediment nematode faunas can be explained as a short-term consequence of greater habitat stability; the greater diversity may well be a consequence on an evolutionary time-scale, the greater habitat stability allowing specialization on narrower niches, and hence the evolution of a greater number of species.

CHAPTER 5

EXPERIMENTS ON FEEDING IN MONHYSTERA DISJUNCTA

5.1. METHODS

5.1.1. Isolation and examination of bacteria

On 8th February 1978 one L. saccharina plant was collected from the Wishing Well in the way described in Chapter 3. About 6g consisting mainly of decomposing tissue was cut from the frond tip with sterile scissors. This was homogenized (4 bursts of 30s with 1 min. cooling time between bursts) in 40cm^3 sterile 3.2% NaCl solution, in a bench homogenizer (MSE, ref. 7700A), the bulb of which was surrounded with ice. Serial dilutions (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}) of the homogenate were made, again using sterile saline. From each dilution 4 plates were prepared by spreading 0.1cm^3 over the surface of a 9cm plate of Zobell's 2216 marine agar (Difco Laboratories). The plates were incubated at 20°C for 4 days. After this time the plates from the 10^{-4} dilution each had ∿100 well separated colonies. Five colonies of obviously different morphology were picked off and plated out separately on marine agar. Repeated subcultures were made until pure strains had been obtained. Stock cultures were maintained on slopes of marine agar, kept in a refrigerator and subcultured every 3 months. These five strains were named ML01, ML03, ML04, ML05 and ML07.

The colony morphology of each strain was described from cultures grown on marine agar for 1 week at 20°C. Cell size and shape were described from smears made from the same cultures and stained with methylene blue in seawater; no heat fixation was used. Gram stains were performed on other smears from the same cultures.

5.1.2. Broth cultures of bacteria

(a) Unlabelled bacteria

50cm^3 broth cultures were grown in 100cm^3 conical flasks on a magnetic stirrer in an incubator at 22°C. Before autoclaving the media a magnetic "flea" was put into each flask. Two precautions were taken to avoid overheating of the cultures: compressed air was passed continuously over the stirrer motor, and a 2cm thick disc of expanded polystyrene with a depression to fit the base of the flask cut into it was placed between the flask and the stirrer.

Strain ML01 was used in tests to find a suitable liquid medium. Three media which proved unsuccessful were:

(i) glucose medium

glucose	0.5g
K_2HPO_4	0.005g
$FePO_4$	trace
seawater	75cm^3

distilled water to 100cm^3. pH adjusted to 7.0-8.0

(ii) glycerol medium

As for glucose medium but with glucose replaced by 0.5cm^3 glycerol.

(iii) glucose/amino-acid medium

As for glucose medium but with the addition of an amino-acid mix:

alanine	0.8g	methionine	0.06g
aspartic acid	0.9g	phenylalanine	0.04g
glutamic acid	1.0g	proline	0.04g
histidine	0.2g	tryptophan	0.05g
leucine	0.05g	tyrosine	0.04g
lysine	0.45g	valine	0.05g

All these quantities per 50cm^3 mix which was then used at 5cm^3 per 100cm^3 medium. The relative proportions of the different amino-acids are based on those given by Aaronson (1970).

The only medium in which ML01 grew was a peptone-phosphate broth:

Bacto Peptone (Difco)	0.5g
K_2HPO_4	0.005g
Fe_3PO_4	trace
aged seawater	$75cm^3$

distilled water to $100cm^3$. pH adjusted to 7.0-8.0

Strains ML03, ML04 and ML05 also grew satisfactorily in this medium. No liquid medium was found in which strain ML07 would grow satisfactorily.

For the feeding experiments it was decided to harvest the bacteria in the early part of the plateau phase of growth, when the cells were no longer actively dividing but before they had started to degenerate. To determine the growth time required to reach this stage, the growth of each strain in the peptone-phosphate broth was followed using both haemocytometer counts, to give the "total count", and plate counts on marine agar, to give the "viable count". The growth curves of the four strains were very similar; that for ML01 is shown in Fig. 5.1. as an example. Harvesting after between 16 and 20h will be satisfactory; after 24h the viable count began to drop.

(b) Labelled bacteria

The amount of K_2HPO_4 in the peptone-phosphate broth was reduced to 250mg (5% of the usual amount) and 1mCi of carrier-free ^{32}P-orthophosphate in dilute HCl (Radiochemical Centre, Amersham, Bucks.) was added to each $50cm^3$ of medium in its flask before inoculation with the bacteria. It can be calculated from the results presented below that 10-12% of the ^{32}P was incorporated into the bacteria, giving an activity of 0.01 dpm/cell.

5.1.3. Harvesting and washing bacteria

Bacteria were spun down from the broth cultures in a Heraeus Christ Simplex bench centrifuge with swing-out head, for 10 mins. at 3800 r.p.m. They were then washed eight times by pipetting off the supernatant, resuspending in 0.4μm Millipore filtered seawater (MFSW) and spinning

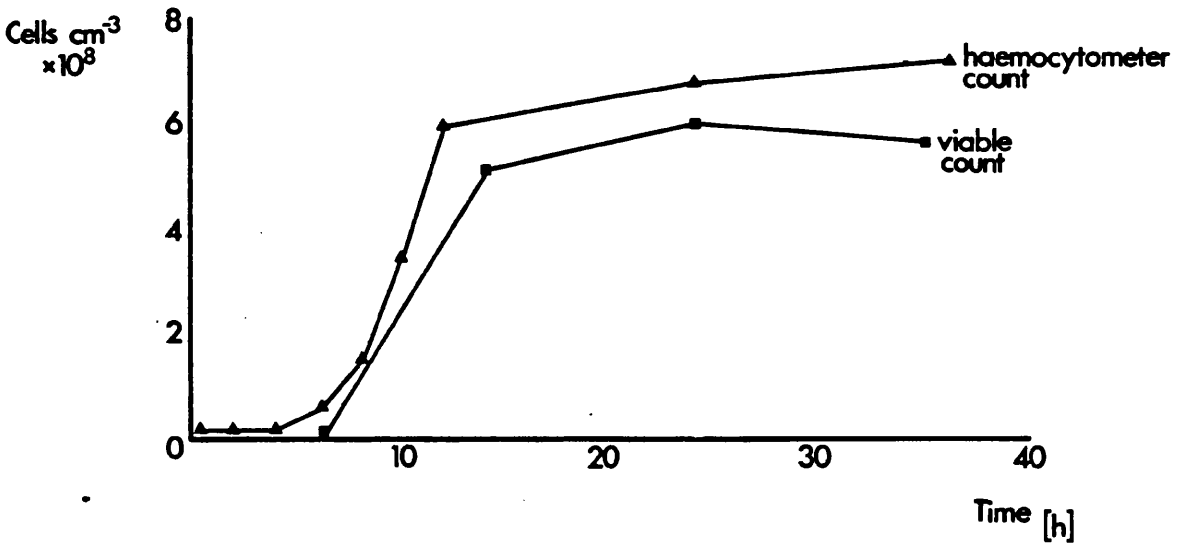


FIG. 5.1. Growth of ML01 in peptone-phosphate broth.

down again. After the final wash, serial dilutions were prepared, a haemocytometer count made on appropriate dilutions, and the cell density adjusted in preparation for presentation to the nematodes. In the case of labelled bacteria, Geiger-Muller counts were made on three appropriate dilutions (for method see section 5.1.7.).

5.1.4. Culture of nematodes

(a) Monhystera disjuncta

In October 1978 gravid females were picked out from the decomposing tissue on L. saccharina frond tips. They were narcotized with $MgCl_2$ and their identity checked under the compound microscope. They were then placed on individual 5cm plates of sloppy seawater agar with an overlay of filtered seawater (Whatman Gamma 12 0.3µm on-line filter). The best results were obtained with a 0.2% seawater agar with 1% of the nutrient concentration of Zobell's marine agar, which was made up as follows:

marine agar	0.055g
plain agar (BDH Ltd.,)	0.185g
filtered seawater	99cm^3
distilled water	1cm^3

If agar with a higher nutrient concentration was used, bacteria grew rapidly and the nematodes died, while on a stiffer agar most nematodes remained on the surface and those which did burrow often died within the agar. Initially a small piece of decomposing Laminaria tissue was added to each culture to provide an inoculum of suitable food microorganisms; the cultures were checked to see that no contamination with other nematodes had resulted from this. Bacteria, flagellates and ciliates were present in the cultures. Once cultures were established, subcultures could be made simply by transferring a small piece of the agar from an old culture, complete with nematodes and microorganisms, to the new plate. This was done every two months; the cultures were still healthy after two years.

(b) M. refringens and C. nudicapitata

Neither of these species survived for more than a few days under the conditions in which M. disjuncta was successfully cultured. An attempt was made to culture C. nudicapitata, both in sloppy agar and in seawater, by adding every three days a few drops of a suspension of the diatom Phaeodactylum tricornutum and the chlorophyte Dunaliella salina as a supplementary food source. These cultures survived for about 3 weeks, during which time one batch of eggs was laid and hatched, but the juveniles died before reaching maturity.

5.1.5. Harvesting and washing nematodes

M. disjuncta of body length 600-700μm were individually picked out from stock cultures with a sharpened quill. They were taken through two washes of MFSW before transfer to the experimental dishes.

5.1.6. Setting up feeding experiments

The experiments were carried out in 5cm diameter disposable plastic Petri dishes. A standard volume ($5cm^3$) of the bacterial suspension was used throughout. When a suspension in seawater was used, the nematodes were placed in $4.5cm^3$ MFSW in the dish and $0.5cm^3$ of bacterial suspension of 10x the required final cell density was added. Suspensions in agar were prepared by melting sterile 0.4% seawater agar (plain agar in 0.3μm Gamma-12 filtered SW), allowing it to cool to 40^oC, placing $2.5cm^3$ in a previously warmed dish and adding $2.5cm^3$ of a bacterial suspension of twice the required final cell density at 40^oC. Preliminary tests had shown that the viability of ML01 was not affected by a short period at this temperature. The nematodes were introduced when the agar had cooled. A bacterial density of $\sim 10^8$ cells cm^{-3} and a nematode density of 15 per dish were used throughout. The dishes were incubated at 20 ± 2^oC.

5.1.7. Geiger-Muller counting

No liquid scintillation counting equipment was available in Millport, so a G-M system was used. This consisted of a Mullard ZP1470 mica end-window G-M tube, a Panax "Reigate" Series counter (Modules GSA-11, TIM-10, SON-10 and PSU-13), a Panax PN1-6 printer and a Panax Betaplan 50 automatic sample changer. All samples were counted on 23mm internal diameter dimpled aluminium planchets (Gallenkamp).

For bacterial suspensions and filtrates, counts were made from serial dilutions (usually 10^{-3}, 10^{-4}, 10^{-5} were appropriate for the initial washed bacterial suspensions). A volume of $0.1cm^3$ was used and the planchets dried on a hot-plate before counting.

At the end of the incubation period in a feeding experiment, nematodes which showed normal active movements were removed from the dish with a sharpened quill and transferred to a drop of 4% formalin in seawater. Nematodes were then taken individually through six washes in Millipore filtered seawater and one brief rinse in distilled water, all transfers being made with clean pins. They were then placed in drops of distilled water on individual planchets and dried on the hot-plate. Preliminary tests had shown that killing in formalin led to no loss of label or of gut contents.

A counting time of 40 secs. was used throughout, and three or four replicate counts were made from each planchet. Background counts were recorded for each experiment.

The main problem with G-M counting, compared with liquid scintillation counting, is that it gives only a relative count. If the conclusions drawn below are to be valid, the efficiency of detection must have been the same when counting nematodes as when counting bacterial suspensions or filtrates. The main cause of differences in efficiency will have been self-absorption. This is less of a problem with a relatively high energy β-emitter such as ^{32}P (Emax 1.71 MeV)

than with, for example, ^{14}C (Emax 0.155 Mev). A dried M. disjuncta will present a thickness of $\sim$ 1mg cm^{-2}, while 0.1cm^3 of a bacterial suspension in seawater (a 10^{-4} dilution of an initial worked concentrate) will present a thickness of $\sim$ 17.5mg cm^{-2}. Reading these values off the nomogram given by Faires and Parks (1973) it appears that in the case of the nematode, self absorption will be < 1%, in the case of the bacterial suspension $\sim$ 5%. It is therefore probable that errors introduced by using the results without correction for self absorption will not be large.

5.1.8. Controls

Dead M. disjuncta were used as controls in all feeding experiments, to allow for the effect of any adhesion of labelled bacteria, or adsorption of dissolved label, to the cuticle. These controls were nematodes of the same size as the experimental animals, previously killed by heat or with formalin.

5.1.9. Treatment of G-M results

Means were taken of all replicate counts, and the background count subtracted from all experimental counts. From the haemocytometer and G-M counts on dilutions of an initial washed bacterial suspension, the 40 sec. count per cell could be calculated. The count for each nematode could then be expressed as a "cell equivalent" (CE), i.e. the number of bacterial cells which would give the same 40 sec. count as the nematode.

5.1.10. Observations on feeding

The feeding activities of M. disjuncta both in the stock cultures and in suspensions of ML01 in 0.2% agar were observed using the compound microscope with a x10 objective and a combination of incident and transmitted light. Observations were also made of nematodes in suspensions of Indian ink, in seawater and in 0.2% agar.

5.2. EXPERIMENTAL PROCEDURES AND RESULTS

5.2.1. Examination of bacteria

The results of gram staining and examination of the cell and colony morphology of each of the five strains of bacteria are shown in Table 5.1.

5.2.2. Uptake of label from the different bacteria

The idea of this experiment was to present *M. disjuncta* with each of the four strains of bacteria at the same cell density and to measure the amount of label taken up from each strain.

Procedure

A labelled broth culture of ML01 was grown (section 5.1.2.) and the bacteria were harvested and washed (section 5.1.3.). Haemocytometer and G-M counts were made on dilutions of the washed suspension and the cell density was adjusted to $\sim 10^9$ cells cm^{-3}. *M. disjuncta* were picked out from one-month-old cultures and washed (section 5.1.5.) and 15 were placed in $4.5cm^3$ MFSW in each of 4 dishes (section 5.1.6.). One dish of 10 control (dead) nematodes was also prepared. $0.5cm^3$ of the bacterial suspension was added to each dish. This procedure meant that the nematodes were without food for a maximum of 30 mins. After 24h, active nematodes were removed, killed and washed, and G-M counts were made. (section 5.1.7.). This procedure was repeated using strains ML03, ML04 and ML05. Table 5.2. gives details of the experiments.

STRAIN	GRAM REACTION	SHAPE	CELL SIZE/um	COLOUR	COLONY	COLONY SHAPE (SURFACE VIEW)	COLONY SHAPE (PROFILE)	COLONY TEXTURE
ML01	-	Rod	2 x 1	White	0.4-1.0	Round, edge regular	Convex	Smooth shiny
ML03	+	Coccus	1.5	Bright yellow	0.4-0.9	Round, edge regular	Convex	Faintly granular, shiny
ML04	-	Rod	2 x 1	Pale yellow	0.2-0.3	Round, edge irregular	Shallowly convex	Faintly granular, shiny
ML05	-	Rod	2.3x1.5	White	0.2-0.6	Roundish, edge irregular and ± indistinct	Shallowly convex, wavy	Granular, dull
ML07	-	Rod	1x1.5	Pink	0.1-0.3	Round, edge regular	Convex, drop-like	Smooth, shiny

TABLE 5.1. Cell and colony morphology of the five strains of bacterium isolated from L. saccharina fronds.

STRAIN	CELL DENSITY IN DISHES (CELLS $cm^{-3} \times 10^8$)	G-M COUNT $CELL^{-1}$	INITIAL NO. OF NEMATODES	NO. ACTIVE AFTER 24h	NO. WITH COUNT >(BACKGROUND +10)
ML01	1.20	0.011	60	43	25
ML03	1.25	0.014	60	48	19
ML04	1.34	0.017	60	51	3
ML05	1.29	0.008	60	36	6

TABLE 5.2. Details of experiment to measure uptake of label from the different strain of bacteria.

The results are presented in Fig. 5.2. None of the control nematodes took up label above background +10 counts. Only small numbers of nematodes took up label from ML04 and ML05, and those which did, took up only small amounts, a maximum of 3×10^3 CE from ML04 and 8×10^3 CE from ML05. Rather more nematodes took up label from ML03 (19 of 48 active after 24h) but the amounts taken up were again small, a maximum of 6×10^3 CE. In the experiment with ML01 most of the nematodes took up label (25 of the 43 active after 24h), and uptake was much greater: 20 took up more than 10×10^3 CE, 15 took up more than 20×10^3 CE, and the maximum uptake was 36×10^3 CE.

5.2.3. Uptake of label from ML01 presented in seawater and in 0.2% agar

M. disjuncta placed in a suspension of bacteria in seawater showed rather aberrant movements and tended to produce clumps of mucus and remain within them. In 0.2% agar the nematodes moved continuously through the agar and did not produce clumps of mucus. The purpose of this experiment was to see whether these differences in behaviour resulted in differences in uptake of label.

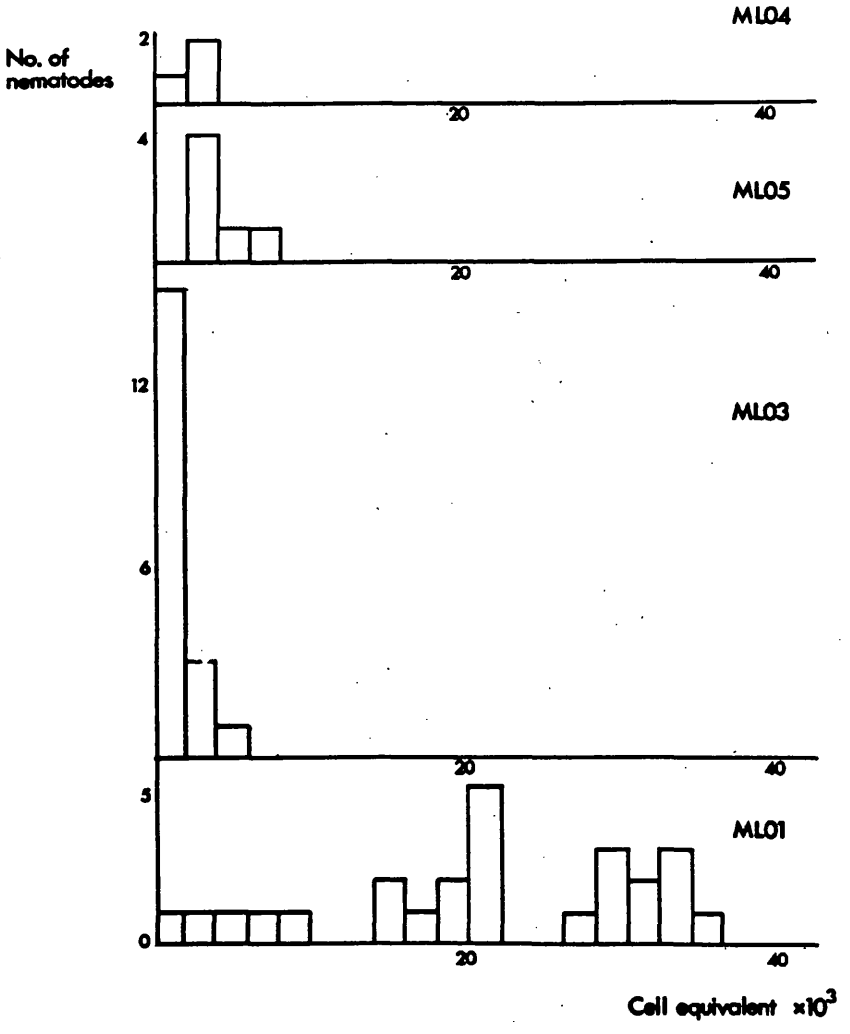


FIG. 5.2. Uptake of label by M. disjuncta from suspensions of four strains of bacteria.

Procedure

The suspension of labelled ML01 prepared for the experiment described in section 5.2.2. was also used for this experiment. Two dishes containing a suspension of these bacteria in 0.2% agar were prepared (section 5.1.6.) and 15 M. disjuncta were washed and placed in each. The details are shown in Table 5.3.; the details of the ML01 dishes in the experiment described in section 5.2.2. are included for comparison.

STRAIN	MEDIUM IN WHICH SUSPENDED	CELL DENSITY IN DISHES (CELLS $cm^{-3}x10^8$)	G-M COUNT $CELL^{-1}$	INITIAL NO. OF NEMATODES	NO. ACTIVE AFTER 24h	NO. WITH COUNT >(BACKGROUND +10)
ML01	0.2% SW agar	1.20	0.011	60	43	25
ML01	SW	1.20	0.011	30	23	17

TABLE 5.3. Details of experiment to compare uptake of label from suspensions of ML01 in seawater and in sloppy agar.

Results

The results are presented in Fig. 5.3., with the results previously obtained for M. disjuncta feeding on ML01 in SW shown for comparison. There is no obvious difference between the two sets of results. It thus appears that the aberrant locomotion shown in SW did not inhibit feeding, and that the presence of mucus threads is not necessary for the uptake of label. This latter point is discussed below (section 5.3.).

5.2.4. The time-course of uptake of label

Comparison of the gut retention time with the time-course of the uptake of label can reveal whether the observed uptake is the result of assimilation into the body or merely of accumulation in the gut. Retention times were estimated from observations of defaecation (see section 5.2.9.);

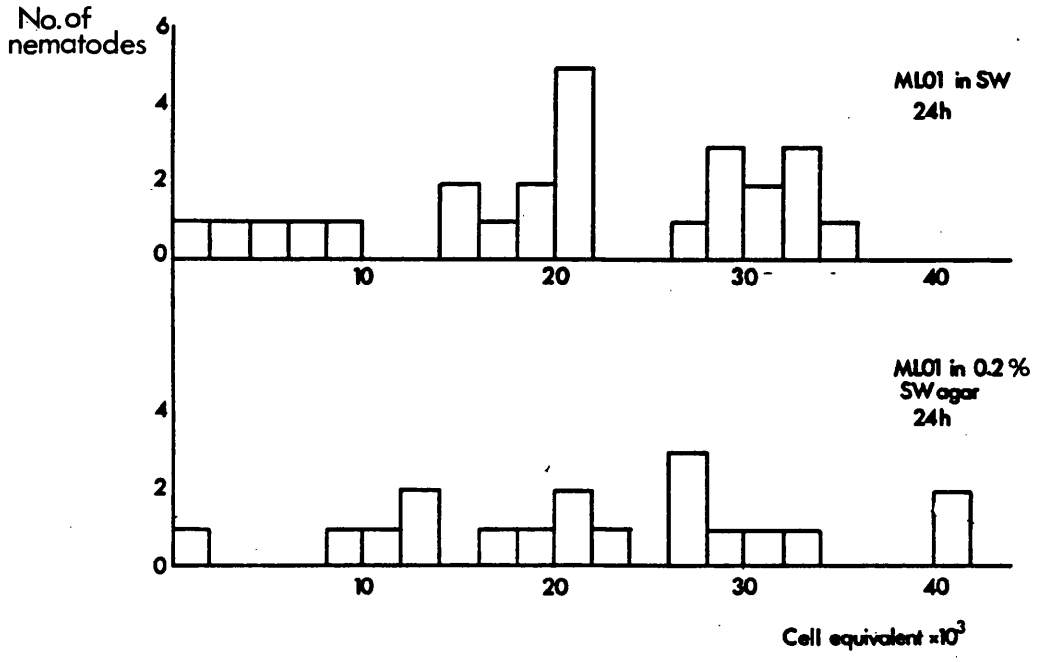


FIG. 5.3. Uptake of label by M. disjuncta from ML01 suspended in seawater and in sloppy agar.

the experiments described in this section followed the time-course of uptake.

Procedure

In the first run of this experiment the uptake of label was followed over 24h. The counts appeared to be increasing right up to 24h, but for some unknown reason many of the nematodes died, and numbers which survived to be counted were too low to allow any definite conclusions to be drawn.

In the second run M. disjuncta was allowed to feed on labelled ML01 for 48 and 72h. The procedure was as described in section 5.2.2., and the results for the 24h experiment described in that section can be used for comparison. Details are given in Table 5.4.

Results

The results, including those from the 24h experiment described in section 5.2.2. are presented in Fig. 5.4. Uptake was clearly greater after 48h than after 24h, and still greater after 72h. It appears that a similar amount of label was taken up in each of the three 24h periods. After 48h and 72h almost all the active nematodes had taken up some label.

5.2.5. The time-course of loss of label

In this experiment, M. disjuncta was fed on a suspension of labelled ML01 in seawater for 24h, then transferred to a suspension of unlabelled ML01 in seawater at the same density. Active nematodes were removed after various times and G-M counts made. It was hoped that as well as providing information on the time-course of loss of label, which could be compared with the gut retention time in the same way as that on the time-course of uptake, this experiment might give another measure of retention time. One would expect an initial period of rapid loss, when label was being both excreted and defaecated, followed by a longer period of slower loss when all the label had been eliminated from the gut and excretion was the only means of loss.

STRAIN	MEDIUM IN WHICH SUSPENDED	TIME (h)	CELL DENSITY IN DISHES (CELLS $cm^{-3} \times 10^{8}$)	G-M COUNT $CELL^{-1}$	INITIAL NO. OF NEMATODES	NO. ACTIVE AT END OF INCUBATION	NO. WITH COUNT >(BACKGROUND +10)
ML01	SW	24	1.20	0.011	60	43	25
"	"	48	1.20	0.009	60	40	38
"	"	72	1.20	0.009	60	40	36

TABLE 5.4. Details of experiment to measure uptake of label from ML01 over 24, 48 and 72h.

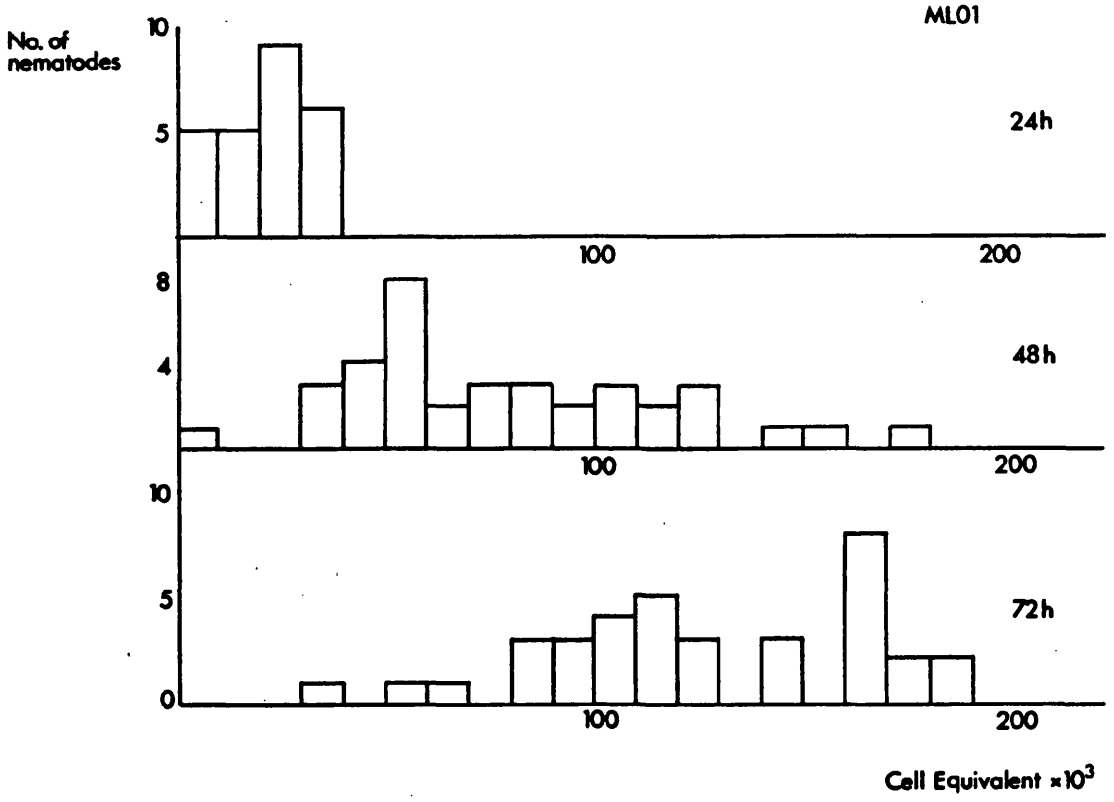


FIG. 5.4. Uptake of label by M. disjuncta from ML01 over 24, 48 and 72h.

Procedure

Details of the bacterial suspensions used are given in Table 5.5.

STRAIN		CELL DENSITY IN DISHES (CELLS $cm^{-3} \times 10^8$)	G-M COUNT $CELL^{-1}$
ML01	labelled	1.30	0.010
ML01	unlabelled	1.32	-

TABLE 5.5. Details of experiment to follow time-course of loss of label.

Nematodes were removed from the unlabelled bacterial suspension after $\frac{1}{2}$, 1, $1\frac{1}{2}$, 2, 3, 4, 6, 8, 10, 12, 14, 16, 18 hours, killed and washed in the normal way and G-M counts made.

Results

The results are shown in Fig. 5.5. The number of nematodes used was rather small, but three points emerge. Firstly, the uptake of label over the 24h in the suspension of labelled ML01 was similar to that found in the experiment described in section 5.2.2. Secondly, in the unlabelled suspension the nematodes lost label only slowly, a high proportion of that initially present remaining after 18h. Thirdly, no discontinuity in the rate of loss, indicating the time at which all label was lost from the gut, was seen. This was probably partly because the numbers of nematodes used were small; also, because the retention time was fairly short (see section 5.2.9.) and the assimilation efficiency may well have been high (see discussion), the amount of label defaecated was probably only a small proportion of that initially present.

5.2.6. Distribution of label in dishes between cells and solution; uptake of label from solution

There was a possibility that the uptake observed in the above experiments was of label which had been released into solution by the

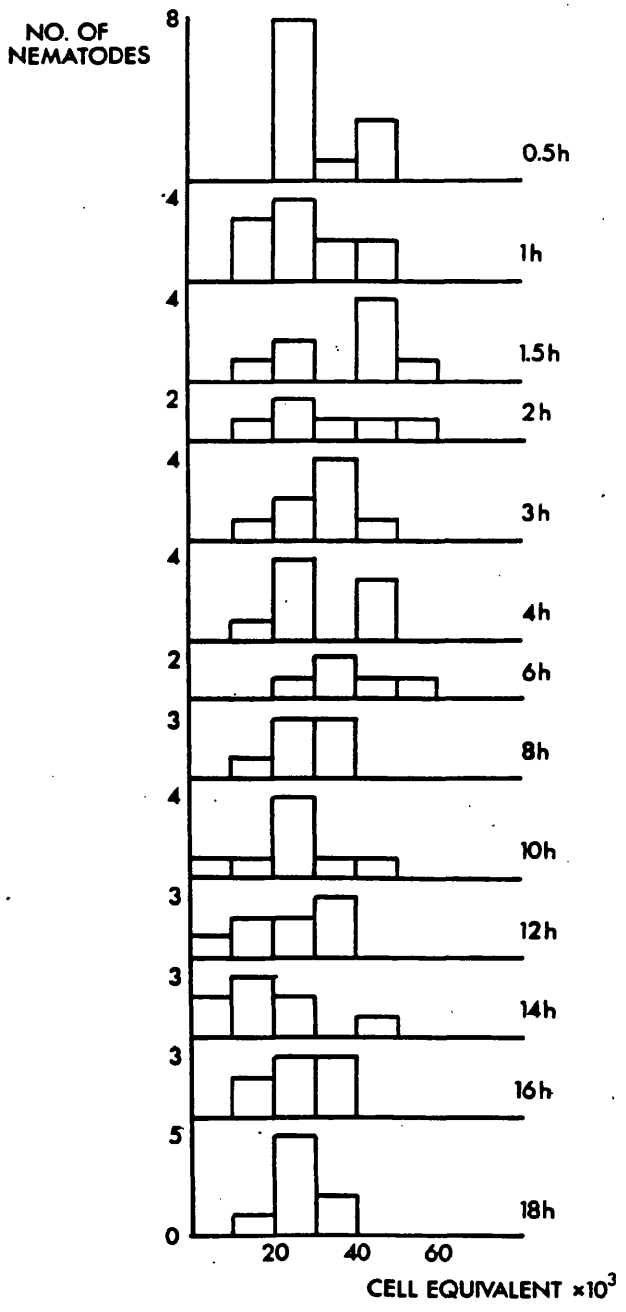


FIG. 5.5. Time-course of loss of label by M. disjuncta.

bacteria. The two parts of this experiment investigated this possibility. In the first part, nematodes were left in a suspension of labelled ML01 for 24h at the end of which the amount of label present in solution was measured. In the second part, fresh nematodes were kept in this solution for 24h and the amount of label they took up was measured.

Procedure

A culture of ^{32}P-labelled ML01 was grown, harvested and washed. M. disjuncta were picked out from stock cultures and washed, and 15 were placed in 4.5cm^3 MFSW in each of 4 dishes. 0.5cm^3 of bacterial suspension was added to give a cell density in the dishes of $\sim 10^8$ cells cm^{-3}. After 24h the contents of each dish were passed through two 0.4µm Millipore filters. A G-M count was made on dilutions of the filtrate from each dish. The remaining filtrate from all 4 dishes was combined, and 5cm^3 put into each of 2 more dishes. 15 fresh M. disjuncta were washed and placed in each dish. After 24h those which were active were removed, killed and washed, and G-M counts were made. Table 5.6. shows the details of the first part of the experiment.

STRAIN	INITIAL CELL DENSITY IN DISHES (CELLS cm^{-3}x10^8)	INITIAL G-M COUNT CELL^{-1}
ML01	1.10	0.012

TABLE 5.6. Details of experiment to investigate distribution of label between bacteria and solution and uptake of label from solution.

Results

The results of the first part of the experiment are shown in Table 5.7. The mean percentage of label in the filtrate was 2.6%. In the second part of the experiment, 19 of the 30 nematodes were active after 24h, and none of these showed G-M counts of greater than background +10. It is unlikely that a significant proportion of the ^{32}P taken up by M. disjuncta in the previous experiments came from solution. This point is considered further in section 5.3.

DISH	INITIAL G-M COUNT cm^{-3} IN DISH	G-M COUNT cm^{-3} FILTRATE	% OF LABEL PRESENT IN FILTRATE
1	134161	19183	1.4
2	"	28170	2.1
3	"	54999	4.1
4	"	38902	2.9

TABLE 5.7. Amount of label present in filtrate.

5.2.7. Passage of viable bacteria through the gut

The differential uptake of label from the different strains of bacteria (section 5.2.2.) could have been the result of selective ingestion or selective digestion. The passage of large numbers of viable bacteria through the gut would suggest that the latter was at least partly responsible. This experiment made use of the fact that the different strains of bacteria form colonies of different colours (see Table 5.1.).

Procedure

This experiment did not involve the use of labelled bacteria. Unlabelled broth cultures of strains ML01 and ML04 were grown, and a suspension in MFSW was made of ML07 grown on marine agar. In all cases the bacteria were washed and the cell density adjusted to $\sim 10^8$ cells cm^{-3}. Colonies of ML01, ML04 and ML07 are white, yellow and pink respectively. Thirty M. disjuncta were picked out from stock cultures and washed, and 15 were placed in each of 2 dishes containing $5cm^3$ ML04 suspension. After 24h the nematodes were transferred to 2 dishes of ML07 suspension where they were left for a further 3h. During this 3h period the ML01 suspension was heated to 55^oC for 20 mins., then cooled; it was hoped that this would kill the cells but leave them intact. At the end of the 3h period the nematodes were removed from the ML07 suspension, taken individually through 6 washes of MFSW and transferred to 2 dishes of the "killed" ML01 suspension. After 12h they were removed and serial dilutions of the suspension remaining in each of the 2 dishes were prepared and plated out on marine agar. The dilutions used were 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6}, and 3 plates were made of each dilution from each dish. The plates were incubated at 22^oC and examined after 1, 2, 3 and 4 days to look for yellow (ML04) and pink (ML07) colonies. A control was provided by a dish of 15 nematodes with which the same procedure was followed except that the nematodes were killed by heat between the incubations in ML04 and ML03.

Results

The main problem with this experiment was that the heat treatment failed to kill all the ML01, which meant that a dense growth of white ML01 colonies made it difficult to see pink or yellow colonies on the plates. However, the three 10^{-2} plates from dish 1 had 6, 1 and 3 recognizable yellow colonies. On none of the plates from either dish 2 or dish 3

(the control) were any yellow or pink colonies seen. While this result is not at all conclusive, it does suggest that viable ML04 may be passed through the gut. The need for heat killing could be avoided if strains with different nutritional requirements or different sensitivities to a particular antibotic were used.

5.2.8. Uptake of label by juvenile M. disjuncta and by M. refringens

As an additional part of one experiment, uptake of label by juvenile M. disjuncta and by M. refringens presented with labelled ML01 for 24h was measured.

Procedure

A labelled culture of ML01 was grown, harvested and washed, and two experimental dishes were set up, as described in section 5.2.2. In one dish were placed 20 M. disjuncta of body length ∿ 300μm, which had been picked out from stock cultures and washed in the normal way. In the second dish were placed 3 adult M. refringens obtained from the field. After 24h, active nematodes were removed, killed and washed, and G-M counts were made. Details of the experiment are given in Table 5.8.

	CELL DENSITY IN DISHES (CELLS cm^{-3})	G-M COUNT $CELL^{-1}$	INITIAL NO. OF NEMATODES	NO. ACTIVE AFTER 24h	NO. WITH COUNT >BACKGROUND +10
M. refringens (adult)	1.12	0.006	3	2	1
M. disjuncta (juvenile)	1.12	0.006	20	13	8

TABLE 5.8. Details of experiment to measure uptake of label from ML01 by M. refringens and juvenile M. disjuncta.

Results

One of the three M. refringens took up 1.1 x 10^4 CE of label. This is within the range observed for adult M. disjuncta, though less than the median uptake for this species. In eight of the juvenile M. disjuncta uptake was in the range 0.2-1.0x10^4 CE. In neither M. refringens nor juvenile M. disjuncta was the retention time or the time-course of uptake investigated.

5.2.9. Observations on feeding

While feeding, the body is moved slowly back and forth while the anterior end is rotated about the longitudinal axis of the body at a frequency of 1-2 revolutions s^{-1}. As far as could be judged by direct microscopic observation, the pumping rate of the oesophagus was usually 1-2s^{-1} with a maximum of $\sim$ 5s^{-1}. It appeared that only one particle or clump of particles was moving down the oesophagus at any one time. Back and forth movements of the gut contents were occasionally observed, but it seemed that little longitudinal mixing resulted.

Defaecation was an obvious event. The gut contents were first displaced anteriorly, then moved posteriorly. Material was squirted from the anus, emptying the posterior part of the gut, and the section of the body which contained this part of the gut shortened. 1-3s later this body section extended again as gut contents moved back to fill the posterior part of the gut. The volume expelled at each defaecation appeared to be fairly constant, and was estimated as 10-15% of the gut volume. Examination of M. disjuncta did not reveal the presence of a prerectal valve of any type (see discussion).

To determine the frequency of defaecation, individual nematodes in a suspension of ML01 in SW agar ($\sim 10^8$ cells cm^{-3}) were observed continuously for up to 2h. Examples of typical observations are:

(i) Adult female. Defaecations after 10, 52, 88 mins.

(ii) Adult male. Defaecations after 15, 40, 70, 101 mins.

Eight nematodes and 21 defaecations were observed. The mean time between successive defaecations was 35 mins. If the mean proportion of the gut contents voided at one defaecation is taken as 12.5%, this gives an estimate of 4h 40 mins for the retention time under these conditions.

Observations were also made using Indian ink particles. These were about 1μm long, approximately the same size as the bacteria, and though in SW some clumps of up to 15μm diameter were formed, large numbers of single particles remained. When put into a suspension of Indian ink in seawater, M. disjuncta ingested these particles, but the lumen of the oesophagus and/or the oesophago-intestinal valve soon became clogged. One feature which the use of Indian ink did demonstrate was the copious production of mucus by M. disjuncta from the mouth and the caudal glands. The ink particles adhered to the mucus, making it easily visible. If put in seawater, the nematodes made mucus aggregates within which they usually remained. Often two or more nematodes tangled together within such an aggregate. Similar behaviour was observed in the feeding experiments where nematodes were presented with a suspension of bacteria in seawater. When provided with 0.2% agar the nematodes burrowed through it continuously and mucus aggregates were not formed.

5.3. DISCUSSION

The direct observations described above show that M. disjuncta does ingest particles of bacterial size. Bennet-Clark (1976) discussed the mechanics of the nematode oesophageal pump. The pumping rate observed in M. disjuncta ($1\text{-}5s^{-1}$) is similar to those recorded for other nematodes, e.g. $1\text{-}2s^{-1}$ in Panagrellus and up to $5s^{-1}$ in Rhabditis (Mapes, 1965). Doncaster (1962) described the mechanism by which two rhabditids, Rhabditis and Pelodera fed on bacteria. He considered that

these nematodes were in effect filter feeders. They have a deep cylindrical buccal cavity with a glottoid apparatus at its base. The oesophagus consists of an anterior corpus, of which the posterior part (the metacorpus) forms the first bulb, a median isthmus and a posterior bulb (the second bulb). Dilation of the corpus draws in water, together with any suspended particles, as far as the anterior end of the isthmus. Closure of the lumen then expels water, leaving particles trapped behind the glottoid apparatus or the first bulb. These particles are then moved posteriorly by subsequent dilations of the corpus. Doncaster suggested that the flaps of the second bulb break up aggregates of food particles. Mapes (1965) suggested that they act primarily as valves. In M. disjuncta the oesophagus is not differentiated into corpus, isthmus and bulb but is roughly cylindrical with a slight posterior swelling. Chitwood and Murphy (1964) observed two minute denticles at the base of the buccal cavity in some specimens; these were mot seen in the present study. It is not clear whether a simple oesophagus of this type could function as a filter in the way described for rhabditids. The buccal cavity of M. disjuncta is much smaller than that of Rhabditis or Pelodera which suggests that there may be more precise selection of individual food items. This would presumably lead to ingestion of less excess water and hence to less need to strain out food particles. A cinemicrographic study would provide useful information.

Doncaster (1966) described movements of the gut contents of two Ditylenchus species, apparently similar to those observed in M. disjuncta, which were the result of localized comtractions of the posterior body wall. In M. disjuncta the movements seemed to result in agitation of the gut contents rather than in significant longitudinal mixing.

Defaecation in nematodes has not been studied as intensively as has feeding. Crofton (1966) stated that in Ascaris the anal dilator muscle opens the anus and the gut contents are forcibly expelled by the

high internal turgor pressure (Harris and Crofton, 1957). In other species the process is more complicated. Seymour and Doncaster (1973) used cinemicrography to study defaecation in Aphelenchoides blastophthorus. In this species there is a prerectum, separated from both the rectum and the posterior intestine proper by complex folded valves. Before defaecation the posterior part of the body shortens, displacing the gut contents anteriorly. Defaecation is coordinated by a posteriorly passing wave of re-elongation followed by localized shortening, which opens and closes the various valves. The faeces are isolated in the prerectum when the prerectal valve closes, pass through the rectal valve as the anterior prerectum closes, and finally are ejected when the anus opens. After defaecation the posterior part of the body re-elongates and the prerectal valve and prerectum open. The prerectal valve limits the amount of material eliminated in one defaecation. In Caenorhabditis briggsae defaecation again involves an initial anterior displacement of the gut contents caused by localized contraction of the posterior body wall. This is followed by a rapid posterior movement of the oesophagus, caused by a contraction of the cephalic musculature, which displaces the gut contents posteriorly, filling the hind gut and rectum. The final evacuation of the rectum follows almost immediately; the prerectum is not emptied. The process of defaecation in M. disjuncta (section 5.2.9.) is clearly similar to that described for these two species. There is apparently no prerectal valve; cinemicrography would again provide information on the details of the process.

In the present study the retention time in the M. disjuncta gut was estimated from the frequency of defaecation (about once every 35 minutes) and the fraction of the gut contents voided at each defaecation (10-15%). Values of these quantities recorded for other nematodes are shown in Table 5.9.

	SOURCE	INTERVAL BETWEEN SUCCESSIVE DEFAECATIONS	VOLUME EXPECTED PER DEFAECATION
Panagrellus silusiae	Mapes (1965)	30 mins.	10% of body volume
Ascaris lumbricoides	Crofton (1966)	3 mins.	-
Aphelenchoides blastophthorus	Seymour & Doncaster (1973)	-	9% of gut volume
Plectus palustris	Duncan *et al* (1974)	2-3 mins.	-
Caenorhabditis briggsae	Croll & Smith (1978)	0.5-1 min.	-
Adoncholaimus thalassophygas	Lopez *et al* (1979)	several hours	-

TABLE 5.9. Results of previous studies of defaecation in nematodes.

The species with high defaecation rates must also have high rates of ingestion; in Plectus palustris the time taken to fill the gut was estimated at 3-10 mins. (Duncan et al, 1974). There is clearly considerable variation in the rate at which food is passed through the gut, but it cannot be seen from the limited data available whether particular taxa or ecological groups consistently show high or low rates. The implications of retention time for digestion and the possible use of dissolved organic matter for food are discussed below.

Crofton (1966) stated that in Ascaris lumbricoides defaecation is initiated by a simple reflex: feeding increases the body volume which produces elongation (Harris & Crofton, 1957) and a stretch receptor in the tail then triggers the contraction of the anal dilator muscle. The frequency of defaecation is directly related to the oesophageal pumping rate. A similar system appears to operate in Panagrellus silusiae, where defaecation occurs when feeding has increased the body volume to a critical value (Mapes, 1965). In contrast to this, Croll & Smith (1978) found that in Caenorhabditis briggsae defaecation frequency was independent of feeding rate, and that components of the defaecation cycle continued to appear in isolated heads and tails. They concluded that the cycle is at least partly controlled by an endogenous pacemaker. A system with a pacemaker would seem most suitable for a nematode with a short retention time. M. disjuncta may well have a system similar to that in Ascaris and Panagrellus.

Comparison of the retention time in the M. disjuncta gut (section 5.2.9.) with the time-course of uptake (section 5.2.4.) and loss (section 5.2.5.) of label indicates that ^{32}P was being assimilated rather than merely accumulating in the gut. If after 24h feeding, label in the gut formed a high proportion of the total body label, then, even allowing for the fact that the assimilation rate will not reach its maximum until the gut is full of labelled food, the increase in label

during the first 24h feeding, during which the gut is filled, would be greater than that during subsequent 24h periods. In fact, in the experiment described in section 5.2.4. a very similar amount of label was taken up in each of the three 24h periods. This suggests that the results of even a 24h feeding experiment will approximate more closely to assimilation than to ingestion. This would be expected if the retention time is as short as 4h 40 mins and if assimilation efficiency is of the same order as the 59.8% recorded for Pelodera by Marchant and Nicholas (1974).

The ^{32}P assimilated by M. disjuncta in the present study must have come either from the bacterial cells or from solution. Uptake of ^{3}H or ^{14}C from solutions of labelled glucose has been demonstrated in the oncholaimids Pontonema vulgaris (Chia and Warwick, 1969) and Adoncholaimus thalassophygas (Lopez et al, 1979). On the other hand, Tietjen and Lee (1975) found that Rhabditis marina did not take up ^{14}C from solutions of several labelled compounds. Chia and Warwick (1969) used autoradiography to show that in P. vulgaris uptake was via the gut rather than through the cuticle. It is likely that in the present study dissolved ^{32}P was in the form either of inorganic ions or of polar organic molecules. The cuticle of Aphelenchus avenae has very low permeability to such substances (Castro and Thomason, 1973). It therefore seems probable that any assimilation of dissolved ^{32}P by M. disjuncta will have been via the gut; autoradiography would be the best way of confirming this.

The possibility of uptake of dissolved label was investigated in the experiment described in section 5.2.6. In the first part of this experiment uptake was not measured, but it is reasonable to assume that it was similar to that measured in the experiments described in 5.2.2. and 5.2.5., and to take a figure of 2.5×10^{4} CE for uptake over a 24h period. If in the experiment described in section 5.2.6., ^{32}P was uniformly distributed throughout the suspension, this amount of label

was present in a volume of 2.27×10^{-4} cm^3. Using Andrassy's formula (Andrassy, 1956).

$$G = \frac{a^2 b}{16 \times 10^5}$$

where G = body weight in µg

a = greatest body width in µm

b = greatest body length in µm.

and assuming a density of 10g cm^{-3}, the volume of a M. disjuncta of body length 600um and maximum body width 25µm can be estimated as 0.27×10^{-6} cm^3. An upper estimate of the gut volume is 30% of the body volume, i.e. 0.08×10^{-6} cm^3. So if ^{32}P was uniformly distributed in the suspension, and uptake was only via the gut, then even if assimilation efficiency was 100%, the gut would have had to be filled 2837 times in 24h, i.e. once every 30 secs., if the observed uptake was to be achieved. If label was only taken up from solution and the final value of 2.6% of the label in solution is assumed to have held throughout the 24h, and if assimilation efficiency for dissolved label was 50%, then the required rate of filling the gut becomes once every 0.4 secs. This clearly was not occurring. Another way of looking at these results is that if the retention time was 4h 40 mins., the gut volume was 8×10^{-8} cm^3, 2.6% of the label was in dissolved form and was assimilated with an efficiency of 50%, and the suspension was ingested unselectively, then 0.0023% of the observed uptake of label could have been obtained from solution.

The nematodes must therefore have been obtaining label in a concentrated form, presumably either from the bacteria or from mucus threads. Label in both these forms will have been retained on the Millipore filters in the experiment described in section 5.2.6. It seems unlikely for two reasons that much of the label assimilated came from mucus threads. Firstly, in the second part of this experiment, nematodes placed in a

solution containing label at the concentration at which it was present at the end of the first part, which was probably the maximum concentration reached during the 24h period, failed to take up ^{32}P despite producing copious mucus. Secondly uptake over 24h was similar whether the bacteria were presented in SW or in 0.2% SW agar. In the latter case, the nematodes moved continuously through the agar and did not form mucus aggregates (section 5.2.3.).

It thus appears that M. disjuncta can assimilate ^{32}P from bacterial cells. Tietjen et al (1970) and Tietjen and Lee (1973, 1977) have used a similar technique to study feeding in Monhystera denticulata, Rhabditis marina, Chromadora macrolaimoides and Chromadorina germanica. In these studies, results were expressed as consumption in µg food nematode^{-1} day^{-1}. The results obtained with M. disjuncta suggest that these figures will in fact be closer to assimilation than to ingestion. Tietjen and Lee's experiments were conducted over 24h when bacteria were the food and 72h when algae were used. The retention time M. denticulata is probably similar to that in M. disjuncta, and short retention times have been recorded for rhabditids (see discussion above). Retention times in chromadorids have never been measured, but in small nematodes such as C. macrolaimoides and C. germanica are likely to be much less than 24h. For the purpose of comparison, the results for M. disjuncta can be expressed in the terms used by Tietjen and Lee. If the size of a bacterial cell is 1.5x1.0x1.0µm (Table 5.1.), and its density is 1.0g cm^{-3}, then the daily consumption ($2.5x10^4$ cells) weighs $3.75x10^{-2}$µg.

The most relevant comparison is between M. disjuncta and M. denticulata. Tietjen and Lee (1977) used 10 strains of bacteria suspended in SW at a density of 10^6-10^8 cells cm^{-3}. "Consumption" varied from zero with some strains to 0.36 µg nematode^{-1} day^{-1} with others, with an average of 0.057µg nematode^{-1} day^{-1}. With only one strain did "consumption" exceed 0.035µg nematode^{-1} day^{-1}. M. denticulata also "consumed" diatoms

and chlorophytes, at rates varying from just above zero to 0.2µg nematode^{-1} day^{-1}. Algae were not used in the experiments with M. disjuncta because there was evidence that these were not consumed in the field (section 4.2.11.).

Tietjen and Lee (1977) used the same 10 bacterial strains to study feeding in R. marina. The maximum "consumption" rate was 0.035µg nematode^{-1} day^{-1}, the average over the 10 strains was 0.018µg nematode^{-1} day^{-1}. Tietjen et al (1970) presented different strains to this species; here the maximum rate was much higher, 0.81µg nematode^{-1} day^{-1}. R. marina also consumed diatoms and chlorophytes, at up to 1.6µg nematode^{-1} day^{-1}, with average "consumption" of 0.23 and 0.4µg nematode^{-1} day^{-1} in two sets of experiments (Tietjen and Lee, 1977).

C. macrolaimoides and C. germanica consumed only very small amounts of bacteria, but "consumption" of algae (diatoms and chlorophytes) was high, the maximum rate and the average rate over the strains used being 14.0 and 2.2µg nematode^{-1} day^{-1} for C. macrolaimoides and 4.7 and 2.16µg nematode^{-1} day^{-1} for C. germanica (Tietjen and Lee, 1973, 1977).

Duncan et al (1974) studied feeding of the freshwater nematode Plectus palustris on the bacterium Acinetobacter. The bacteria were labelled with ^{14}C, washed and presented to the nematodes at a density of 5-10x10^{9} cells cm^{-3}. Feeding was for very short periods, 2-5 min., so that in this case ingestion rather than assimilation was being measured; it was estimated that the time taken to fill the gut was between 3 and 10 minutes. Extrapolation of the measured rate of consumption gave $\sim$9.75µg nematode^{-1} day^{-1}. Mercer and Cairns (1973) used respirometry and calorimetry to obtain an energy budget for the rhabditid Pelodera chitwoodi. The rate of ingestion of the food (a gram-negative bacterium) was calculated from these results as $\sim 10^{6}$ bacteria day^{-1} for an adult male (1-1.5µg nematode^{-1} day^{-1}). Soyza (1973) used measurements of the oesophageal pumping rate and the volume pumped at each contraction to estimate the rate at which Aphelenchus avenae ingested the fungus

Botrytis cinerea. The calculated rate was 26% of the body weight day^{-1} (0.4-0.5µg nematode^{-1} day^{-1}), but comparison with the other components of the energy budget indicated that this was probably an underestimate.

In these last three studies ingestion rather than assimilation was measured. Knowledge of the assimilation efficiency is necessary if the results are to be compared with those of Tietjen et al (1970), Tietjen and Lee (1973, 1977) and the present study. As mentioned above, Marchant and Nicholas (1974) measured the assimilation efficiency in a Pelodera sp. feeding on bacteria as 59.8%. If a figure of 60% is assumed to apply to the other species, then assimilation rates were: 5.85µg nematode^{-1} day^{-1} for P. palustris, 0.6-0.9µg day^{-1} for P. chitwoodi, and 0.24-0.3µg day^{-1} for A. avenae.

A comparison of the results of these various studies leads to several conclusions. The two chromadorids, feeding on algae, had much higher maximum assimilation rates than did any of the bacterial feeders. In the experiments by Tietjen and Lee, bacteria and algae were used at the same cell density (10^6-10^8 cells cm^{-3}); in most natural situations algae would be present at much lower densities than bacteria. Very different assimilation rates were obtained with different algae; the phenomenon of selective feeding is discussed below.

There is also considerable variation among the values obtained for assimilation rates in bacterial feeders. The results for M. disjuncta are similar to those for M. denticulata, and come at the bottom end of the range for bacterial feeders. Part of this variation is again likely to be due to selective feeding; to obtain a realistic estimate of maximum feeding rates a wide range of bacteria should be tested. Another probable source of variation is the difference in the density of the bacterial suspensions used by different workers. The highest feeding rates were those measured by Duncan et al (1974) who used a suspension 50 to 100 times as dense as those used in the present study. Nicholas et al (1973) found that when Caenorhabditis briggsae was fed on aqueous

suspensions of E. coli, the decrease in the number of bacteria with time followed a negative exponential equation when bacterial multiplication was restricted. This indicates that a constant volume of suspension was filtered/ingested per unit time over a wide range of bacterial densities, and thus that the cell ingestion rate was directly proportional to cell density. A third factor is that the nematodes used in the various studies were of different sizes; body wet weights range from ∿0.3µg for M. disjuncta to ∿ 1.8µg for A. avenae.

Further studies are needed before it will become clear how much of the observed variation in feeding rate between different species is the result of differences in experimental technique and how much is real, and how any real differences are related to differences in feeding mechanism or are characteristic of particular taxonomic groups.

It is not known whether the differential uptake of label from different food organisms seen in the experiments by Tietjen and Lee and in the present study is the result of selective ingestion, selective digestion or both. If selective ingestion occurred in the experiments with M. disjuncta, selection must have been on the basis of chemical differences, or possibly differences in surface texture, since very different levels of uptake were recorded from different bacteria of similar size and shape. It would be interesting to run a series of choice chamber experiments with these bacteria, to see if M. disjuncta will move towards those strains from which uptake was greatest.

Though inconclusive, the experiment described in section 5.2.7. did suggest that viable cells of the bacterium ML04, from which M. disjuncta did not assimilate much label (section 5.2.2.), were passed through the gut. Since in monhysterids breakdown of the food must be largely chemical rather than mechanical, any selective digestion which occurs is probably the result of chemical differences in the cell walls of different food organisms rendering them more or less susceptible to attack by the nematode's digestive enzymes. One major difference in bacterial cell

walls is between those of gram-positive and gram-negative bacteria. The one gram-positive strain used in the present study was ML03. Assimilation from this strain was low, of the same order as from the gram-negative strains ML04 and ML05, while assimilation from ML01, also gram-negative, was much higher. It does not therefore seem that the gram reaction is correlated with suitability as food for M. disjuncta. Nothing further is known about the cell wall composition of these four strains. No study was made of enzymes in the M. disjuncta gut. Jennings and Deutsch (1975) found a positive reaction for β-glucuronidase in a group of cells at the junction of the oesophagus and the intestine in M. denticulata, and concluded from the position of these cells and their merocrine glandular nature that they were secreting this enzyme which then acted extracellularly in the lumen of the gut. In Diplolaimella sp. (Monhysteridae), Deutsch (1978) found ultrastructural evidence that the oesophageal gland cells, the cells of the oesophago-intestinal valve and the anterior four cells of the intestine were secretory. However the only enzyme activity which was demonstrated was non-specific esterase activity present throughout the lumen of the intestine. If Diplolaimella was well fed and then starved, the intestine was still full of undigested food cells after several days, indicating that the rate of luminal digestion was low. There was an unusual fibrillar matrix through which food molecules had to pass to enter the intestinal cells. The final stages of digestion were intracellular, involving lysosomes. "Pigment granules", probably similar to those in Diplolaimella some of which were demonstrated to be lysosomes, are present in the intestinal cells of M. disjuncta.

The digestive physiology of two other marine nematodes has been investigated. In Chromadorina germanica, Jennings & Deutsch (1975) found no β-glucuronidase activity, which seems appropriate for a nematode in which the cell walls of the food are broken in the buccal cavity and only the cell contents ingested. Deutsch (1978) demonstrated only esterase

activity in the gut of this species; the final stages of digestion were again intracellular, involving a complex set of lysosomes. Jennings and Colam (1970) suggested that in the oncholaimid Pontonema vulgaris the oesophageal glands produced an esterase, the acidophilic and basophilic cells secreting separate precursors which reacted to give the active enzyme when mixed in the gut. In both these species the intestinal cells appeared to pass through a secretory phase, but it is not known what the secretions contained.

A wide range of enzymes is known from nematode parasites of plants and animals (see e.g. Lee, 1965; Fairbairn, 1969; Rogers, 1969). The limitations of the normal histochemical techniques may be the cause of the failure to demonstrate a greater variety of enzymes in the guts of marine nematodes, particularly if the amounts of enzymes secreted are as small as is suggested by the observations of Deutsch (1978) on the slowness of luminal digestion in Diplolaimella (see above). Given the present state of knowledge it is difficult to judge whether selective digestion could account for the large differences in the rates with which marine nematodes assimilate label from apparently similar food microorganisms. It has been shown that selective digestion of bacteria occurs in estuarine oligochaetes, and furthermore that each of three sympatric species preferentially digested different bacteria (Brinkhurst and Chua, 1969; Brinkhurst, 1970; Wavre and Brinkhurst, 1971; Chua and Brinkhurst, 1973).

Uptake of label by juvenile M. disjuncta from a suspension of ML01 was demonstrated in the experiment described in section 5.2.8. The time course of uptake and the retention time in juveniles were not investigated, but it is probable that assimilation was occurring - the retention time is unlikely to be longer in juveniles than in adults. The juveniles used were of the smallest size present in the stock cultures and were presumably newly hatched. It seems possible that by the time the food reserves in the egg are exhausted, juvenile M. disjuncta are just at the size where the buccal cavity is large enough to allow them to feed on bacteria.

CHAPTER 6

FIELD AND LABORATORY STUDIES OF DECOMPOSITION

6.1. FIELD STUDY

6.1.1. Methods

On 11th October, 1979 twenty 0-class (1979) L. saccharina plants were collected from the deeper part of the kelp zone at the Wishing Well by carefully detaching the holdfasts from the substratum. These plants were then taken back to the laboratory in baths of seawater. The frond length of each plant was measured and the plants were numbered by tying around the base of the stipe a label, 15x10cm in size, cut from a sheet of flexible white plastic and numbered with a felt-tip pen. At the Wishing Well a line had previously been laid along the bottom perpendicular to the shore from -1m to -28m, anchored at each end with oil drums filled with concrete. The twine which attached labels to the stipes was also used to attach the plants to the line, about 40cm apart at a depth of 18-20m. Attached by the stipe and lying on the bottom, the plants were free to stream out in the current.

After $3\frac{1}{2}$, 6, 10 and 14 weeks the frond lengths of all remaining plants were measured. On each occasion "disc" and "decomposing tissue" nematode samples were taken from one plant as described in Chapter 4, and the same plant was then cut from the line and taken back to the laboratory, where naked-eye observations, fluorescence and brightfield microscopy were used to study the epiphytes as described in Chapter 3.

6.1.2. Results

6.1.2.1. Decrease in length of the fronds

The measurements of frond length taken after $3\frac{1}{2}$, 6, 10 and 14 weeks are plotted in Fig. 6.1. The number of plants remaining to be measured was smaller at each successive sample. This was because some plants tore through at the frond-stipe junction. For as long as plants remained attached to the line, decomposition involved gradual distal loss of tissue. The rate of loss varied both between plants and, for each plant, between the four intervals between samples. This was probably because tissue was lost in large pieces in the way described in Chapter 2. Overall, the rate of loss was fairly constant throughout the 14-week period, at $\sim$5cm $week^{-1}$.

6.1.2.2. Distribution of decomposing tissue

Scale drawings of the plants used for the nematode and epiphyte samples are given in Fig. 6.2.

0 weeks

Wing and dimpled tissue was missing above 45cm. The central tissue had holes and areas without meristoderm as far back as 40cm. As far back as 15cm the wing tissue showed damage, having many small holes and pits. Around the tip, the decomposing areas were small, extending in for only 2-3mm from the frond edge.

$3\frac{1}{2}$ weeks

Only the central tissue remained above 40cm; this central tissue had one large hole (1x1.4cm) at 52cm and small areas without meristoderm as far back as 20cm. Decomposing areas around the frond tip were again small. As far back as 15cm the wing tissue showed a similar amount of damage to that present at 0 weeks, and there was no extensive development of decomposing tissue around the points of damage.

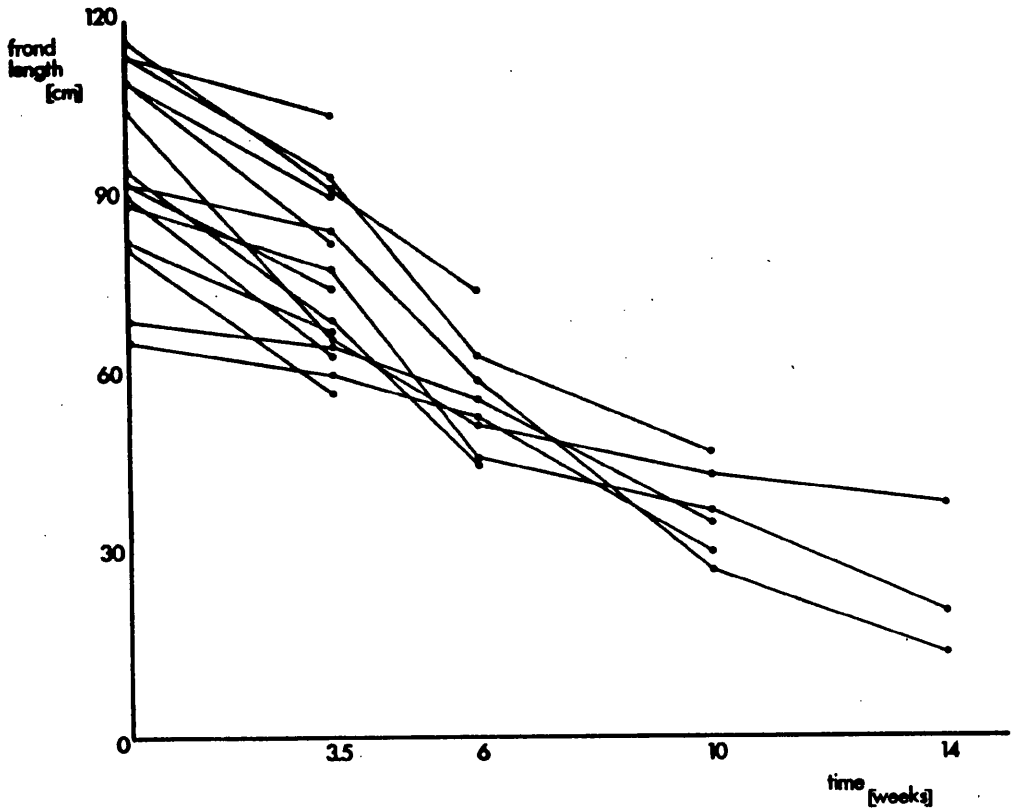

120
frond
length
[cm]
90
60
30
0
3.5
6
10
14
time [weeks]

FIG. 6.1. Decrease in length of L. saccharina fronds tied to line.

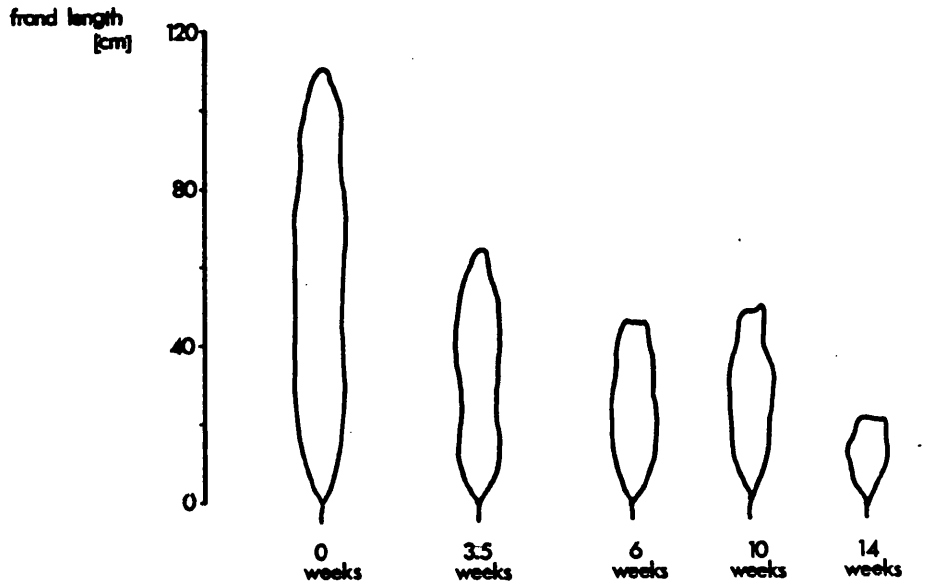


FIG. 6.2. Scale drawings of plants used for nematode and epiphyte samples.

6 weeks

The tip of this frond was almost square, as though it had recently torn across. The wings and dimpled tissue were missing above 35cm. Holes and pits were present in the wing tissue as far back as 20cm. Decomposing areas, both at the frond tip and around points of damage,were again small.

10 weeks

The wing and dimpled tissue had been lost above 25cm. The remaining wing tissue showed some areas of damage back to 15cm. The decomposing areas around the frond edge were small, as in the $3\frac{1}{2}$ and 6 week samples. At the extreme frond tip, however, the situation was different. The tip was again rather square, suggesting that tearing-off had occurred. In addition to the usual small decomposing areas there was a zone where the meristoderm was intact but the tissue was soft and apparently decomposing, (Fig. 6.3.).

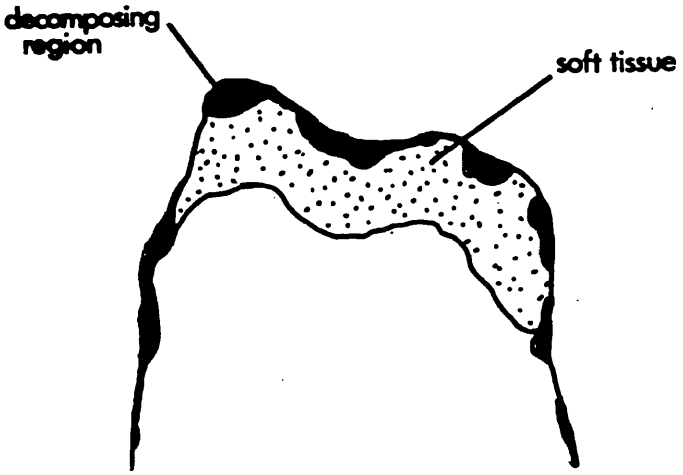


FIGURE 6.3. Tip of the 10-week frond, showing distribution of decomposing areas and soft tissue.

14 weeks

The wing and dimpled tissue had been lost above 10cm. Areas of decomposing tissue around the tip and edges were larger than in previous samples, and soft tissue was more extensive than in the 10-week sample.

6.1.2.3. Frond surface - large animals

When laid down, the fronds had a small number of Lacuna vincta and Gibbula cinerarea (see section 3.3.). These gastropods remained on the fronds for the first 6 weeks; none were seen on the 10-week and 14-week plants. A small number of Spirorbis were also present when the fronds were put down. Further colonization occurred while the plants were tied to the line. After 10 weeks the density on the frond tip was 5-8cm^{-2}; this decreased down the frond and none were seen on the basal 10cm. Neither the Spirorbis initially present nor that which settled during the experiment was identified, so it is not known whether they were the same species. Colonies of the bryozoan Membranipora were present on the upper parts of the frond at the start of the experiment (see section 3.9.). The tissue carrying these colonies was soon lost, and no further colonization by bryozoans took place. Several animals which were never seen in the kelp zone colonized the fronds. Young specimens of the queen scallop Chlamys opercularis (L.) were found attached by byssus threads; 4 on the 6-week plant and 1 on the 10-week plant. Young specimens of the starfish Asterias rubens L. were also found; 2 on the 3½-week plant, 1 on the 6-week plant and 1 on the 10-week plant. One large spider crab Hyas araneus (L.) was found sheltering under the 6-week plant. A mucus tube probably belonging to a polychaete was attached to the 14-week plant, but its inhabitant was not seen.

6.1.2.4. Frond surface - algal epiphytes

0 weeks

Small stands of the diatom Licmophora were present at the frond tip as described in section 3.5. The size of stands, the density of stands and the density of cells within stands all decreased down the frond and none were seen below 40cm. A small number of clumps of ectocarpoid filaments (see section 3.6.), most of the filaments of which were dead, were present above 75cm.

3½ weeks

A few small stands of Licmophora were present at the frond tip and scattered cells were seen back to 30cm. Two small clumps of ectocarpoid filaments, mainly dead, were seen.

6 weeks

A few small stands of Licmophora were seen at the frond tip, the overall density in this region being ∿ 20 cells mm^{-2}; no Licmophora were present below 35cm. No ectocarpoid filaments were seen. One notable feature was the presence over most of the frond surface of small numbers of sediment-mucilage aggregates. A typical example was 70x20um in size and contained 18 sediment grains in the size range 1.2-9.0μm.

10 weeks

Only a few scattered Licmophora were present at the frond tip, and no ectocarpoid filaments were seen. Sediment-mucilage aggregates, as described above, were present on all parts of the frond.

14 weeks

No Licmophora or ectocarpoid filaments were seen, but sediment-mucilage aggregates were present.

6.1.2.5. Frond surface - bacteria

As on plants growing in the kelp zone (section 3.4.), the bacterial flora of intact frond surfaces consisted almost entirely of short rods. Fluorescence counts were made as described in section 3.1.3., and the results are presented in Fig. 6.4. The bacterial densities were similar to those recorded from plants in the kelp zone (Fig. 3.4.). More bacteria were present on the frond tip than on the meristem; there was no obvious change in density during the course of the 14-week period.

6.1.2.6. The community on decomposing tissue

In all plants examined, the community on the decomposing tissue was similar to that seen on the decomposing tissue of plants living in the kelp zone (see section 3.10.). Rod-shaped bacteria were always present; these included short rods, some with orange-fluorescing capsules, and longer rods, some curved (probably Flectobacillus). Filamentous bacteria included small unicellular and multicellular filaments, and also Leucothrix; the maximum abundance of Leucothrix on growing plants coincided with the early part of the experimental period. Ciliates (predominantly Holosticha and Euplotes) and flagellates were usually abundant. An apochlorotic Nitzchia species appeared within the first three weeks of the experiment and was seen in all subsequent samples, reaching a density of $\sim 20 mm^{-2}$ in the 6-week sample. This was apparently the same species as later (January 1980) colonized the decomposing tissue of plants in the kelp zone; apochlorotic diatoms are discussed in section 3.10.3.

The surfaces of the areas of soft tissue which developed in the later part of the experiment (see section 6.1.2.2.) did not carry a microbial community noticeably different from that of other intact frond-tip surfaces. If the "softness" was the result of decomposition, this must have been internal.

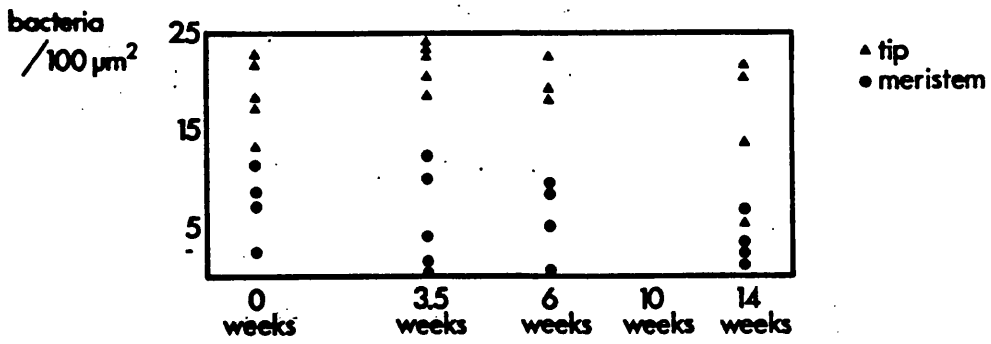


FIG. 6.4. Results of fluorescence counts of bacteria during the decomposition experiment.

6.1.2.7. The nematodes

Table 6.1. shows the results of the disc samples; the species composition of the decomposing tissue samples is shown in Fig. 6.5. The nematode fauna was dominated by Monhystera disjuncta, M. refringens and C. nudicapitata, the three species which were abundant on living plants during the same period. The species composition of the fauna of the 0-week plant was, as would be expected, intermediate between that of the September and November samples in the bimonthly series (Chapter 4). Over the 14 weeks of the experiment the relative abundance of M. disjuncta increased; this paralleled the change on the living plants during this period. As in the bimonthly samples, nematodes were almost absent from the meristem and mid-frond surfaces. On the frond-tip surface the nematode density, $0.4cm^{-2}$, was similar to those recorded in the September and November bimonthly samples. Length-frequency histograms for M. disjuncta from each sample are given in Fig. 6.6. The only remarkable feature of these is the unusually high proportion of very large individuals in the 6-week sample; for which there is no obvious explanation.

Discussion

The rates of basal growth and distal tissue loss in living L. saccharina plants were discussed in section 2.2.3. From these results it can be estimated that over the 14-week period of the experiment, small 1979 plants in the kelp zone would have shown 20-25cm of basal growth and 60-70cm of distal loss. The site of the decomposition experiment, at a depth of 18 to 20cm below C.D., was well below the lower limit at which L. saccharina grows ($\sim$-7m, see Chapter 2), so basal growth in the experimental plants will have been minimal, and the observed decrease in length can be equated to distal tissue loss. This was again 60-70cm over the 14-week period. Since wave action was presumably less at the experimental site than in the kelp zone, the extension of decomposing areas may have contributed more to the process of tissue loss, but the

SAMPLE	3 TIP DISCS COMBINED		MID-FROND DISC	MERISTEM DISC
0 week	*M. disjuncta*	3	*C. nudicapitata* 1	-
	M. refringens	6		
	C. nudicapitata	2		
$3\frac{1}{2}$ week	*M. disjuncta*	2	-	-
6 week	*M. disjuncta*	3	-	-
	oncholaimid	1		
10 week	*M. disjuncta*	5	-	-
14 week	*M. disjuncta*	14	No sample	No sample

TABLE 6.1. Nematodes found in tip disc samples taken during the decomposition experiment.

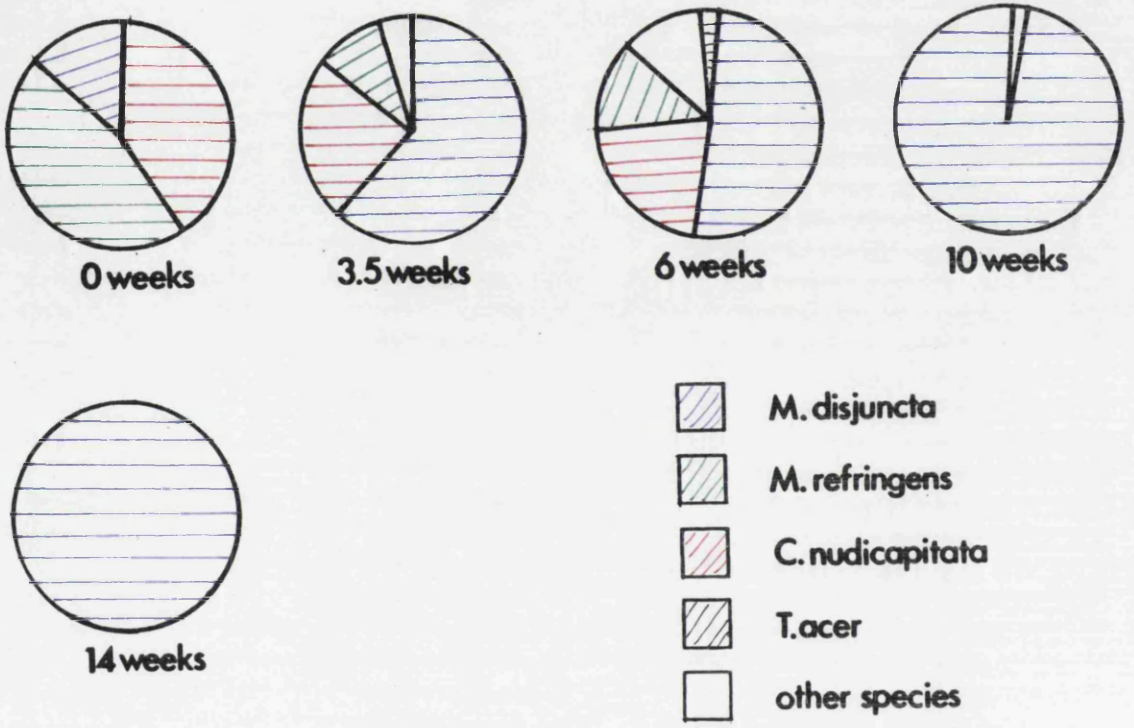

0 weeks
3.5 weeks
6 weeks
10 weeks
14 weeks
M. disjuncta
M. refringens
C. nudicapitata
T. acer
other species

FIG. 6.5. Proportion of the different nematode species in the decomposing tissue samples taken during the decomposition experiment.

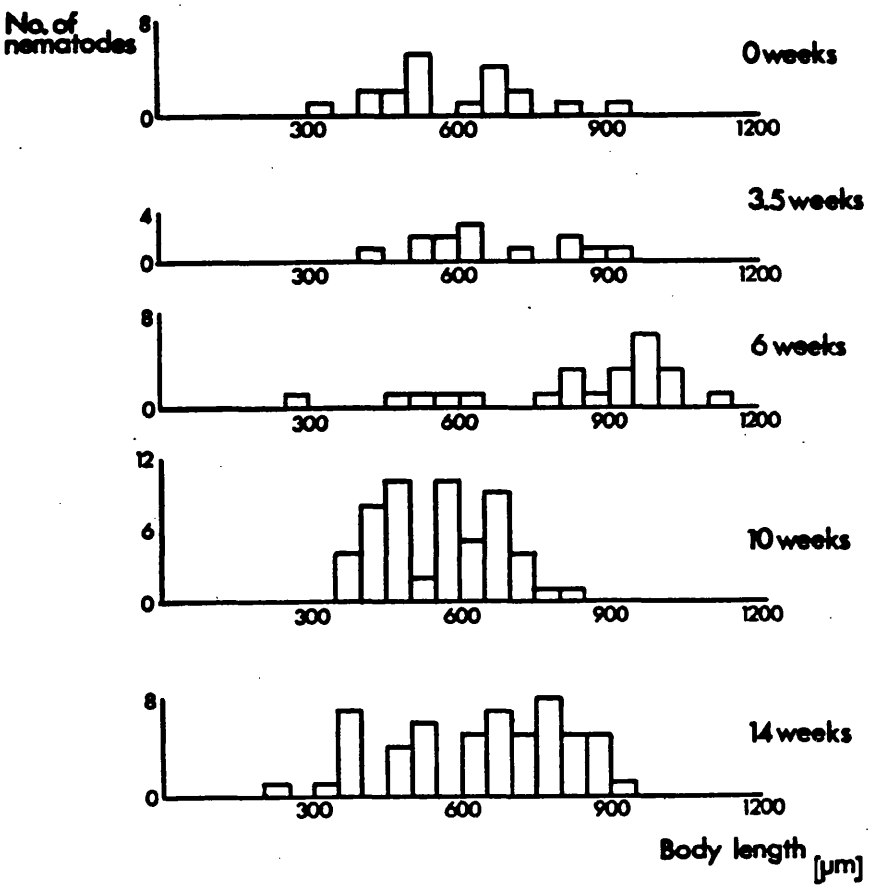


FIG. 6.6. Overall length-frequency histograms for Monhystera disjuncta from each sample taken during the decomposition experiment.

process and rate of decomposition were essentially the same as the process of distal tissue loss in living plants.

Kelp tissue is generally considered to decompose more rapidly than detritus derived from angiosperms such as Spartina or seagrasses (Mann, 1976).

The observations on Licmophora and ectocarpoid filaments on the experimental plants are consistent with the hypothesis that these epiphytes were lost with distal tissue and that no reproduction or colonization occurred. After 14 weeks the tissue at the base of the experimental plants was of approximately the same age as the tissue at the tip of plants remaining in the kelp zone. The latter had both Licmophora and ectocarpoid filaments, the former had neither. An alternative explanation for this is that the growth of these epiphytes is limited by inhibitory substances (see section 3.13.) the effect of which is always greater in the basal tissue of a frond, even when, because basal growth is slow or zero, the basal tissue is old, i.e. that the gradient of effect is position-dependent rather than age-dependent.

In the case of frond surface bacteria, the hypothesis of a position-dependent gradient in inhibitory effect does not entirely explain the observed results. Over the 14-week period the density of bacteria on the basal parts of the experimental fronds increased. On the other hand, the density in this region at the end of 14 weeks was less than that on the tip-surface of living plants, so some positional effect may be involved.

The greater abundance of sediment-mucilage aggregates on the experimental plants than on living plants is probably the result of the presence of finer sediment at the deep-water site.

There was no colonization by nematode species other than those present in the 0-week sample. Gerlach (1977) found that Sabatieria migrans Jensen and Gerlach was attracted to pieces of sardine buried in the sediment. The decomposing Laminaria fronds were in contact with the

sediment for much of the time, but to colonize them, a sediment nematode would have had to move into a habitat with completely different physical properties. Confirmation that M. disjuncta is not dependent on diatoms or on ectocarpoid filaments is provided by the observation that the seasonal increase in the abundance of this species which was seen on the living plants also occurred on the decomposing plants, despite the progressive decrease in the abundance of these epiphytes. The decrease in the abundance of M. refringens and C. nudicapitata cannot be taken as evidence that they were dependent on such epiphytes because at the same time the abundance of these two nematodes on the living plants decreased in a similar way despite the lack of a decrease in the abundance of these epiphytes.

6.2. LABORATORY EXPERIMENTS ON DECOMPOSITION

These were designed to investigate the effect of nematodes, particularly M. disjuncta, on the rate of decomposition of discs of L. saccharina frond tissue in the laboratory. Oxygen consumption of decomposing discs was used as a measure of the decomposition rate.

6.2.1. Design of the respirometer (Fig. 6.7.).

The body of the chamber was made from Perspex, the base from a tube and a sheet, the lid from a solid block. The lid and base screwed together and an O-ring (A) provided an airtight seal. Tubes B and C were 1/16 inch internal diameter stainless steel. The conical shape of the upper part of the chamber allowed air to be displaced through tube C by running in water through tube B while gently tapping the respirometer. The plastic jacket (D) of the oxygen electrode (see below) was cemented into the lid. A removable shelf (E) which separated the specimen from the magnetic "flea" was made from plastic drainpipe ("Durapipe") and plastic netting ("Netlon"). With the shelf and the "flea" in place the chamber

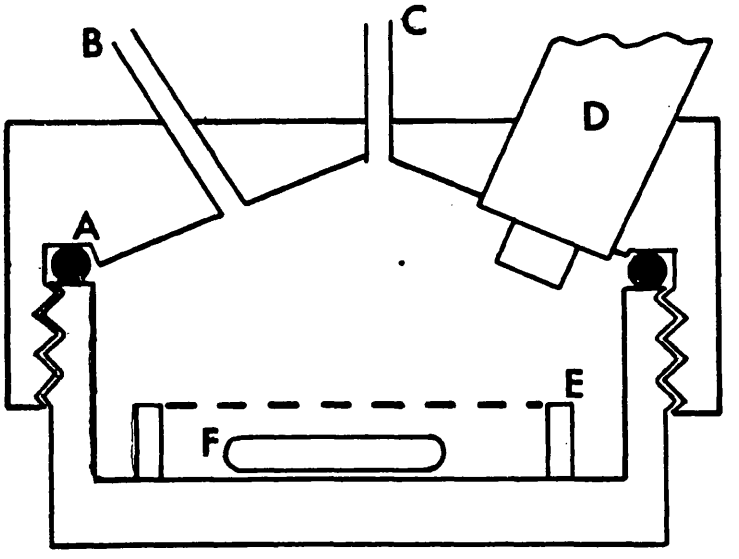


FIG. 6.7. The respirometer.

held 46.3cm^3 of water. The electrode used was a Radiometer E5046 pO_2 electrode, which was connected to a Radiometer PHM71 Mk2 pH meter and a chart recorder (Smiths RE520.20).

All experiments were carried out in a water bath at 15°C. Between experiments the electrode was kept in distilled water at 15°C. It was calibrated daily, using seawater aerated to saturation at 15°C, the oxygen concentration of which was checked with a Winkler titration, and a zero-pO_2 solution (a pinch of solid Na_2SO_3 added to a 0.01M $Na_2B_4O_7$ solution).

To measure the oxygen consumption of a Laminaria disc, the base of the respirometer was filled with oxygen-saturated MFSW at 15°C and the disc was laid on the shelf. The lid was screwed on and more saturated MFSW run in through a flexible plastic tube attached to B, displacing air through C. The plastic tubes attached to B and C were then clamped shut and the respirometer was placed in the water-bath on a submersible magnetic stirrer. The water bath was then covered with black plastic to exclude light, and the chart recorder was switched on. Recording was continued until enough of a linear trace was obtained for its slope to be measured; this usually took ∿30 minutes. After this time the stirrer and recorder were switched off, and the clamps on the tubes attached to B and C were removed, allowing water to siphon out through C, being replaced by fresh aerated water through B. When the chamber had been completely flushed out, the clamps were replaced, the stirrer and chart recorder were switched on again and a second recording made. A third recording was made in the same way.

Between runs the discs were kept in flowing filtered (Whatman Gamma-12 0.3μm on-line filter) aerated seawater at 15°C. The inside of the respirometer was wiped between runs to prevent a build-up of bacteria.

6.2.2. Results

Unfortunately no very useful results were obtained. Two main difficulties were encountered with these experiments. Firstly, the Laminaria discs soon broke up, either during incubation or in the respirometer, even though the stirrer speed was kept to the minimum necessary to ensure complete mixing. This might have been avoided in part by enclosing the discs in a bag made from non-biodegradable mesh. Secondly, in most runs the measured rate of oxygen consumption was lower in each of the three successive replicate measurements. A possible explanation for this is that some of the organisms whose respiration rate was being measured were sensitive to lowering of the oxygen concentration, despite the fact that this was never allowed to fall below about 20% of saturation level at 15°C.

For these reasons, further experimental effort was directed into the feeding experiments described in Chapter 5, which were producing results with fewer problems. A method similar to that used by Fenchel (1976) would probably prove more satisfactory for investigations of decomposition. ^{14}C could be incorporated into L. saccharina discs by allowing them to photosynthesize in seawater with added $NaH^{14}CO_3$. The discs could then be incubated in seawater through which a stream of air was bubbled, and decomposition could be followed by monitoring the release of $^{14}CO_2$.

6.2.3. Discussion

It would be expected from the results of previous studies that nematodes would increase the rate of decomposition. There are several mechanisms by which this might occur. Nematodes of the three smaller species (M. disjuncta, M. refringens and C. nudicapitata) burrow into the decomposing tissue. In so doing they must disrupt the tissue and expose fresh surfaces for colonization by bacteria. This effect would be similar to that described for larger invertebrate detritivores

such as amphipods (Fenchel, 1970; Hargrave, 1970). As well as exposing new surfaces, nematodes could transfer an inoculum of bacteria to these surfaces, either on the body or, in the case of bacterial feeders, via the gut. In the latter case, selective digestion and the passage of viable bacteria of particular types through the gut could influence the composition of the microbial flora which subsequently developed on the new surfaces. Another possible effect is through increasing bacterial metabolism. Johannes (1965) found that bacterivorous protozoans increased the rates of decomposition processes. He suggested that in the absence of grazing, nutrients become locked up in bacterial biomass. By feeding and excretion the protozoans regenerate dissolved nutrients which are then available for further bacterial growth. The study by Barsdate et al (1974) however, did not confirm this hypothesis. In the microcosms used in Barsdate's study there was rapid turnover of phosphorus in both grazed and ungrazed populations. This cycling was somewhat faster and the rate of mineralization was four times greater in grazed than in ungrazed systems. However, excretion by the protozoan grazers accounted for only 4% of the phosphorus regeneration of grazed systems. It cannot be totally ruled out that grazers excrete some growth-promoting substance, but it seems more likely that the mechanical activity of the grazers leads to micro-turbulence and thus increases the availability of nutrients or oxygen in the immediate vicinity of the bacteria, or that grazing selects for quickly growing forms among the mixed assemblage of bacteria. (Barsdate et al, 1974; Fenchel, 1976; Fenchel and Harrison, 1976).

The studies discussed above have used protozoan grazers. It is likely that nematode grazing can have similar effects. The experiment described in section 5.2.6. indicates that the rate of phosphorus excretion by M. disjuncta is low. On decomposing Laminaria tissue, protozoans (ciliates and flagellates) may well be more important than nematodes as consumers of bacteria. The main effect of the nematodes is probably

therefore through the mechanical disruption of the Laminaria tissue.

Any increase in the rate of in situ decomposition at the frond tip will lead to an increase in the rate of leakage of DOM, the rate of production of fine particulate detritus, and, through an increase in the rate of spreading of decomposing areas, the rate of loss of large pieces of tissue (Fig. 3.3.).

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A NEW SPECIES OF GONIONCHUS (NEMATODA : XYALIDAE) FROM THE FIRTH OF CLYDE, WITH A REDESCRIPTION OF ENOPLOIDES SPICULOHAMATUS SCHULZ (NEMATODA : ENOPLIDAE). [1]

M. P. BENWELL

University Marine Biological Station, Millport,
Isle of Cumbrae,
Scotland, KA28 OEG.

RESUME

Deux espèces de Nematodes libres marins du sable sublittoral a Tomont End, Isle de Cumbrae, Ecosse, sont décrites. Gonionchus cumbraensis est une nouvelle espèce et Enoploides spiculohamatus Schulz était jusqu' ici mal décrite. La taxonomie des genres Gonionchus et Xyala est discutée.

1. Typescript of paper in press (Cahiers de Biologie Marine.).

INTRODUCTION

As part of a study of the role of free-living nematodes in the decomposition of kelp, a sample was taken by SCUBA diving of the sediment at a depth of 6m off Tomont End, Isle of Cumbrae, Scotland. This sediment is a medium coarse sand containing a large number of broken mollusc shells. Of the eighteen nematode species in the sample, one, Gonionchus cumbraensis sp. nov., proved to be new and another, Enoploides spiculohamatus Schulz, has previously been only poorly described.

Descriptions have been made from glycerine mounts and the material deposited at the British Museum (Natural History). Measurements have not been assembled into ratios (de Man or Filipjev formulae) as this practice is often unhelpful. Curved structures have been measured as the arc and not the chord.

ENOPLOIDES SPICULOHAMATUS Schulz 1932 (Fig. 1)

Material studied

2♂♂, 2 juveniles
B.M. (N.H.) Reg. nos. 1979.2.7. to 1979.2.10.

Measurements

In µm, in order $♂_1$; $♂_2$; juv_1; juv_2

Body length	2682	2840	1725	896
Maximum diameter	78	83	68	40
Head diameter	47	46	35	26
Labial setae	20	20	14	9
Long cephalic setae	48	48	34	25
Short cephalic setae	24	25	15	11
Anterior end to nerve ring	151	151	143	-
Oesophagus length	578	560	421	267
Diameter at base of oesophagus	70	74	65	40
Supplement length	27	25	-	-
Supplement to cloaca	94	89	-	-
Spicule length	335	356	-	-
Gubernaculum length	45	42	-	-
Cloacal (anal) diameter	59	54	50	26
Tail length	176	167	170	118

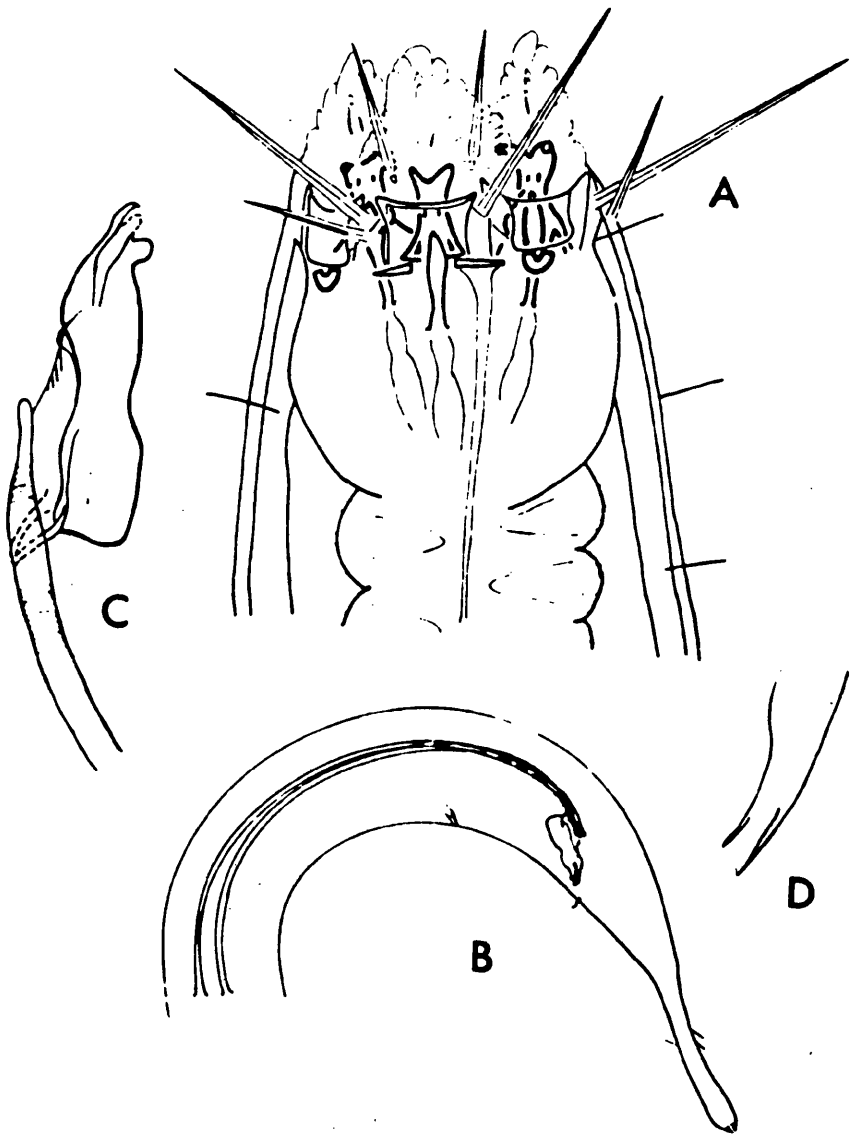

FIG. 1. Enoploides spiculohamatus Schulz.

A: Male head; B: Male tail; C: Gubernaculum;
D: Supplement.
All drawn from ♂1

Description

Three high lips, with distinct striations on their inner surfaces. Each with two labial setae. Ten cephalic setae: six longer anterior setae and four shorter setae immediately posterior to the dorso-lateral longer setae. Clawed mandibles projecting anterior to the cuticularized plates. Each with two short linear perforations in the anterior part and each bearing a small tooth, these teeth equal in size. Immediately posterior to each mandible is a pair of transverse cuticularized bars. Outside the mandibles are two layers of cuticularized plates. The outer layer consists of six plates of two types, one type in the labial positions, the other in the interlabial positions. The cephalic setae are attached over the spaces between these plates. The anterior margin of the inner layer runs slightly posterior to the anterior margin of the outer layer in the labial positions, and in the interlabial positions runs diagonally posteriorly to a heavily cuticularized crescentic structure. The amphids could not be seen. Oesophagus typically enoploid, three files of glands down its length. Tail with a few scattered setae but no long terminal setae.

Male: Gubernaculum a pair of plates joined by a process on each at the proximal end. Distal end of each plate grooved, with a claw-shaped projection on the dorsal side of the groove and two equal rounded projections in the ventro-lateral positions. Spicules equal, long, transversely striated, open at proximal end, distal end of each joined by a muscle or ligament to the blunt dorsal process on the respective gubernacular plate. Supplement small, tubular, thickened at the distal end. A pair of S-shaped spines 13μm posterior to the cloaca.

DISCUSSION

The only definite differences between the specimens described above and Schulz's (1932) description of E. spiculohamatus are in the distance from the supplement to the cloaca and the length of the spicules, and these do not warrant recognition of a separate species. Unfortunately Schulz's description does not show the cuticularized structures of the head or the shape of the gubernaculum very clearly, and there appear to be no type specimens. For the present the above specimens are assumed to be E. spiculohamatus, but collection from Schulz's site might reveal a different species fitting his description, in which case they will have to be renamed. The description and preservation of these specimens is a step towards sorting out the taxonomy of this difficult species group.

There has been confusion over specimens hitherto named as E. spiculohamatus, E. labiatus and E. longispiculosus. E. labiatus Butschli 1874 was described from a female, and is correctly regarded by Wieser and Hopper (1967) as a species inquirenda. Stekhoven (1935) and Bresslau and Stekhoven (1940) figure males under the name E. labiatus with which they consider E. spiculohamatus to be synonymous. This synonymy cannot be proved and should be abandoned. Bresslau and Stekhoven's species may be E. spiculohamatus but the description is poor. Stekhoven's species is not E. spiculohamatus but the description is not good enough to serve as a description of a new species.

Riemann (1966) records E. aff. labiatus which he considers is probably a complex species. Skoolmun and Gerlach (1971) record E. spiculohamatus. In neither case is a description given.

Warwick (1971) and Platt (1977, 1977a) record E. spiculohamatus from the British coast. Examination of specimens provided by Dr. Platt and a description provided by Dr. Warwick has shown that in both cases the species concerned is E. longispiculosus rather than E. spiculohamatus.

Wieser and Hopper (1967) give a good key to the species of Enoploides with long spicules and S-shaped gubernacula. To this must be added E. longispiculosus and also E. delamarei Boucher (1977). E. caspersi Riemann 1966, E. disparilis Sergeeva 1974, E. ponticus Sergeeva 1974 and E. alexandrae Uzunov 1974 do not belong to this species group.

GONIONCHUS CUMBRAENSIS sp. nov. (Fig. 2)

Material supplied

3♂♂ (Holotype and 2 paratypes), 3♀♀ (paratypes).
B.M. (N.H.) Reg. nos. 1979.2.1. (Holotype) to 1979.2.6.
Additional specimens in the author's possession.

Measurements

In µm, in order (Holotype)	$♂_1$	$♂_2$	$♂_3$	$♀_1$	$♀_2$	$♀_3$
Body length	1320	1370	1510	1474	1526	1358
Maximum diameter	52	54	58	52	61	54
Head diameter	17	19	20	20	22	23
Long cephalic setae	18	18	17	18	17	19
Short cephalic setae	10	10	11	12	11	13
Amphid distance	25	25	28	28	29	27
Amphid diameter	13	12	14	9	11	10
Diameter at level of amphid	23	26	26	22	26	34
Anterior end to nerve ring	112	-	126	140	-	-
Oesophagus length	297	324	400	386	391	337
Diameter at base of oesophagus	44	50	50	50	60	44
Anterior end to vulva	-	-	-	1007	1033	957
Spicule length	40	42	42	-	-	-
Cloacal (anal) diameter	33	36	38	34	35	32
Tail length	238	232	281	289	310	280

Description

Cuticle annulated, annules 2.5µm wide. Six high lips, each with three fine longitudinal striations on its outer surface. The anterior part of each lip forms a delicate flap apparently hinged to the rest of the lip. Short (3µm) labial setae prominent. Around the buccal cavity

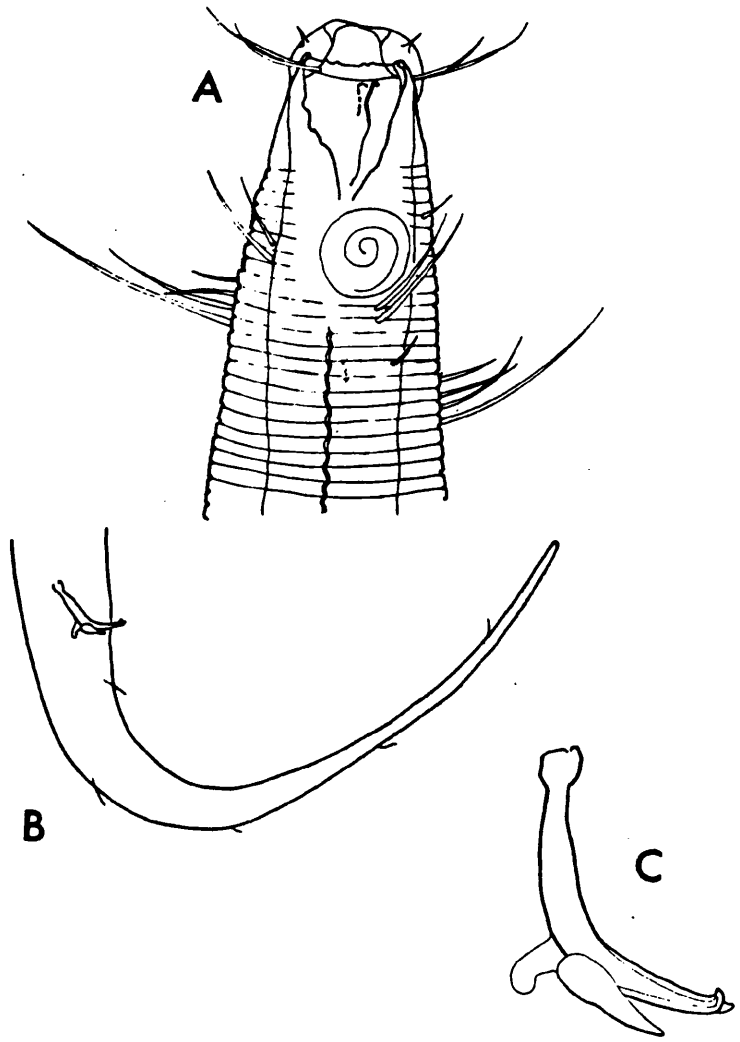

FIG. 2. Gonionchus cumbraensis sp. nov.

A: Male head; B: Spicules and gubernaculum; C: Male tail. Drawn from ♂1, (holotype).

runs a cuticularized band from which a double peg extends anteriorly into the base of each lip. In the buccal cavity are two subventral cuticularized flanges. In the female ten cephalic setae: six longer setae and four shorter setae adjacent to the submedian longer setae. In the male two additional setae just ventral of the lateral long setae. Four short (6 µm) subcephalic setae just anterior to the amphids. Amphids indistinct, circular in outline with faint spiral structure. Larger in the male than in the female. Cervical and somatic setae long and numerous. Apart from a few short single setae, cervical setae arranged in groups of three or four. Setae in each group in a longitudinal row, one on each of successive annules of the cuticle. The most conspicuous groups are of four setae, with a progressive increase in length from the anterior (10-15µm) to the posterior, (35-40µm). As far as the anus, somatic setae more widely spaced, 10-45µm long. Tail long, tapering in the anterior part, almost cylindrical posteriorly. Caudal setae only sparse and short (5-15µm long). Two 15µm terminal setae in some specimens.

Male: spicules paired, equal, proximally cephalate. Walls very thick distally, becoming progressively thinner proximally. Spicules distally bifid, each with an outwardly-turned lateral process and a slightly narrower median process. Gubernaculum with weakly cuticularized, paired, 6µm long, dorsocaudal apophyses. Two testes, the anterior larger and lying to the left of the gut, the posterior, much smaller, lying to the right of and dorsal to the gut. Seminal vesicle conspicuous 110-130µm anterior to the cloaca. Immediately anterior to the cloaca lies a pair of glands, 20-30µm long, one on either side of the vas deferens, each with a duct leading to the cloaca.

Female: ovary single, anterior, lying to the left of the gut.

DISCUSSION

G. cumbraensis sp. nov. is close to the type species, G. villosus Cobb 1920, and to G. inaequalis Warwick and Platt 1973. It can be distinguished from both of these by the bifid spicules, and also from G. villosus by the possession of gubernacular apophyses and from G. inaequalis by the shorter body setae and equal spicules. These three species form a homogeneous group.

The species remaining in Xyala after Lorenzen's (1977) revision of the Xyalidae are X. striata Cobb 1920 X. riemanni Boucher and Helleouet 1977, and X. imparis Boucher and Helleouet 1977. These species form a second homogeneous group.

The problem in the taxonomy of these two genera is the placing of G. longicaudatus (Ward 1972), which Lorenzen transferred to Gonionchus from Xyala, and G. sensibilis Lorenzen 1977. These species resemble each other closely. They have neither teeth within the buccal cavity nor a cuticularized band around it. Their cuticles have longitudinal ridges as well as transverse annulation. The body setae are not exceptionally long. In all these respects these species resemble Xyala rather than Gonionchus. Lorenzen places them in Gonionchus because he considers that they have high lips of a type otherwise found only in the other Gonionchus species and that these lips characterize a monophyletic group.

Examination of specimens of G. cumbraensis, G. inaequalis, G. longicaudatus, X. striata and X. riemanni has shown that the lips of all these species have the same basic structure. There is a terminal flap, often longitudinally striated, separated from the posterior part of the lip by a septum anterior to the labial setae. This is best shown, for the three species groups, in the descriptions of G. cumbraensis above, G. sensibilis in Lorenzen (1977) and X. riemanni in Lorenzen (1978). The lips of the three species groups do differ, but there is no strong reason to classify G. longicaudatus and G. sensibilis with Gonionchus rather than

with Xyala. It seems preferable to use a classification based on several characters any of which can reasonably be given more weight than can the structure of the lips: the teeth, the cuticularized band, and the cuticular ornamentation.

It is therefore proposed that G. longicaudatus be returned to, and G. sensibilis transferred to, Xyala, as X. longicaudata Ward 1972 and X. sensibilis (Lorenzen 1977) respectively. An alternative would be to erect a separate genus for these two species. This seems to be unnecessary at the present but could be done later, particularly if additional species are discovered.

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SUMMARY

Two species of free-living marine nematode from a sublittoral sand at Tomont End, Isle of Cumbrae, Scotland are described. One of these, Gonionchus cumbraensis sp. nov. is new to science, the other Enoploides spiculohamatus Schulz was previously only poorly described. The taxonomy of the genera Gonionchus and Xyala is discussed.

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