THE ROLE OF HYALINE HAIRS IN FUCUS

Thesis submitted in accordance with the requirements of the University of Liverpool for the degree of Doctor of Philosophy

by

Robert Stephen Galvin

Department of Marine Biology
University of Liverpool
Port Erin
Isle of Man.

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DAMAGED TEXT IN ORIGINAL

To my Mother; and to the memory

of my Father.

CONTENTS

ACKNOWLEDGEMENTS

In time honoured tradition I would like to initially give thanks to my supervisor, Professor Trevor Norton, for his help and, above all, his patience throughout the course of this work ("If ever a man suffered..."). The problem now, however, is to acknowledge the people who spent time and energy, not to mention money, in helping me to complete this thesis. Over a period of many years it is easy to forget the helping hands which gave the necessary support, whether physical, moral or alcoholic, at any given time. The following list, therefore, is not, and never will be, complete. If anyone is missed out please forgive my ailing brain and accept that I appreciated everyone. Some names refer to more than one person (a good get out clause eh!).

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Love and thanks to you all.

"I get by with a little help from my friends"

Lennon and McCartney.

CHAPTER ONE - GENERAL INTRODUCTION

The occurence of hyaline (colourless) hairs in many algal genera has been well known for many years (e.g. Huber, 1892; Rosenvinge, 1911; Fritsch, 1935, 1945; Ginsburg-Ardre, 1966). "Hairs" in the eukaryotic algae generally refers to a sterile filamentous outgrowth from the surface of the thallus (DeBoer and Whoriskey, 1983). A survey of the literature, shows that hairs are generally assumed to have an assimilatory function, increasing the uptake of inorganic ions for incorporation into organic cellular components (e.g. Sinclair and Whitton, 1977; Whitton and Harding, 1978; DeBoer and Whoriskey, 1983; Wallentinus, 1984). It is somewhat surprising therefore that little quantitative work has been carried out on the ecological role of these hairs in adult plants.

The intertidal brown algae of the genus *Fucus*, all exhibit hyaline hairs in both the adult and embryonic forms. Laboratory culture studies on the early stages of morphogenesis in *Fucus* spp., have mentioned the formation of multicellular hyaline hairs at the apical tip of the developing embryo (Fritsch, 1945; Burrows and Lodge, 1953; Galun and Torrey, 1969; Schonbeck and Norton, 1981). The first hair is pushed out from the embryonic *Fucus* by an intercalary meristem, this being followed by several other hairs from the same source. At the base of these hairs, one cell will ultimately become the apical meristematic cell of the adult plant.

Galun and Torrey (1969), regarded hair formation as being a vital phase in differentiation of the developing *Fucus*. They suppressed the

formation of hairs by the use of 5-Fluoracil, and embryos of *Fucus vesiculosus* L., failed to progress onto any further developmental stages.

Given the importance of these hairs not only in a phase in differentiation but also in a potential assimilatory role, a great deal of work has recently been carried out on their different aspects (e.g. DeBoer and Whoriskey, 1981,1983; Livingstone and Whitton, 1983; Livingstone et al, 1983; Gibson and Whitton, 1986,1987). It is all the more surprising therefore to find that almost no attention whatsoever has been paid to the role of hairs in adult *Fucus*. Again the literature mentions the assimilatory role of hyaline hairs in the uptake of nutrients by *Fucus* (Schonbeck and Norton 1979) but there is apparently no quantitative data to support this view.

This study aims to establish whether or not the hairs do actually have an assimilatory function and the subsequent effects this may have on the growth of *Fucus* plants. The wider ecological implications of bearing hairs will also be examined.

CHAPTER TWO - AN EXAMINATION OF HYALINE HAIRS IN *FUCUS*

2.1. INTRODUCTION

The hyaline hairs and the cryptostomata from which they emerge were examined by means of Scanning Electron Microscopy and photographs of histological sections.

2.2. METHODS

2.3. SCANNING ELECTRON MICROSCOPY

Pieces of algal thallus were cut horizontally into 1 cm strips and fixed in 5 %. formaldehyde-seawater for a minimum of 24 hours. The algae may be stored for longer periods at this stage without any obvious deleterious effects (G. Russell pers. comm.), although this was avoided whenever possible. The portions of thallus were washed in distilled water before being dehydrated in an acetone series of 2.5, 5, 7.5, 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90, 100, 100 %. Changes were made every twelve hours so that the samples were dried slowly to minimise cell wall distortion (Russell and Veltkamp, 1984). Following this initial dehydration process the algae was kept in 100 % acetone and transported to the Botany Department in Liverpool.

Samples were further dried in a Polaron E3000 critical point dryer before being glued to aluminium stubs and sputter coated in 60 %

gold-palladium. The specimens were then viewed using a Philips 501B Scanning Electron Microscope.

2.4. HISTOLOGY

Samples to be sectioned were initially fixed in 5% formalin-seawater for 24 hours. Following this the alga was slowly dehydrated in alcohol through progressive stages from 10% to 70% increasing the alcohol in 10% steps every twelve hours. The samples were then immersed in a solution of 50% of 70% alcohol and 50% of tertiary butyl acid (TBA) for 24 hours. This stage was followed by a further 24 hours in 50% of 90% alcohol and 50% TBA before three daily changes of 50% absolute alcohol and 50% TBA. The samples and the final change of this solution were floated on top of solid wax in a pyrex container prior to being placed in an oven at 60 °C. The solution of absolute alcohol and TBA evaporated off in the oven, leaving the algal material in the wax. The wax was changed twice at 24 hour intervals before the samples were embedded and sectioned. Sections were floated onto albumen smeared glass slides and dried slowly for a minimum of twelve hours.

The slides with the sections attached were placed in two changes of histoclear for five minutes each before spending a further five minutes in each of a descending alcohol series of 100%, 100%, 90%, and 70% prior to five minutes in two changes of distilled water. The

material was stained for three minutes in Gill's Haematoxalin and blued in running tap water for 30 minutes. Counterstaining was carried out in Eosin for two minutes before the slides were taken rapidly back through the alcohol series of 70%, 90%, 90%, 100%, 100% and left in the histoclear for a minimum of five minutes. Coverslips were mounted on the slides, whilst still wet from the histoclear, using DPX mountant.

2.5. DISCUSSION OF RESULTS

The three species of the genus *Fucus* commonly found intertidally on British coasts are *Fucus spiralis, F. vesiculosus* and *F. serratus*. All three are characterised by parenchymatous thalli with apical growth from a single apical cell (Fritsch, 1945). The three species possess small cup shaped structures called cryptostomata, situated in the thallus wings and open to the exterior, (Plate 2.1). The cryptostomata are steep sided pits similar in structure to the specialised reproductive conceptacles of *Fucus* but they do not contain either antheridia or oogonia (Clayton, 1984). As a result of this similarity to the reproductive bodies they have previously been termed sterile conceptacles by many authors (e.g. Dangeard, 1933; Fritsch, 1945; Chapman, 1962).

Clayton (1984), has found gametes in conceptacles which had developed from cryptostomata in *Hormosira*. She considers, however,

that the distribution of conceptacles in this genus is a primitive condition. In *Splachnidium* and *Notheia* there is also a transformation of the cryptostomata into reproductive structures but this transformation does not occur in *Fucus* (Clayton, 1984). Multicellular hyaline hairs protrude from the thallus of *Fucus* after growing out from the cryptostomata (Plates 2.2 to 2.5). The cryptostomata are formed below the apical tip (Plate 2.6) and when formed and open, the hairs protrude beyond the ostiole (Plate 2.7). Once produced by the *Fucus* the hairs remain visible along the length of the thallus as apical growth continues (Plates 2.8 and 2.9).

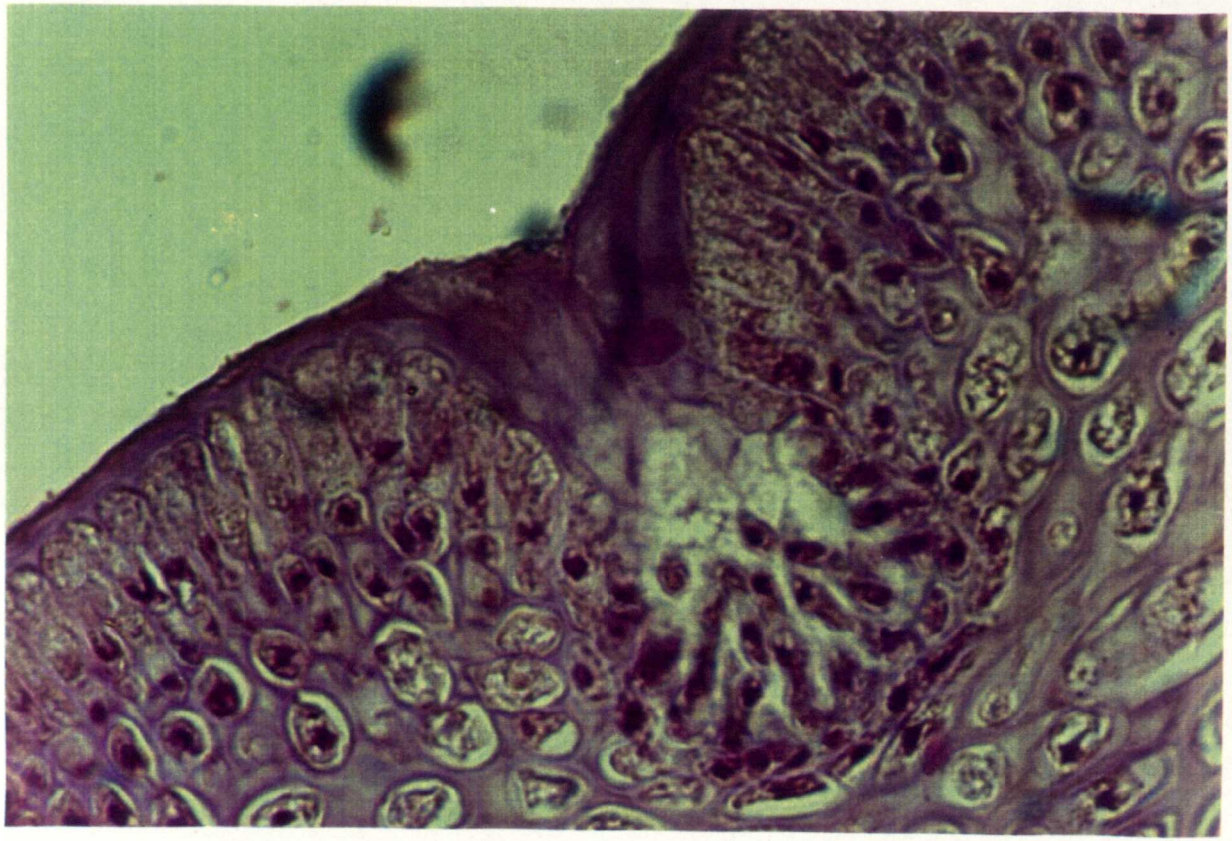
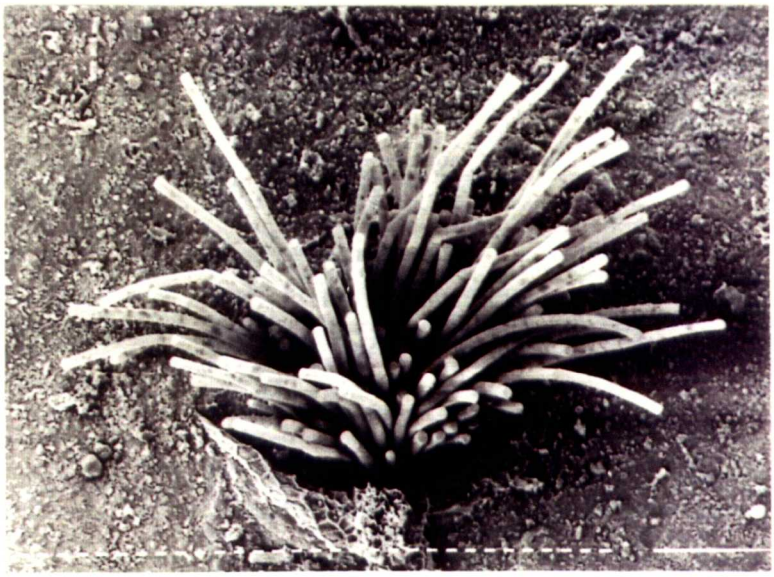
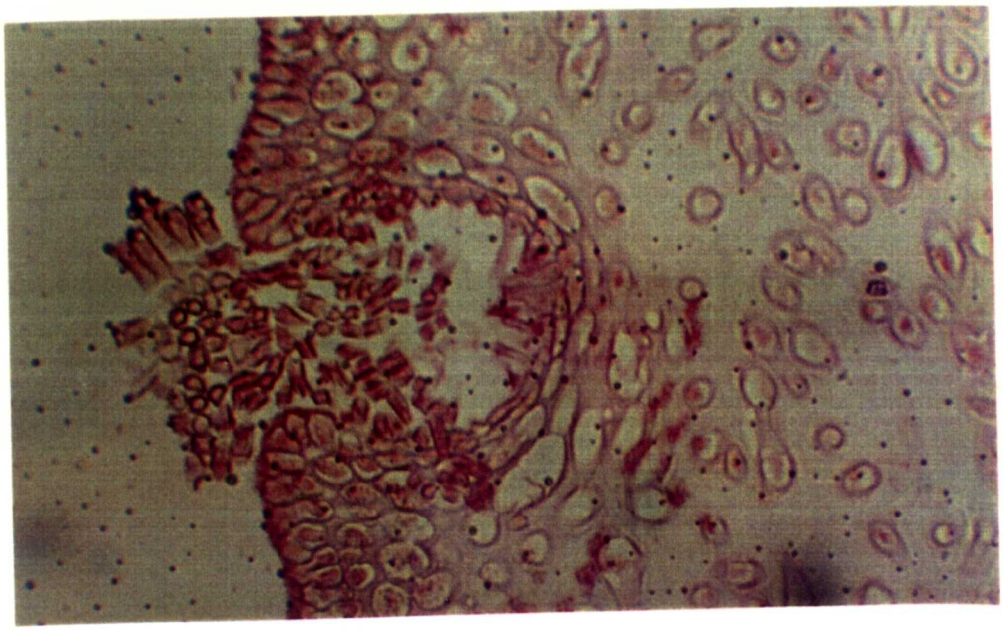
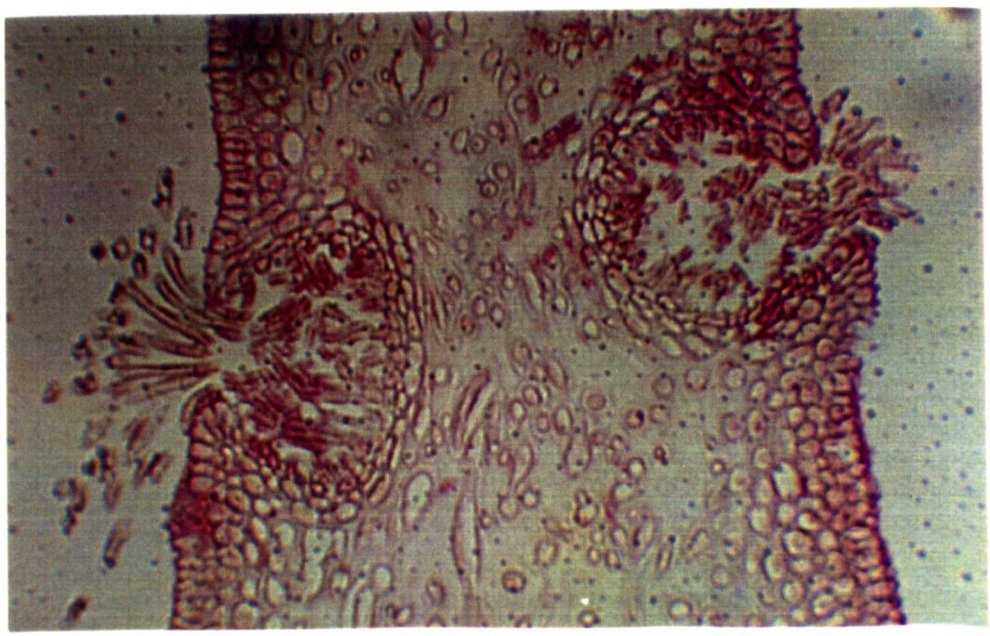


Plate 2.1: Newly formed cryptostoma (x800)





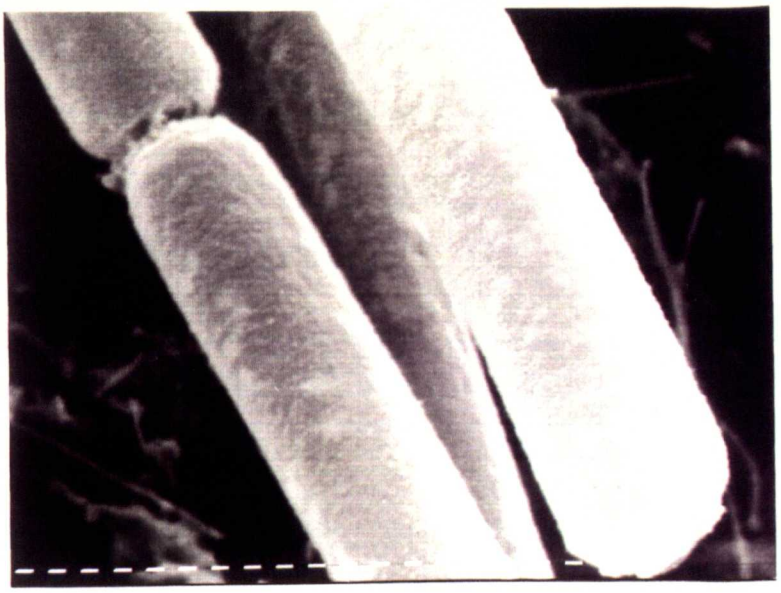


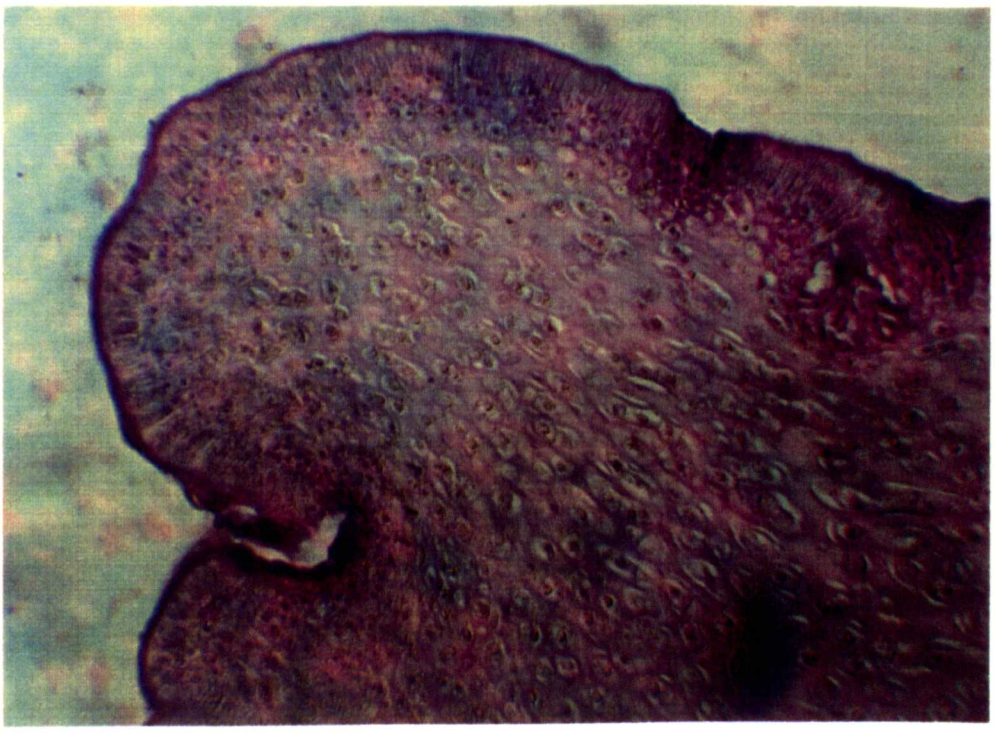
Plates 2.2 to 2.4: Hairs emerging from the cryptostomata. Plate 2.2 scale bars = 10μm, Plate 2.3 x125, Plate 2.4 x100.

Plate 2.5: The multicellular hyaline hairs (10mm)

Plate 2.6: Cryptostoma forming behind the apical tip of *Fucus* (x160)

Plate 2.7: Apical tip of *Fucus* spp.illustrating the formation of the cryptostomata and growth of hairs (100mm)





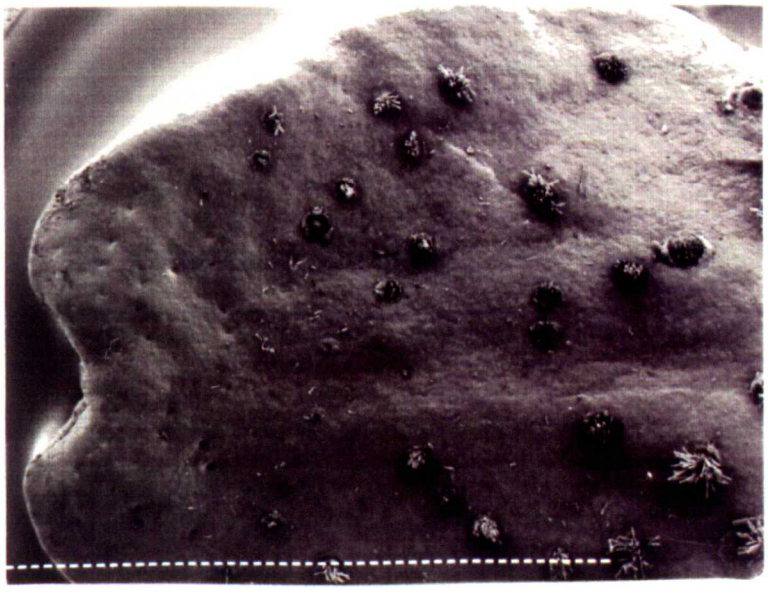


Plate 2.8: *Fucus spiralis* bearing hairs (x1)

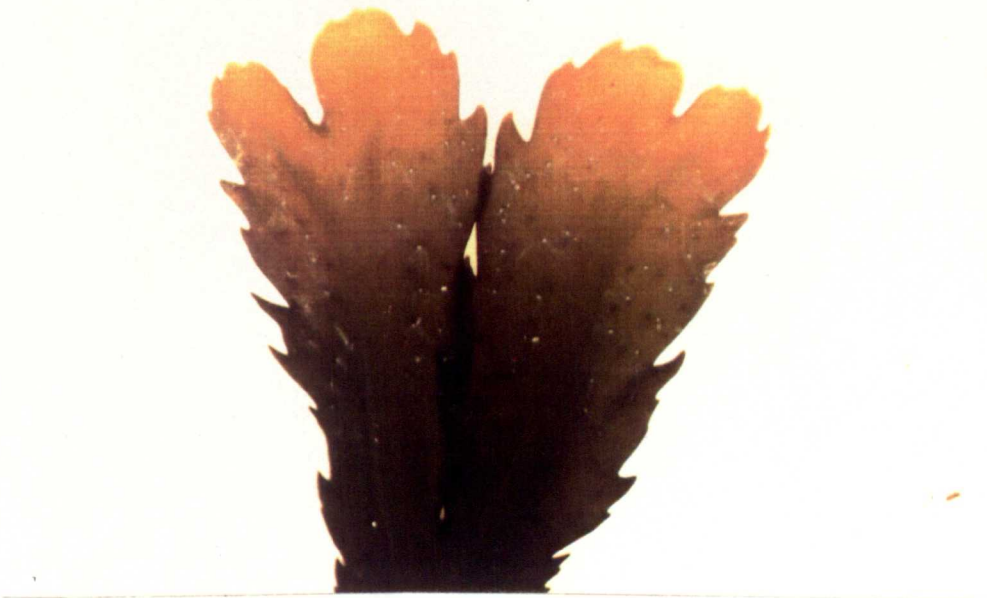


Plate 2.9: Hairs on *Fucus serratus* (x1)

CHAPTER THREE - THE OCCURENCE OF HAIRS IN INTERTIDAL *FUCUS*

3.1 INTRODUCTION

Casual observations in the field had revealed a distinctive seasonal cycle in the occurrence of hyaline hairs in all of the common intertidal *Fucus* spp. The hairs seemed to occur in spring and they remained visible on the plants throughout the summer before disappearing in the autumn. Such seasonal changes are commonplace, e.g. Knight and Parke (1950), describe a morphological rhythm in frond development, reproduction and defoliation in *Fucus vesiculosus* L. and *F. serratus* L.. Luning (1982) examined the effects of environmental factors on gametophyte development and sporophyte periodicity in *Laminaria* spp..

The seasonal changes in hair occurrence, however, is hardly mentioned in the literature for mature plants and only in passing for germlings of *Fucus spiralis* (Schonbeck and Norton, 1979). It was decided therefore to undertake an autecological study of the genus *Fucus* with respect to hair formation and disappearance in an attempt to clarify this phenomenon.

3.2. FIELDWORK METHODS

3.2.1. FIELDWORK SITE

The site chosen for the majority of the field work was a small island off the south-east coast of the Isle of Man (O.S. ref. 674296). The island is officially called St. Michael's Island but is known locally as "Fort Island" due to the 16th century fortifications built to protect the nearby harbour at Derbyhaven. The island is connected to the mainland by a short bridge over a narrow channel. St Michael's Island is a convenient site for field work as it has both exposed and sheltered shores within easy walking distance. In addition it is less than one Kilometre from Ronaldsway airport from where meteorological data may be obtained.

3.2.2. PERMANENT TRANSECTS

A permanent vertical transect was set up on each of three shores around the island. The shores were selected as they met the criteria laid down by Ballantine (1963), for sheltered, semi-sheltered and exposed shores. The height of each transect was marked at 1/2 metre intervals by cementing a pre-stamped plastic marker in place using Hawke Quick-set Cement. The height of the transect stations was determined by the use of a hydraulic levelling device developed for use in biological field work by R. V. Tait (Pers. Comm.) (Fig. 3.1.). As no Ordnance Survey bench mark was located nearby, the heights were determined, on two successive calm days, by taking the predicted low

tide height from Tide Tables as a base line for the transect and working up from low-tide level. The transects were then cross checked against each other by means of the hydraulic device. The error between them amounted to no more than 4 cm; an indication of the accuracy of the equipment used.

3.2.3. HAIR SEASONALITY, TAGGING EXPERIMENT

Two algal monitoring experiments were set up in the field to follow the development of hairs in *Fucus* spp. in Spring and their subsequent loss in Autumn.

Five stations were selected at tidal heights of 3.0m 3.5m 4.0m 4.5m and 5.0m above Lowest Astronomical Tide, on transects SS (Semi-Sheltered), and E (Exposed), at St Michael's Island. On transect S (Sheltered) only two stations (3.0m and 5.0m) were set up to assess the *Fucus* spp. because the extensive mid-tidal cover of *Ascophyllum nodosum* (L.) Le Jolis. Twenty five individual *Fucus* plants were tagged with plastic cable ties at each station. In the spring experiment the first 25 plants to the right of the transect were tagged and in autumn 25 to the left were selected. The first 25 plants encountered were tagged to avoid preference being given to larger or smaller plants or those of a particular species. This was important as it was the effect of shore height on hair formation which was being studied and not any inter or intraspecific differences

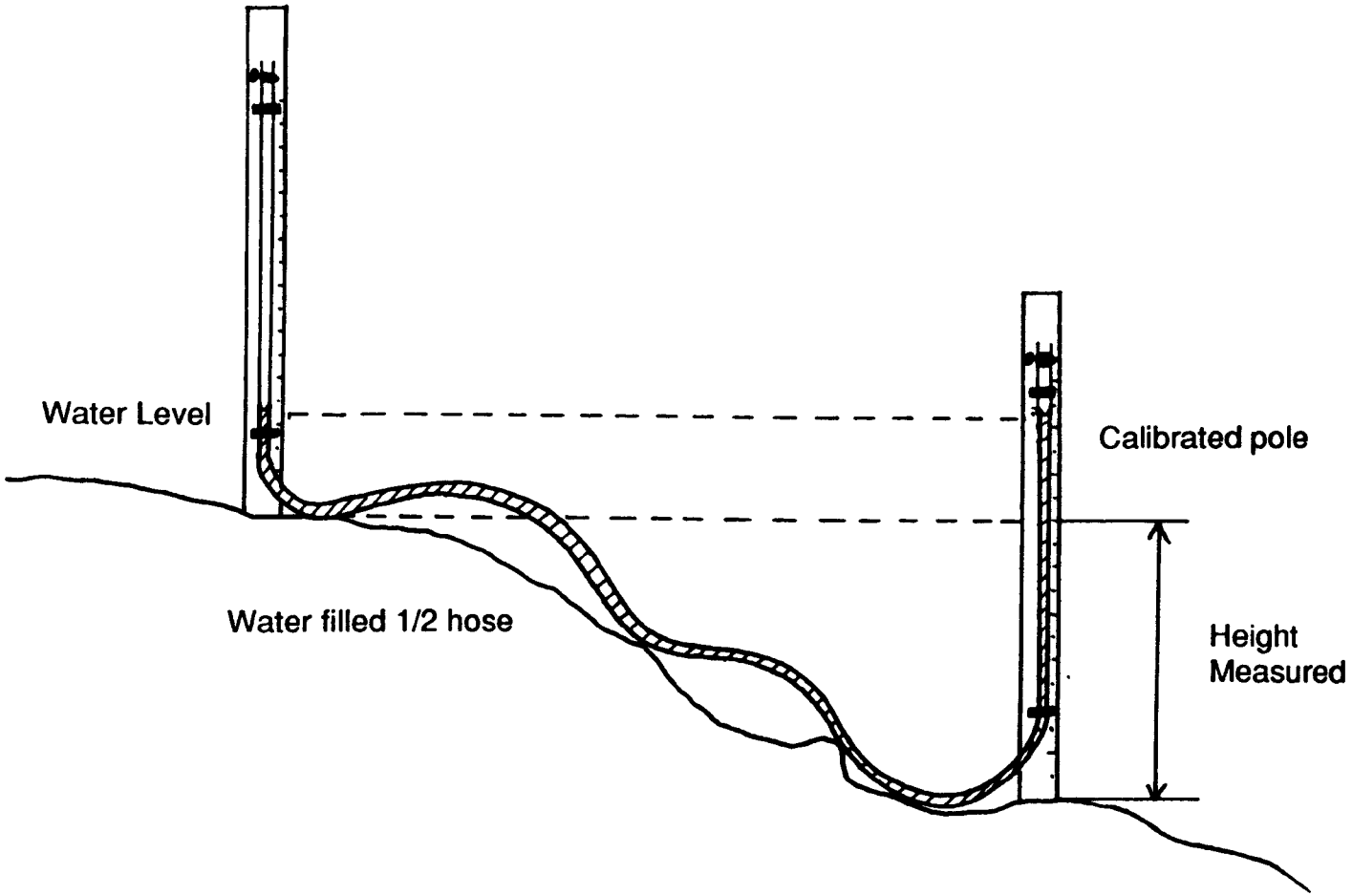


Figure 3.1 Hydraulic shore leveling device used to obtain Transect heights. The difference between the water level readings on the calibrated scales is equivalent to the variation in height.

between the plants. The range of stations chosen, however, ensured that on transect SS, the three main intertidal *Fucus* species, *F.serratus, F.vesiculosus* and *F.spiralis* were included in the survey. On transect E, only *F. vesiculosus* var *evesiculosus* were tagged as it was the only *Fucus* species to occur within the chosen tidal range..

All plants tagged during the spring experiment were initially glabrous, with no visible hairs emerging from the cryptostomata. At each station five additional plants were removed and brought back to the laboratory for microscopic confirmation that hairs were not in fact present. Conversely, the autumn tagged plants all had clearly visible hyaline hairs at the onset of the experimental period as hairs are in evidence on all *Fucus* spp. throughout the summer. Although the appearance of hairs in the spring had been noted in the previous year, the exact date when the plants would initiate hair production was not known. To ensure that the period of hair formation would be covered in the experiment, the plants were tagged in mid-January 1986 although the results shown only cover the time when hairs were actually seen to protrude from the cryptostomata. The tagged plants were checked on the shore every two to three days when low tides occurred during daylight hours but this interval occasionally stretched to five days. In spring it was the appearance of hairs which was noted, whilst in autumn the cessation of hair production was observed.

On each visit a seawater sample was taken for analysis of nitrate, nitrite and phosphate levels. The 500ml samples were collected, from the low-water mark on transect SS, in a glass container and brought immediately to the Marine Station. The method used for phosphate estimation was taken from Murphy and Riley (1962), whilst the nitrate and nitrite analysis followed the method outlined in Strickland and Parsons (1960). The two methods are in common usage for the preparation of samples which were then analysed in a Unicam SP500 spectrophotometer.

3.3. RESULTS

3.3.1. HAIR SEASONALITY

Figure 3.2. shows the results of the spring tagging experiments on the three transects. In each case it may be seen that the hairs are produced on all 25 plants at all stations within a period of only seven or eight days. Table 3.1, shows no significant difference between time of onset or rate of hair formation at any height on the shore.

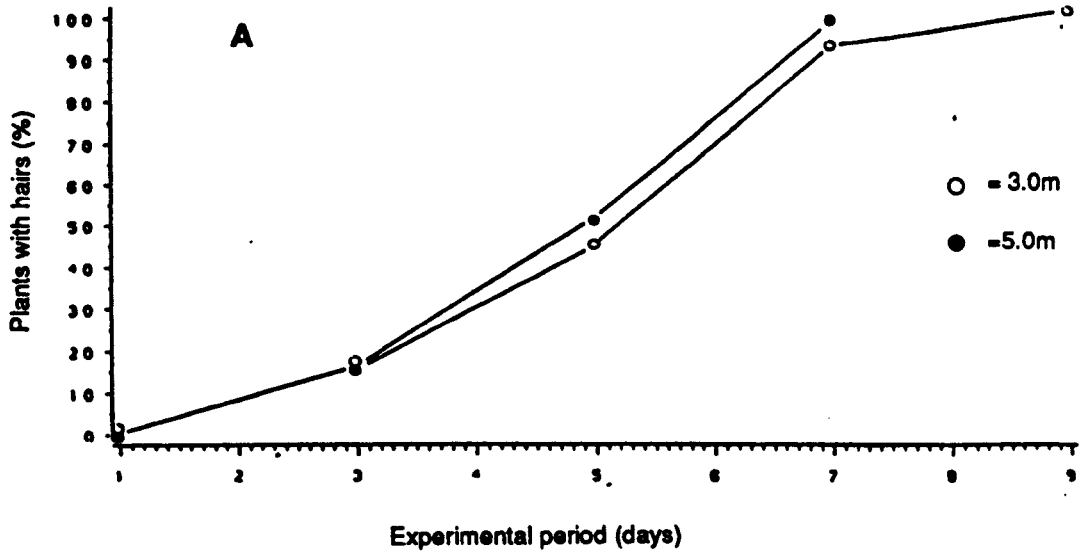
The results for the autumn tagging experiment show marked differences from those obtained during spring. It can be seen that

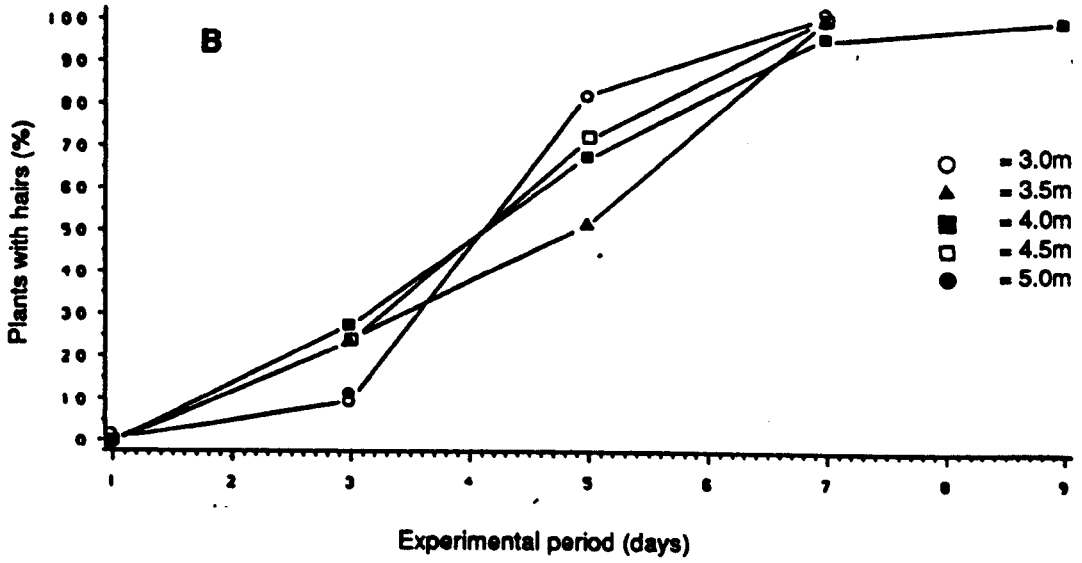
Figure 3.3 and Table 3.2, show a significant difference in the rate of *Fucus* spp. hair loss at each of the measured heights. The obvious trend shown by the autumn figures is for plants higher up the shore to maintain hair production for longer than plants lower on the shore. This means that the cessation of hair production begins with plants at the lower shore levels and progresses slowly to those at higher levels. The halting of hair production in the *Fucus* spp. is therefore a continuous process in the intertidal throughout the whole autumn period.

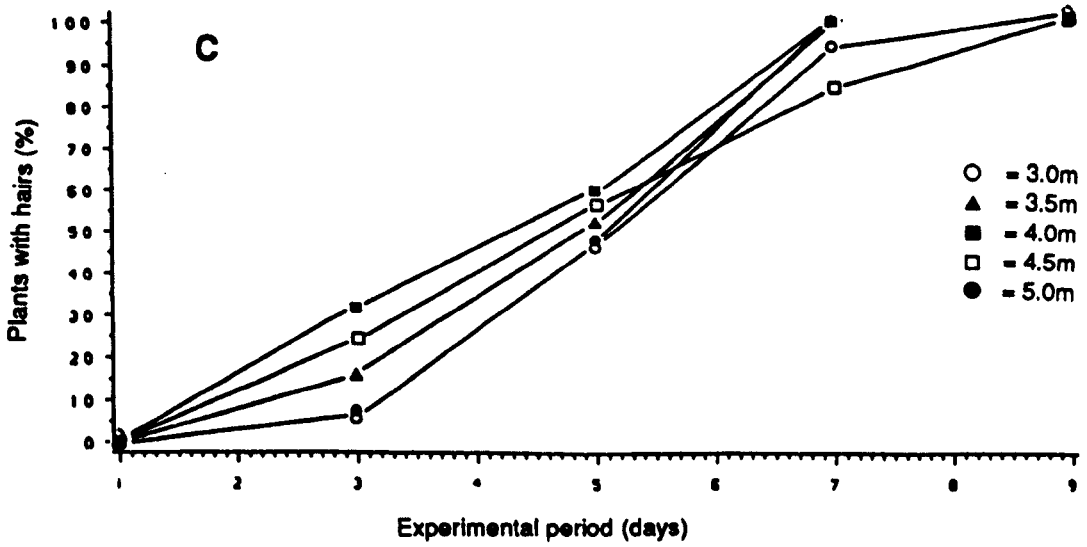
An inter-shore comparison of the autumn figures from the three transects is given in Figure 3.4 with statistical analysis in Table 3.3. The results suggests that at 3.0m there is little difference between transects SS and E but that the period of hair production is extended for plants on transect S. The results for the three transects at the higher level of -5.0m indicates that the extended period of hair production for the sheltered shore is again in evidence but in addition there is also a significant difference between the exposed and semi-sheltered shores. At this height the plants on transect SS, are also seen to be producing hair for longer than those plants on the exposed transect E. The difference in the experimental period for the graphs in Fig. 3.4 should be noted when comparisons are being drawn.

The underlying trend in autumn, therefore, is that hair production ceases in the plants at about the same time for any given height on

Figure 3.2 The formation of hyaline hairs in spring at differing shore heights on the three transects.
A = Transect S.
B = Transect SS.
C = Transect E.







Note: Day one = February 22nd 1986

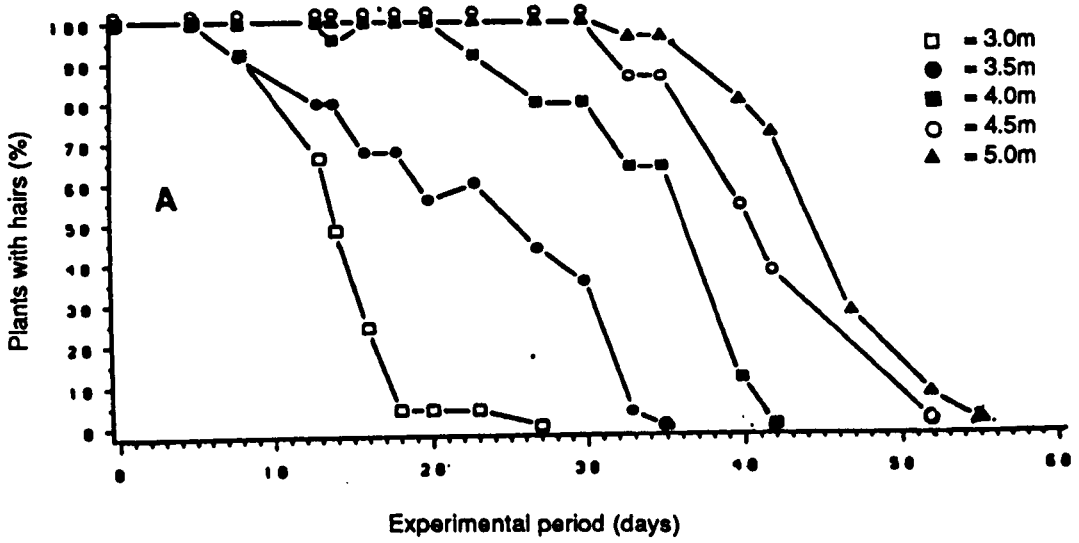
Table 3.1: Statistical treatment for the formation of hyaline hairs in spring. (N/S = not significant).

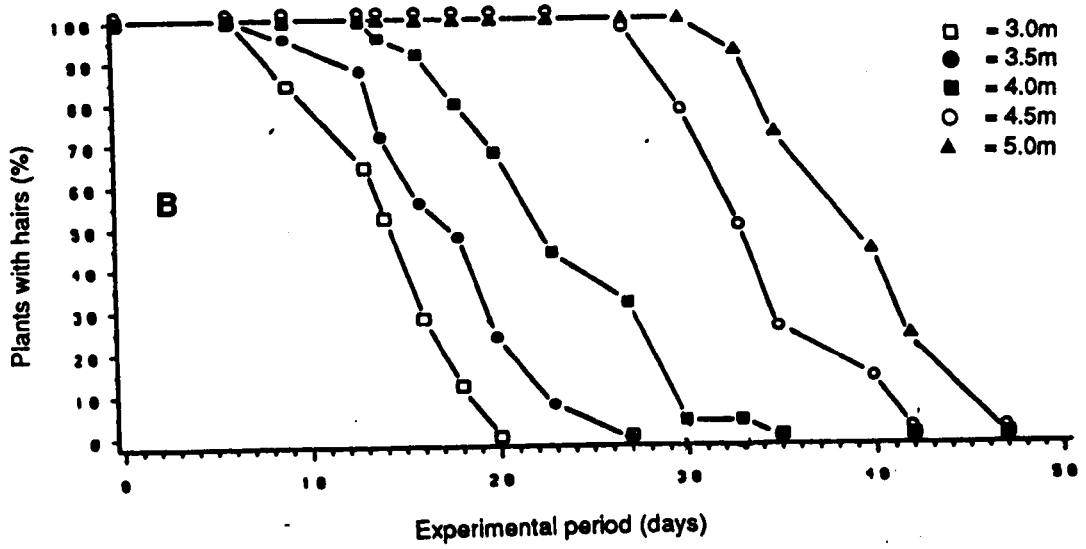
Transect.	Test.	Value.	Significance.
Sheltered	Wilcoxon	z = 1.41	N/S
Semi-sheltered	Kruskall-Wallace	H = 1.08	N/S
Exposed	Kruskall-Wallace	H = 6.57	N/S

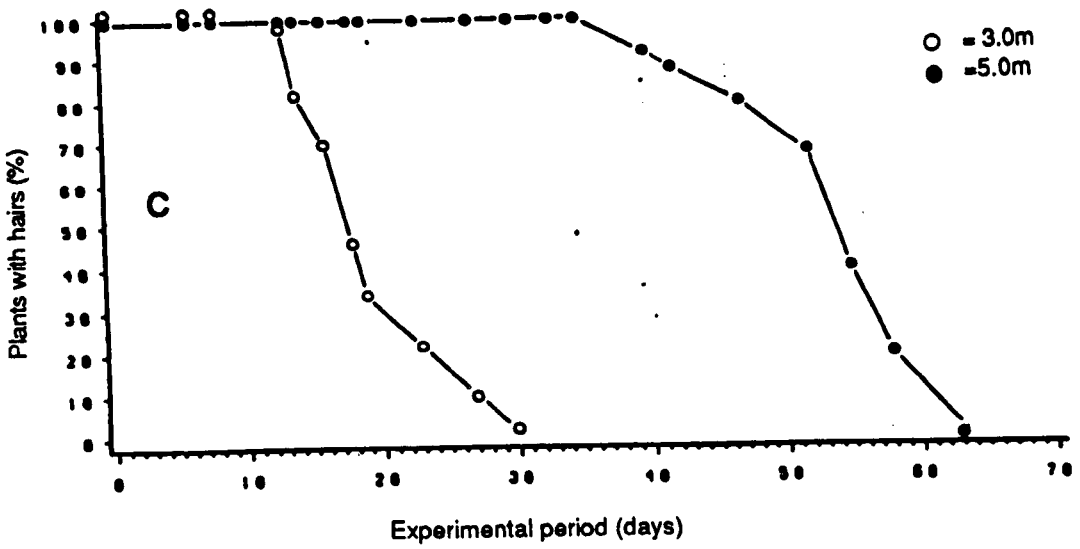
Table 3.2: Statistical tests on the cessation of hair production in autumn on the three transects (***=P<0.001).

Transect.	Test.	Value.	Significance.
Sheltered	Wilcoxon	z = 3.87	***
Semi-sheltered	Kruskall-Wallace	H = 48.03	***
Exposed	Kruskall-Wallace	H = 40.44	***

Figure 3.3 The cessation of hair formation at differing shore heights on the three transects.
A = Transect E.
B = Transect SS.
C = Transect S.







Note: Day one = September 22nd 1986

Figure 3.4a: Cessation of hair formation in autumn. Comparison of the three transects at **5.0m.**

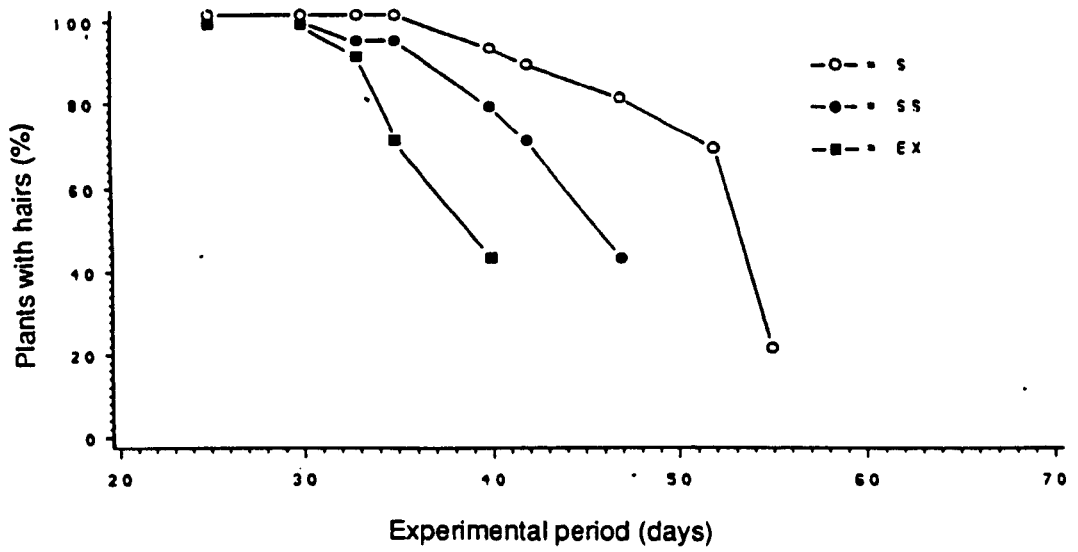
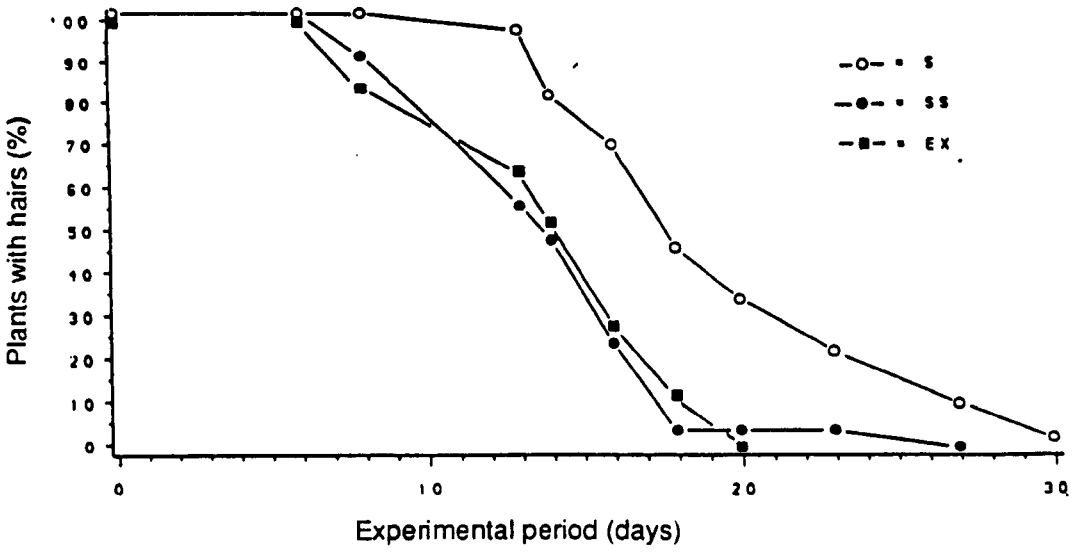


Figure 3.4b: Cessation of hair formation in autumn. Comparison of the three transects at **3.0m.**



Note: Day one = September 22nd 1986

Table 3.3: Comparative statistical analysis of the cessation of hair production on the three transects at 3.0m and 5.0m (Chisquare values, **=0.001<P<0.01, *=0.01<P<0.05, N/S=Not Significant)

Five Metres

	Sheltered	Semi-Sheltered	Exposed
Sheltered	-	2.24 *	1.73 *
Semi-Sheltered	-	-	1.73 *

Three Metres

	Sheltered	Semi-Sheltered	Exposed
Sheltered	-	2.45 **	2.45 **
Semi-Sheltered	-	-	0.82 N/S

the shore. The degree of wave exposure however, or some factor associated with it, influences the rate at which individual plants halt production, such that those in more sheltered conditions are seen to maintain hair production for longer than plants on exposed shores.

Table 3.4, shows some environmental parameters in the week leading up to hair formation in late February and throughout the period when the hairs were emerging. The levels of nitrate, nitrite and phosphate are relatively stable throughout this period whilst the air temperatures, although fluctuating, are slowly rising towards their higher spring levels. Sea temperature falls during the time of hair production before recovering to its previous level. These figures for individual days however may easily mask underlying long term changes. Table 3.5 gives a greater overall picture of environmental fluctuations by indicating means for the three week period leading up to hair formation and throughout the experimental period itself.

Again nutrient levels are seen to remain quite stable in the period leading up to the onset of hair formation. The sea temperatures do indicate a fall during the experimental period itself as was suggested in Table 3.4 but they can be seen to remain reasonably steady in the weeks leading up to hair appearance. Air temperatures however show a fall both before and during the time the hairs appeared. Daylength, as would be expected, showed a steady increase whilst sunshine hours also rose to a peak of 6.74 hours while hairs were forming.

Table 3.4: Environmental parameters measured during the Spring experiment. The temperatures given were obtained from the Meteorological Office at Ronaldsway Airport and are used with permission.

Date.	Experiment Day	Day Length (hrs.mins)	NO_3	NO_2	PO_4 ($\mu g\text{-at.}l^{-1}$)	Temperatures (° C). Air. Median Day	Air. Median Night	Sea
21/2/86	--	10.15	5·4	0·10	0·76	0·8	- 4·0	5·6
26/2/86	3	10.36	5·1	0·10	0·78	0·5	- 0·6	4·7
28/2/86	5	10.45	5·0	0·09	0·74	3·2	2·1	4·4
2/3/86	7	10.53	5·3	0·01	0·76	- 0·1	- 3·2	4·7
4/3/86	9	11.05	5·4	0·09	0·75	8·2	6·3	5·6

Table 3.5: Mean daily figures in the period leading up to, and during, the Spring experiment.

Week Commencing	Day Length (hrs.mins)	Sunshine (hours)	NO_3	NO_2	PO_4 ($\mu g\text{-at.}l^{-1}$)	Temperatures (° C). Air. Median Day	Air. Median Night	Sea
3/2/86	9.19	0·9	5·32	0·11	0·7	2·42	1·54	5·38
10/2/86	9.47	1·61	5·44	0·12	0·7	2·18	1·15	5·43
17/2/86	10.10	4·26	5·36	0·09	0·7	1·26	- 0·43	5·30
24/2/86	10.40	6·74	5·37	0·10	0·7	1·50	- 0·42	4·64
3/3/86	11.14	4·20	5·41	0·11	0·7	6·58	4·78	5·78

Table 3.6: Environmental data for the period of hair disappearance in *Fucus* species during autumn.

Date.	Experiment Day	Day Length (hrs.mins)	Sunshine (hours)	NO_3	NO_2	PO_4 ($\mu g\text{-at.}l^{-1}$)	Temperatures (° C). Air. Median Day	Air. Median Night	Sea
19/9/86	--	12.10	9·6	1·2	0·21	0·38	13·05	7·45	12·2
25/9/86	3	12.02	5·8	1·3	0·28	0·45	10·05	11·4	12·2
30/9/86	8	11.41	0·0	***	0·24	0·50	12·4	11·9	12·5
3/10/86	11	11.28	7·8	2·9	0·26	0·61	13·5	9·75	12·5
7/10/86	15	11.12	4·9	2·6	0·20	0·54	13·6	13·7	12·8
14/10/86	22	10·43	2·9	2·8	0·21	0·58	11·5	12·3	12·5
20/10/86	28	10.18	5·7	2·8	0·18	0·57	8·8	7·8	11·7
27/10/86	35	9.50	0·0	***	***	***	13·4	12·1	11·4
3/11/86	42	9.22	6·5	3·9	0·20	0·71	9·7	9·05	11·1
11/11/86	50	8.53	4·0	3·1	0·14	0·65	8·5	8·55	11·1
17/11/86	56	8·33	2·7	4·0	0·10	0·64	7·1	8·1	10·8

*** = Results Lost.

Environmental parameters were also considered for the autumn period and because observations were made over a longer period, there is a much greater seasonal change taking place. These changes can be seen in Table 3.3., which shows the results for nitrate, nitrite and phosphate levels in the water samples taken on each visit to St Michael's island. Both before and throughout the experimental period, the levels of nitrate and phosphate rose towards their winter peaks, whilst nitrite levels fell.

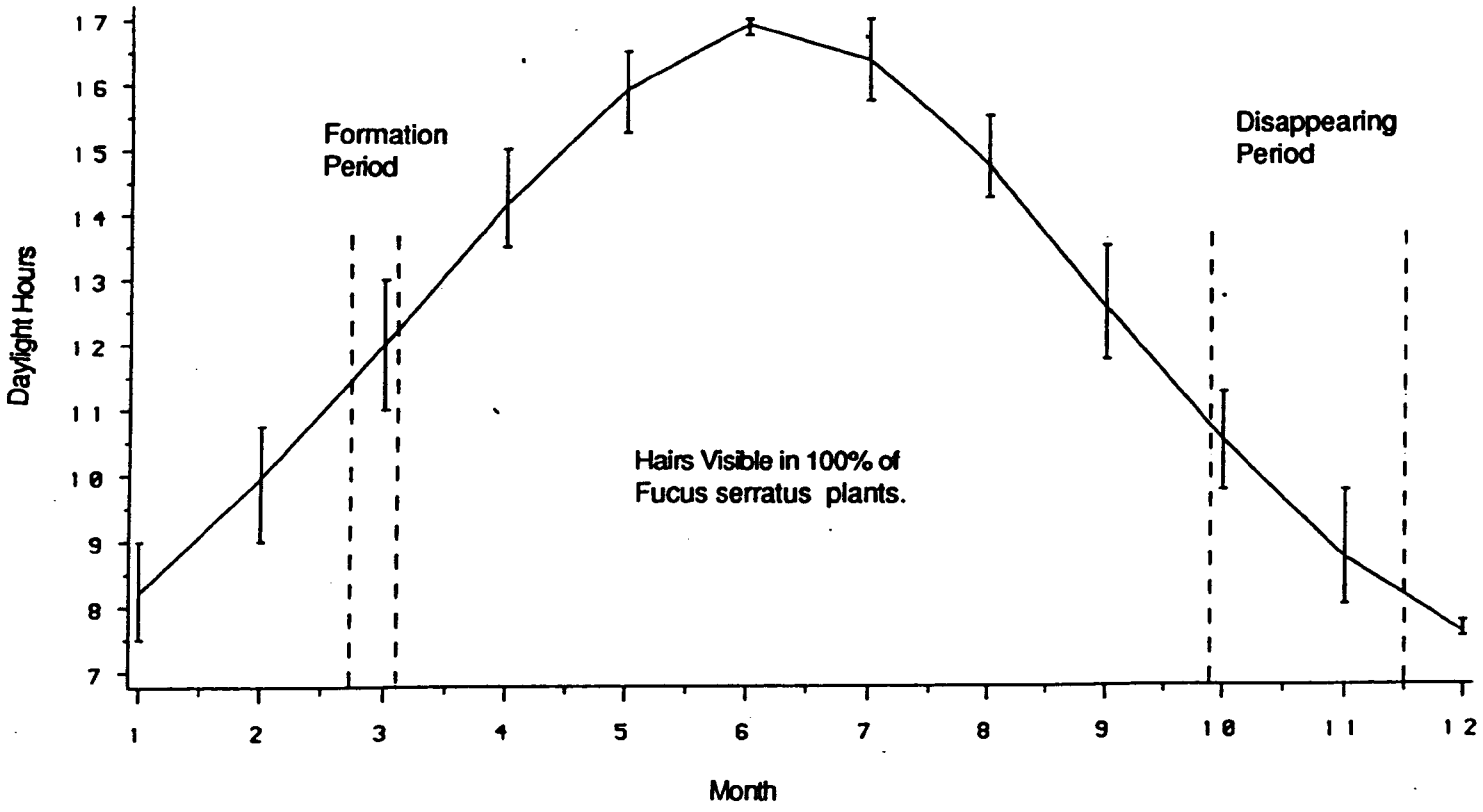
Figure 3.5 is included to illustrate more clearly the time of year when hairs are visible. *Fucus serratus* has been chosen simply as a representative example of the fucoids studied since the others follow a similar pattern. The daylength means for each month have been superimposed to indicate that the average number of daylight hours, at times of hair formation and subsequent disappearance, differs considerably.

3.4. DISCUSSION OF SEASONALITY IN HAIRS

3.4.1. SPRING

The results of the spring tagging experiments indicate a striking

Figure 3.5: The relationship between annual daylength and hair occurrence in the field.



Light data source : Lavers Tide Tables 1986.

synchrony of hair production in intertidal *Fucus* spp. (Fig. 3.2). The short amount of time (7 to 9 days), from onset of hair production in the first of the seaweeds to their emergence in all 25 plants at each level, suggests a highly effective stimulus or synchronised control mechanism.

Table 3.5, gives a list of environmental factors which could have potential bearing on the rapid formation of hyaline hairs in *Fucus* spp. The stability of the nutrient levels has already been mentioned. Indeed the seasonal fall in nutrient levels from the winter peaks rarely begins in Manx waters until the end of March with the major reduction corresponding with the spring phytoplankton outburst in April or May (Slinn and Eastham, 1984). It is unlikely therefore that ambient nutrient levels play a part in triggering hair formation in the field.

The temperature fluctuations over the experimental period are what would be expected for late winter and early Spring. The average air temperature shows a steady climb to a mean daily maximum of 8.0 °C in March, following that of 3.8 °C for February (Ronaldsway Met Office) but the hairs are very much in evidence by this time suggesting that their appearance pre-empts the amelioration in the weather.

Temperature change can be an effective stimulus for biological response in algae. The gametophytes in two species of *Desmarestia* and six species of the laminariales become fertile within a given

temperature range irrespective of daylength (Luning, 1980). Despite variability in recorded air temperatures in Table 3.5, there does not, however, seem to be a progressive change in such a way as to trigger hair formation. The only other recorded environmental parameters which can be seen to change continuously in the period leading up to hair formation therefore, is daylength, or conversely, nightlength, and the total amount of light received as measured by sunshine hours.

The change in daylength suggests the possibilities of a photoperiodic response in *Fucus* resulting in the appearance of hairs. Photoperiodism in terrestrial plants was initially demonstrated by Garner and Allard in 1920. It was not until 1967 however, that a clear photoperiodic effect in algae was discovered by Dring (1967), and Rentschler (1967), working on *Porphyra tenera* Kjellman. This revealed the possibility of similar effects in the life histories of other marine algae. As late as 1973, Russell, in his review of developments in the Phaeophyta, stated that brown algae do not usually register any marked photoperiodic responses. This was the case until Bird and McLachlan (1976), first recorded a photoperiodic response in the Fucales. Their work on *Fucus distichus* L. subsp. *distichus* found that a 12L:12D and 8L:16D light regime would induce receptacle formation but not 16L:8D. Terry and Moss (1980) found that 8L:16D and 12L:12D light regimes would also initiate receptacle formation in *Ascophyllum nodosum*. What the work of Bird and McLachlan (1976) and Terry and Moss (1980), suggests therefore, is that a physiological mechanism

in fucoids for the photoperiodic control of hair formation is theoretically possible.

Hillman (1979) stated that photoperiodism is "the control of some aspect of a life cycle by the timing of light and darkness". The problem with hair formation therefore is to determine whether or not the appearance of hairs is caused by a change in the time of light and dark in a 24 hour cycle, or by the total amount of light quanta received. This question cannot be answered by the results obtained in the field alone. Kain (1971) discusses the difficulties involved in accurately recording the total solar radiation reaching the sea surface and in particular the amount penetrating to immersed algae. Whether or not hair formation is a photoperiodic response can only be established in the laboratory.

3.4.3. AUTUMN

Unlike the synchronised emergence of hairs in Spring, the disappearance of hairs from *Fucus* in Autumn is slow and variable. The loss of hairs from the lower shore plants before a progressive removal, with time, further up the intertidal (Figure 3.3) suggests a very different controlling mechanism from that which determines the synchrony of hair production in spring.

The plants in autumn did not lose hairs from the thallus, at least not initially, but simply stopped producing them in newly formed cryptostomata at the apical tip (Fig. 3.6). There was therefore an unquantifiable time lag between the hair production being halted and this event being observed in the field. It is considered that this is not of great importance since the time lag would have amounted to no more than a few days in each plant.

In some ways, the results obtained are misleading since they suggest hairs will disappear from all intertidal plants by November. In fact, hairs were seen to remain on some untagged *Fucus spiralis* plants higher on the shore for a longer period than is indicated. Indeed, in the very sheltered areas of St Michael's Island, *F. spiralis* were observed to possess hairs during the winter months.

The environmental data for Autumn given in Table 3.6, shows a number of parameters changing over the period when the hairs were disappearing. Due to the length of time from the onset of the hairs being seen to disappear, to the final loss of hairs from the high shore *Fucus spiralis*, there is less possibility of a distinct environmental trigger being responsible for hair loss. It is far more likely that the observed changes are due to small differences in these parameters or others not measured. On this basis, it would seem that the decrease in daylength, the rise in nitrate and phosphate levels and the reduction in both air and sea temperatures are all potentially of importance.

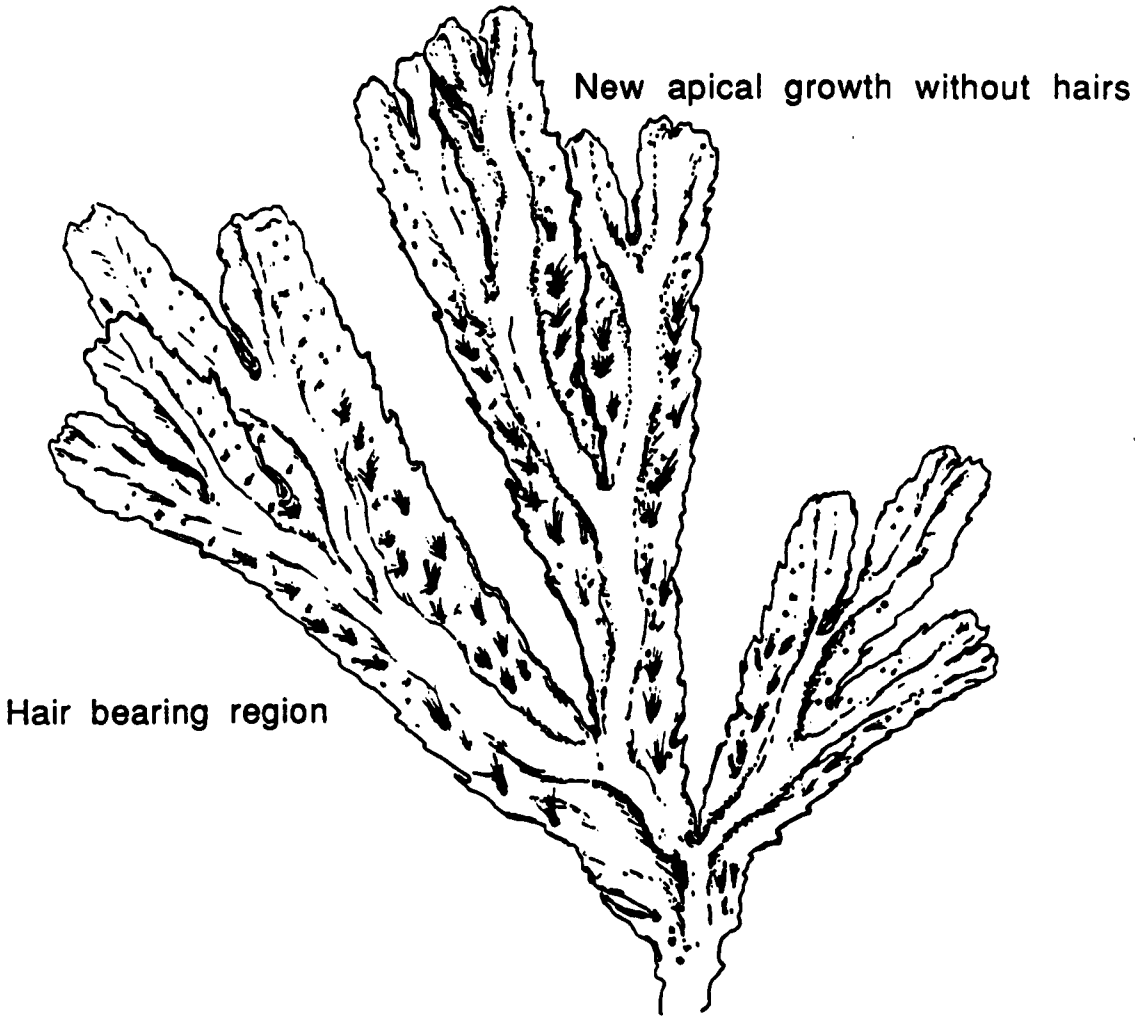


Figure 3.6 Diagram illustrating the distribution of hairs on *F.serratus* following apical growth after the cessation of hair production in autumn.

The extreme range of temperatures which can be experienced by intertidal algae over short time periods suggest that, intuitively, temperature alone is unlikely to trigger hair loss. It is, however, quite possible that the gradual decrease in temperature throughout the period when the algae were losing hairs could be having the overall effect of slowing plant metabolism.

The rise in nutrient levels throughout the Autumn period gives rise to the possibility that the algae are reaching a point where they are no longer nutrient deficient. This is important if the hairs are linked in some way with nutrient uptake. The argument has been put forward that submarine discharge of groundwater in coastal areas means that the inshore nutrient levels are enhanced due to the high nitrate levels of the groundwater (Johannes, 1980). The results given in Table 3.6 indicate that this is not occurring off the SS transect on St Michael's island. Nitrite levels at this time fell, as is common for waters around the Isle of Man where nitrite normally reaches its maximum in September and October before falling away through the winter (Slinn and Eastham, 1984). Allied to the noted increase in nutrient levels in the Autumn is the reduction in daylength, which would reduce the level of photosynthesis and consequently reduce the nutrient requirements of the algae.

It would appear therefore that the disappearance of the hairs is potentially a complex interaction between environmental parameters. The results obtained indicate that hairs begin to disappear from the

low shore *Fucus*, which suggests that nutrient availability, which is highest in the lower intertidal, might be important relative to light availability. The problem with this suggestion is that the *Fucus* spp. may have differing nutrient uptake rates and would not necessarily be expected to show such a clear, progressive spatial disappearance of hairs if nutrient availability was the only factor. In addition, the role of water movement and its effects upon nutrient availability may well be an important parameter to consider. The comparisons of the 3 transects at shore heights of 3.0 and 5.0m (Fig. 3.4) emphasises this latter point.

The field results obtained are insufficient to comment further on the underlying mechanisms involved in hair disappearance and laboratory experiments under controlled conditions are essential if the effects of individual parameters are to be understood.

3.5 ALGAL TRANSPLANTS

3.5.1. INTRODUCTION

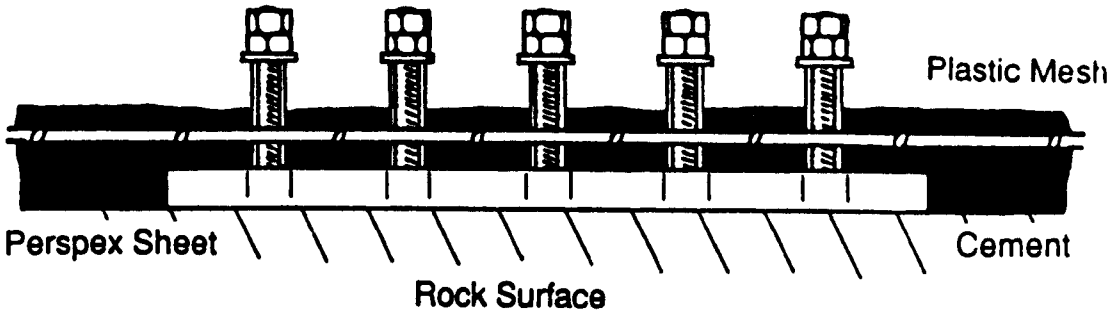
The differences in hair loss relative to shore height as shown in the autumn figures and the regular zonation of the fucoids, poses the possibility that the hair loss response may, to some extent, be species specific. This argument is supported by observation of

Himanthalia elongata (L.) S.F. Gray in the field. This member of the Fucales, though not of the family Fucaceae (Clayton, 1984), was seen to possess clearly defined hairs on its reproductive tissue in late November and well after they had completely disappeared from the thallus of *Fucus serratus* plants situated above it on the shore. This in itself is not surprising as the ecological requirements of *Fucus* and *Himanthalia* are not necessarily matched. It was considered important, however, to examine the response of hair bearing plants which were transplanted out of their customary intertidal zones during this autumn period. This would help to indicate whether the overriding factor in *Fucus* hair loss was shore position, or the ecological requirements of an individual species at a particular shore height.

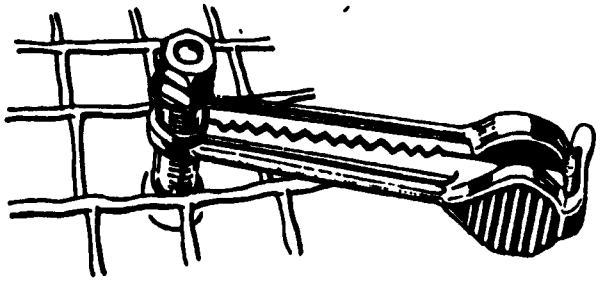
3.5.2. TRANSPLANT METHODS

A series of transplant holders (Fig. 3.7.) were cemented to the shore at St Michael's Island alongside two of the existing three lines of the permanent transects. The holders consisted of a perspex sheet held on the shore by means of plastic netting and quick setting cement. Steel bolts covered by plastic hypodermic syringe barrels, protruded from the perspex and blue plastic clips were attached to the bolts. The algae were wrapped in foam sponge and clamped firmly into each clip.

Figure 3.7. Schematic diagram of a transplant holder with detail of a blue clip used to hold the algae.



Blue Plastic Clip attached to bolt

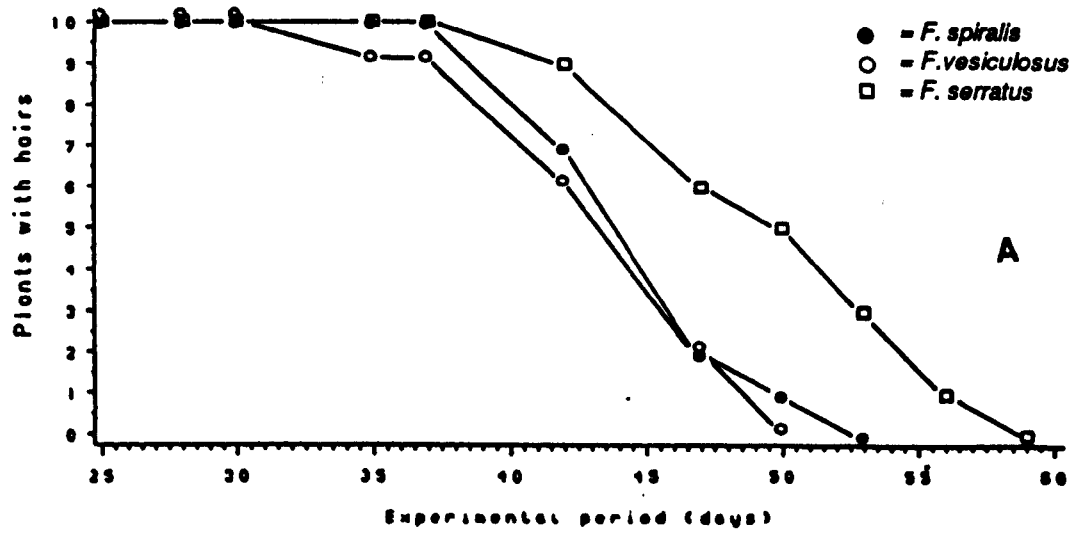


Three of the transplant holders were attached by each transect at tidal heights of 3.0m 4.0m and 5.0m to correspond with the zones of *F.serratus, F.vesiculosus* and *F.spiralis*, respectively. This meant that a total of thirty plants could be attached at any one time. Ten plants from each of the three *Fucus* species were attached to each transplant holder in late august 1986.

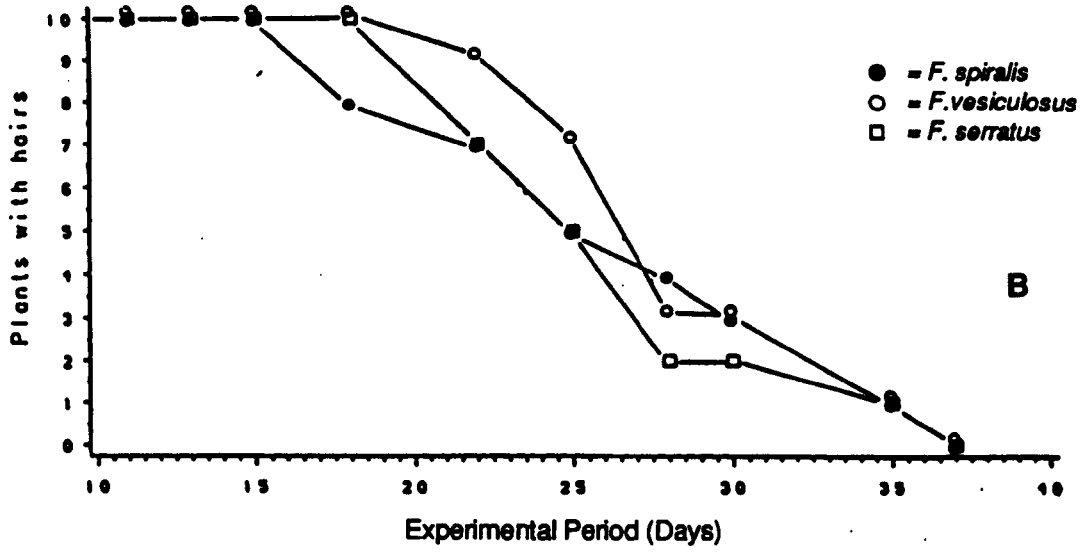
3.5.3. RESULTS

The results of the transplant experiments are given in Figures 3.8. and 3.9 with statistical analysis in Table 3.7. There is no significant difference between the hair loss of transplanted algae at shore heights of 3.0m, and 4.0m on both the Semi-sheltered and Exposed transects. At 5.0m however, there is a difference for both transects. On transect SS, *Fucus serratus*, which has been transplanted to this higher shore level, loses hair significantly slower than *F. vesiculosus* and *F. spiralis*. The 5.0m transplants on the exposed transect (E), show that the *F. evesiculosus* transplanted up from the low shore lose their hairs at a significantly slower rate than plants from both 5.0m and 4.0m. The transplant holder at 5.0m on transect E was lost after 42 days and a number of plants were lost from the 4m transplant holder; again on transect E.

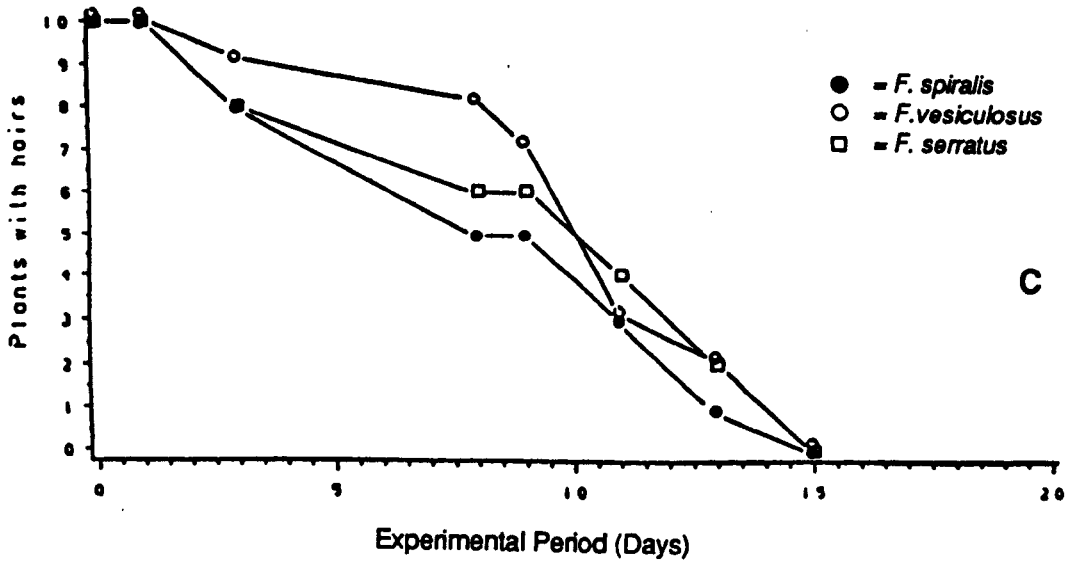
Figure 3.8 Cessation of hair formation in autumn. Comparison of three transplanted species of *Fucus* on the Semi-Sheltered transect (SS) at different shore heights.
A=Five Metres.
B=Four Metres.
C=Three Metres.



Note: Day 25 = October 17th 1986.

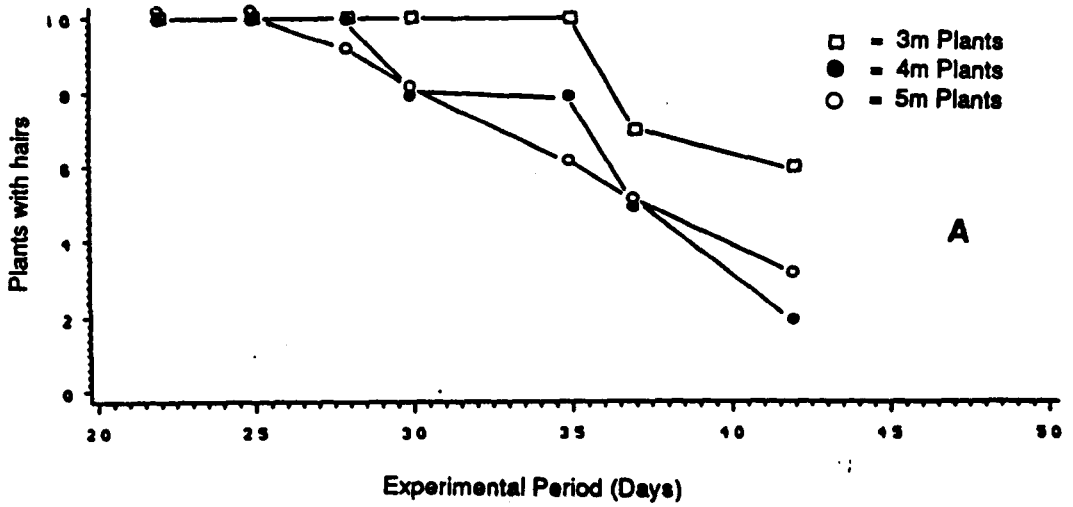


Note: Day 10 = October 2nd 1986.

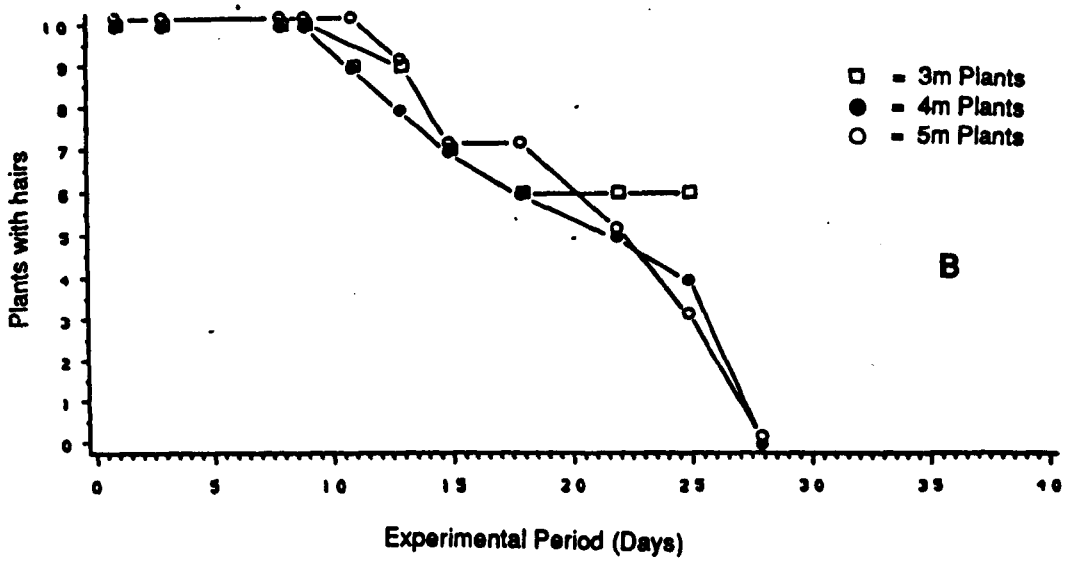


Note: Day one = September 22nd 1986

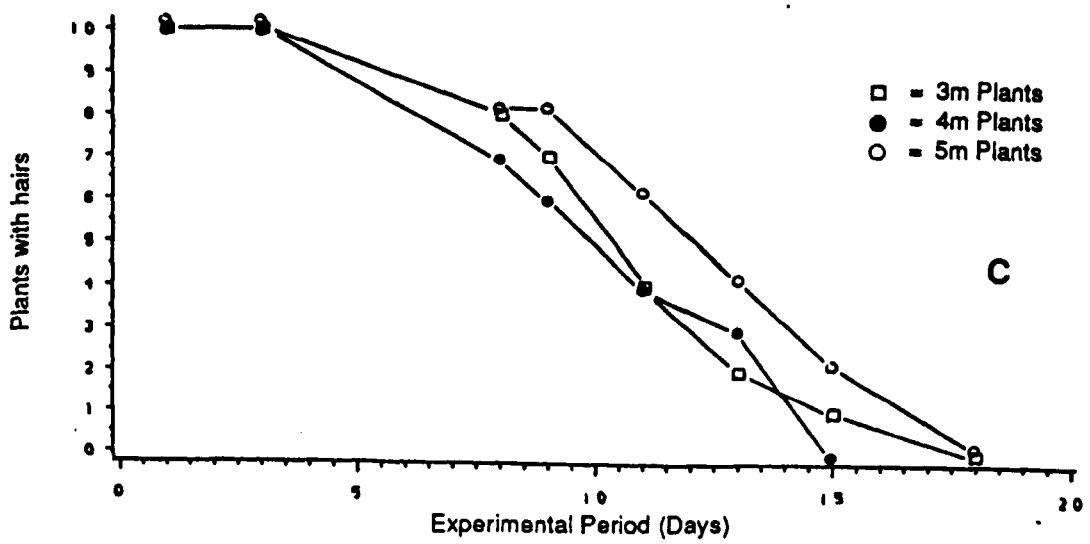
Figure 3.9 The cessation of hair formation in Autumn. Comparison of Fucus vesiculosus var vesiculosus, transplanted between three shore heights on the Exposed transect (E).
A = Five metres.
B = Four metres.
C = Three metres.



Note: Day 20 = October 12th 1986
Transplant holder lost Day 42



Note Day one = September 22nd 1986



3.5.4. DISCUSSION

The results of this experiment show two complementary features. The first is that *F. serratus* loses hairs at a slower rate than *F. vesiculosus* or *F. spiralis* when transplanted above its zone. The second, is that despite the logical conclusion that this is a species specific reaction, the lower rate of hair loss in low shore *F. evesiculosus* when transplanted above its zone is indicative that the differences may be due to intertidal positional factors. Whether this is a genotypic or a phenotypic reaction is open to question.

3.6. LABORATORY STUDIES

3.6.1. PHOTOPERIOD RESPONSES

3.6.2. INTRODUCTION.

There had been a suggestion from the environmental data that a photoperiodic response could be triggering hair formation in glabrous fucoids. Photomorphogenesis is a term given for those responses in which light acts as a signal which will trigger a change in metabolism or morphological development (Luning, 1981). Due to the synchronicity of hair formation during spring, the possibility that this was a photomorphogenic response needed to be examined. As there had been

no significant difference between the *Fucus* species, in terms of hair formation under the same environmental conditions, only one species, *Fucus serratus* was used in the experiments.

3.6.3. METHODS

Forty five glabrous *Fucus serratus* were freshly collected and weighed. Five individuals were placed in each of nine, 5 litre, aquaria such that a similar biomass occurred in each tank. Three tanks were then placed under each of the following light regimes; 8L:16D, 12L:12D and 16L:8D. In this and all following laboratory cultures, the tanks were placed in a constant temperature room at 10° C ±1° C. under cool-white fluorescent tubes at 70 $\mu E/m^2/s$ ±3. The time hair formation commenced for each alga was noted and the results given in Table 3.8.

3.6.4. RESULTS

The results given in Table 3.5., show a decrease in the time taken for the algae to produce hyaline hairs with an increase in "daylength".

Table 3.8: Time of hair formation in *Fucus serratus* under differing light regimes (with 95 % confidence limits, n = 15).

	Light Regime		
	8L:16D.	12L:12D.	16L:8D.
Time of hair formation (days)	15·97	11·10	8·93
95 % Confidence limits	± 0·39	± 0·33	± 0·51

Table 3.9: Mean time of hair production (with standard deviations) under different levels of irradiance (Kruskall-Wallace, *** = P<0·001).

	Levels of Irradiance ($\mu E.m^{-2}.s^{-1}$)				Inference
	70	62	53	41	
Time of hair formation (days)	8·4	10·4	13·1	19·3	***
Standard deviation	0·42	0·82	1·14	1·99	

3.6.5. DISCUSSION

Photoperiod effects are due to the length of uninterrupted darkness and not the length of the light period (Lobban et al., 1985). The range of photoperiod chosen from 8L:16D to 16L:8D, would suggest that it is not a photoperiodic response which elicits hair formation. In the U.K., days averaging at least eight hours of light occur in every month (Fig. 3.5) and if a photoperiodic response triggered formation, then it would be expected that hairs would occur throughout the year. It is thought unlikely from these results, that the hairs are produced by a photoperiodic response. The effects of total amount of light received, therefore, would seem to be of importance. Consequently, glabrous *F. serratus* were cultured in differing irradiance levels to ascertain if either the level or the duration of irradiance, were critical to hair formation.

3.7. THE FORMATION OF HAIRS UNDER DIFFERENT IRRADIANCE LEVELS.

3.7.1. METHOD

Twenty glabrous *F. serratus* were collected, weighed and placed in four, 5 litre, aquaria such that there was a similar amount of algae in each tank. The tanks were then covered with fine netting in different amounts so that a different amount of light would penetrate

into each tank. The amount of light passing through each quantity of netting was measured with a Crump quantum radiometer-photometer. The aquaria were then placed in a constant temperature room, without added media, under a 16L:8D light regime and the time when each plant produced hairs was noted.

3.7. RESULTS

It can be seen from Table 3.6., that there is a highly significant relationship between the amount of light reaching the algae and the time of hair formation (Kruskall-Wallace, $H=17.91$ $P<0.01$) .

3.7.2. DISCUSSION

Returning briefly to the field data given in Table 3.5, it can be seen that before hairs are produced in the intertidal there is an increase in sunshine hours. The conclusion is, that the hairs are produced when the amount of light received by the algae rises beyond a certain metabolic threshold. This conclusion, however, may only be part of the answer since an increase in light will increase photosynthesis and raise the nutritional demands of the plant. Since the levels of nitrate and phosphate remained fairly constant throughout the period of hair formation in the field, it is reasonable to suppose that there may be a relationship between the amount of

nutrients available and the amount of light received by the plants which will affect hair growth. It was decided, therefore, to grow algae under the same light conditions but with differing amounts of available nutrients to see if this would affect the production of hairs.

3.8. HAIR PRODUCTION UNDER DIFFERENT NUTRIENT LEVELS

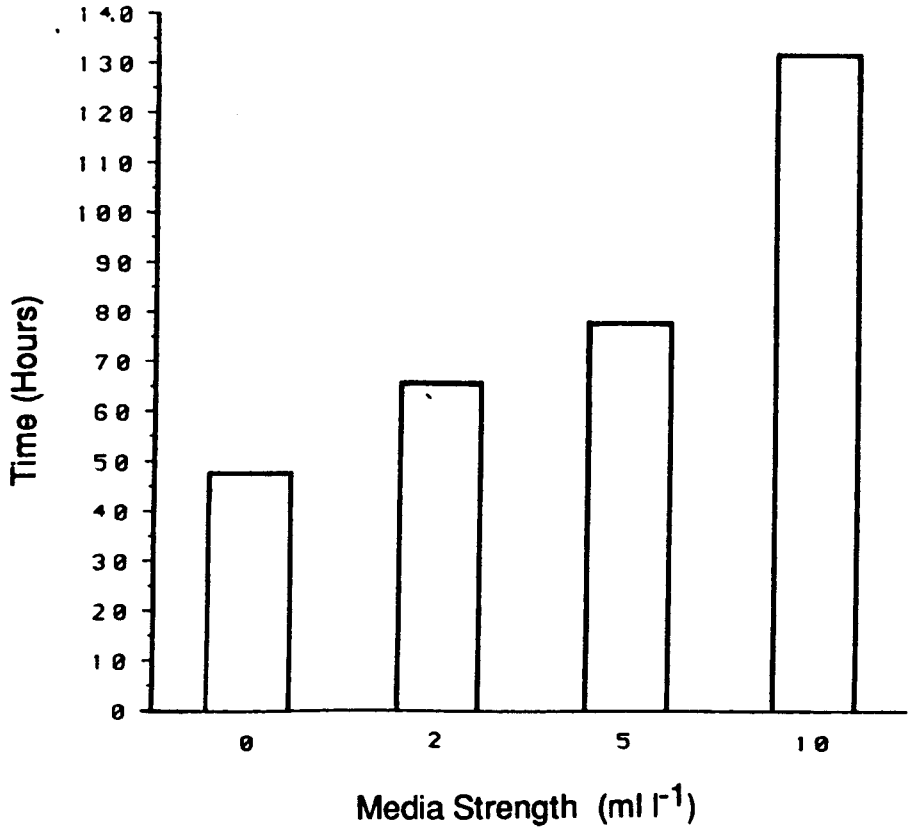
3.8.1. METHODS

Five glabrous *F. serratus*, of similar weight, were collected in late January 1986 and placed in each of four aquaria. Each tank contained 12.55g ±0.06g of algae and Provasoli's Enriched Seawater growth medium was added to three tanks in concentrations of 2, 5, and 10ml/l, with the fourth tank having no added enrichment. The tanks were then placed under the same light source at 16L:8D in the constant temperature room and the time of hair formation noted for each alga.

3.8.2. DISCUSSION

It may be seen from Figure 3.10, that an increase in the concentration of medium enrichment results in a corresponding increase in the amount of time before hairs are formed. This would suggest

Figure 3.10: Effects of growth media concentrations on hair formation in glabrous Fucus serratus. Light=70mE m^{-2} s^{-1}, Media=Provasoli's E.S.



that hair formation is caused by a starvation response in the algae and that the function of the hairs is to increase the uptake of nutrients. If this were true, it would also explain why the disappearance of hairs in Autumn is faster on the more exposed transect than on the sheltered transect (Fig. 3.4.). The disappearance could be caused by the greater wave action removing the hairs (although the presence of hairs throughout seven months of the year make this an unlikely explanation on its own), or the fact that increasing the water movement around the thallus of the algae will break down surface laminar layers of still water and increase nutrient uptake. The increased nutrient uptake would mean that the plant had less need for the hairs and they would no longer be produced. If this was indeed the case then exposing glabrous plants to greater degrees of water movement in a closed system with finite nutrients, would cause hairs to be produced earlier. It was decided therefore to place glabrous algae in still and moving water to see if this would affect hair formation.

3.9. THE EFFECT OF WATER MOVEMENT ON HAIR PRODUCTION.

3.9.1. INTRODUCTION

The importance of water movement as a means of supplying nutrients to algae is well documented (i.e. Gerard, 1982; Parker, 1981; Gerard and Mann, 1979; Neushul, 1972). As water moves over an algal thallus,

the plant surface creates drag which has the effect of reducing water velocity nearer to the surface. This will create a layer of motionless water, the boundary layer, very close to the surface of the plant (Lobban et al, 1985; Neushul, 1972). This layer will form resistance to the passage of nutrients between the cell and the surrounding water body. The resistance being inversely proportional to the thickness of the boundary layer which itself is inversely proportional to the velocity of the water movement (Neushul, 1972).

Conover (1978) used a range of current velocities and found an increase in growth in *Zostera marina* L. with increasing current. This he attributed to enhanced dissolved nutrient uptake from the rapidly moving water. Gerard and Mann (1979) confirmed this in kelp but also concluded that high exposure proved more stressful than beneficial to some populations of benthic marine plants.

With the probability of increased nutrient uptake with higher water velocities, it was decided to examine the formation of hair under differing levels of water movement. The assumption being that high nutrient uptake, with increased water movement in a closed system, would result in the plants becoming nutrient deficient sooner and thus lead to earlier hair formation.

3.9.2. WATER MOVEMENT USING AIR STONES.

3.9.3. METHOD

It had been noted, in pilot experiments, that in all *Fucus* species studied, glabrous plants produced hairs in the top few centimetres of the apical branches when deprived of nutrients. It was further noted that excised branches behaved in exactly the same way as whole plants in that, under similar culture conditions, they produced hairs. It was decided therefore that given space limitations on culture conditions, a greater number of individuals could be examined by using branches rather than whole plants. The dichotomous branching on *Fucus* spp. is such that a dominant and a subordinate branch will usually be formed. For reasons, which will be made clear in Chapter Four, only the dominant branches were used in all experiments.

To create differing degrees of water movement, submersible pumps were placed in tanks but their rate of flow was too intense. Since the pumps being used were not adjustable to give lower flow rates, it was decided to create differing flow conditions by using air stones. This is not a very satisfactory method since it brings in a further variable i.e. differential availability of gases in the water. However, as this seemed to be the only available method for producing small amounts of water movement it was decided to continue; with the proviso that the results would be treated with caution.

Sixty dominant branches were therefore collected from 60 separate adult *F. serratus* plants. Twenty branches were then placed in each of three tanks with one tank containing three air stones, one containing one stone and no aeration at all in the third tank. The tanks were then placed in the constant temperature room and the time of emergence of hairs noted for each alga. The results are given in Figure 3.11..

3.9.4. RESULTS

Figure 3.11 and Table 3.10, show the significant differences in times of hair formation with different degrees of water movement. The plants in the tanks with three air stones produced hairs significantly quicker than those plants in tanks with one air stone and without water movement. The plants in static conditions produced hairs significantly sooner than those plants in tanks with one air stone.

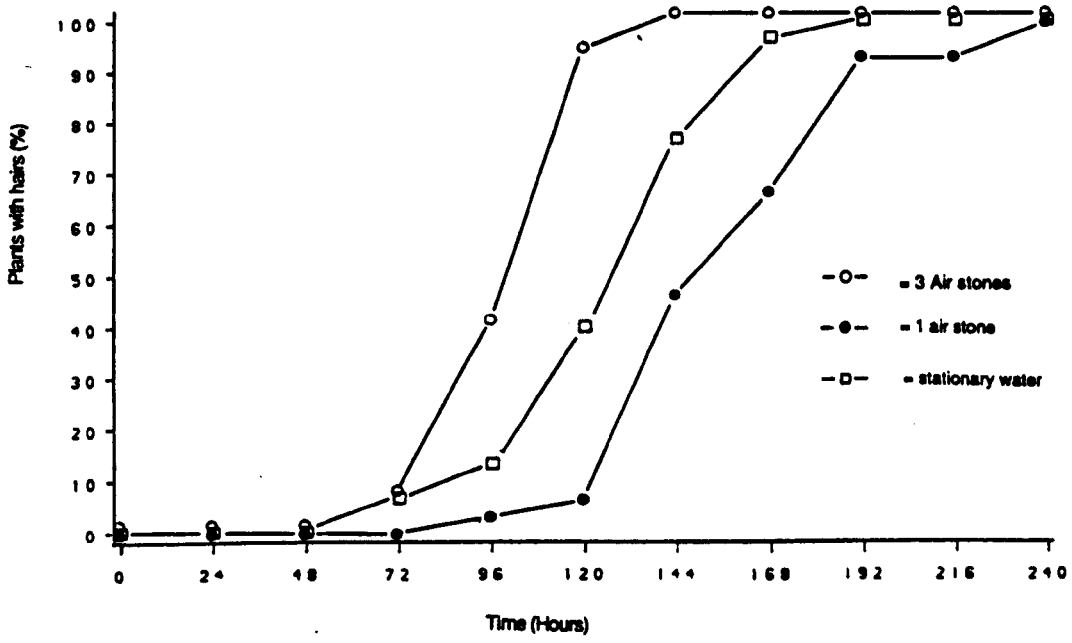
3.9.5. DISCUSSION

The more rapid production of hairs in both stationary and well aerated culture, as compared to the culture with intermediate water motion, are probably both due to nutrient starvation. In stationary medium, nutrient deficient boundary layers around the plants might lead to a physiological lack of nutrients although they remain abundant in the medium. In contrast, turbulent conditions favour

Table 3.10: Statistical comparison of the time of hair formation in varying degrees of water movement, produced by three and one air stones, and static water (Wilcoxon Paired Ranks **=0.001<P<0.01, *=0.01<P<0.05).

	Static Water	1 air stone	3 air stones
Static Water	-	z=2.65 **	z=2.00 *
1 air stone	-	-	z=2.65 **

Figure 3.11 Hair formation with different degrees of water movement produced by air stones.



rapid nutrient uptake resulting in a depletion of the nutients in the culture.

Whitford and Kim (1966) found that a number of species, including *F. vesiculosus*, will grow faster in a culture if the medium is agitated. In a stationary medium, the plants might seriously deplete the supply of gases and nutrients in their immediate vicinity, allowing a layer lacking these substances to form around the surface of the plant. If there is movement in the surrounding water, this layer will be disturbed allowing nutrients to reach the thallus. The result will be that turbulent water will be richer in physiologically available nutrients than static water even if the concentrations in both will be equal. Matsumoto (1959) found that increased agitation of ordinary seawater had the same effect on growth of *Porphyra tenera* as enriching the nutrients in slower flowing water.

Ions will enter cells by moving across the boundary layer of water to the cell surface. They will then pass through the cell wall and plasmalemma into the cytoplasm. The thickness of the boundary layer, therefore, can determine the rate of uptake by limiting the rate of diffusion (Lobban et al, 1985). It is believed that the hairs grow and protrude through the boundary layer enhancing nutrient uptake at their tip. This view is supported by the fact that hairs are visibly longer on sheltered shore algae than on those found under more exposed conditions. It is recognised, however, that this decrease in length

on exposed shores could be due to attrition of the hairs by increased wave action.

3.10. NUTRIENT DEFICIENCIES

3.10.1. INTRODUCTION

Having established that nutrient deficiency, at a given irradiance and level of water motion, will induce hair formation, it was decided to attempt to isolate the specific nutrient deficiency that will produce hairs. Gibson and Whitton (1987) have shown an increase in hair formation under nitrogen and phosphorous deficiency in the Chaetophoraceae. It was suspected, therefore, despite the great taxonomic differences with the family Fucaceae, that the phosphate and nitrate components of the added media would also be the most likely to cause a deficiency response if removed from *Fucus* cultures. In addition, Fe deficiency had been noted to cause hair production in strains of the Rivulariaceae (Whitton and Harding, 1978) and therefore, Fe was also removed from one experimental medium.

3.10.2. METHOD

Fifty glabrous, apical sections of each of *Fucus serratus*,

F. vesiculosus and *F. spiralis* were placed, ten to a tank, in fifteen, 5 litre glass aquaria. The plants had been blotted, weighed and sorted such that each tank contained 12.6g of algae. Provasoli's E.S. was added to the filtered seawater in each tank and they were placed in culture at 10°C with a light source of $70\mu E/m^2/s$ for 24 hours prior to the experiment to standardise the nutrient state of each batch of algae. The plants were then removed and placed in fresh aquaria. Three types of media, each deficient in one of P, N, or Fe was prepared by ommitting the appropriate chemicals. One type of media was then added to a tank containing either *F. spiralis, F. vesiculosus* or *F. serratus* and this was repeated for all media and all species. The resulting combination of species and media was such that each tank contained one species of *Fucus* and one media deficient in one element. Three further tanks each containing ten apical sections of one of the *Fucus* species, had full media, without any deficiencies, added and three more tanks, again each containing a *Fucus* species, had no added media at all. The deficiencies were obtained by omitting chemicals; Na-glycerophosphate for P deficiency, $NaNo_3$ for N deficiency and Fe was omitted from both the chelating agent stock and the PII trace metals.

The plants were reweighed every ten days and fresh media, with the appropriate deficiencies if necessary, was added every two days.

3.10.3. RESULTS

Results have only been given for those tanks in which algae produced hairs during the 30 days in which the experiment was carried out. It can be seen from Table 3.11 that hair formation is induced only in those plants deficient in P and N. Those plants deficient in phosphorous produced hairs slightly, but not significantly, earlier than those deficient in nitrogen. The plants in the tanks lacking any enrichment produced hairs significantly earlier than those plants deficient in N or P.

3.10.4. DISCUSSION

It is clear from the results that hairs are produced as a result of nutrient deficiency. What is also interesting is the fact that those control plants without any added enrichment produced hairs significantly faster than plants with deficient media. This suggests that the situation is more complex than a simple N or P deficiency triggering hair growth. The results obtained however, only allow speculative comments and further work is necessary to understand fully the mechanisms underlying the processes involved. DeBoer (1981), points out three general responses of algae to nutrient deficiency. These being; a decrease in the content of photosynthetic pigments, the accumulation of C-storage compounds and a decrease in proteins and amino acids. The possibility that one of these may be acting as

Table 3.11: Results of nutrient deficiency experiment. The mean time in days (with standard errors) for hairs to form in each tank . Controls with no added nutrients. Results have only been given for those tanks in which hairs formed within the 30 days of the experiment.

Species.	Deficient nutrient	Hair formation (days)	Standard error
F. spiralis.	N	13·75	0·21
	P	13·75	0·19
	Control	8·00	0·15
F. vesiculosus.	N	18·35	0·21
	P	18·05	0·25
	Control	10·45	0·19
F. serratus.	N	14·95	0·20
	P	14·80	0·20
	Control	9·05	0·24

Table 3.12: Fresh weight mean growth rates over 30 days, of the *Fucus* species used in the nutrient deficiency experiments. (Rates $x10^{-2)}$. Controls with no nutrients added.

Species.	Growth rate when the deficient nutrient is: N	P	Control
F. spiralis	1·14	1·86	1·07
F. vesiculosus	0·83	0·97	0·65
F. serratus	0·96	1·04	0·62

a biochemical trigger for hair formation could be the basis of future study.

A further difficulty when discussing the comparative results for N and P deficiencies, is the different growth rates found with each (Table 3.12). It is possible to say however that a reduction in growth rate, as seen in the N deficient tanks, does not of itself lead to the immediate production of hairs.

3.11. GENERAL DISCUSSION

The production of hyaline hairs in *Fucus* appears to occur in response to a nutrient deficiency in the surrounding water. It has been noted that many seaweeds appear to respond to nutrient deficiency by the production of hairs from the surface of the thallus and it has been suggested that their role is akin to that of root hairs in vascular plants (Lobban et al, 1985). In algae, the hairs would aid in taking up nutrients by piercing the boundary layer of still water at the plant surface and increasing the total plant surface area.

It appears clear that the quantity of light received by the algae is a fundamental driving factor in hair formation. It is the amount of light received which controls the rate of photosynthesis and subsequently determines the nutrient requirements of the plants. Light primarily affects nutrient uptake in an indirect manner. One

of the main effects is that growth rates are increased which also increases nutrient uptake and light will also provide the energy for active transport (ATP production by photosynthesis) (Lobban et al, 1985).

There is some evidence in the work of Dring and Luning (1975), that the wavelength of the light received may also have some effect. They found that hair formation in sporelings of the brown alga *Scytosiphon lomentaria* was greater in blue light than red. They concluded that this was a photomorphogenic response not attributable to the different effects of blue and red light on photosynthesis or growth.

Investigations of this type, into the effects of differing wavelengths of the light received, have not been carried out in this study. However, light received by the *Fucus* is polychromatic during the period of emersion and, with this and the synchronised appearance of hairs in spring, it is unlikely that the effects of different regions of the light spectra will be of great ecological significance.

CHAPTER FOUR - THE FUNCTION OF HYALINE HAIRS IN *FUCUS*

4.1. GENERAL INTRODUCTION

In Chapter Three, the seasonal occurrence of hyaline hairs on the intertidal *Fucus* species was examined and the point was made that their appearance was due to the algae undergoing nutrient stress relative to the amount of light received. Having isolated the environmental conditions under which the hairs appear, it remains to elucidate the role which they play in the ecology of the plants and to quantify the suggestion that they increase nutrient uptake (i.e. Adamich et al, 1975; Schonbeck and Norton, 1981).

Sinclair and Whitton (1977), have commented that the deficiency of a nutrient, especially nitrate, has been the most commonly quoted factor favouring increased hair formation. They also postulated that the hairs in the Rivulariaceae have a role in the uptake of nutrients present in low concentrations.

This chapter will concentrate upon the part the hairs play in algal growth and nutrient uptake rates, leaving the remaining chapters to study other effects which the hairs have upon the ecology of the *Fucus* species.

4.1.1. GENERAL METHODOLOGY

Unless otherwise stated, all experiments took place in a constant temperature room at 10°C ±1° under a 16L:8D light regime which inhibits receptacle formation (Evans et al, 1982) and encourages the highest growth rates (Bird et al, 1979). The lights used were "Cool White" fluorescent tubes producing light levels of 70μE / m^2 / s ±3. Bird et al (1979) have stated that 100μE/m^2/s, is necessary for light saturation in *Fucus serratus* but due to a number of problems it was not possible to achieve such high light levels.

The growth media used throughout was based upon Provasoli's Enriched Seawater, the composition of which is detailed in Appendix 1. All plants were freshly collected and free from macroepiphytes. Following collection they were lightly brushed and rinsed with filtered seawater to remove excessive surface microepiphytes.

As Round (1973) points out, it is comparatively rare for experiments to be carried out on material collected directly from nature. The great bulk of algal physiology experiments being undertaken in unialgal, bacteria free cultures. This should not be considered too detrimental to the results obtained however since the comparative nature of the growth experiments means that the uncontrollable variables such as bacteria infestation, may be considered to equally affect plants under all experimental conditions as collection and pre-experimental transport and storage was similar for all plants.

In *Fucus* spp., terminal branching often results in a dominant and subordinate branch. Where apical sections, as opposed to whole plants, were used only the dominant branches were employed in all experiments to help standardise methodology. The use of apical sections to study growth, as opposed to whole plants, allows for greater sample sizes in limited experimental space. It is not considered that the growth of excised apical tips is wholly representative of the growth of entire plants. Despite this, there are other advantages since the concentration on the growth of only one part of a plant reduces the variability seen in the growth of different parts of the thallus. Niell (1976) showed that the rate of production of a given alga is inversely related to its C:N ratio, with older thallus parts having a greater C:N ratio than younger parts. Apical sections are easy to cultivate, a point illustrated by Moss (1965), who cultured those of *Fucus vesiculosus* for a period of four months.

Littler (1979) has pointed out that there are problems with cut thalli since increased respiration has been recorded in algae for several hours following a cut. He also comments however, that some of the observed effect may be due to phenolic compounds being released by the algae and the subsequent oxidation affecting results. To reduce the loss of material from the cut thallus, blue plastic clips (See Fig. 3.2.) were fitted across the cut which helped to both seal the damaged area and hold the algae on the bottom of the aquaria during culture.

The plants were weighed from the hydrated state, being removed from the water, blotted dry and weighed. This was done for three plants at a time, to ensure the minimum amount of weight loss from desiccation effects. The leathery surface of the fucoids reduced water loss over this short exposure time and, in general, made this an accurate, non-destructive method to use. Reweighing the algae after one hour of rehydration gave an error of ± 2 percent.

Pilot experiments had shown that *Fucus* kept submerged for long periods of time would begin to exhibit necrosis of the outer thallus cells. They also show other signs of deterioration and exude copious mucus. To prevent this, all plants were removed from the water for six hours per day but always remained under the light source. The removal was timed so that three hours of the removal occurred under dark conditions and three hours under light. Fulcher and McCully (1969a) also found that a period of emersion was important for *Fucus vesiculosus* in culture, although the slow growth rates of their plants indicates that they were, in any case, growing in sub-optimal conditions.

Initially, all measurements taken to establish growth were based upon three separate parameters: fresh weight, surface area and linear extension. However, when using the dominant terminal branch of *Fucus serratus*, the correlation between these three parameters was so close that the duplication of time and effort seemed groundless. Figure 4.1., shows the relationship between the three parameters. A similar

Figure 4.1a: Relationship between Fresh Weight and surface area of Fucus serratus thallus tips. Linear regression with 95% confidence limits.

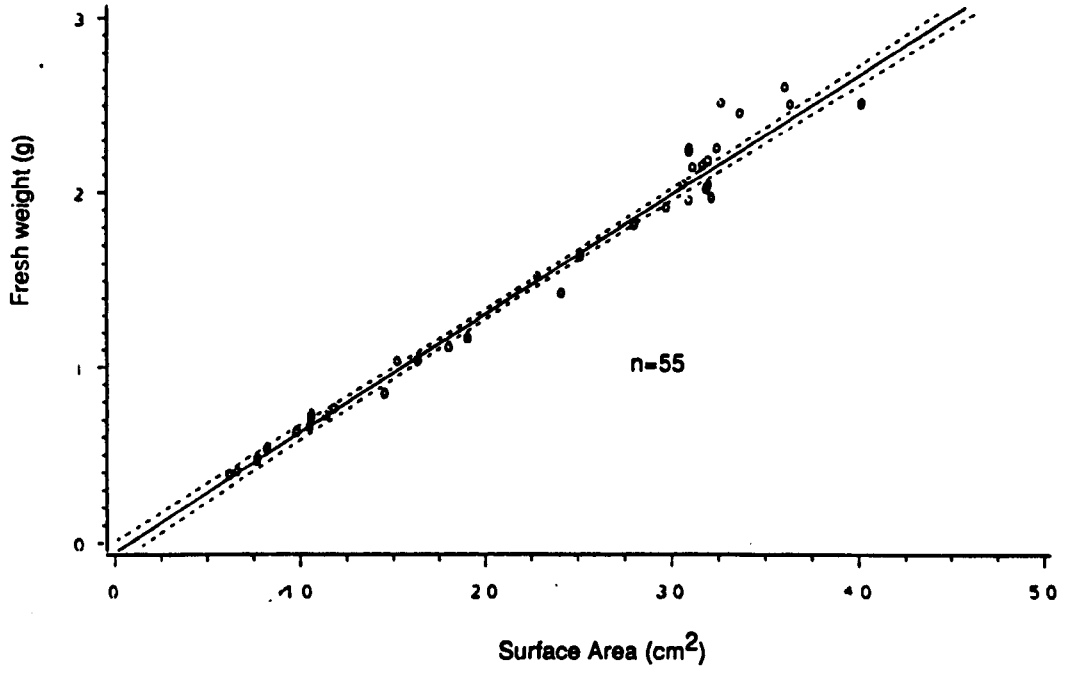
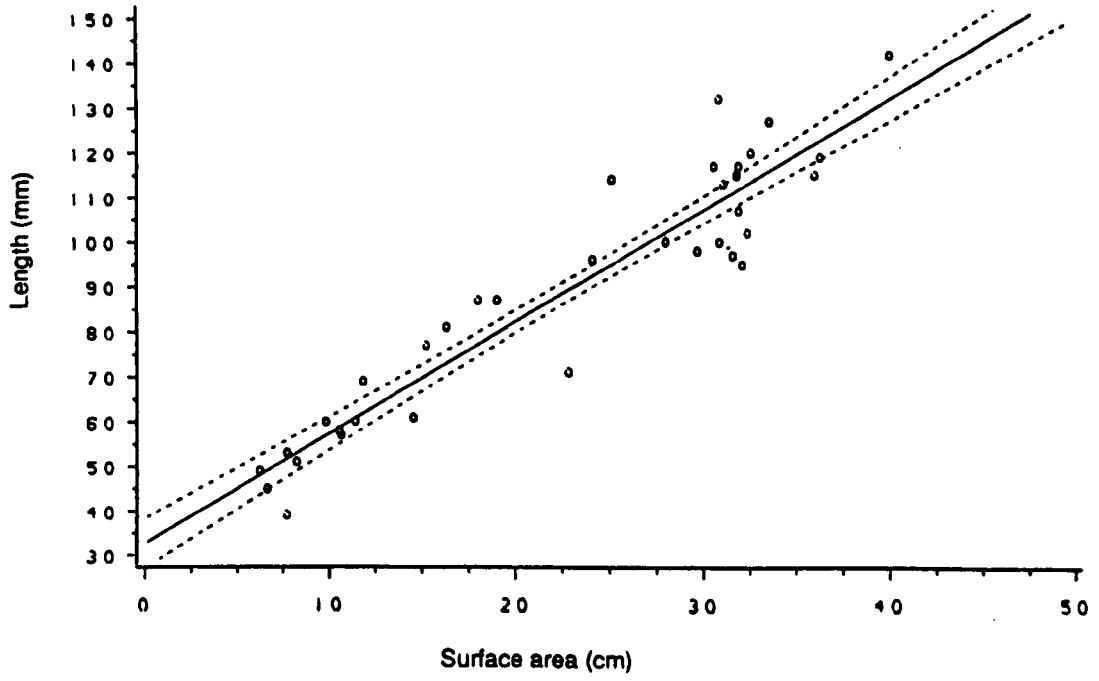


Figure 4.1b: Relationship between length and surface area of Fucus serratus thallus tips. Linear regression with 95% confidence limits.



relationship was found with $\log_{10}$ transformed data between fresh weight and thallus area, for whole plants of *Fucus vesiculosus*, by Russell (1978). The linear relationship for thallus sections shown in Figure 4.1. is probably, therefore, a function of their small size. It was decided to only measure fresh weight, as this method proved to be slightly more accurate, and less time consuming, than the other two. Although intuitively it might be considered that linear extension would prove to be more accurate the results obtained gave a higher error in replicated trials.

The method of calculating the mean growth rates is based upon the formula:

$$R = \frac{\log_e Wt_2 - \log_e Wt_1}{t_2 - t_1}$$

Where: Wt_1 = Initial Fresh Weight.
Wt_2 = Final Fresh Weight.
t_1 = Time Experiment Commenced.
t_2 = Time Experiment Concluded.

See Hunt (1978) for theoretical background

4.2 UPTAKE OF NUTRIENTS

4.2.1 GENERAL INTRODUCTION

Over fifty chemical elements may be found in, or on the surface of, seaweeds, although the mere presence of an element is not, in itself, evidence that it is essential to an organism. The normal biochemistry of plants requires the availability of 14 - 21 specific elements for their main metabolic processes (De Boer, 1981). These elements may be considered to be those required by any alga to sustain itself. Lobban et al (1985) have laid out the criteria for an absolute requirement of an element as being, "1) A deficiency of the element makes it impossible for the alga to grow or complete its vegetative or reproductive cycle, 2) It cannot be replaced by another element and 3) The effect is direct and not due to interaction with (e.g. detoxification of) other nonessential elements, stimulation of the epiflora, or the like".

As O'Kelly (1974) points out however, the inherent heterogeneity of algae makes generalisations as to their individual requirements extremely difficult. Alternatively, Lobban et al (1985), consider it possible to infer that nitrogen, phosphorus and iron are potentially limiting as they occur in algae at much higher concentrations than in seawater. In addition the argument that N. P. Fe. Cu. Zn. Mn. and C. might limit algal growth is due to the fact that these elements vary in concentration in seawater due to biological activity (Lobban et al, 1985). Of these elements, nitrogen has been found, in a number of studies, to be the one most likely to limit algal growth i.e. in

Fucus spiralis (Topinka and Robbins, 1976), *Macrocystis pyrifera* (Jackson, 1977) *Codium fragile* ssp. *tomentosoides* (Hanisak, 1979), *Gracilaria tikvahiae* (Lapointe and Duke, 1984) and phytoplankton (Ryther and Dunstan, 1971).

The appearance of hyaline hairs has been quite clearly linked with low levels of inorganic nutrients in seawater and the suggestion has been made that hair occurrence is a factor linked with nutrient stress. For this reason it was decided to examine the way in which the presence of hairs affected nutrient uptake rates and any subsequent effects this may have upon the overall growth rates of hairy and glabrous plants.

4.3. THE EFFECT OF HAIRS ON NUTRIENT UPTAKE

4.3.1 INTRODUCTION

Harrison and Druehl (1982), have outlined three main techniques for measuring nutrient uptake. These being 1) Radioactive isotope uptake, 2) Stable isotope uptake and 3) Disappearance of nutrients from the medium measured colorimetrically. Lobban et al (1985), consider the measurement of the loss of a radioactive tracer from the culture medium to be a fourth. In order to compare the rate at which nutrients are taken up by both hairy and glabrous plants, the third

and most commonly used method, disappearance of nutrients from the medium, was employed. The uptake of phosphate and nitrate was measured since they are the salts which have the greatest effect upon the growth of algae and if the hairs are related to nutrient uptake and growth, it is their relationship to the uptake of these salts which will be the most important.

4.3.2. METHODS

Apical sections of *Fucus serratus*, 4 cm in length, were excised from both glabrous and hairy plants in Autumn 1986. Apical sections were used, rather than whole plants, because it was considered important to avoid the variation in uptake rates which can occur in different parts of the thallus (Wallentinus, 1984). It is recognised that using apical parts alone may well overestimate the total nutrient uptake of the plants (Topinka, 1978; Wheeler, 1979), but this was considered to be equally valid for both types of algae.

Thirty apical sections of each type were placed, ten to a tank, in three tanks with an equal biomass in each tank. There were, therefore, three tanks containing ten glabrous algae, three containing ten hairy algae and three control tanks without algae The tanks were then filled with 7l of filtered seawater and 1/10th strength Provasoli's E.S. was added to each. The control tanks without algae were also filled with filtered seawater and had media added at the

same concentration as the experimental tanks. A water sample was taken from each tank and analysed for nitrate and phosphate using the method outlined in Chapter Three (3.2.3.). The four tanks were then placed under the usual culture conditions and 150ml water samples taken from each tank every 12 hours for analysis.

Both nitrate and phosphate were supplied in high concentrations. This was considered important since the hairs are produced as a result of a nutrient deficiency. Low nutrient levels may well have triggered hair formation in the glabrous algae and ruined the comparative nature of the experiment. Ammonia, which may be toxic to some seaweeds in high concentrations (Waite and Mitchell, 1972), was not used.

With algae which have a history of nutrient deficient conditions, a rapid short-term uptake of nutrients can occur due to an ionic depletion of the diffusion free space (which does not require energy to replenish) and reduced intracellular nitrogen pools. The diffusion free space is exterior to the plasmalemma and ions can be readily removed from it by washing the alga or storing it in nutrient depleted water (Lobban et al, 1985). However, under saturated media conditions, the replacement of ions would take place within the first hour of culture, as would the replenishment of intracellular nitrogen pools (Lobban et al, 1985). These pools would possibly be depleted in the hairy algae since these could be nutrient stressed prior to the experiment. It was considered that the use of 1/10th concentration Provasoli's media and the long term comparison of uptake

rates would render these factors irrelevant for the purposes of this experiment.

4.3.3 RESULTS

Figure 4.2. shows clearly that the added nutrients are disappearing at a greater rate from those tanks containing hair bearing algae. This is the case for both phosphate and nitrate, with the uptake rates differing significantly between glabrous and hairy plants. Table 4.1 gives the significance levels of the illustrated differences. The nitrate in the experimental tanks was above the detection limits for the equipment used for the first 24 hours and only results obtained after this time are shown in Figure 4.2. The control tank nitrate levels were always above the detectable level.

Nutrient concentrations are given as µg-at/l (ppb). To convert to the other commonly used unit of measurement, µM, multiply µg-at/l by the atomic weight of the element.

4.3.4 DISCUSSION

The increased rate of nutrient uptake exhibited by hair bearing *Fucus* (Fig. 4.2.), is of great ecological importance to the plant. The ability to respond to falls in ambient nutrient concentrations

Figure 4.2a: Phosphate uptake with time in *F. serratus* hairy and glabrous plants (With confidence limits).

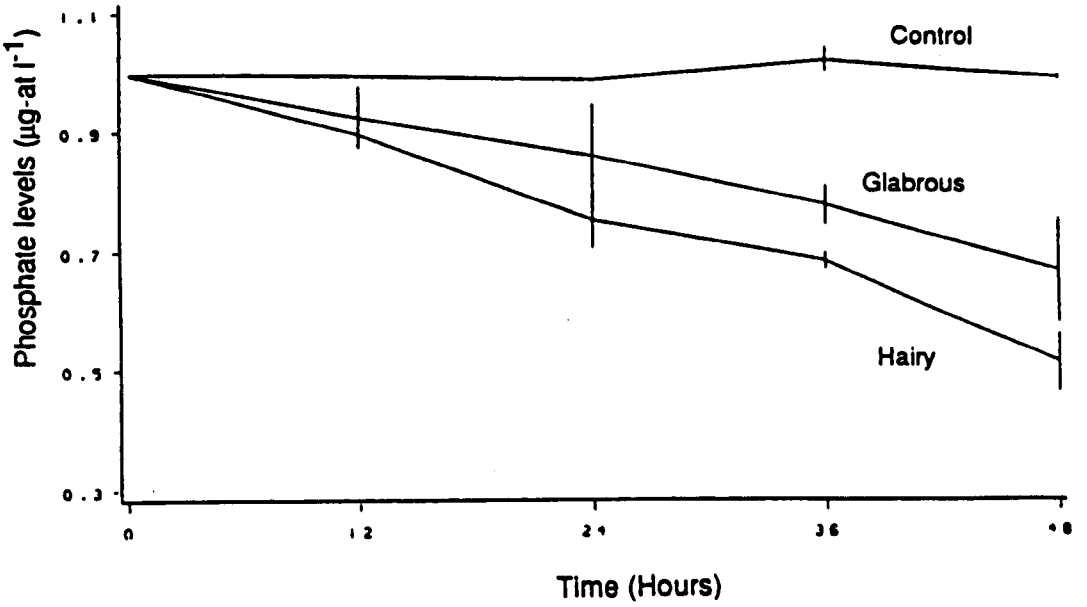


Figure 4.2b: Nitrate uptake with time in *Fucus serratus* hairy and glabrous plants (with confidence limits).

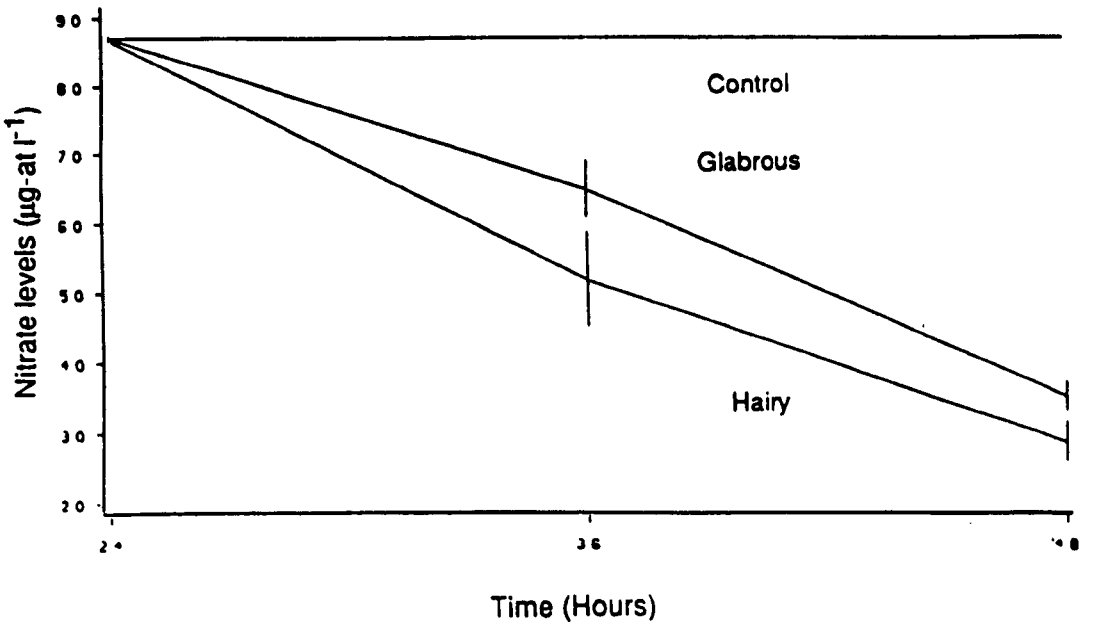


Table 4.1: Statistical comparison of phosphate and nitrate uptake by Pilose and Glabrous plants (Chisquare analysis at 48 hours, ***=P<0.001, **=0.001<P<0.01, *=0.01<P<0.05).

Phosphate

	Glabrous	Pilose	Control
Glabrous	-	3.27 ***	3.94 ***
Pilose	-	-	3.94 ***

Nitrate

	Glabrous	Pilose	Control
Glabrous	-	2.94 **	2.95 **
Pilose	-	-	3.09 ***

is extremely significant in habitats where variations occur on a regular annual basis. The appearance of hairs in response to nutrient limitation enables plants to increase nutrient uptake and counteract, to some extent, the nutrient deficiency. As the hairs do not immediately cease to function when nutrients again become available, any localised rise in the ambient nutrient levels leaves the *Fucus* plants in an ideal condition to exploit this to the full. For example, Johannes (1980) has commented on the increased nutrient availability from surface water runoff after heavy rains.

Despite the advantage of bearing hairs, there will inevitably be energy costs in their production and maintenance. As light levels fall in the autumn and nutrient levels in seawater increase, the costs will potentially outweigh the benefits and hairs will not be produced.

It was previously considered that nutrient availability for benthic algae in coastal waters was not limiting. Indeed Ryther (1963), maintained that despite the low concentrations of nutrients in the water, the renewal by tides and currents provided an inexhaustable supply of essential materials. It is now considered however, that the growth of macroalgae is, at times, limited by the availability of nitrogen (Hanisak, 1983; Topinka and Robbins, 1976).

There is growing evidence that hyaline hairs are produced in a diverse range of algal species as a response to nutrient deficiency. Schonbeck and Norton (1979) noted tufts of apical hairs on *Fucus*

spiralis germlings growing on the shore. The hairs were conspicuous on the germlings in all seasons except winter, a fact attributed by them to the higher nutrient concentrations during the winter months.

The vegetative form of the unicellular green alga *Acetabularia mediterranea* Lamour, has a series of segmented cellular extensions, termed "whorls", at the end of a stalk projecting from a basal rhizoid. Under conditions of nutrient limitation, these whorls undergo hypertrophy and greatly augment the cell surface (Adamich et al, 1975). The suggestion is that the increased surface area facilitates the uptake of nitrogenous traces from the water. Gibson and Whitton (1987) have also commented that colourless hairs are widespread in taxanomically diverse algae, being found particularly in the Chaetophoraceae. Whitton and Harding (1978) noted that 11 strains of *Stigeoclonium* and one of *Chaetophora incrassata* formed hairs in deficiencies of both nitrogen and phosphorus.

The production of hairs in *Fucus* would appear therefore, to follow a commonly seen morphological response in many plants to low nutrient conditions. The increased nutrient uptake found in hair-bearing algae would be expected to elevate growth rates. A series of experiments were devised, therefore, to test this hypothesis.

4.4 GROWTH RATES

4.4.1 INTRODUCTION

The growth of seaweeds in common with that in other plants is dependant upon favourable environmental conditions. Optimal growth may be limited by several environmental factors primarily light, temperature and nutrients (DeBoer, 1981). The ability of plants to approach optimal growth levels will affect their biological fitness in terms of their ultimate reproductive capabilities (McLachlan, 1982).

As the uptake rates of nitrates and phosphates were seen to be greater in plants with hairs compared to glabrous plants, it was felt that there must also be a difference in growth rates. It was decided, therefore, to grow glabrous and hairy *F. serratus F. spiralis* and *F. vesiculosus* under the same culture conditions so that this assumption could be tested.

4.4.2 METHOD

Apical sections, 4 cm in length, of each of *Fucus spiralis, F. vesiculosus* and *F. serratus* were collected from the semi-sheltered (SS) transect at St Michael's Island in autumn 1986 and brought back to the laboratory. The sections were separated into glabrous and hairy algae with ten of each type, for each species, being placed in

one of six 5l tanks. Enrichment was added to the filtered seawater in each tank at the start of the experiment and again after seven days. The algae had been sorted in such a manner that a comparative fresh weight of a species was present in both conditions; hairy and glabrous. The plants were then grown under the culture conditions outlined earlier, for fifteen days, being weighed every three days.

4.4.3 RESULTS

Table 4.2 shows that, under the same culture conditions, plants of the same species with hairs, will grow at a faster rate than those without.

4.4.4 DISCUSSION

The results shown in Table 4.2 are expected following the increased nutrient uptake rates shown in hairy *Fucus serratus*. In addition, the hairs which are initially produced as a result of nutrient deficiency do not appear to cease functioning when placed in nutrient rich media.

This in itself, is a logical evolutionary outcome for the algae. A response to low nutrients, in terms of rapidly producing hairs which will increase the uptake rate, holds obvious advantages. Having devoted energy to hair production, it would seem to be

Table 4.2: Comparative fresh weight growth of hairy and glabrous *Fucus serratus, Fucus vesiculosus* and *Fucus spiralis* over 15 days. H = hairy, G = glabrous. (Mann-Whitney values of u, * = 0·01<P<0·05).

Species.		Initial Measurements. Total weight (g).	Mean weight (g)	s.d.	Final Measurements. Total weight (g)	Mean weight (g)	s.d.	Growth Rate. ($x10^{-2}$)	u	Inference
F. vesiculosus	(H)	17·65	1·76	0·39	19·97	2·00	0·42	0·82	100	*
	(G)	17·63	1·76	0·39	18·97	1·90	0·42	0·49		
F. serratus	(H)	14·32	1·43	0·32	17·33	1·73	0·38	1·27	84	*
	(G)	14·33	1·43	0·31	16·28	1·63	0·34	0·85		
F. spiralis	(H)	9·42	0·94	0·33	13·10	1·31	0·43	2·20	90	*
	(G)	9·36	0·94	0·32	12·08	1·21	0·37	1·70		

counterproductive for those hairs to stop functioning once nutrient levels are increased. The evolutionary pressures therefore would appear to favour a fairly sensitive mechanism to produce hairs under nutrient deficient conditions but would not favour a similar mechanism to remove the hairs. This supposition would appear to be borne out by the growth rates of the *Fucus*.

Examination of Table 4.2, reveals a descending, growth hierarchy of *F. spiralis, F. serratus* and *F.vesiculosus* for both hairy and glabrous algae. This result directly contradicts those of Stromgren (1977) who found that apical sections of *F. vesiculosus* grew faster than those of *F. serratus*. Stromgren, however, used apical tips from only one plant, mixed subordinate and dominant apices and had considerable variation in growth rates for apices of the one *F. serratus* plant used.

4.6 THE EFFECTS OF INDUCED HAIRS ON ALGAL GROWTH

4.6.1 INTRODUCTION

The effects of hairs on algal growth have been compared on pilose and glabrous plants collected from the field. To ensure that the increased growth rates found in hairy plants was not solely a result of environmental preconditioning, it was decided to produce hairs in

glabrous plants in the laboratory. The plants with newly formed hairs would then be grown against glabrous control plants with an awareness of the nutrient pre-history of both types.

4.6.2 METHODS

Glabrous *Fucus serratus* apical tips were freshly collected and weighed before being placed in two experimental aquaria. The first aquarium contained 15 tips, filtered seawater and no added enrichment. The second contained 15 tips, filtered seawater and Provasoli's E.S. media. The aquaria were then placed in culture under the usual temperature and lighting conditions. Medium was added to the second aquarium after seven days but the water was not changed in either tank.

The plants remained in culture for 13 days until all the glabrous plants in the unenriched-medium tank had developed hairs. Following this the plants were weighed and enrichment was added to each tank before they were returned to culture. The plants were weighed periodically throughout the experiment.

4.6.3 RESULTS

Table 4.3 gives an overall view of the changes in fresh weight for plants starved of nutrients and those which had added enrichment.

Table 4.3: Changes in fresh weight and growth rates for glabrous algae grown initially under different conditions. Fifteen plants grown constantly in Provasoli's E.S. (Enriched) and fifteen plants which were starved of enrichment for 13 days, until hairs were produced (Starved). Students t-test values of t ***=P<0.001.

Changes in Fresh Weight (g)

Time (Days)	Enriched			Starved		
	Total weight	mean	s.d.	Total Weight	mean	s.d.
0	27.81	1.85	0.29	27.80	1.85	0.31
13	35.32	2.35	0.36	32.79	2.19	0.37
40	56.73	3.78	0.58	57.75	3.85	0.64

Mean Growth Rate ($x10^{-2}$)

	Enriched	Starved	t	inference
0-13	1.84	1.26	24.84	***
13-40	1.75	2.09	24.39	***

After 13 days, the growth of those plants in media enriched water was significantly greater than that of the starved plants. Following the addition of nutrient to both tanks, the hair bearing plants displayed consistently higher growth rates. This resulted in a higher final fresh weight for those plants which were starved than for those plants which were constantly in high media and therefore did not produce hairs.

4.6.4 DISCUSSION

The quite striking results obtained from this experiment actually show an increased final biomass for those plants which had been initially starved of nutrients. This is a direct reflection of the efficiency of hairs in taking up nutrients, and re-emphasises their importance in the ecology of the *Fucus* spp.

The economic overtones of this result cannot be ignored. If species of algae which can produce hairs are starved before being returned to their normal growing conditions, there is a possibility that a higher biomass will be obtained. At this juncture, this is simply a suggestion since other species may not respond in a similar manner to *Fucus serratus*. However, the resulting higher biomass for starved plants could feasibly be increased if an optimal starvation period is ascertained prior to the plants return to the media. This could be of importance in economically grown algae which are

cultivated under controlled artificial conditions (see Tseng, 1981 for review). It should be emphasised that this is a tentative suggestion but the results in Table 4.2, indicate that future work in this area could be extremely productive.

4.7 DISTANCE OF HYALINE HAIRS FROM APICAL TIP & GROWTH RATES

4.7.1 INTRODUCTION

It has been mentioned in Chapter Three, that the hairs in *Fucus serratus* do not simply disappear in Autumn but simply cease to be produced. This results in the new apical growth appearing glabrous whilst the older parts of the thallus are still bearing hairs. The phenomena whereby remaining hairs exist below hairless new growth, was considered useful for examining the effect which proximity of the nearest hairs would have on the growing apical tip. In effect, the suggestion is, that even if the hairs were continuing to increase nutrient uptake, the increased translocation costs would reduce the growth of those plants with hairs further from the tip.

This seems particularly of interest in view of the observation by De Boer and Whoriskey (1983), working on *Ceramium rubrum*. They noted that cytoplasmic streaming occurred in hairs on the apical (meristematic) regions but decreased in hairs on the older parts of

the plant. Microscopial examination of the hairs in *Fucus*, however, revealed no evidence of cytoplasmic streaming in hairs from any part of the plants.

4.7.2 METHOD

Thirty dominant *Fucus serratus* tips were randomly collected from Transect SS on St Michael's Island in Autumn 1986. Each tip exhibited an area of glabrous new growth above an older thallus which still had hairs. The density of hairs on each apical section was noted as was the distance of the nearest hair to the apical tip. The plants were grown for seven days in a single large glass aquaria under the usual culture conditions.

The number of hairs on the algae ranged from 4 to 34 per sq. cm. (mean 16.7), with the distance from the apical tip being between 6 and 38mm (mean 17.7mm).

4.7.3 RESULTS

There was no significant, correlation between the distance of hairs from the tip and the growth rate of the individual plant (Pearsons Correlation coefficient -0.07334).

4.7.4 DISCUSSION

It would appear from the results that hairs continue to absorb nutrients from the surrounding media whether they are close to the apical tip or not. The lack of a significant difference in growth rates is probably caused by the efficiency of translocation of nutrients in *F. serratus*.

Translocation of substances in large brown algae is well documented, with Clendenning (1971) indicating the efficiency of some transport systems by recording maximum elongation of 50cm / day in *Macrocystis*. Floc'h and Penot (1972) have revealed that Fucales are able to translocate, despite the fact that they do not possess the sieve tube elements which are so well developed in the Laminariales. Floc'h (1982), has pointed out that in all studies dealing with ^{32}P translocation in Fucales, there is a "source to sink" relationship. Translocation, therefore, always occurs from the older tissues towards the younger regions of the thallus. Diouris and Floc'h (1984) indicated that translocation of labelled carbon in Fucales takes place through the filamentous cells of the medulla and experiments on *Fucus serratus* in the field resulted in a calculated transport velocity of 2-4cm / hour.

Whilst it is recognised that transport velocities can be very variable, being dependent upon various environmental factors (Schmitz, 1981), the results quoted from the literature, would indicate that

the efficiency of the hairs in *F. serratus* is not being reduced. The figure of 2-4 cm per hour quoted by Diouris and Floc'h (1984) for translocation in the field for this species, suggests that a growth experiment carried out over seven days would not reveal differences and measurements of hourly growth would be preferable.

4.8. HEAVY METAL UPTAKE BY PILOSE AND GLABROUS ALGAE.

4.8.1 INTRODUCTION

Hairs on *Fucus* spp. have been shown to increase the uptake of nutrients. It is also possible, however, that hairs will increase the internal concentrations of unwanted substances, which are potentially toxic, or have sub-lethal effects on metabolism. Munda and Hudnik (1986) for example, found that the growth of *F. vesiculosus* was inversely correlated with the accumulation of heavy metals. As Sorentino (1979) points out, cationic uptake mechanisms have been developed by algae to absorb and concentrate nutrients from the surrounding medium and these mechanisms may also be used to take up non-essential or toxic elements. As hairs have been shown to increase uptake rates of both phosphate and nitrate, it would appear that heavy metal uptake could also be increased by the presence of hairs. It was decided to test the possible differential uptake, by pilose and glabrous algae, on a range of heavy metals using both pilose and

glabrous algae. There is a local interest in heavy metals on the Isle of Man, as concentrations found in *Fucus* spp. from coastal areas of the Irish Sea, are higher than those of all other areas around the British coast (Preston et al, 1972).

Passow et al (1961), have defined the term "heavy metals" as describing those elements with an atomic number greater than 59 (Fe) or having a density greater than 5g/ml. Wood (1974) has classified elements according to their toxicity and also their availability. The three categories are; 1) Noncritical 2) Toxic but very insoluble or very rare and 3) Very toxic and relatively accessible. It is the metals in this third category which are, therefore, of the greatest interest.

4.8.2. METHODS

Fresh samples of *Fucus serratus* were collected, from the same shore in spring, blotted and weighed. The algae were separated into two groups those with hairs visible and those without. Ten plants with hairs were placed in each of five small glass aquaria (40 X 20 cm) and into five further tanks were placed ten glabrous plants. The algae had been sorted so that the weight of algae in each tank was approximately the same. All ten aquaria were filled with five litres of filtered seawater and aerated but no growth medium was added due to potential inhibition or enhancement of the effect of the metals.

High ambient phosphate levels, for example, are known to decrease the toxicity of metals (Rai et al, 1981) whilst Li (1978) found reduced cadmium toxicity in the presence of nitrate, for the marine diatom *Thallassiosira fluviatilis*. A different metal salt was added to a pair of tanks, each containing either glabrous or pilose algae, so that four pairs of each type of algae each contained one metal, the fifth pair acting as a control with no added metals.

The metals chosen were Cadmium, Lead, Manganese and Zinc, these four being selected for a number of reasons. Lead for example is known to be taken up passively by algae, being adsorbed by charged polysaccharides in both the intracellular matrix and the cell wall (Morris and Bale, 1975; Eide et al, 1980). Conversely, zinc and cadmium are taken up actively against intracellular concentration gradients (Eide et al, 1980). Using metals of both types, therefore, allowed for the possibility of hairs exhibiting an increase in either passive or active uptake rates for the individual plants.

In addition to uptake methods, the metals were chosen to give a range of potential toxicity to the algae, with all four occurring in Wood's (1974) third category of "very toxic". The four chosen metals have a general toxicity order of Cd > Pb > Zn > Mn, although this is dependant upon algal species and experimental conditions (Rice et al, 1973; Rai et al, 1981). Manganese and zinc, although potentially toxic, are essential micronutrients, often being referred to as trace metals and their absence can limit algal growth (Lobban et al, 1985).

However, trace metal toxicity has been shown to be functionally related to the concentration of free metal ions (Rivkin, 1979), which would suggest, that in the amounts added in this experiment both Mn and Zn would be toxic to the algae.

The heavy metals were added at 10 ppm as ions in the following salts; Zinc sulphate, Manganous sulphate, Cadmium chloride and Lead nitrate, one metal being added to a pair of tanks to avoid synergistic or antagonistic effects. Work by Foster (1976), suggests that the presence of copper and zinc in high dissolved concentrations would occupy the uptake binding sites and inhibit the accumulation of cadmium, lead, nickel and chromium. Rebhun and Ben-Amotz (1988), found competitive inhibition between cadmium and manganese in the unicellular alga *Dunaliella salina*. With such factors in mind, therefore, it was decided to examine the effects of each metal separately. The tanks were placed under a 16L:8D light regime (70 μ E / m^2 / s), at 10 ° C and left for 5 days. Leaving the algae for longer than this would have resulted in hairs being formed by the glabrous plants and altered the comparative nature of the experiment.

At the end of this period, the algae was brushed vigorously with a half inch paint brush, in running filtered seawater, to remove any adsorbed metal ions from the surface of the algae (Bryan and Hummerstone, 1973). The plants were then blotted and the fresh weight taken before being placed in a Gallenkamp "Hotbox" oven at 75 ° C and dried to constant weight. Dry oxidation of the plant material to

extract the metals cannot be used with lead due to the volatile nature of the element (Price, 1974). To ensure direct comparability between the results, all four metals were extracted by acid digestion following the procedure outlined by Allen and Parkinson (1969).

200 mg of dry plant material was finely ground using a mortar and pestle and placed in a boiling tube. To the ground algae was added 0.5 ml of concentrated sulphuric acid, 1.0 ml of 60% perchloric acid and 5.0 ml of conc. nitric acid. Glass marbles were used to seal the mouth of the tube which was then placed in a Techne Dri-Block DB3 digesting block. The digestion was allowed to proceed slowly at first, the temperature being gradually increased to 90 ° C. After 48 hours the samples were cooled and filtered through Whatman 542 hardened ashless filters into 50 ml calibrated flasks. Potential problems of chemical interference effects from silicon in the sample was avoided by adding calcium chloride at 0.2 percent weight / volume. The sample was then made up to 50 ml with distilled water and analysis was carried out on a Unicam SP90A series 2 atomic absorption spectrophotometer. The readings were converted to ppm by the use of calibration curves; plotted for each metal from known concentrations.

RESULTS

Table 4.4 indicates that there is a highly significant difference in the uptake of lead and cadmium by glabrous algae compared with hair

Table 4.4: Mean values (ppm with std. dev.) of metals found in the 200mg of dried algal tissue. Statistical analysis is given for the differences in metal concentration found in the hairy and glabrous plants (Students T-Test, **=0.001<P<0.01, *=0.01<P<0.05, N/S=Not Significant).

Metal	Controls				Treatment			
	Hairy	Glabrous	t	Inference	Hairy	Glabrous	t	Inference
Pb	1.97	1.95	0.19	N/S	26.86	33.47	4.23	**
s.d.	0.15	0.29			4.28	2.46		
Zn	1.00	1.05	1.63	N/S	7.15	9.02	2.42	*
s.d.	0.07	0.07			1.04	2.31		
Mn	0.34	0.33	0.29	N/S	9.07	7.57	0.60	N/S
s.d.	0.07	0.08			6.35	4.65		
Cd	-	-			0.94	1.13	5.73	**
s.d.	-	_			0.09	0.07		

bearing algae. There is also significantly higher uptake by glabrous *F. serratus* of zinc but no significant difference in uptake for manganese. No differences were found in the metal content of pilose or glabrous control plants. The levels of cadmium in the controls was below the detection levels of the equipment used.

4.8.4 DISCUSSION

The increased uptake in glabrous plants of three of the tested metals was surprising. As the role of hairs is assimilatory, as has been shown for the uptake of nitrate and phosphate, then the reverse result would be expected. The possibility of a selective uptake mechanism in the hairs is highly unlikely due to their apparent physiological simplicity. Factors which could affect uptake, such as the affinity of metals for alginic acid (Huag, 1961), would be expected to be the same for both the pilose and glabrous algae. Alginic acid levels could however, explain why lead, which has the highest affinity for the acid (Huag, 1961), is taken up in greater quantities than the other metals.

The fact that some of the plants used in this experiment were bearing hairs is indicative that there may well be a difference in their internal composition. As the plants were collected at the same time and from similar levels on the same shore however, their physiological makeup would not be expected to be too dissimilar. This point is supported by the fact that the pilose plants would have been glabrous in the days preceding their collection. It must be

concluded, therefore, that there is no obvious reason for the significantly higher uptake of some metals by glabrous algae and that further work is necessary to ascertain the precise cause of this phenomenon.

All of the treatment plants recorded higher metal levels than their corresponding controls, which is merely indicative of the high levels of metal ions added in the experiment. The increase in uptake at higher metal concentrations however, can be a useful biological tool. Fuge and James (1974) have commented that brown seaweeds may be used to provide a running average of metal contamination in their surrounding waters as they cannot regulate their uptake of trace metals. However, the recorded differences in mean values for lead (33.47 ppm) and cadmium (1.13 ppm), in glabrous algae would suggest that some regulation of uptake is occurring. As all metals were added to the treatment tanks at 10 ppm, the suggestion is that selective uptake is producing the observed difference in results. For example, brown algae have a high selectivity for divalent metals (Eide et al, 1980) which are taken up from seawater with the release of monovalent or Mg ions (Myklestad, 1969). Zinc, Cd and possibly Mn exist in seawater as divalent cations with much of the Mn existing as particulate matter (Fuge and James, 1973). The pathways of ion entry into the algae therefore, can lead to the differential levels of metal accumulation. Lead will be taken up passively by the algae (Morris and Bale, 1975) whilst cadmium is taken up actively against concentration gradients (Eide et al, 1980).

Munda and Hudnik (1986) found a sequence of accumulation for single metal applications of Zn-Mn-Cu-Co-Ni-Cd. The inhibitory effects on growth followed the sequence Mn-Co-Zn-Cd-Ni-Cu. They concluded that the growth effects were primarily determined by the biological role of the metals in algal tissue. The accumulation sequence in this study was Pb-Mn-Zn-Cd for both glabrous and hairy algae, which would agree with the findings of Munda and Hudnik (1986), that the biological role of the metal is of great importance in uptake, with the very obvious exception of lead.

Zinc, for example, is an important micronutrient for growth and metabolism in various algae (Rai et al, 1981) although in high concentrations the growth of algae is inhibited (i.e. Whitton, 1980; Rana et al,1971). Manganese is also known to be required for growth (i.e. Harvey,1947), although again in high concentrations growth will be reduced (Christensen et al, 1979). The physiological and metabolic roles or requirements for heavy metals such as lead and cadmium, however, are not properly understood (Rai et al, 1981). Although the physiological essentiality of lead is not known, there are extensive reports of its accumulation in living systems. Lead reduces algal growth rate and has weakly toxic effects on photosynthesis, respiration and cell division (Rai et al, 1981). Wong et al (1979), working on green freshwater algae found cadmium to be acutely toxic, inhibiting photosynthetic $^{14}CO_2$ uptake. Cadmium uptake was reported in *Dunalliella tertiolecta* Butcher, by Jennings and Rainbow (1979) but the exact location of cadmium in the cell was not resolved. An

increase of Cd levels in *Fucus* spp. till March followed by a decrease through the summer as found by Fuge and James (1973), is supported by the ability of glabrous plants to take up Cd at a significantly higher rate than hairy plants.

Whatever the cause of increased metal uptake in glabrous algae, it would suggest that, given similar seasonal seawater metal concentrations, plants should have higher levels of metals in the winter months when they are not bearing hairs. The effect would be enhanced by a lower winter growth rate since concentrations relative to the amount of algal tissue would be higher. As Fuge and James (1973) point out, if it is true that seaweeds cannot regulate their rate of trace element uptake, then concentrations should rise in the dormant wintering plants up to the point where growth resumes in the spring. Concentrations will then be lowest in the new growth and subsequently concentrations in the plant as a whole will fall as it grows (Fuge and James 1973). The same authors also found seasonal variation in the concentrations of Zn, Cu, Cd, Fe and Co, in both *Fucus vesiculosus* and *F. serratus*. The concentrations were at their highest in spring and lowest in autumn. Munda(1986) supports the theory of Fuge and James (1973) that metal concentrations will rise in winter plants, since the metal accumulation of receptacles in *Fucus spiralis*, which appear in the summer months, was diminished relative to the vegetative parts of the thallus. Conversely, Drude de Lacerda et al (1985) reported higher concentrations of Cu, Cr, Cd, Zn, Co, and Pb, during the summer in five species of tropical marine algae,

with lower concentrations during the winter, suggesting that large variations in uptake can exist between species.

In *Ascophyllum nodosum*, which does not bear hairs, Eide et al (1980) found that uptake of zinc, cadmium and to a lesser extent lead, was high in the summer but low in the winter. As this is the opposite of the situation found in *Fucus* it may confirm that the presence of hairs could reduce metal uptake.

The levels of lead found in the control plants was higher than the recorded figures for the other three metals, with cadmium concentrations being negligible. Whilst this would suggest that the influence of seepage from the many defunct Manx lead mines in the surrounding area could be affecting the levels found in the shore algae, there is insufficient data to make any more than tentative suggestions. Indeed, Morris and Bale (1975) have pointed out that the extrapolation of single point data to describe the surrounding local conditions is fraught with dangers. There does however appear to be scope for a future survey on metal pollution on Manx shores.

The use of seaweed for such a survey would appear to be ideal. Trace metal concentrations in benthic algae are not subject to the erratic and transitory fluctuations which may be found in seawater. The consistency of results which can be obtained for seaweed, emphasises their value in long-term monitoring of metal pollution; a point borne out by their use in studies by Preston et al, (1972) and

Nickless et al, (1972). Eide et al (1980) showed how *Ascophyllum* responds to changes in the surrounding seawater concentrations by transplanting seaweed between shores with recorded high differences in levels of metal pollution. The ability of the algae to release heavy metals when transferred to low level environments and increase uptake in high concentrations, further suggested that the use of algae as a temporal monitor of heavy metal pollution is invaluable (Eide et al, 1980).

4.9 GENERAL DISCUSSION

It is clear from the results of the experiments in this chapter that the presence of hyaline hairs is of great importance to *Fucus* spp.. The hairs have been shown to increase the uptake of phosphates and nitrates and under equal culture conditions, plants bearing hairs will grow faster than those without. Since the hairs in *Fucus* are not shed for some time when the plants are returned to high nutrient conditions, the plants bearing hairs are ideally placed to take advantage of localised increases in nutrient levels. This is potentially of great importance to the summer growth of the algae particularly in view of the work of Johannes (1980) which has suggested that additional nutrients from terrestrial run-off may be available to the algae at this time of year.

Keser and Larson (1984), studying three species of *Fucus*, found that their growth patterns were very similar, being low in winter, increasing in spring, rising throughout the summer and reaching a maximum in autumn. Figure 3.2. reveals that hairs are formed in spring, when Keser and Larson (1984) revealed an increase in growth and the irradiance levels are increasing.

Autumn, however, is the only season when hairs will be present on the algae at a time which coincides with higher nutrient levels. It is tentatively suggested, therefore, that the presence of hairs, which have been shown to increase growth in raised nutrient levels, are a prime factor in the autumn growth maxima referred to by Keser and Larson (1984).

Much of the work presented here has made comparisons between the uptake and growth rates of hairy and glabrous algae. This has been necessary because the role which the hairs play may only be understood by examining the situation which exists when they are not present. That this is an artificial comparison however, is obvious when one realises that glabrous and pilose algae will only occur together for brief periods of the year. Nevertheless, artificial manipulations of natural populations has always been a useful biological tool. Due to the fact that hairy and glabrous algae rarely coincide, no attempt has been made to examine their relative competitive abilities although hairy algae are known to outgrow their glabrous counterparts when cultured together under high nutrient conditions.

The result of the experiment whereby starved glabrous seaweeds developed hairs and proceeded to outgrow algae which had remained in high nutrient conditions, is of great interest. Although it is always unwise to make exaggerated claims, the fact that starved algae can outgrow algae fed with nutrients could be an extremely useful economic discovery. Plants grown commercially are often "pulsed" with added nutrients. The possibility that higher growth rates may be obtained without the addition of expensive chemical supplements, is, therefore, an attractive idea.

As Wallentinus (1984) has pointed out, nutrient uptake by macroalgae has long been a neglected field with the majority of work being mainly devoted to species of economic interest. Her paper on nutrient uptake rates is important in that it details the sampling season and the presence of hairs on the thallus. The uptake rate of *Fucus* with and without hairs have been shown to differ significantly and this may well be the case for other algal species which produce hyaline hairs when nutrient stressed. It is vital therefore, that all studies on nutrient uptake rates should contain information on the status of any hairs seen on the species under investigation. Further, it would appear that past studies without this information should now be re-examined with a critical eye.

The preferential uptake of heavy metals in glabrous algae was very surprising given the assimilatory function of the hyaline hairs. As this study is the only comparative work on uptake of metals in pilose

and glabrous plants, it is not possible to examine the literature for the comments of other authors. Any conclusions reached from these results,therefore would be purely speculative. It is interesting to note, however, that there is indirect support for increased metal uptake in glabrous *Fucus* in the seasonal peaks found in the winter by Fuge and James (1973).

CHAPTER FIVE - GRAZING

5.1. GENERAL INTRODUCTION

The grazing of algae by gastropods can potentially be a major determinant of intertidal community structure. Lubchenco (1978) has demonstrated that the mesogastropod *Littorina littorea* L., has a controlling influence on the type and abundance of algae in high intertidal rock pools on the New England coast. Sze (1980), also working on pools, found *Enteromorpha intestinalis* (L.) Link, in high abundance in the absence of *L. littorea*, with a mixed flora occurring when the snail was present. As Watson (1983) points out however, the feeding ecology of littorinid molluscs has, in general, received scant attention; a point echoed by Hughes (1980) who stressed the shortage of relevant data to test optimal foraging theories.

The grazing of *Littorina obtusata* was examined in this study as observation of its grazing behaviour in the field had led to a view that the hyaline hairs of the *Fucus* spp. were being selectively removed by the snail. It was decided therefore to examine this grazing behaviour in greater detail by means of Scanning Electron Microscopy so as to look at both hair grazing and potential grazing of the epiphytic microflora. A number of authors (e.g. Lubchenco, 1982; Reimchen, 1974) have made the assumption that flat winkles use the epiphytic microflora on the surface of fucoids as their primary source

of food. By use of the electron microscope it was hoped to evaluate this claim for both *Littorina obtusata* and its sibling species *L. mariae*.

5.2. ELECTRON MICROSCOPY

5.2.1. METHOD

Over 100 dominant branches bearing hyaline hairs were excised from different *Fucus serratus* plants on the sheltered transect (S) at St Michael's Island. At the same time 100 adult *Littorina obtusata* and 100 adult *L. mariae* were collected from the same shore level as the *F. serratus*. On returning to the laboratory, each branch was examined closely for grazing marks or abrasion damage. Fifty two undamaged branches were then selected and cut so that each of the branches was four centimetres long. Each 4cm tip was then placed in a clean, white plastic coffee cup and covered with filtered seawater. The plastic cups had been standing in running freshwater for 48 hours prior to the experiment to allow any toxins to leach out.

Two adult *Littorina obtusata* were then placed in each of 20 cups and two *L. mariae* in 20 others. Twelve cups were left with algae only, to act as controls. The cups were then placed in a constant temperature room at 10°C ±1° in a 12L:12D light regime.

Small aeration holes were bored in the centre of petri-dish lids and the lids were then placed over the cups to prevent the littorinids from escaping. A small aeration tube was pushed through the hole in the petri-dish lid into the water as it had been noted in pilot experiments that littorinids were more likely to graze when there was a certain degree of water movement.

Two control plants were removed from their cups at the start of the experiment and four 1cm wide, horizontal strips cut from each. These were then fixed in 5% formaldehyde-seawater for 24 hours before being dehydrated in the acetone series outlined in Chapter Two.

The cups were then left for 24 hours, after which time a further ten plants, two control plants, four plants being grazed by *L. mariae* and four grazed by *L. obtusata* were removed, fixed and started on the acetone series. This process was repeated every 24 hours until all the algal tips had been removed.

These samples were again taken to the main campus at Liverpool University for SEM examination and treated in the manner previously outlined in Chapter Two.

5.2.2. RESULTS

Examination of the electron micrographs reveals an interesting set of time-series photos for grazing in *Littorina obtusata*. Plate 5.1 shows a typical control plant at the start of the experiment and reveals undamaged hairs emerging from the cryptostomata. Plates 5.2 to 5.6, are photomicrographs indicating that there is a set pattern to the way in which *L. obtusata* grazes *F. serratus*. The 24 hour plate (5.2) shows an undamaged algal surface (the fissures seen on the left side of the alga were caused during excision). It is clear however that a number of the cryptostomata have had their hyaline hairs removed. Plates 5.2 to 5.6, show a progressive removal of the hairs prior to extensive grazing of the epithelial cells of the *Fucus*. A further important point to note here, is the way in which grazing always appears to commence at the cryptostomata once the hairs have been removed. This is shown clearly in Plates 5.7 to 5.11.

Plate 5.12, is typical of all those taken for *L. mariae* throughout the course of this experiment. There appears to be no grazing damage to either the hairs or the underlying algal cells. There is a slight physical compression of the hairs seen in most of the algal samples exposed to *L. mariae*, which is probably due to the snails moving over the hairs whilst foraging.

Further photomicrographs were taken at increased magnification to determine whether or not *L. mariae* or *L. obtusata* were grazing the

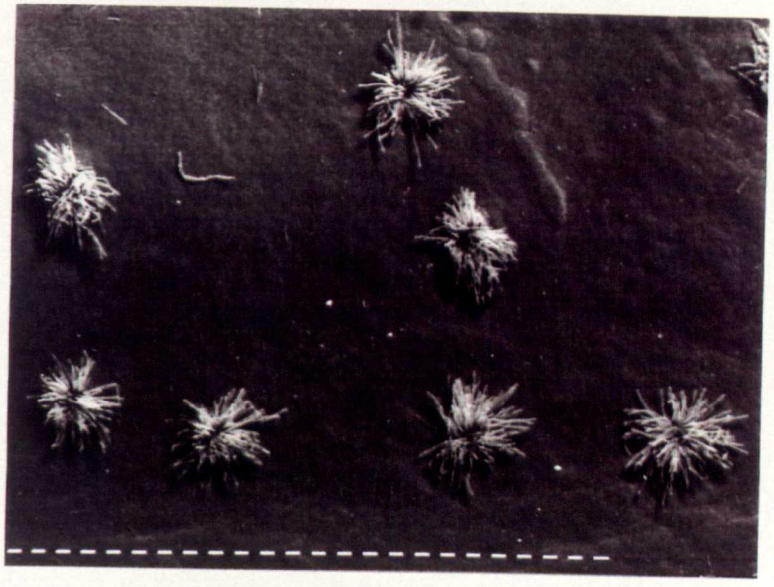


Plate 5.1: Control plant showing undamaged hairs (scale bars=100μm)

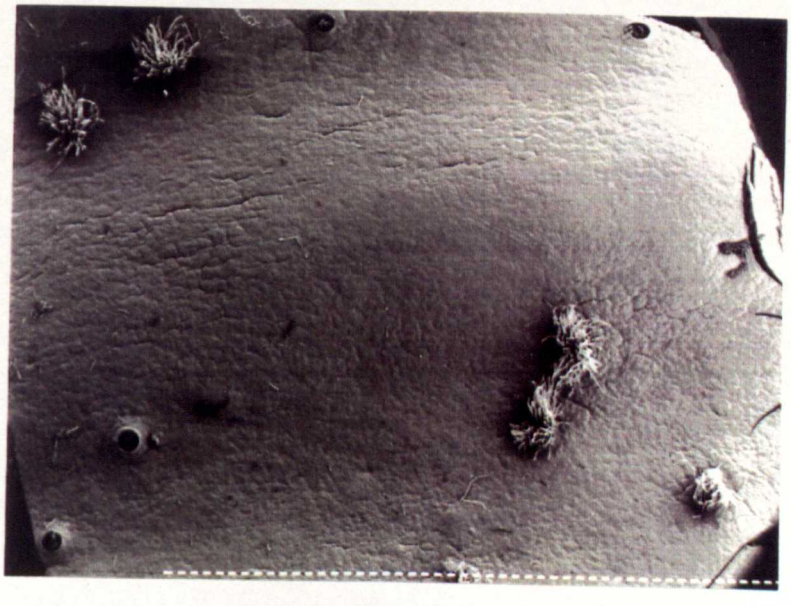
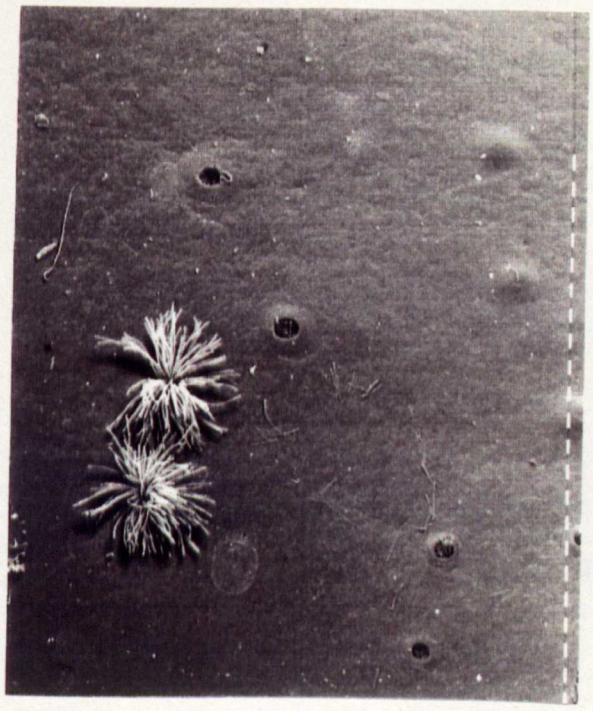
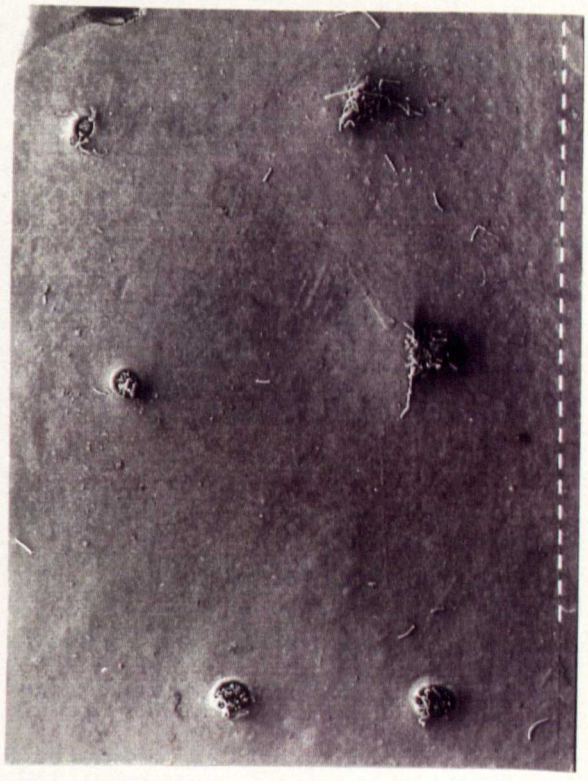


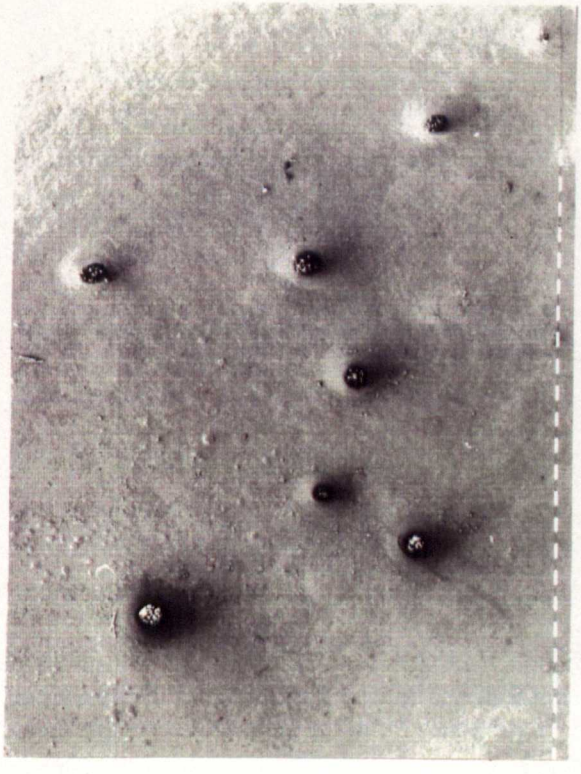
Plate 5.2: Plant grazed by *Littorina obtusata* for 24 hours. Note the exposed cryptostomata when the hairs have been removed (100μm).



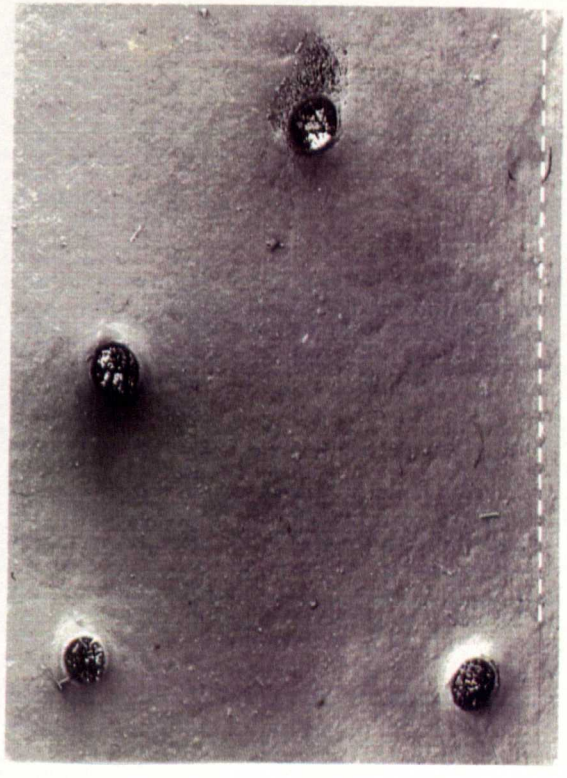
5.3



5.4



5.5



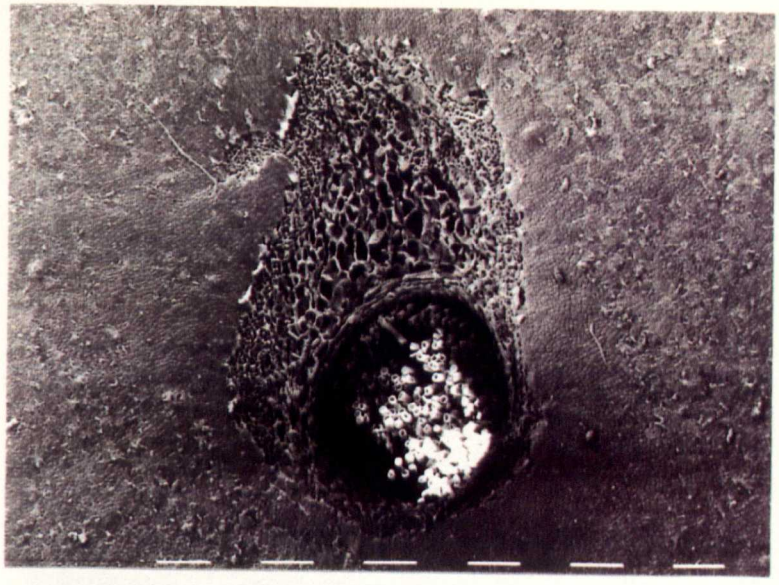
5.6

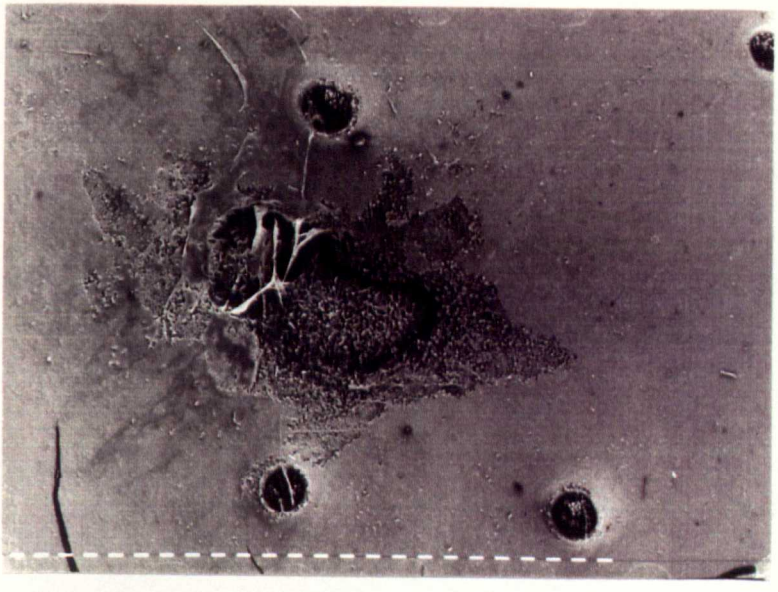
Plate 5.3 to Plate 5.6: Progressive removal of the hairs. (48, 72, 96, 120 hours).

Plate 5.7: Grazed cryptostomata (100μm).

Plate 5.8: Grazed tissue of *Fucus serratus* (100μm).

Plate 5.9: Increased magnification of Plate 5.8, to highlight the damaged cells (10μm).





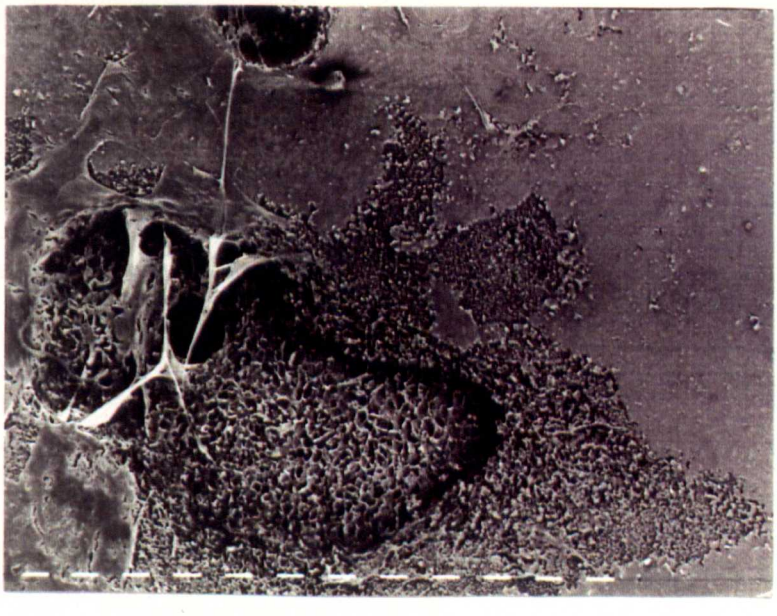
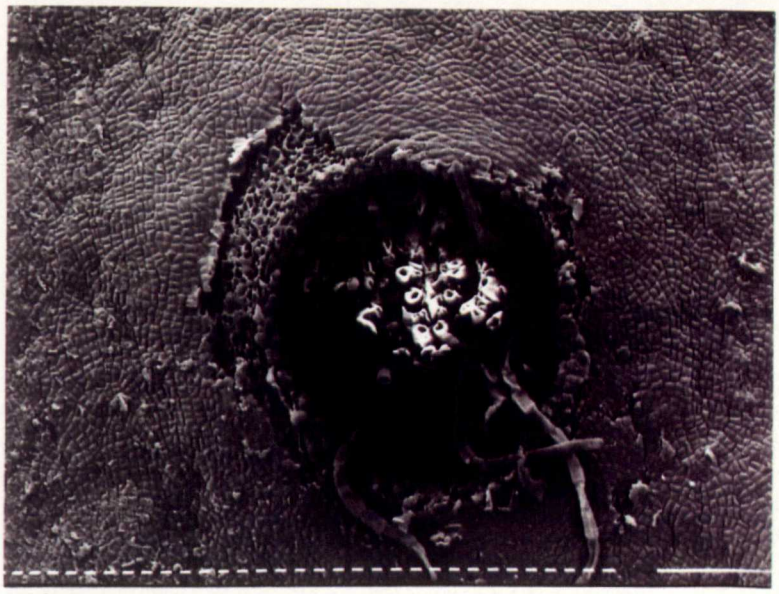
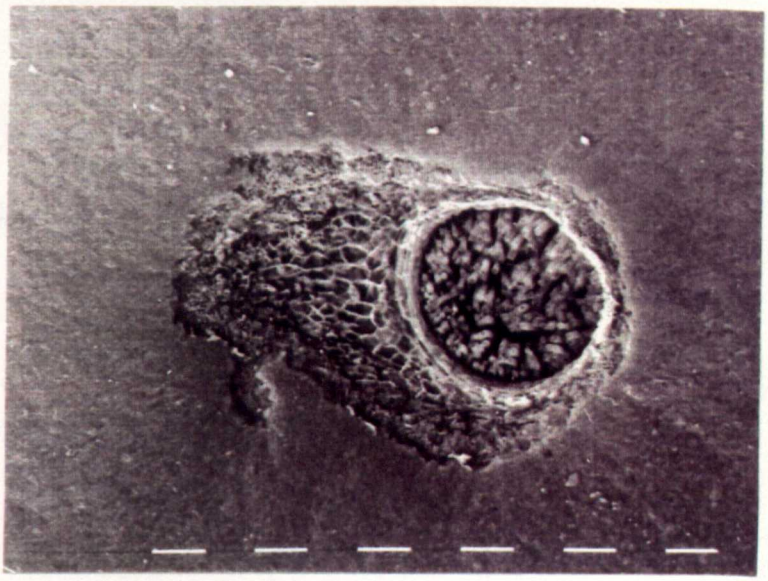


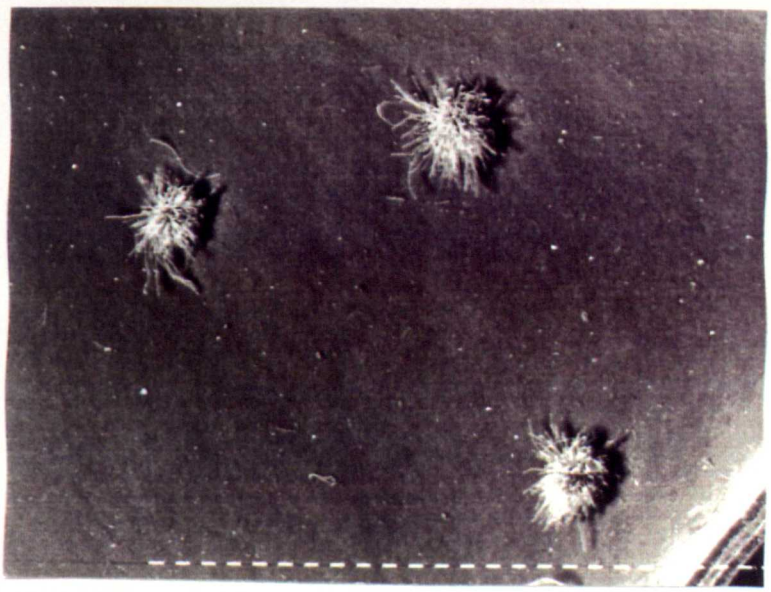
Plate 5.10: Cryptostomata showing grazed hairs and damage to the thallus tissue (10μm).

Plate 5.11: Grazed cryptostomata (100μm)

Plate 5.12: Hairs undamaged by *Littorina mariae* grazing (100μm).







surface of the algal cells. Comparisons between plates 5.13 and 5.14 and the control plates 5.15 and 5.16, fail to give any clear indication of this occurring. This was the case with all samples examined.

5.2.3. DISCUSSION

The series of electron photomicrographs show a clear trend in the grazing strategy of *Littorina obtusata*. The hyaline hairs are initially removed from the fucoid surface which exposes the edge of the cryptostomata. The snails then appear to use the cryptostomata as a breach in the well protected integumentary cells of *Fucus serratus* to begin grazing the main body of the alga.

The grazing sequence shown, indicates that hyaline hairs are initially preferred to the underlying algal cells as a food source by *L. obtusata*. If this is indeed the case then the ramifications of this preference need to be explored further.

The subject of preference by grazers is of quite considerable interest to ecologists on both a theoretical and practical level. Lubchenco (1978) points out that preference will affect the physiological condition of the grazer and ultimately its biological fitness. It will also affect the plant being grazed in terms of the plant's competitive abilities, life history and physical tolerance to the environment. Hughes (1980) extends this statement when he

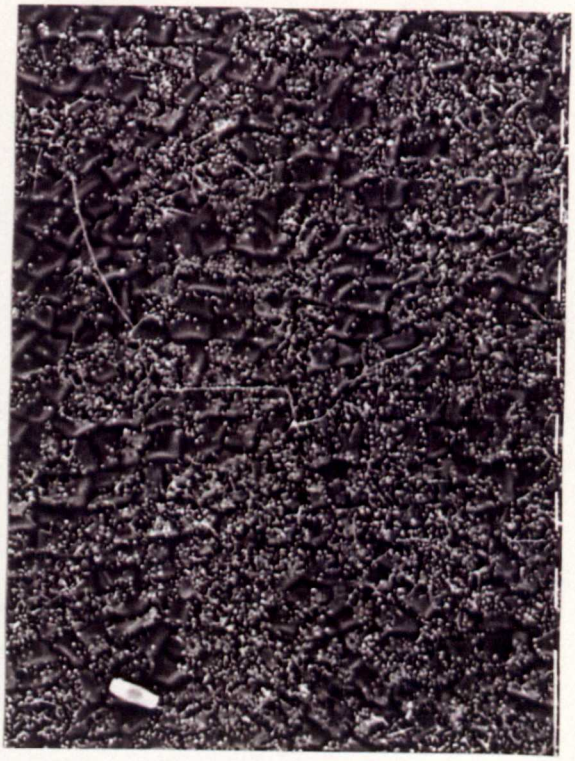
Plate 5.13: Thallus grazed by *Littorina obtusata* for 120 hours (10µm).

Plate 5.14: Thallus grazed by *Littorina mariae* for 120 hours (10µm).

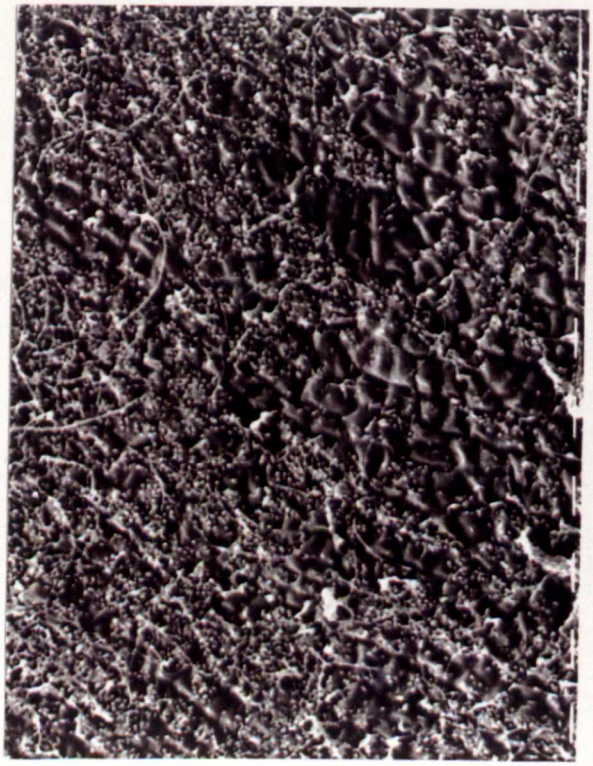
Plate 5.15: Surface of a control plant after 120 hours (10µm).

Plate 5.16: Control plant after 120 hours (10µm).

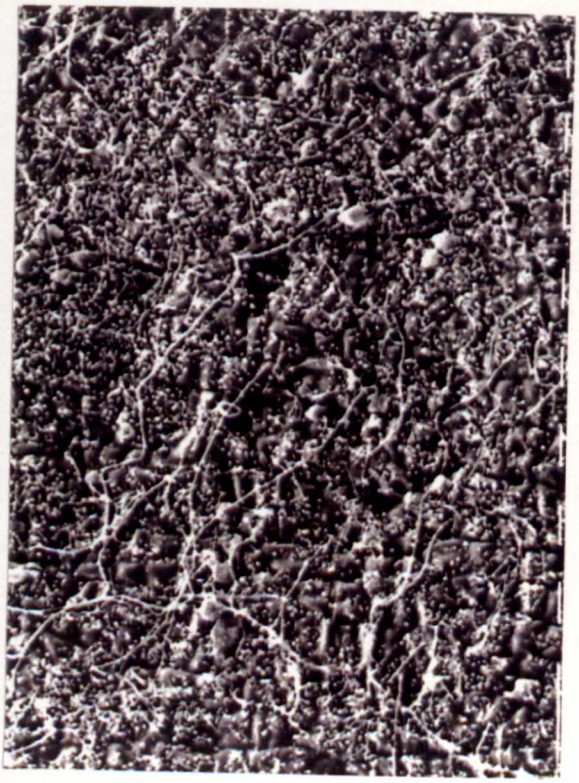
5.14



5.16



5.13



5.15

suggests that the feeding activities of animals have consequences which ramify deeply into theoretical and conceptual developments of population, community and evolutionary ecology.

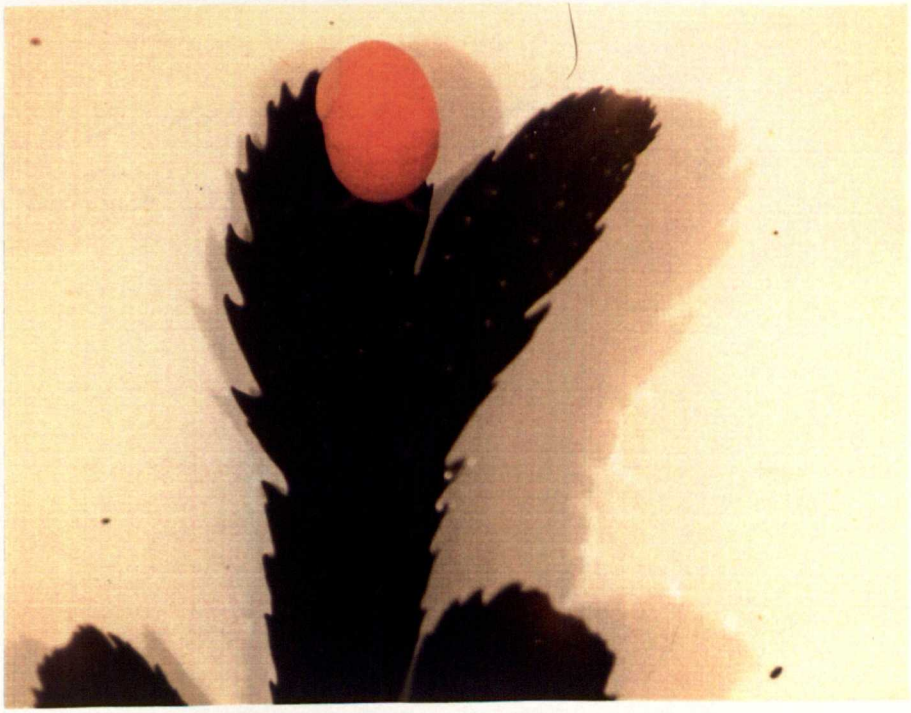
It has been stated in Chapter Four, that the hyaline hairs increase the growth rate of algae and it therefore follows that removal of these hairs by grazers may well have the effect of depressing algae populations. Grazing by littorinids on fucoid algae has previously concentrated on the tissue damage which can be seen following intensive grazing. If, however, as seems to be the case, there are two types of grazing in *L. obtusata*, termed in this study "hair grazing" and "cellular grazing", which are not apparently conducted simultaneously, then the question arises as to whether or not the snails actively forage for hairs before settling for cellular grazing as a second choice.

To confirm that hair grazing was indeed taking place before cellular grazing, a series of photos were taken in the laboratory, using fresh pieces of *Fucus serratus* and *Littorina obtusata* which had been freshly collected and then starved for 48 hours. These photos (Plates 5.17 to 5.22) clearly show a snail grazing the hyaline hairs from the surface of the algae. No cellular grazing took place in the time the photos were being taken (approx. one hour). These plates clearly show that *L. obtusata* is grazing hairs in preference to cellular grazing. Preferrential hair grazing appears to be the case

Plate 5.17: Plant at outset of experiment. Note the prominent hyaline hairs

Plate 5.18: Snail is introduced onto the thallus and begins to forage.

Plate 5.19: Five minutes after the introduction of the snail and hairs have been removed from the left hand branch.



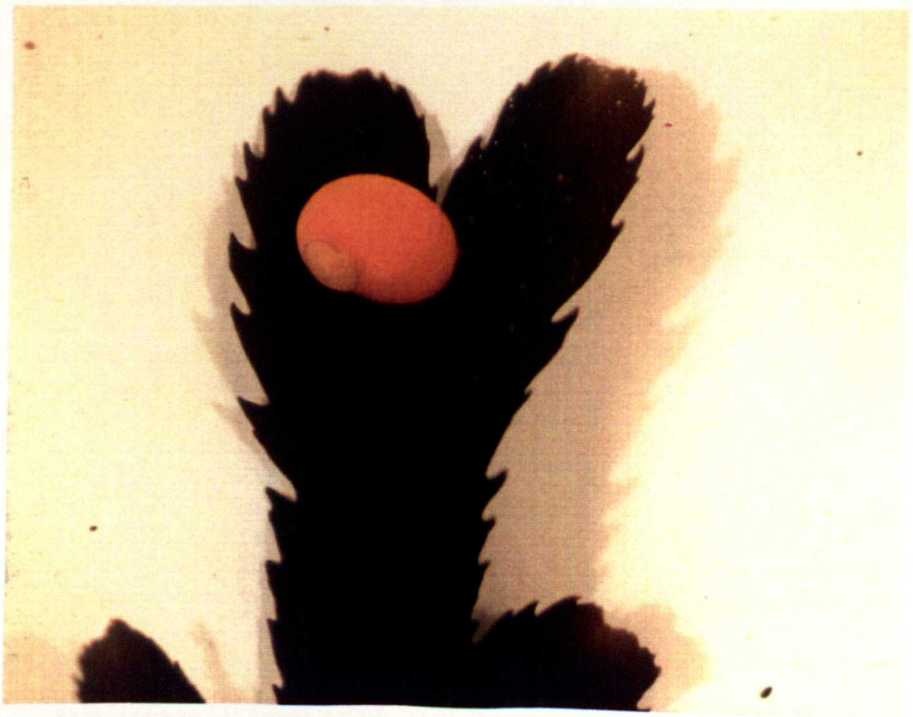
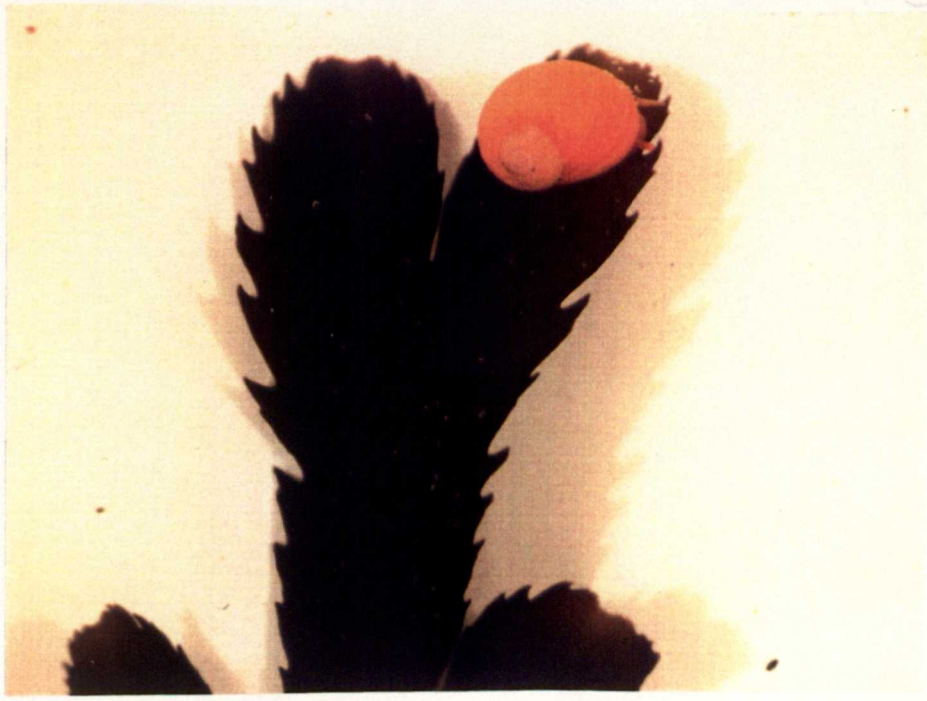
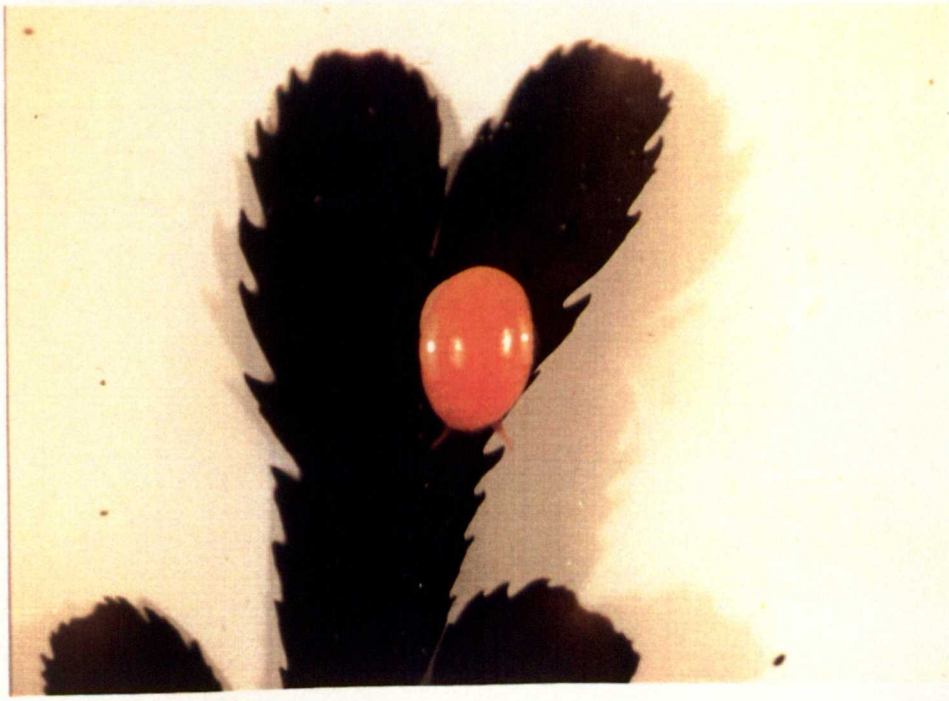


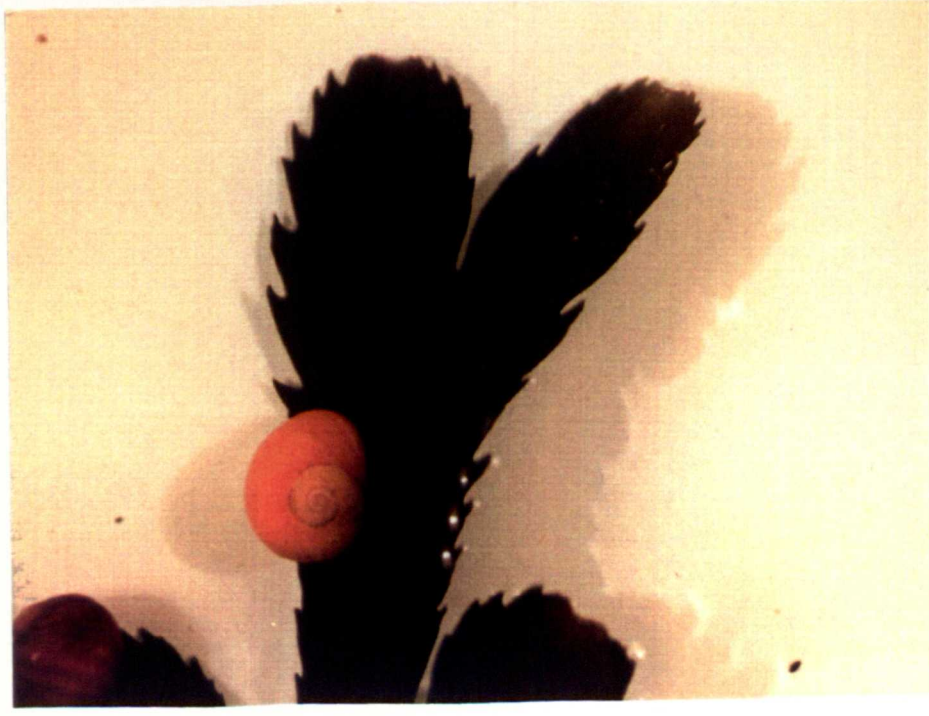
Plate 5.20: Ten minutes the introduction of the snail.

Plate 5.21: Twelve minutes after the introduction of the snail.

Plate 5.22: Fifteen minutes after the commencement of grazing and the snail has clearly removed the majority of the hairs. The snail remained stationary at this point, for a further 45 minutes.







whether or not the snails are starved, as here, or freshly collected as in the SEM experiment.

5.3. THE REGROWTH OF HAIRS FOLLOWING GRAZING

5.3.1. INTRODUCTION

Grazing of the hairs may result in damage being done to the assimilatory capabilities of the plant but this cannot be judged from the photographs. For this reason a number of plants were exposed to grazing by *Littorina obtusata* and their hairs monitored for any subsequent growth.

5.3.2. METHOD

Twenty five undamaged *Fucus serratus* plants were placed in five aquaria, filled with 5l of filtered seawater, and twenty *L. obtusata* added to each tank. The littorinids were allowed to graze until the hairs were removed from each plant. The snails were then removed and the plants, still in their respective aquaria, placed in a constant temperature room at 10°C under cool white tubes at 50μE/m^2/s in a 16L:8D light regime. The plants were monitored every 12 hours to determine whether or not the grazed hairs had grown again. A plant

was considered to have regrown its hairs when 75% of its cryptostomata had hairs emerging above the algal surface.

5.3.3. RESULTS

The combined results from the five tanks showed that the hairs were regrown in a mean time of 5.8 days (± S.D.= 0.73)

5.3.4. DISCUSSION

Grazed plants were seen to regrow hairs fairly rapidly, suggesting that this type of grazing damage causes only a temporary problem for the algae unless further grazing occurs. The ability to regrow the hairs following grazing is an obvious advantage to the plant. It is also interesting that the hairs of *Fucus* are multicellular. It would be expected that hairs whose function was totally assimilatory would tend to be unicellular, to increase nutrient flux and avoid plasmodesmata linkage problems. It is possible that the multicellular hairs in *Fucus* are an adaptation to grazing or abrasion allowing a more rapid recovery following damage. What is not clear however, is the extent to which loss of hairs will affect the growth of the plant and in addition, why the snails apparently prefer to graze the hairs rather than the underlying algal tissue.

5.4. GROWTH OF ALGAE FOLLOWING HAIR GRAZING.

5.4.1. INTRODUCTION

Having established that hairs are preferentially grazed by *Littorina obtusata*, it is necessary to ascertain the effects which this grazing has on the plants. It has been shown in Chapter Four, that the growth rate of plants without hairs is significantly lower than pilose algae. Hairs will however, re-establish themselves following grazing, as has been demonstrated in Section 5.3. What this does not reveal however is the effects of differing grazing pressures, which would be expected to occur in the field. An experiment was devised, therefore, whereby plants would be grazed by different numbers of snails, in a closed laboratory system and the effects on growth rate monitored.

5.4.2. METHOD

Nine 7 litre glass tanks had a fine plastic mesh placed vertically in such a way as to separate the tank into two halves. Five, large *Fucus serratus* apical sections, 8 cm in length, were then placed in each half of a tank such that there was an equal weight of plants in each. *Littorina obtusata* were then placed in one half of each tank in the following series: Two *Littorina* in tanks 1-3, four in tanks

4-6 and eight in tanks 7-9. It was envisaged that this would ensure a sufficient range of grazing pressures upon the plants to allow growth effects to be determined.

By growing the ungrazed, control plants in the same tank as the experimental grazed plants, it was hoped to standardise the growing conditions for each type. This was felt to be important to alleviate media enrichment of the water in the grazed tanks alone by any littorinid faecal material. As the droppings of *L. obtusata* would only be present in one half of the tank, aeration was provided to both halves to enhance water movement and aid nutrient mixing. The plants were weighed and examined for grazing damage, every three days for fifteen days

5.4.3. RESULTS

There are highly significant differences in the growth rates of plants in all treatments relative to their controls (Table 5.2). As grazing pressure is increased from two to eight snails, there is a corresponding fall in growth rates. The control plants for each condition showed a significant increase in growth when higher numbers of snails were grazing in the experimental half of the tanks. Cellular grazing only occurred in those tanks containing eight snails whilst hair grazing took place in all tanks.

Table 5.1: Growth rates of the algae in each half of the experimental tanks. Rates are the mean growth, in fresh weight, per treatment over the fifteen days of the experiment (with standard deviation).

Tanks	Mean Growth Rate (x 10^{-2})			
	With Snails		Without Snails	
	Mean	S.D.	Mean	S.D.
2 snails	0·92	0·03	1·06	0·04
4 snails	0·83	0·05	1·10	0·03
8 snails	0·42	0·02	1·13	0·03

Table 5.2: Statistical analysis of the growth rates of *Fucus serratus* when grown with differing numbers of grazers. (Mann-Whitney Test, values of U for each condition. * = 0·01<P<0·05, ** = 0·001<P<0·01, *** = P<0·001, n/s = not significant).

	2 Snails	4 Snails	8 Snails	2 Snails Control	4 Snails Control	8 Snails Control
2 Snails	---	207 **	225 **	225 **	---	---
4 Snails	---	---	225 **	---	225 **	---
8 Snails	---	---	---	---	---	225 **
2 Snails Control	---	---	---	---	174 n/s	217 **
4 Snails Control	---	---	---	---	---	180 *

5.4.4. DISCUSSION

The growth of algae is determined by many factors, the main ones being light and nutrient availability. By standardising light for all tanks in this experiment, the remaining effects on the growth rates would be nutrient supply and the addition of littorinid grazers. There is an increase in the growth rate of the controls with respect to the grazer numbers in the other half of the tank. It is likely that nutrients are added to the water by the snails. The enrichment of the culture by *Littorina*, suggests that the corresponding reduction in growth rates of the experimental algae is an underestimate and the quoted figures would be lower if the faecal material of the snails was not a factor.

It is hardly surprising that increased grazing will reduce algal growth but the observation that only hair grazing took place in those tanks with two and four snails and growth was significantly reduced, indicates the importance of hairs to the algae. The significantly higher growth rates of algae in the tanks with two snails, compared with those with four snails, indicates that all the hairs were not being grazed in the two snail tanks and are able to perform their customary assimilatory function.

The previously ascertained ability of the plants to extend hairs beyond the lip of the cryptostomata, in a mean time of 5.8 days, would suggest that hairs were being constantly replaced following grazing.

This would imply that the snails added at densities of two and four per tank did not need to indulge in cellular grazing as their preferred food source, hairs, were being constantly replenished. It is reasonable to suppose that hairs became limited at the higher grazing density of eight snails per tank, as they were being grazed faster than they were replaced by the algae. This would lead to the observed cellular grazing which would certainly reduce the final fresh weight of the algae and result in the lowest growth rate of the three grazing densities.

5.5. DIFFERENTIAL GRAZING OF GLABROUS AND PILOSE ALGAE

5.5.1. INTRODUCTION

Observation of grazing behaviour in the laboratory, had suggested the possibility that there may be a difference in the amount of cellular grazing carried out on glabrous and hairy plants of *Fucus serratus*. In an attempt to quantify this difference, the two types of algae were grazed under controlled conditions.

5.5.2. METHOD

Twenty five glabrous and twenty five pilose, undamaged apical

sections of *Fucus serratus*, were placed in plastic coffee cups under the conditions previously described. Two *Littorina obtusata* were then placed in each cup and the algae examined every 24 hours for cellular grazing damage. The time when initial cellular grazing was observed was recorded for each plant and the experiment was replicated three times.

5.5.3. RESULTS

Figure 5.1. reveals a distinct preference for cellular grazing by *L. obtusata* on hair bearing plants.

5.5.4. DISCUSSION

Both pilose and glabrous algae possess cryptostomata, which have been shown to be the entry point for cellular grazing. It would not be expected, therefore, that a difference would occur in the time cellular grazing would be initiated in each type. That there is a marked difference, suggests that factors other than the presence of the cryptostomata, are inducing the exhibited grazing behaviour. The damaged hairs, which had been grazed prior to the cellular grazing, may well have exuded cytoplasmic material which could have acted as an attractant to the snails. Alternatively, the physical presence of hairs just below the lip of the cryptostomata, may also have induced

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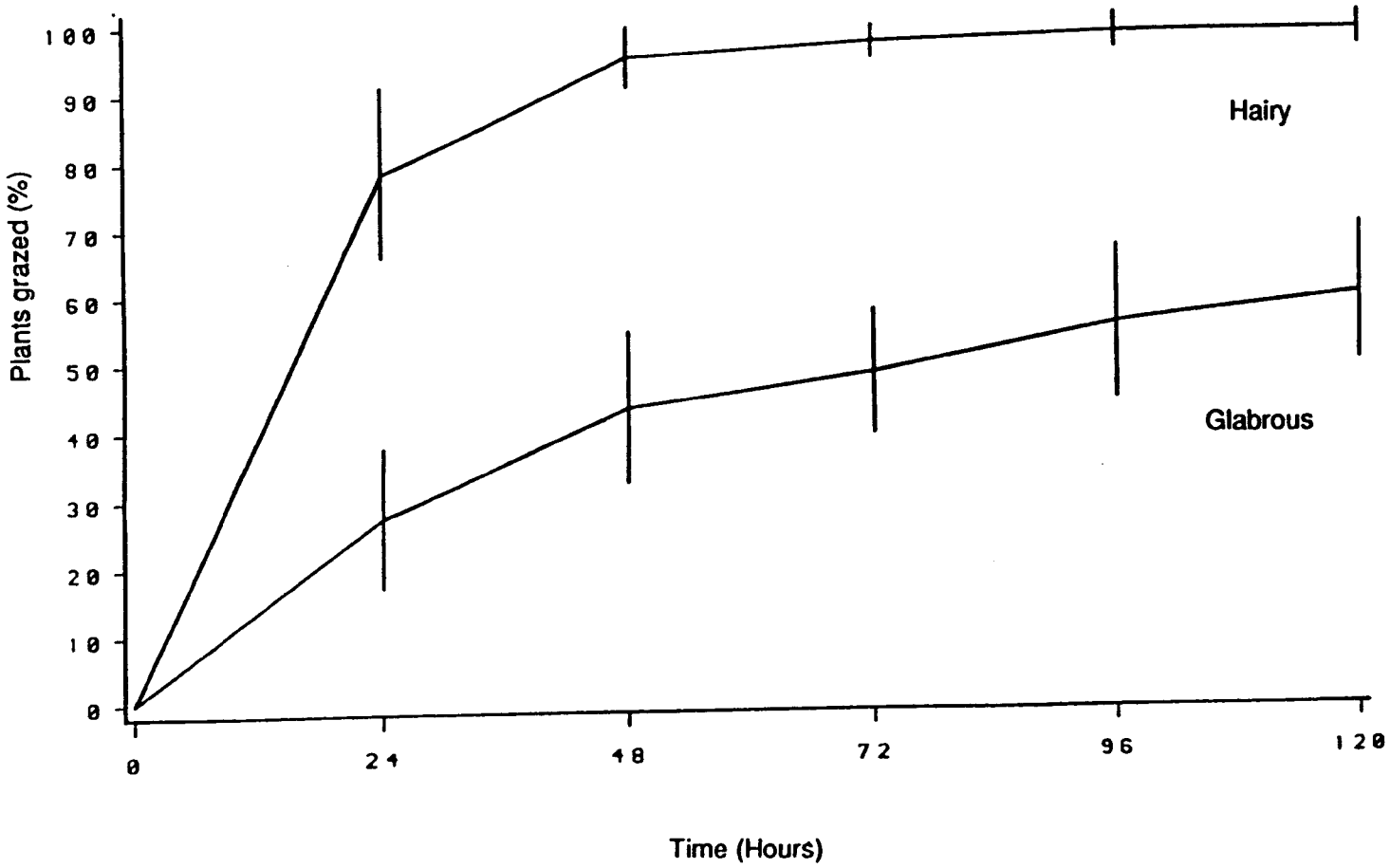
Figure 5.1: Differential cellular grazing by *Littorina obtusata* on *Fucus serratus* with and without hyaline hairs.



the snail to commence cellular grazing in an attempt to graze hair remnants.

5.6. THE SWITCHING FROM HAIR GRAZING TO CELLULAR GRAZING

Since it has been established, by the scanning electron micrographs, that *L. obtusata* is capable of grazing the underlying epithelial cells, then it remains to elucidate why the snails graze hairs in preference to thallus tissue.

The ability to recognise different food sources on a qualitative basis is referred to as switching. Crawley (1983) points out that switching occurs when a previously avoided food becomes a preferred food as a result of a change in its abundance or relative abundance. Thus, it would appear that in a closed laboratory system *L. obtusata* is reducing hair abundance and switching to cellular grazing.

Prior to examining the behavioural or nutritional changes in *L. obtusata*, to explain possible reasons for dietary switching. it is worthwhile referring to the physical structure of the littorinid radulae in order to examine whether there may be a simple physical reason for the apparent dietary preference for hairs.

5.7. RADULAE STRUCTURE

Littorina obtusata like most mesogastropods, possesses a taenioglossan radula. It differs from the rhipidoglossan radula in that it has fewer teeth and a less complex musculature (Fretter and Graham, 1976; Graham, 1973). The taenioglossan radula has fewer marginal teeth and this is accompanied by a loss of ancillary muscles which are used to adjust radula tension and position (Steneck and Watling, 1982). Graham (1973), has suggested that the differences in musculature between these two radulae types is reflected in a change from a "sweeping" action to a "scraping or rasping" mode of feeding. This has led Steneck and Watling (1982) to describe the taenioglossan radulae as "rakes" and rhipidoglossan radulae as "brushes". They suggest that less emphasis is put on positional adjustments of the taenioglossan radula and more on the force with which it is applied to the substratum being grazed. Juch and Boehschoten (1980) point out a further difference between the radulae, stating that the central, or rachidian, tooth is only commonly used for grazing in taenioglossan radulae.

Such differences alone would not appear to be consistent with the hair grazing exhibited by *L. obtusata* but Steneck and Watling (1982) point out that the propensity of taenioglossan grazers for grazing microalgae and filamentous algae is probably facilitated by the splaying of the pluricuspid marginal teeth to produce a greater surface area for collecting particles. In addition, inward raking and,

possibly, cutting of algal filaments occurs as the teeth converge towards the central axis of the radula during retraction. The latter suggestion means that the taenioglossan radula of *L. obtusata* is well suited to remove the filamentous hairs from *Fucus* spp.

Steneck and Watling (1982) however, have also commented that the robustness of the radular teeth in taenioglossan radulae may explain how they graze a greater proportion of tough leathery macrophytes and articulated coralline algae than do rhipidoglossans. This point emphasises the versatile nature of the taenioglossan radula. The need for a robust radula however, may not necessarily be applicable to the same degree in *Littorina obtusata*, if grazing is usually confined to the hyaline hairs.

It would appear that the taenioglossan radula is not specifically suited to grazing hairs although it is capable of doing so. However, a closer study of the grazing strategy and foraging behaviour of *L. obtusata* is necessary to elucidate the mechanisms behind the switching from hair to cellular grazing.

Kamil et al (1987), have stated that a better understanding of foraging behaviour will contribute to resolving a number of important ecological issues. In addition, they recognise that one of the most important factors in the recent interest in foraging behaviour has been the development of Optimal Foraging Theory. The theory remains controversial with critics who claim that it is tautological,

misguided and fruitless, whilst supporters believe it to be programmatic progressive and fruitful (Kamil et al, 1987).

Both of these extreme views are to be avoided since it is clear that Optimal Foraging Theory in itself is only a descriptive tool. Reference in this study to Optimal Foraging Theory is made simply to allow experimental results to be considered within a defined framework, with the intention of highlighting the processes involved in the grazing strategy of *L. obtusata*. It is not intended to propose a view in favour of, or against, Optimal Foraging theory *per se* but simply to use the theory as a method of enhancing the understanding of exhibited grazing behaviour. To do this a brief outline of the ideas behind the theory are given.

5.8. OPTIMAL FORAGING THEORY

MacArthur and Pianka (1966) made a differentiation between the exploitation of prey, which they considered as a discrete item that is captured and consumed completely, and patches. Patches are thought of as being clumps of food or heterogeneities in prey distribution. This initial separation has led to the development of two basic maximising models, the Prey and Patch models. Both of these assume sequential encounters whereby patches or prey are encountered one after another and that foraging consists of repetitions of; Search-Encounter-Decide (Stephens and Krebs, 1986). The models attempt

to predict the decision made following an encounter. They differ in that they analyse different decisions. In the Prey model the question is whether to eat or continue searching. In the Patch model it is how long the animal will stay in a given patch.

Much Optimal Foraging Theory is based upon carnivore predator/prey models. However, Lubchenco (1978) and Hughes (1980), believe that the theory is equally applicable to both herbivores and carnivores since, in an ecological context, it is only the trophic level of their prey which differs. Indeed Hughes (1980), prefers a behavioural classification of predators based upon Browsers, Filter-Feeders, Deposit Feeders and Hunters.

MacArthur (1972), argued that on perceiving a prey item, a predator has the choice of either pursuing that item or continuing to search for better prey. He assumed that the predator should choose the option which yields the most resource (usually assumed to be energy in OFT) per unit time. He divided the total time a predator spends actively foraging into Search Time, during which the predator scans the habitat for prey, and Pursuit Time, which is the time spent pursuing and capturing an individual prey item. The two categories led to his classification of predators into searchers and pursuers.

Searchers covered the wide variety of animals which actively search for food items but which normally do not spend much time pursuing or handling prey. The total time spent pursuing or handling prey is short

relative to the time spent searching during a foraging bout. Pursuers on the other hand are considered to be predators which have morphological and behavioural properties which enable them to feed on "difficult" prey. These prey items have some measure of protection from other predators by virtue of defence or escape mechanisms. In order to feed on such "difficult" prey, a predator must usually invest a considerable amount of time pursuing or handling each prey item and on this basis is expected to have a specialised narrow diet. It is perhaps counter intuitive to regard slow moving snails as pursuers but by definition pursuers include any case where pursuit or handling time is long relative to search time (Hughes, 1980) .

If the above is applied to *Littorina obtusata*, it would appear that the dietary switching behaviour of the snail is such that it will cross the Pursuer/Searcher theoretical boundaries depending upon whether it is consuming hairs or the underlying epidermal cells. When consuming hairs the snail appears, from *ad hoc* observations, to behave as a searcher spending little time handling the food item once it has come across it. Once the hairs have been removed the behaviour switches to that of a pursuer, with a longer handling time spent [illegible] algal surface cells. In order to quantify the observed differences in the handling time spent by *Littorina obtusata* on both hairs and cellular grazing, the snail's grazing behaviour was studied in the laboratory.

5.9. HANDLING TIME

5.9.1. METHOD

Thirty adult *L. obtusata* were collected and starved for 48 hours to ensure standardisation of appetite. They were then introduced into a glass tank, the sides of which had been covered in black plastic sheet to exclude light. The tank contained ten, freshly collected *Fucus serratus* plants, bearing obvious hyaline hairs. The plants had been carefully selected to ensure that they exhibited no sign of grazing or abrasion damage. The behaviour of the snails was noted and as a hair clump was approached by a snail the amount of time spent before the hairs were completely consumed, or feeding ceased, was measured with a stop watch. Once all, or the vast majority of hairs were removed, the alga was taken from the tank and once again examined for grazing damage. The method was repeated 15 times with fresh snails and fresh pieces of alga being used each time.

The plants without cellular grazing damage, were then washed in filtered seawater to remove any mucus trails or other surface debris. A fresh snail, starved for 72 hours, was then placed on one of the plants and its behaviour observed. Following a period of foraging movement, the snail remained stationary and no visible attempt was made to indulge in cellular grazing. After one hour on the

F. serratus, the snail left the algae and began to climb the wall of the tank. Similar behaviour was observed in all fifteen replicates.

The reluctance of the littorinid to graze when no hairs were present on the thallus was a typical reaction which had been commonly observed. In order to quantify cellular grazing times therefore, it was found necessary to increase the numbers of snails on a given piece of algae and to ensure that they remained on the plant. Following hair removal by grazing, in the manner described above, ten *L. obtusata*, starved for 96 hours, were placed on one piece of *Fucus serratus*. Those snails that left the algae were returned to the thallus. The experiment was carried out in five tanks simultaneously so that there were, in total, 50 snails grazing on 5 pieces of algae.

After five hours, cellular grazing was seen to be taking place and an attempt was made to quantify the time taken to breach the algal surface. Again, in every case, the grazing snail entered the alga through the cryptostomata and did not attempt to breach the epidermis directly. It was not possible to discern clearly when the snail had commenced grazing the outer cells of the algae but by observing radula movement and counting the number of radula strokes, prior to removing the snail from the algae to inspect the damage, it was possible to estimate the average number of strokes needed. Only when obvious grazing had taken place and the meristoderm cells around the cryptostomata were clearly damaged, was a result recorded. From this

a fairly accurate estimate of the time taken, to graze into the algae, was obtained.

5.9.2. RESULTS

It may be seen from Table 5.3., that less time and effort, in terms of radula strokes, is required by *L. obtusata* to consume hairs than to penetrate the cryptostomata.

5.9.3. DISCUSSION

The reduced amount of time needed to graze hairs would suggest that a lower handling time is necessary for hair grazing as opposed to cellular grazing. A further interesting point to note is the mean figures for the number of strokes per second. The figure of 1.077 in hair grazing is considerably higher than the 0.633 strokes per second found with cryptostomata grazing. This suggests that either fatigue is playing a part and the snail is slowing down its feeding rate or that cellular grazing is more difficult for the snail. Whichever is correct, and they are not mutually exclusive, the end result is the same in that it takes longer for the snail to obtain food by grazing on the thallus rather than on hairs.

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Table 5.3: *Littorina obtusata* mean grazing times, strokes per second and mean number of radula strokes needed to graze hairs and breach the cryptostomata of *Fucus serratus.*

	Mean Number of Radula strokes	Standard Deviation	Mean Time (seconds)	Standard Deviation	Strokes.sec^{-1}.
Hairs.	4·28	0·87	3·97	0·97	1·077
Cryptostomata.	28·42	3·37	44·92	14·69	0·633

To establish if the starvation period for the snails was having any effect on their grazing, the experiment was repeated with freshly collected snails. There was no significant difference between the mean times taken to graze the hairs or to breach the cryptostomata (hairs 3.92 seconds, cryptostomata 43.84 seconds) nor in the number of strokes per second.

Concentrating upon a food source with a low handling time is probably important for *L. obtusata* which grazes mainly when immersed by the tide (Watson, 1983). The limited feeding time allowed by the tidal cycle means that there are environmental pressures at work upon *L. obtusata* beyond the normal dietary requirements of the animal.

A basic premise of Optimall Foraging Theory, is that foraging behaviours which maximise the net rate of energy intake confer a selective advantage (Energy Maximisation Premise) (Hughes, 1980). In reality however, there will arise a variety of constraints which will cause a violation of the Energy Maximisation Premise from time to time. A foraging behaviour may deviate from what would be predicted as optimal due to the influence of predators or competitors. Alternatively, at any given time resources other than energy e.g. specific nutrient requirements, may be of greater immediate importance. Usually, however, the net rate of energy intake is of central importance (Stephens and Krebs, 1986).

To establish if there were calorific differences between the hairs or thallus cells, five samples of 1g dry weight were collected of each. Each sample was then placed in a bomb calorimeter but due to a fault in the equipment, the results were so variable that they have not been quoted.

The switching in the grazing behaviour of *L. obtusata* is conceivably of major ecological importance not only to the snails but also to the *Fucus* populations. An explanation of the possible theoretical basis for this switch can be found in the Marginal Value Theorem outlined by Charnov (1976).

5.10. MARGINAL VALUE THEOREM (CHARNOV, 1976)

If a mobile animal forages for food it will encounter suitable food in discrete habitat patches. Theoretically therefore, a wide ranging predator is faced with the choices of which habitat patches to feed in and how long to stay in each. Many authors have commented on this problem (e.g. Royama, 1970; MacArthur and Pianka, 1966; Pulliam, 1974; Smith and Dawkins, 1971; Tullock, 1970; Emlen, 1973; Krebs, 1973; Krebs et al, 1974; Charnov et al, 1976), and the model of Charnov (1976) gives a convenient explanation of the underlying theoretical considerations.

Charnov's model makes the familiar basic assumption that the choice of habitat and the time spent in each should maximise the average net rate of energy intake E/T where:

E = Net gain of Energy

T = Time spent foraging

Further, the time taken to travel between patches is given by Tr. This is an average figure and it is assumed that no feeding takes place during this time. A further assumption is that the rate of food intake in a patch will decline with time. This is represented by P_i which equals the proportion of patches that are of type i (where $i = 1,2,...n$), each patch type having a unique value in terms of prey availability and individual prey items are assumed to be of equal value in all patches.

Further:

E_r = the energy cost incurred per unit time in travelling between patches.

E_{si} = the energy cost per unit time while searching for food in patch type i.

T_{si} = Time spent foraging for food in patch type i.

E_{ti} = Net energy gain after searching for T_{si} time units in patch type $_i$. This is the total energy intake minus costs incurred in Searching, Pursuing, Handling and digesting prey.

Thus, the average time, T, taken by the predator to use a single patch, which will include the time taken to get to the patch from a previous one, is:

$$T = T_r + \Sigma P_i \cdot T_{si}$$

The average net energy gain, E, from a patch is:

$$E = \Sigma P_i \cdot E_{ti} - T_r \cdot E_r$$

Thus, E/T is assumed to be:

$$\frac{E}{T} = \frac{\Sigma P_i \cdot E_{ti} - T_r \cdot E_r}{T_r + \Sigma P_i \cdot T_{si}}$$

To find how long a predator should stay in each patch type, T_{si}, may be found by setting $d\ (E/T)\ /\ d\ T_{si}$ to zero for all patch types simultaneously and solving the equations. This gives:

$$\frac{d\ E_{ti}}{d\ T_{si}} = \frac{E}{T} \quad \text{for all i.}$$

This will mean that the predator should leave a patch when its net rate of energy intake, $d E_{ti} / d T_{si}$, falls to the net rate of energy intake for the habitat, E/T.

If a habitat is foraged persistently then the value of E/T is depressed such that the predator should start to feed in patches that were previously ignored (Charnov, 1976). The outlined theoretical model could explain why, in the laboratory, *L. obtusata* switches from grazing hairs to concentrating on the underlying algae. The closed system will potentially increase the value of T_r to infinity thus raising E_{si} and reducing E_{ti}. More simply, the net gain of energy (E) is lowered and the time spent foraging (T) is raised causing the perceived switch in diet.

It should be emphasised here, to help clarify the terminology used, that switching in its accepted sense in optimal foraging models normally refers to the ability of a predator to change from one prey type to another. Switching in the sense in which it is used in this study only refers to a dietary change viz, the switching from grazing hairs to grazing the epithelial cells of the alga. To illustrate the point further, Krebs (1978) regards predator switching as being important in stabilising the fluctuations in prey population densities. Krebs (1978), is clearly not referring to the switching from browsing one part of a fucoid thallus to another part. Indeed, Begon and Mortimer (1981) regard switching as only being of importance in vertebrate predators due to their ability to learn from

experiences. For invertebrates, the ease of exploitation of the prey is a much commoner factor in determining predator preference (Begon and Mortimer, 1981).

Begon and Mortimer (1981) have commented upon the existence of a "search image" which is the theoretical ability of predators to specialise upon the commonest prey item within a given habitat. The development of a search image by *L. obtusata* has not been evaluated in this study and therefore cannot be discounted as a factor in the preference for hairs. A search image is based upon a learning ability in the predator whereby previously captured prey enhances the possibility that the predator will continue to forage for that prey item. Thus the commoner the prey, in this case hairs, the more will be grazed and the stronger the effects of the search image. This will result in the grazer concentrating more upon the "image prey" to the exclusion of the non-image prey. The difference in the grazing of *L. obtusata*, is that we are talking about easier handling of the prey item being the controlling factor and not its frequency; since clumps of hairs and cryptostomata are, by definition, available in equal numbers.

What is not clear however is why there is obvious cellular grazing damage on *Fucus* spp. in the field if the number of hairs is not obviously limited in the way it is in a closed system in the laboratory. One possible answer lies in the fact that the physically stressful intertidal habitat can cause considerable abrasion damage

to the *Fucus*. It is, therefore, possible that the snails could be taking advantage of the damaged integumentary cells of the *Fucus* and switching from hair to cellular grazing as the overall handling time of the latter is reduced by a breach in the plant's physical defences.

In an attempt to look at the effect of damage in greater detail, it was decided to artificially cut *Fucus* plants, thus allowing easier access to the main body of the algae for the snails. It was envisaged that this would give a clearer example of the true dietary preference of *L. obtusata* since the relative handling time between hair grazing and cellular grazing should be reduced.

5.11. ARTIFICIAL DAMAGE

5.11.1. METHOD

One hundred, undamaged, hair bearing, apical branches of *Fucus serratus* were collected and cut into 4cm lengths. On 50 of these branches three deep horizontal scratches were made across their surface on one side of the thallus. The 100 tips were then placed in 100 plastic cups under the conditions described in section 5.2.5 and two *L. obtusata* which had been starved for 48 hours were placed in each cup. The algae were examined every 12 hours for signs of grazing damage. The apparent point of entry into the thallus was also

noted. The experiment was replicated three times and the results are given in table 5.4. and Fig 5.2.

5.11.2. RESULTS

Figure 5.2. shows clearly that the plants undergo cellular grazing significantly quicker when artificially damaged (Students T-Test, T=9.37, p=0.00). The point of ingress for grazers on the damaged plants (Table 5.4.) showed a highly significant preference by *Littorina obtusata* for the damaged thallus over the cryptostomata (T=15.66, p=0.00).

5.11.3. DISCUSSION

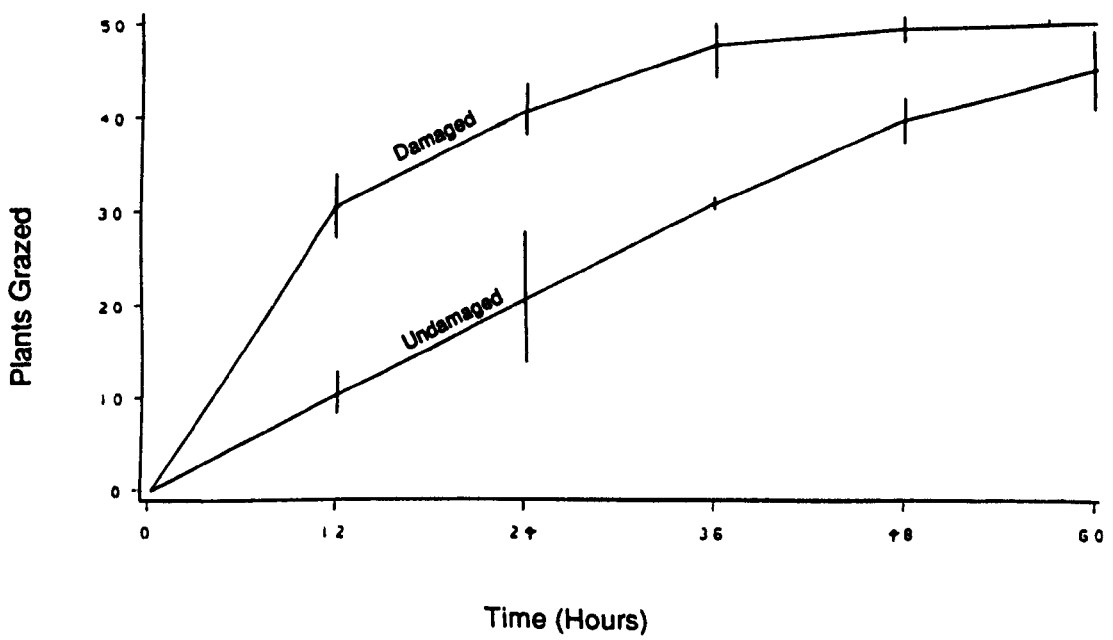
The results of this experiment suggest strongly that the preference for hairs is a factor of handling time and when snails are presented with an obvious breach in the alga "defensive wall", hair consumption becomes of secondary importance. Figure 5.2., shows clearly that cellular grazing takes place far more readily when the cell wall of the *Fucus* is damaged although Table 5.4 indicates that grazing through the cryptostomata, although not significantly so, is still a factor even in damaged plants. One surprising observation is that the damaged plants, which were still grazed through the cryptostomata rather than the cut, were still bearing a number of hairs. An

Table 5.4: Site where grazing commenced on the damaged plants *. (cumulative mean totals).

Time. (hours)	Site of Damage Cut	Cryptostomata
12	25·3	5·0
24	34·0	6·7
36	39·0	8·0
48	41·3	8·0
60	41·7	8·3

* all grazing on the undamaged plants commenced at the cryptostomata.

Figure 5.2: Differential cellular grazing by *Littorina obtusata* on *Fucus serratus* with and without damage to the thallus. n=150 per category.



observation which is directly contradictory to the result obtained when the plants were not damaged. There is no obvious reason for this to occur although possible chemosensory stimulation caused by the seepage of excessive cellular material through the cuts could have affected the behaviour of the snails. This seems unlikely however since the branches in the previous grazing experiments were also cut, albeit only at the base.

The results indicate that external damage to the thallus of *Fucus serratus* is a major factor in determining which foraging strategy is employed by *L. obtusata*. The plants however were damaged experimentally and as such might prove to be far more attractive to the littorinids than plants which had been allowed time to heal the wounded tissue. To test for possible differences in grazing behaviour in the snail when presented with damaged plants directly from the field, the following experiment was carried out.

5.12. FIELD DAMAGED PLANTS

5.12.1. METHOD

A selection of *F. serratus* plants were collected during a storm from the semi-sheltered transect (SS) at St Michael's Island. Their

apical sections were excised and visually sorted into three categories: undamaged, freshly damaged and 'healed' plants. It was important to collect the plants during a period of heavy wave action as any damage to the thin, perforated cross-walls of the medullary filaments would be plugged after about six hours (Fulcher and McCully, 1969b, 1971). In the freshly damaged plants, fresh cellular material could be clearly seen through breaches in the algal surface. The plants which were designated as 'healed', were those where regeneration of plant tissue had clearly healed the underlying cells at sites of prior abrasion. Twenty plants from each category were placed in the white plastic coffee cups in the manner described earlier, and two *Littorina obtusata*, starved for 48 hours, placed in each cup. The cups were placed in a 12L:12D light regime at 10°C and aerated. The algae were examined every 12 hours for evidence of further cellular grazing damage. The experiment was replicated 3 times.

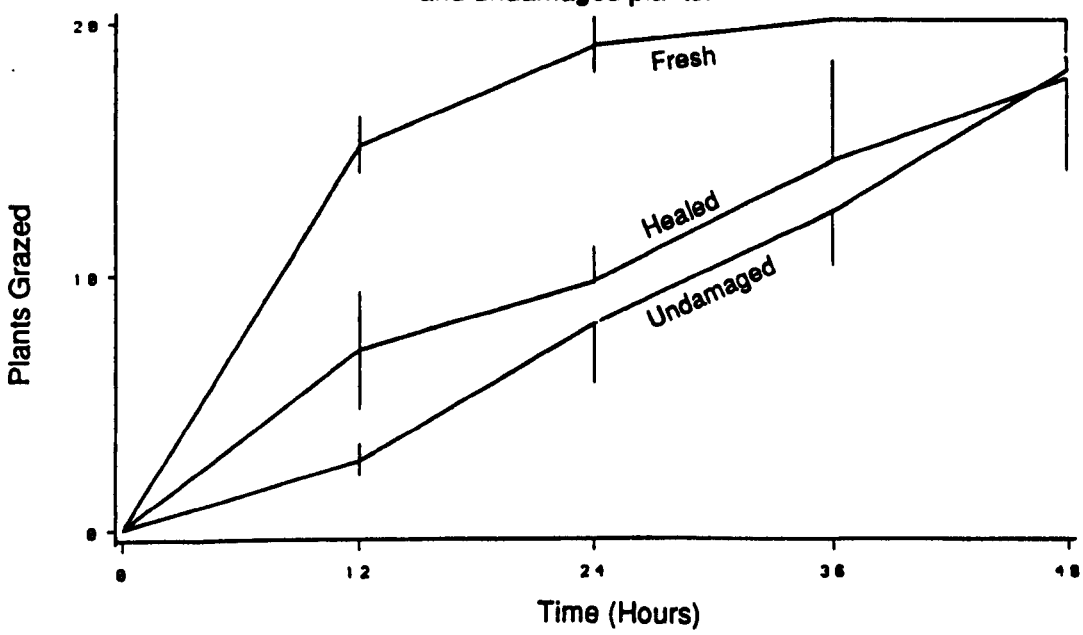
5.12.2. RESULTS

The results in Figure 5.3 indicate that freshly damaged plants are far more susceptible to grazing than both undamaged plants and those which have undergone a degree of wound healing and regeneration. Table 5.5., shows highly significant preferences for freshly damaged plants over both undamaged and healed plants. There is also a

Table 5.5: Statistical analysis of the differences in grazing found between Freshly Damaged, Undamaged and Healed plants. (Wilcoxon signed rank test, * = 0·01<P<0·05, ** = 0·001<P<0·01).

	Fresh Damaged.	Healed.	Undamaged.
Fresh Damaged.	—	z = 3·81 **	z = 4·06 **
Healed.	—	—	z = 2·11 *

Figure 5.3: Differential cellular grazing by *Littorina obtusata* on *Fucus serratus* with healed and freshly damaged tissue and undamaged plants.



significant preference for grazing healed plants when compared with undamaged plants.

5.12.3. DISCUSSION

The significant preference by grazing *Littorina obtusata* for freshly damaged plants suggests considerable ramifications for *Fucus serratus* in terms of the external damage caused by wave action, abrasion against rock surfaces and previous grazing. The grazing preference also emphasises the need for the plants to expend energy in regeneration of damaged tissue. Moss (1964) and Fulcher and McCully (1969b,1971), have studied wound healing and regenerative process in *Fucus vesiculosus* where the medullary cells adjacent to the damage will produce lateral filaments which branch and protect the wound surface. The cortical cells undergo longitudinal division and the outer cells assume the cytological and functional characteristics of epidermal cells.

The fact that healed plants are still grazed significantly more than undamaged plants is probably due to the irregularities in the surface contour of the thallus following healing, allowing snails easier access. The healed tissue does not seem to be any thinner than undamaged tissue suggesting that it is the raised edges of the healed tissue which allow the snails access to the thallus. It is possible however, that the level of regeneration would be sufficient to reduce

grazing in the field where the snails will have greater access to pilose plants and freshly damaged algae.

5.13. CHOICE EXPERIMENTS

5.13.1. INTRODUCTION

Having determined that the presence of hairs on *Fucus* spp. plays a major part in the foraging behaviour of *L. obtusata*, the effects of the hairs on choice of fucoid, in terms of attractiveness and edibility, was examined.

Nicotri(1980) has pointed out that two distinct components of food preference are commonly recognised; 1) Selection of the prey (Attractiveness) and 2) The rate at which the prey is ingested (Edibility). In addition, Bakker (1959) stressed the importance of giving equivalent choices to the animal in any preference experiment. Two separate experiments were, therefore, devised in which pairs of fucoids were offered to *Littorina obtusata* to test the attractiveness and edibility of each to the snail.

Mann (1972) inferred that there is a seasonal difference in the calorific value of *Laminaria longicruris, L. digitata* and *Agarum cribrosus*. Himmelman and Carefoot (1975) confirmed seasonal calorific

changes in the red alga *Iridaea cordata* (Turner) Bory, and the brown algae *Hedophyllum sessile* (C. Agardh) Setchell and *Lessoniopsis littoralis* (Farlow and Setchell) Reinke. Although no similar studies have been undertaken for the fucoids, the possibility of seasonal differences was considered and the attraction and edibility experiments were, therefore, carried out over a short period in July and August.

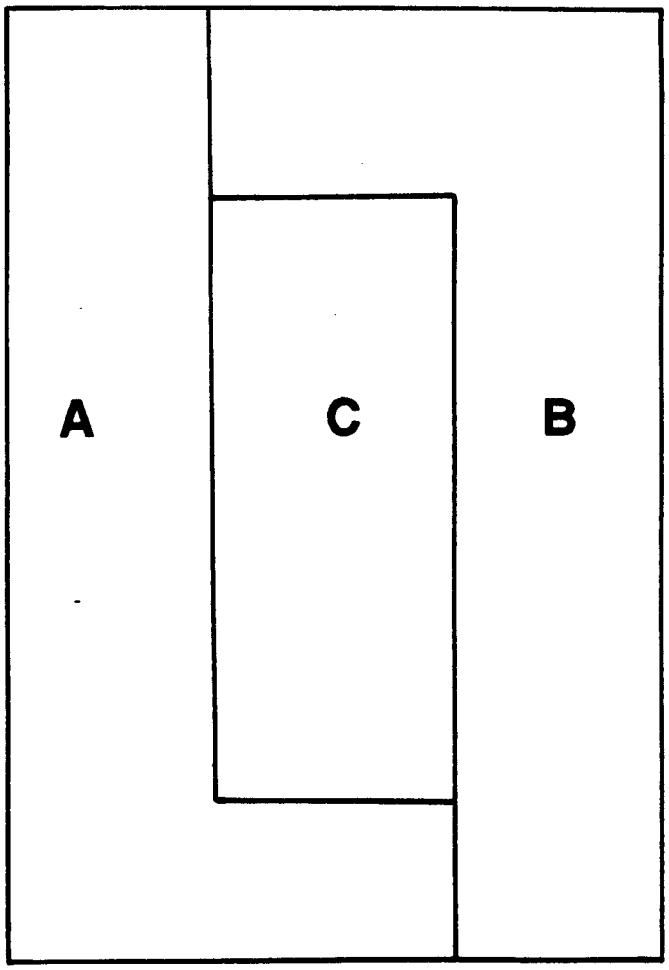
5.14. ATTRACTION

5.14.1 METHODS

The method used to test the attractiveness of the various algae for *L obtusata* was largely based upon that used by Lubchenco (1978), and Watson and Norton (1985a). A 10 litre glass aquarium was covered in black polythene to reduce the external stimuli to the snails which would be placed in it. The importance of this has been pointed out by Watson and Norton (1985a) since the crawling direction of littorinids is affected by light source. The base of the tank was separated into three distinct areas marked A, B, and C (Fig. 5.4.).

The algae were compared in pairs by placing each of the two species being tested in either zone A or B and 20 adult *L. obtusata*, which

Figure 5.4: Schematic diagram of the base of the tank used in the attraction experiments. The snails were placed in the central zone (C), and the test algae in zones A or B.



had been starved for five days, into the central zone C. The snails were starved as the alga acts as both habitat and food source and it was attraction in terms of diet, and not shelter, which was being studied. Sufficient filtered seawater to cover the snails and algae was then added to the tank. The snails were placed in such a way that they were alternately pointing towards one or the other species of algae. This was done to avoid the initial orientation of the snails affecting the ultimate choice of seaweed. Once the snails had been placed in the central area, a black polythene lid was placed upon the tank and it was left for one hour. At the end of this time, the lid was raised and any snails found on the walls of the tank were returned to the central area, the lid replaced and the tank left for a further two hours. Pilot experiments had shown that the movement of the snails settled down after this length of time. The position of each snail in terms of the zone it was found in was noted, the tank cleaned out and fresh algae and snails used in 10 replicates per pair of algae. The importance of cleaning the tank after each experiment must be stressed since it is absolutely essential to reduce any possibility of trail following in the snails.

Trail following has been well documented in many gastropod species, with its suggested purpose being to find a mate (Wareing, 1986), homing behaviour (i.e. McFarlane, 1980) or locating prey (Cook and Cook, 1975). Watson and Norton (1985a) have commented that mucus trail following may have biased previous results for the attractiveness of macroalgae to gastropods. For this reason the

bottom of the experimental tanks were thoroughly scrubbed and rinsed in filtered seawater after every trial.

A count was also made of the number of hyaline hairs on each algal species. As the plants differed considerably in size, it was decided to count the number of hairs in the top 4cm from the tip of each thallus branch on ten plants chosen at random.

The results of the attractiveness experiments are detailed in Table 5.6. It may be seen from the table, that the most preferred fucoid for *L. obtusata*, is *Fucus spiralis* followed by *F. serratus*, *F. vesiculosus* and *Ascophyllum nodosum*. Table 5.7. indicates that a similar ranking occurs in the mean number of hairs found on the apical sections of the *Fucus* spp..

5.14.3. DISCUSSION

Although a very similar methodology was used in this study and that of Watson and Norton (1985a,1987), there are distinct differences in the results obtained. These could have been caused by the manner in which the snails were placed in the central area of the experimental tank. In this study, I deliberately polarised the starting direction of the snails so that initial orientation had as little bearing as possible on the preference shown. The placement of the snails by Watson and Norton (1987) was random in that all snails were simply

Table 5.6: Attractiveness of fucoid algae to *Littorina obtusata*.

Preference Ranking. (Chi-square analysis)	Alga.	% of snails choosing each alga in all comparisons.
I	*F. spiralis.*	33·3
II	*F. serratus.*	28·3
III	*F. vesiculosus.*	24·2
IV	*Ascophyllum.*	14·2

Table 5.7: The mean number of hairs (cm^{-2}) found on the apical 4 cm of ten randomly collected apical sections of *Fucus spiralis*, *Fucus vesiculosus* and *Fucus serratus* (with standard deviations).

Fucus spiralis.		*Fucus vesiculosus*		*Fucus serratus*	
mean	s.d.	mean	s.d.	mean	s.d.
56·12	5·17	9·21	2·75	17·12	7·10

put into the central area (Watson, pers. comm.). Whether this is sufficient to cause the difference in results is debatable but Table 5.8., gives an indication of the way methodology differences have given quite contradictory results in five separate studies of *L. obtusata* attraction for the fucoids.

It is clear from Table 5.8, that there are quite considerable differences in the results obtained from the different attraction experiments. Whilst this in itself does not infer an intrinsic fault in attraction experiments *per se*, it does suggest that the methodology for future studies should be standardised. Further discussion of the Attractiveness results is continued in combination with those of the Edibility results.

5.15. EDIBILITY

5.15.1. METHOD

Sixty undamaged sections of each of *Fucus serratus, F. spiralis, F. vesiculosus* and *Ascophyllum nodosum* were carefully cut and weighed such that there were six batches of ten plants for each species. The plants were sorted by weight to allow a similar biomass of each species to be compared. The plants were then placed in aquaria so that ten from one species were placed in a tank with ten from another species until each combination of two species were together i.e.:

Table 5.8: Comparison of the results of five attraction experiments using *Littorina obtusata*. (Rank 1 = most preferred).

	This Study	Watson & Norton (1987)	Bakker (1959)	Van Dongen (1956)	Barkman (1955)
F. spiralis.	1	2	2	2	3
F. serratus.	2	4	4	3	1
F. vesiculosus.	3	1	1	1	4
Ascophyllum.	4	3	3	4	2

Fucus serratus v F. vesiculosus

F. serratus v F. spiralis

F. serratus v Ascophyllum nodosum

F. spiralis v F. vesiculosus

A. nodosum v F. spiralis

A. nodosum v F. vesiculosus

This combination of species was then duplicated to give a control tank, containing two species of algae, for each experimental tank. Each control tank contained the same fresh weight of algae as each species in the corresponding experimental tank.

Twenty *Littorina obtusata*, which had been starved for five days, were then placed in each experimental tank and allowed to graze for 5 days. At the end of this time the number of plants of each species which had been grazed was noted and the plants were blotted and reweighed. The difference between the experimental plants before and after grazing, adjusted for any weight gain or loss in the controls, was considered to be the total amount grazed.

5.12.2. RESULTS

It can be seen from Table 5.9., that there exists a general order of edibility in the fucoids with *F. spiralis* being the most edible, followed by *F. serratus, F. vesiculosus* and *Ascophyllum*. This ranking also applies to the total number of plants grazed in all comparisons:

Table 5.9: The amount of algal tissue consumed per snail (mg.day^{-1}, means and standard deviations) by *Littorina obtusata* in choice experiments. Preferred species in each comparison is in bold. (Wilcoxon signed rank test, n/s = not significant, * = 0·01<P<0·05, ** = 0·001<P<0·01).

Species A.	Comparisons				Species B.
	Plants Grazed	Amount (mg. day^{-1})	Plants Grazed	Amount (mg. day^{-1})	
F. vesiculosus	14	1·49	2	0·03	*Ascophyllum* **
		1·43		0·41	
F. vesiculosus	7	0·84	18	1·53	***F. serratus*** *
		1·29		1·13	
F. vesiculosus	10	1·10	18	2·53	***F. spiralis*** *
		1·54		1·26	
F. serratus	17	1·81	9	0·08	*Ascophyllum* **
		1·29		1·13	
F. serratus	13	1·21	20	2·17	***F. spiralis*** n/s
		0·71		0·98	
F. spiralis	20	3·21	5	0·07	*Ascophyllum* **
		1·14		0·19	

Fucus spiralis 58, *F. serratus* 48, *F. vesiculosus* 31 and *Ascophyllum* 16.

5.15.3. DISCUSSION

The amount of algal tissue consumed by *L. obtusata* differs considerably between the fucoids examined. The high standard deviations recorded for the edibility of the plants is indicative of the high degree of variation seen in the grazing. This variation is to be expected since some plants were not grazed at all, whilst others lost as much as 50 percent of their thallus area to cellular grazing. Such a result is in line with the earlier suggestion that a freshly damaged plant is more susceptible to grazing, since on *L. obtusata* indulging in cellular grazing would leave the plant surface open to further grazing by others following.

What is also interesting is the fact that the edibility results give the same preference ranking as the results for attraction. This is not always the case in such experiments due to the differing motivating factors which attract the snails to the algae. The fact that the fucoids act as both food source and habitat for *L. obtusata* can lead to differences in preferences depending upon the immediate requirements of the snail. The period of starvation could therefore have led to dietary need becoming the overriding factor in the attractiveness choices of the snails.

In a direct comparison of edibility *Fucus spiralis* is clearly preferred to the other fucoids, with *Ascophyllum nodosum* being the least edible for *L. obtusata*.

It should be noted, particularly in the light of this result, that *Ascophyllum* possesses neither hyaline hairs on its surface nor cryptostomata, which have been shown, in this study, to be the access route for cellular grazing by *L. obtusata*. Furthermore, the relative number of hairs for each species given in Table 5.7, gives the same ranking as the edibility and attractiveness experiments. The number of hairs, and therefore cryptostomata, is clearly correlated with the grazing preference of *L. obtusata*. Results also suggest that studies on the relative "toughness" of the fucoids as a barrier to grazing (i.e. Watson and Norton, 1985b) could be inconsequential since the littorinids may not be directly affected by toughness if there is an indirect route into the thallus via the cryptostomata or other damaged areas.

5.16. GENERAL DISCUSSION

Using games theory models, Maynard-Smith (1978) showed how the adoption of a feeding strategy by a predator population must be such that no mutant individual could adopt a strategy which would do better than typical members of that population. This he termed an Evolutionary Stable Strategy. As a working assumption therefore, it

may be considered that natural selection will have ensured the survival in the greatest numbers, of those genotypes which are able to exploit the food resources of their environment most efficiently. This is also the basis of the Optimal Foraging Theory and suggests that optimality will inevitably lead to an Evolutionary Stable Strategy.

It follows that a predator population which would be able to predate its prey at a level where a maximum sustainable yield could be established, would be at a distinct advantage. Maximising the crop taken whilst ensuring that the prey does not become extinct is not proposed as a conscious strategy on the part of *Littorina obtusata* but its evolution would hold considerable advantages. In terms of food availability therefore, this strategy would help to stabilise the population of *L. obtusata* close to the maximum sustainable by the environment. It would also ensure that the fucoid population would be able to support predation at a level where its reproductive potential would not be jeopardised to the point of extinction. This idea suggests the existence of a co-evolutionary strategy occurring and *L. obtusata* interacting with the *Fucus* to develop the Evolutionary Stable Strategy which is now being exhibited. The suggestion of co-evolution is dependent upon the littorinid being perceived as the major grazer of *Fucus*, which appears from strength of numbers to be a reasonable assumption. Krebs and Davies (1981) describe co-evolutionary stability in predator-prey systems as being due to

an "Arms Race" with the successful prey always being one step ahead in the race.

What we may be witnessing therefore is an evolved grazing strategy whereby the main predator of the *Fucus* spp. is reducing the growth rate, and ultimately the fecundity, of the algae but not threatening its long term existence. Following their results from canopy removal experiments, Hawkins and Harkin (1985) concluded that interactions between macrophytes is much more important than grazing in structuring algal communities. However, the hairs are of obvious importance to the growth of the algae and therefore its efficiency in terms of inter and intraspecific competition. Hair removal by the snails will inevitably have a considerable effect but intuitively would not seem to have the same consequences as, for example, a grazed apical cell. A larger comparative amount of algal tissue will, however, ultimately be removed from the plant in hair grazing.

The preference for hairs, by grazing littorinids, could be considered, potentially, as a long-term advantage to the algae since they are readily replaced, whereas extensive damage to the thallus is much more serious. The degree to which hair grazing prevents cellular grazing, is dependant upon the density of snails in a given area and it is certainly snail numbers which will determine the extent and type of any grazing damage; as observed in the laboratory experiment.

Personal observations on the grazing behaviour of *L. obtusata* indicate that it only actively grazes in the Spring, Summer and early Autumn. During the Winter, it appears to migrate to the rock surfaces and become fairly sedentary. Watson (1983), has also noted this behaviour which could possibly explain why the snails grazing undamaged plants take so long (12-48 hours) to switch from hair grazing to cellular grazing in a closed laboratory system. During the peak grazing period for *L. obtusata*, the hairs are ubiquitous on plants at all levels of the shore. As hairs are replaced following grazing, there is apparently little prospect of the snails removing all of the hairs on all, or even most, of the fucoids. It is far more likely that the snails will graze patches of hairs and move on when this resource becomes depleted. A bonus for the snails occurs when, during their foraging for hairs, they encounter a fucoid with a damaged thallus. From the results shown in Figure 5.3., it appears that this changes the attractiveness of the *Fucus* for the snail which will then switch from Hair grazing to Cellular grazing.

From the electron micrographs (Plates 5.13 to 5.16), grazing of epiphytic microflora from the surface of the fucoids by *L. obtusata* does not seem to occur to any great extent, despite the suggestions by Lubchenco (1982) and Reimchen (1974). Indeed, Lubchenco's view, disputed by Watson and Norton (1985a), that *L. obtusata* is a specialised grazer of microepiphytes was not supported at all by results obtained in this study.

It would appear therefore, that a radical change is needed in the way *Littorina obtusata* grazing is currently referred to in the literature. The commonly held view that the visible grazing damage seen on fucoids has the greatest effect upon their growth rate is surely questionable in the light of the results obtained here. As Lobban et al (1985) point out, the effect of grazing an algae depends upon the amount and kind of tissue lost and on the timing, particularly with respect to reproduction. This statement whilst referring to cellular grazing and not hair grazing is still valid. Further work is necessary on the effects hair grazing has upon the long term growth rates and reproductive potential of the algae but the suggestion from this study indicates that it could be much more profound than has previously been realised.

CHAPTER SIX - FURTHER EFFECTS OF HYALINE HAIRS ON THE ECOLOGY OF *FUCUS*

6.1 FUCUS SERRATUS AND THE CLOSING OF CRYPTOSTOMATA

6.1.1 INTRODUCTION

During the formation of hairs by *Fucus* in the field during spring and, also in later nutrient deficiency experiments, a difference was noted in the way hairs are produced by the three species under investigation. *Fucus spiralis* will produce hairs along the full length of the thallus, *F. vesiculosus* will also grow hairs from an area immediately below the apical tip and down beyond the mid-thallus level. In contrast, *Fucus serratus*, will only produce hairs on the upper few centimetres of the apical branches. Closer examination of the three species revealed a difference in the cryptostomata of *F. serratus*, which may have caused the observed differences. The cryptostomata of *F. spiralis* and *F. vesiculosus* remain open over the full length of the thallus. *F. serratus* on the other hand, has open cryptostomata only on the upper portions of the thallus. It was decided therefore to examine the cryptostomata in greater detail by the use of electron microscopy.

6.1.2 METHODS

Sections of the thallus of *F. serratus* and *F. vesiculosus* were cut every 1/2 cm. beginning from the apical tip and continuing to mid-thallus level, where, on *F. serratus*, the cryptostomata could be seen to be visibly closed. They were then treated in the manner outlined in Chapter Two, and taken to the electron microscope in the main campus at Liverpool. Histological sections of *Fucus serratus* were made of cells which could be seen to be closing. The method used has been previously outlined in Chapter Two.

6.1.3 RESULTS

It can be seen clearly from the electron micrographs, Plates 6.1 to 6.6, and the histological section, Plates 6.7 and 6.8, that the cryptostomata of *F. serratus* are progressively closing, with the cells of the meristoderm extending across the opening until they are completely sealed. Hairs will occasionally be seen to to protrude from the cryptostomata whilst the closing is in progress (Plates 6.2, 6.9 and 6.10) but this does not appear to affect the process.

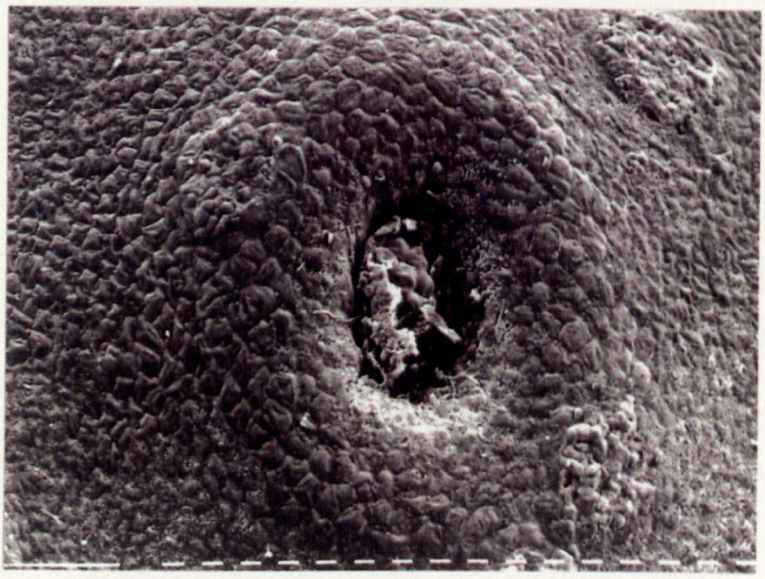
6.1.4 DISCUSSION

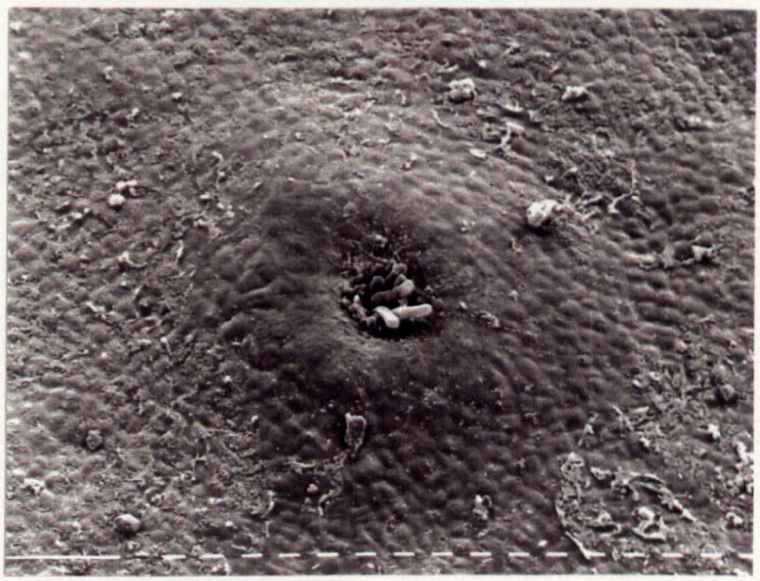
Norton (1969) found that the splitting of the lamina of *Saccorhiza*

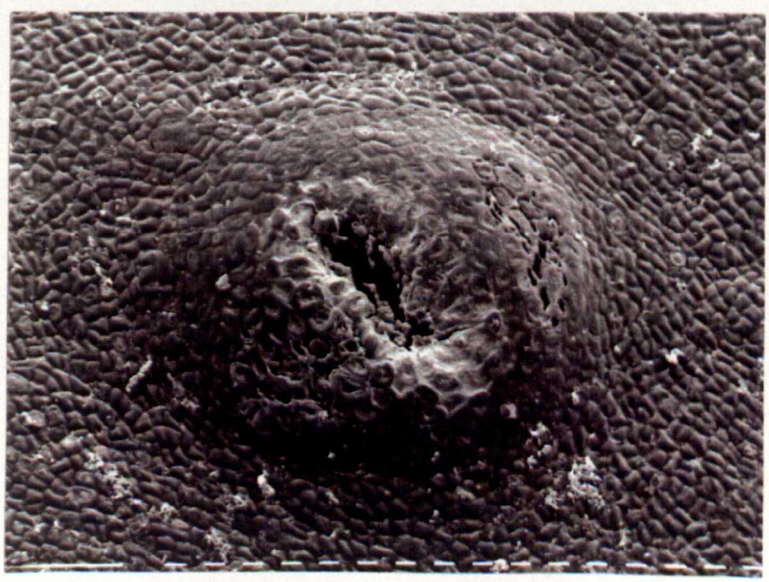
Plate 6.1: Open cryptostomata (scale bars=10um).

Plate 6.2: Closing cryptostomata with hairs protruding (10um).

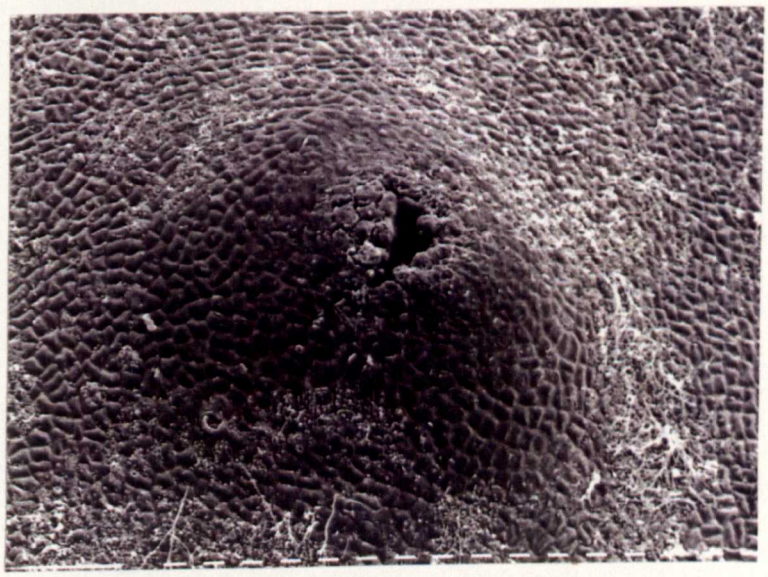
Plate 6.3: Closing cryptostomata with hairs visible internally (10um).

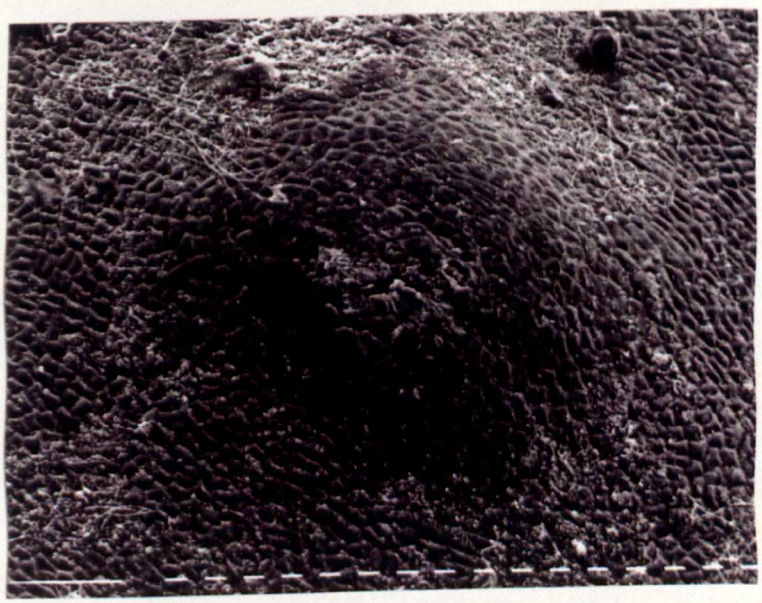


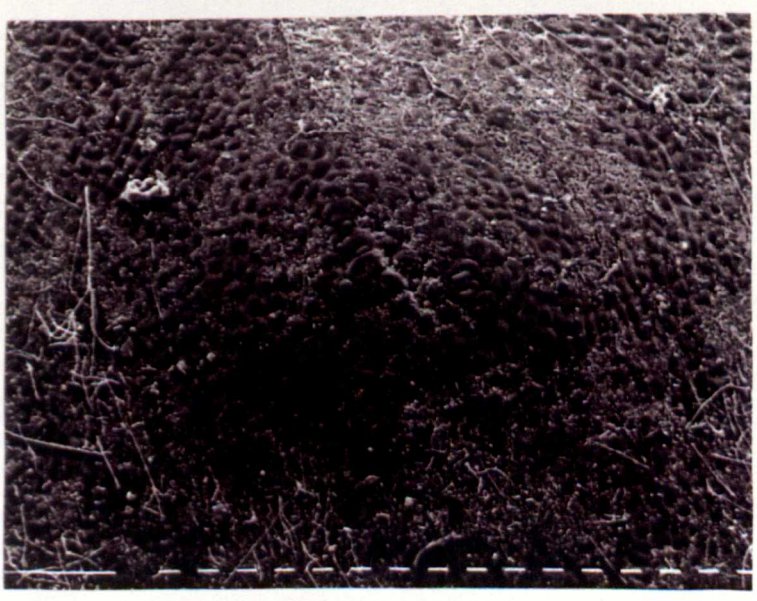




Plates 6.4 to 6.6: Progressive sealing of the cryptostomata in *Fucus serratus*.







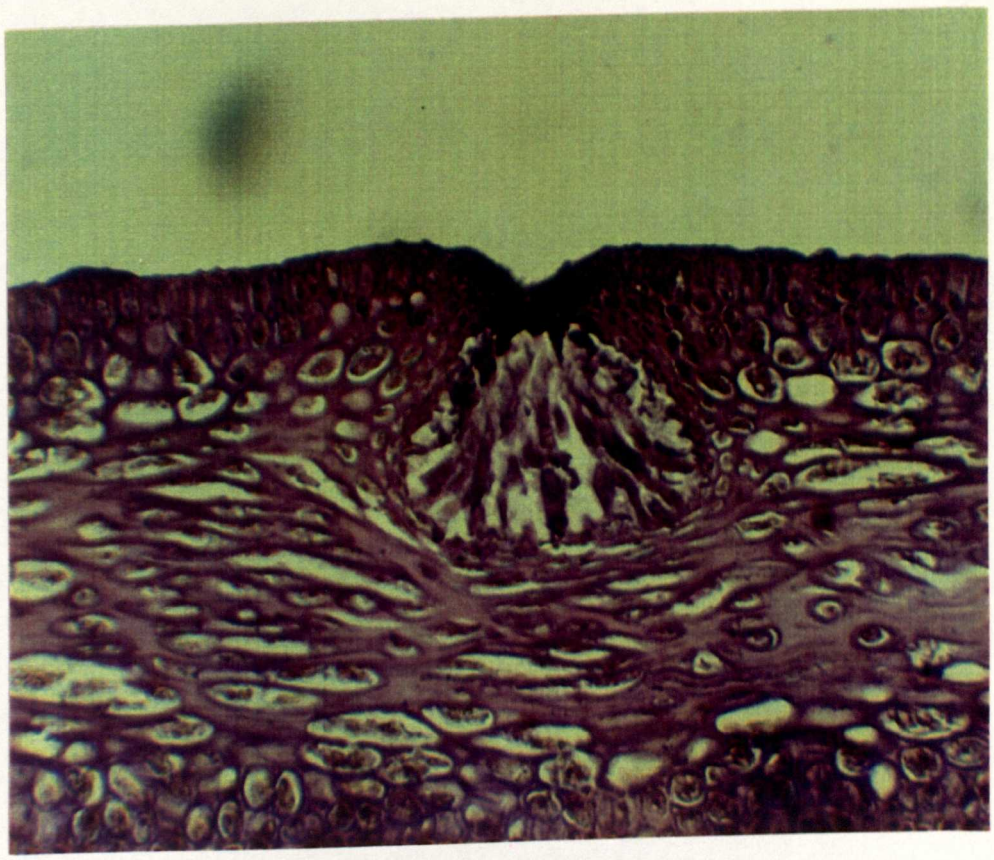


Plate 6.7: Closing cryptostoma (x200).

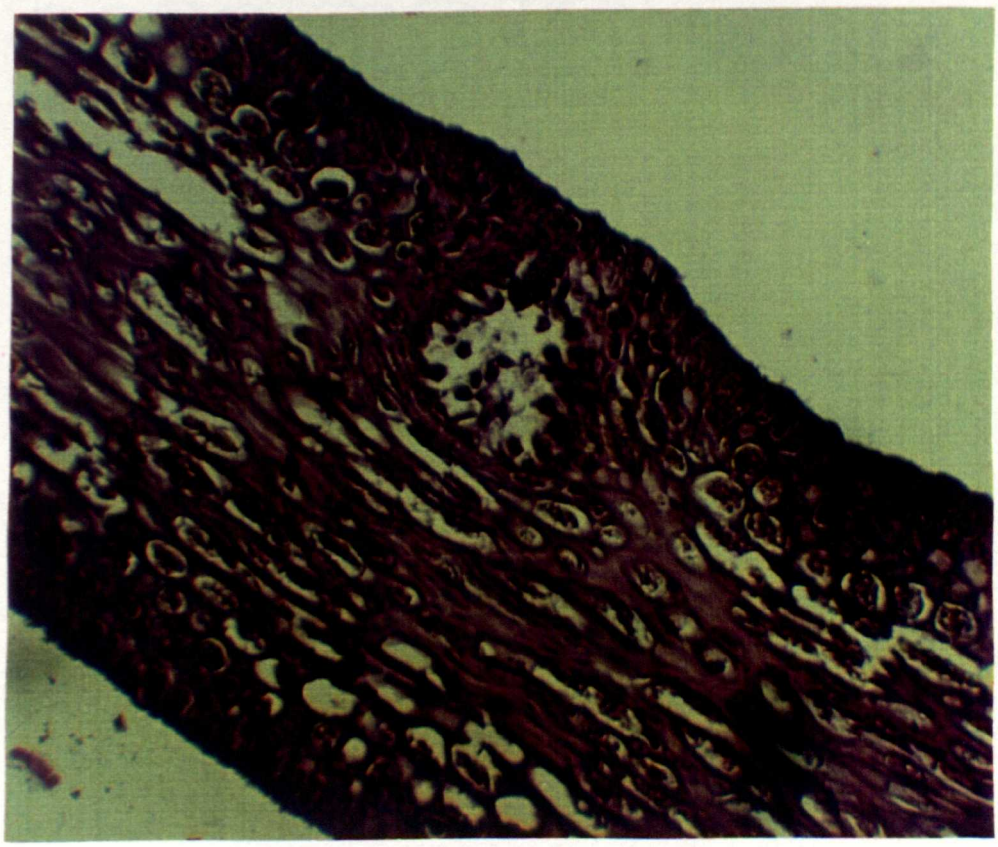
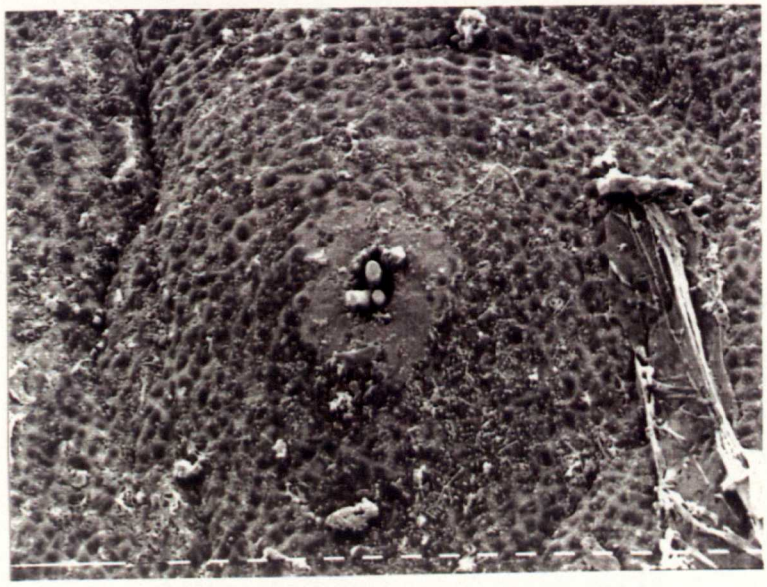
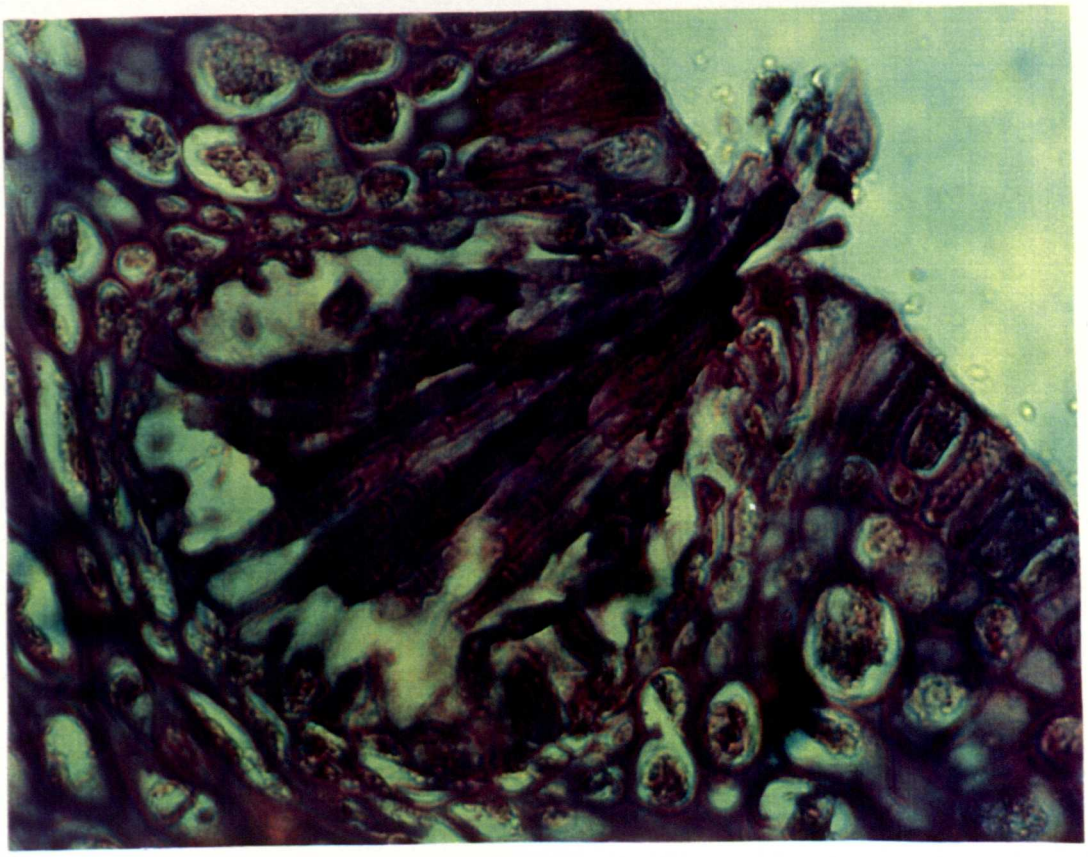


Plate 6.8: Sealed cryptostoma (x200).





Plates 6.9 and 6.10: Protruding hairs being constricted by the closing cryptostomata.

polyschides was preceded by the formation of grooves in the blade. The grooves where the result of disintegration of cortical cells and the activity of the overlying meristoderm, causing invaginations in the surface. An important point to note is that here the activity of the meristoderm is not a wound healing response to damage but is an initiated growth response to an environmental stimulus, namely wave action. Moss (1974), has pointed out that the controlling factors for the splitting and subsequent rapid wound healing in *Saccorhiza*, are unknown.

In *Fucus serratus*, there appears to be division and growth of the superficial meristoderm until the cryptostomata is completely closed. The sealing of cryptostomata in *F. serratus* is obviously an active process with the plant expending resources to ensure that these breaches in its integumentary layer are sealed. The bulk of cell expansion, and therefore growth, occurs behind branch tips and the translocation costs for nutrients absorbed in areas well removed from the areas of high metabolic activity would be increased. As the cryptostomata are also a possible source of ingress for infections and grazers, they are sealed. One obvious reason for expending energy on the closure of the cryptostomata would be to alleviate the cellular grazing damage by littorinids highlighted in Chapter Five. Although this would appear to be a plausible strategy for the plant, it does not explain why a similar morphological alteration of the thallus does not occur in *F. vesiculosus*.

In addition, the timing of the cryptostomata closing, in autumn, occurs after the peak of grazing pressure in the summer has passed (Watson, 1983). The distribution of *Littorina obtusata* on sheltered and semi-sheltered shores will often show a peak in the *Ascophyllum/F.vesiculosus* zone (e.g. Tait, 1981; Watson, 1983). The increased *Littorina* numbers would suggest that the grazing pressure from this snail would be greater on *F. vesiculosus* than *F. serratus*. In very broad evolutionary terms, therefore, it would be expected that *F. vesiculosus* would be reacting to this pressure to a greater extent than *F. serratus* and also closing its cryptostomata to inhibit grazing. Unfortunately, evolutionary arguments of this nature are purely speculative, since any existing ecological situation could be a relatively new phenomenon.

It is recognised that the complexity of the intertidal habitat of these *Fucus* species means that any emphasis upon one environmental parameter (i.e. littorinid grazing), cannot lead to a satisfactory understanding of the ecological processes involved. Therefore, the potential exploitation of the cryptostomata by an algal epiphyte, *Elachista fucicola* Vell. Aresch, was also studied.

6.2 ELACHISTA SETTLEMENT IN THE LABORATORY

6.2.1 INTRODUCTION

Russell and Veltkamp (1984), had revealed that zoospores of *Elachista* will aggregate around the opening of a cryptostoma of *Fucus vesiculosus*. It was suggested by the same authors, that this settlement by-passes the antifouling skin shedding of *F. vesiculosus*. Skin shedding in *Fucus* species has been observed during the current study but only in *Fucus* thalli allowed to remain in static or near static water conditions for more than two weeks. The plants at this time were in very poor condition and skin shedding was not observed on healthy plants during any culture experiments. The settlement work of Russell and Veltkamp (1984), however, was only carried out on the apical sections of *Fucus vesiculosus*. It was decided, therefore, to extend the examination of settlement to both the upper and lower portions of the thallus of *F. vesiculosus* and *F. serratus* so as to cover that region on the latter species where the cryptostomata were seen to be closed. *F. spiralis* was not used as the sparse covering of *Elachista* seen on this fucoid species suggested that the epiphyte would not have the same ecological impact on this species.

Elachista fucicola is an obligate epiphyte found, to a lesser or greater degree, on all *Fucus* spp. on the Isle of Man (Knight and Parke, 1931). The plants consist of a central basal disc with a peripheral cortex of photosynthetic filaments. The filaments consist of long unbranched assimilatory structures and very short branched paraphyses

at the base bearing the reproductive organs. Branches extend from the large inflated cells of the medulla, which is attached to the underlying *Fucus* (Fritsch, 1945; Blackler and Katpitia, 1962; Koeman and Cortel-Breeman, 1976).

6.2.2 METHODS

Fertile thalli of *Elachista fucicola* were obtained from the shore in late November and returned to the laboratory. Following removal from their fucoid host, they were laid out in shallow filtered seawater in a petri dish. Mature unilocular zoidangia were collected following the method of Koeman and Cortel-Breeman (1976), by squashing the fertile plants and picking up the zoids with a micropipette under a kyowa binocular dissecting microscope. When enough of the spores had been collected, 15ml of the zoospore suspension was pipetted across the surface of a large culture vessel containing five clean, apical branches of each of *F. vesiculosus* and *F. serratus*. The branches were no more than 4cm in length to ensure that the area of *F. serratus* where the cryptostomata were closed was not in evidence. The specimens of *F. vesiculosus* had been chosen so that they did not possess vesicles near to the apical tip so that the thalli of both were lying flat.

Larger branches were laid on the bottom of another culture vessel but 4cm of the apical sections were covered with glass sheets. Glass

was also placed at the base of each branch. These sheets only allowed settlement on the mid portion of the thallus and also held down the vesiculated *F. vesiculosus*. In *F. serratus* only closed cryptostomata were available to the zoids of *Elachista* to evaluate if the closed cryptostomata would inhibit settlement. A further 15ml of zoospore suspension was pipetted across the surface of this culture vessel.

Both culture vessels were then held at 10 °C in an 8L:16D light regime under 50μE/m^2/s for 24 hours. The water was then carefully removed from the culture vessels and the plants placed under a dissecting microscope. Zoospores landing within 50μm of the ostioles of the cryptostomata were counted and recorded. The distance of 50μm was chosen as it was considered that zoospores found beyond this limit could not be considered to be attracted to the ostiole, nor would they be sufficiently close for the cryptostomata to provide an anchorage point for the epiphyte rhizoids.

6.2.3 RESULTS

There was a highly significant higher number of *Elachista* zoospores settling around the cryptostomata of *Fucus vesiculosus* for both the apical sections and the mid-thallus region. There was also a highly significant difference in the amount of settlement between the apical and mid-sections of each species. The figure recorded for the mean settlement of *Elachista* on the mid-thallus region of *F. serratus* was

considerably lower than that for any other part of either plant. There was negligible amounts of settlement on the open thallus of both species (<4/sq.cm) but with clumps of zoospores being found around sites of damaged epithelium.

6.2.4 DISCUSSION

The closing of the cryptostomata on *Fucus serratus* is making a significant impact on the settlement of *Elachista* in the laboratory. A small number of zoospores still settled around the closed cryptostomata but this was probably due to the irregularity in the surface contour of the thallus which remained after the cryptostomata were completely sealed. A similar aggregation of zoids, although in much lower numbers, was observed in naturally damaged areas of the thallus. The latter point would agree with Russell and Veltkamp (1984), that recognition of cryptostomata by spores would not necessarily be involved in the observed pattern of settlement. It may be the case that all breaches in the epidermis are areas of enhanced leakage of exudates which might be attractive.

The cryptostomata will bear hyaline hairs in the summer and it is considered probable that the relatively large and growing hairs would form an effective barrier to zoospore settlement within and around the cryptostomata. *Elachista* has been shown to produce zoospores in the winter (Knight and Parke, 1931; Koeman and Cortel-Breeman, 1976)

Table 6.1: The settlement of Elachista zoospores around the cryptostomata of apical and mid-thallus sections of Fucus vesiculosus and Fucus serratus (Students T-Test, ***=P<0.001).

	Fucus serratus		Fucus vesiculosus		t	Inference
	mean	s.d.	mean	s.d.		
Apical	14.51	5.40	19.03	5.60	4.67	***
Mid-thallus	1.95	2.15	13.04	5.25	16.15	***
t	18.74		6.12			
Inference	***		***			

Table 6.2: *Elachista* distribution relative to shore height, on three species of Intertidal *Fucus*. Mean number of *Elachista* in five quadrats at each height, with standard deviations (s.d.)

Shore Height (metres)	*Fucus serratus*		*Fucus vesiculosus*		*Fucus spiralis*	
	No.	s.d.	No.	s.d.	No.	s.d.
4..5	-	-	68.0	31.68	5.2	6.76
4.0	-	-	282.6	63.39	-	-
3.4	135.6	76.08	927.6	435.62	-	-
2.9	31.2	30.8	-	-	-	-
2.4	4.4	6.5	-	-	-	-

when the cryptostomata are free of hairs. The sealing of the cryptostomata in *F. serratus* occurs in autumn coinciding with the cessation of hair production and pre-empting the settlement of *Elachista*.

The above factors and the results of the zoospore settlement experiment might suggest that more *Elachista* would occur on *F. vesiculosus* than on *F. serratus*.

6.3 ELACHISTA IN THE FIELD

6.3.1 INTRODUCTION

Due to the differential settlement of *Elachista* in the laboratory, a shore survey was undertaken to establish if zoospore settlement patterns were reflected in the distribution of the adult plants.

6.3.2 METHOD

A series of 50cm quadrats were taken on the semi-sheltered transect of St Michael's Island. In order to fully ascertain the distribution of *Elachista fucicola* on the intertidal fucoids, it was decided not to take quadrat samples at fixed shore heights as this would not

necessarily have produced the required information. Five quadrats were, therefore, taken at three levels within the observed zonation patterns of each of the three *Fucus* spp., namely high, middle and low. Thus quadrats on the high part of the *F. serratus* zone would overlap with the quadrats of the low *F. vesiculosus* zone. It was foreseen that this sampling method would give the relevant information as to the distribution of *Elachista* both within species, and between species at the same shore height. Shore heights were determined using the hydraulic levelling device detailed in Chapter Three.

6.3.3 RESULTS

It can be seen from Table 6.2, that *Elachista fucicola* is fairly ubiquitous throughout the intertidal with the the peak of distribution occurring at the top of the *Fucus serratus* zone and the lower *F. vesiculosus* zone. An interesting point is that where the host plants overlap, *Elachista* is found in significantly higher numbers on *Fucus vesiculosus* than on *F. serratus*

6.3.4 DISCUSSION

The distribution pattern revealed in Table 6.2 will be the result of a number of environmental and biological factors which determine the niche of this particular alga. Seaweeds readily form bands

running parallel to the shoreline. Such zonation is the result of a host of biological and physical constraints upon the upper and lower limits of the algae (e.g. Coleman, 1933; Lewis, 1964; Schonbeck and Norton 1978, 1979a, 1980; Norton, 1985)

Whilst zonation is very noticeable in the fucoids, the distribution of an obligate epiphyte is highly dependent on that of its host species and should therefore display a similar distribution (Blackler and Katpitia, 1962). If, as in the case of *Elachista*, the distribution at a given shore height (3.4 metres in this study) will vary significantly between two host species, then it would suggest that biological, rather than physical, criteria will determine distribution patterns.

The higher frequency of *Elachista* on *F. vesiculosus* would support the settlement differences found for the zoospores in the laboratory. It was also observed in the field that the distribution of *Elachista* on the individual plants of the *Fucus* spp. differs considerably. The *Elachista* on *F. serratus* is rarely found on the lowest part of the thallus whilst it will often occur on *F. vesiculosus* near to the stipe as well as on the rest of the seaweed.

6.4 ELECTRON MICROSCOPE AND HISTOLOGICAL EXAMINATION OF *ELACHISTA*

6.4.1 INTRODUCTION

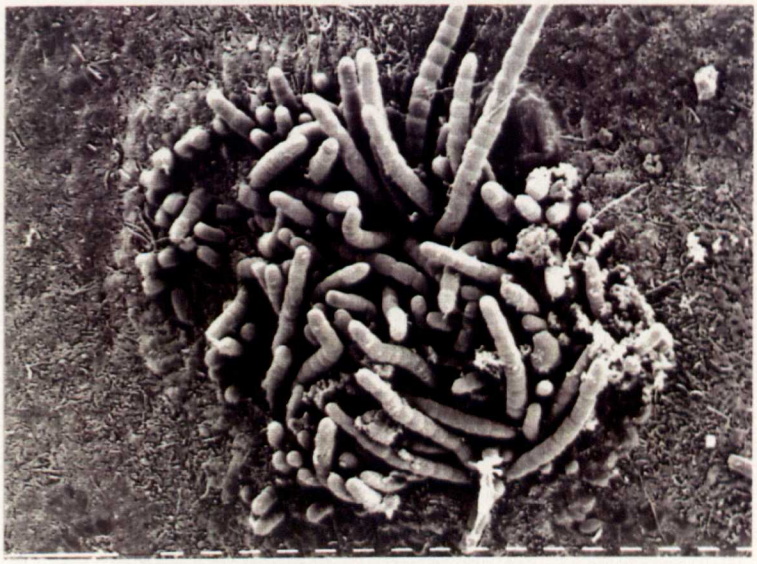
Elachista zoospores are seen to settle around cryptostomata and the adult plants occur in large numbers on the intertidal *Fucus* in the field. Histological sections were therefore taken through the *Fucus/Elachista* interface to evaluate the fixation site of the adult *Elachista* on the thallus of the *Fucus*. Electron microscopical examination of *Elachista* emerging from the *Fucus* surface was also carried out.

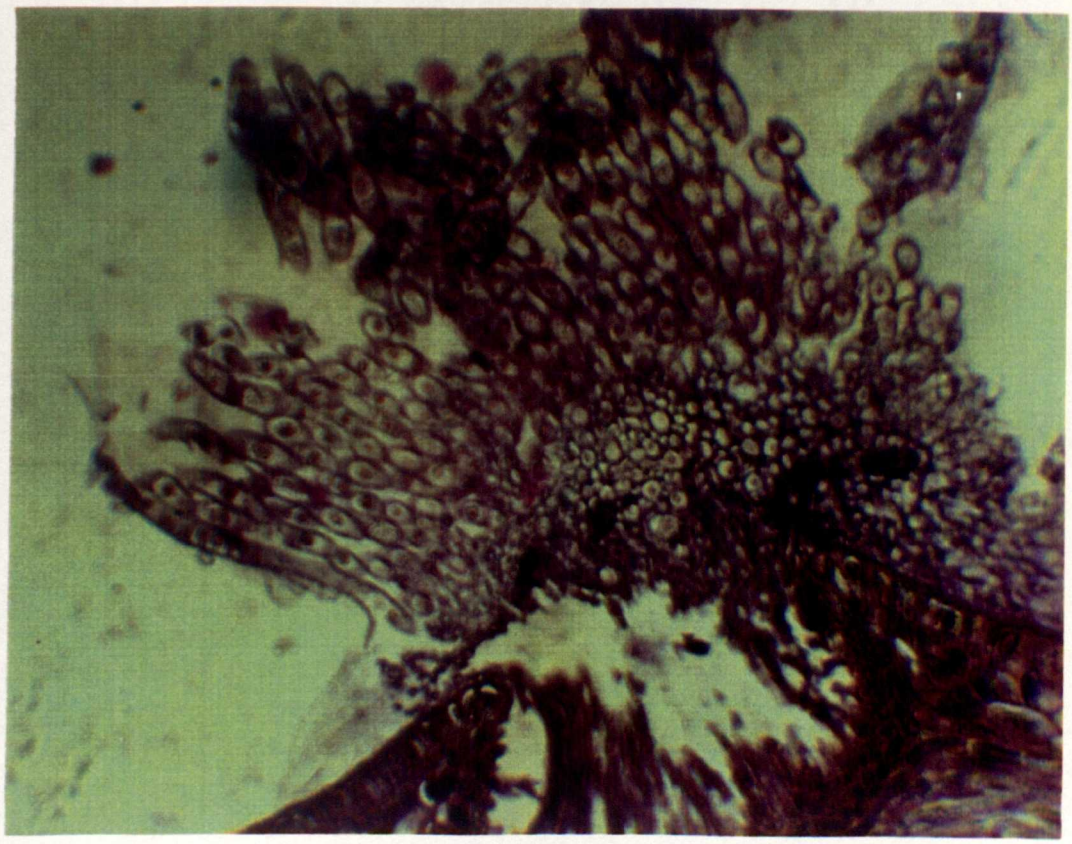
6.4.2 METHOD

Plants of both *Fucus* bearing *Elachista* were prepared for histological sectioning and electron microscopy in the manner detailed in Chapter Two.

6.4.3 RESULTS

Plates 6.11 and 6.12, show *Elachista* emerging from a cryptostoma on *Fucus vesiculosus*





Plates 6.11 and 6.12: Elachista emerging from the cryptostomata (6.11 scale bars=10μm, 6.12 x200).

6.4.4 DISCUSSION

The histological examination of *Elachista* on *Fucus*, confirms that the cryptostomata are used as a site for settlement and growth. Plate 6.12, is indicative of many photos taken of the sections. The microtome sectioning, however, tends to separate the epiphyte from the host species, which leads to the impression that the *Elachista* does in fact use the interior of the cryptostomata as a site for growth. *Elachista sculata*, commonly found on the reproductive straps of *Himenthalia*, are known to germinate in the conceptacles, from which the cushions of the adult epiphyte later project (Fritsch, 1945). *Elachista fucicola* may be seen, in Plate 6.11, emerging from a cryptostoma on the thallus of *F. vesiculosus*.

6.5 ELACHISTA AS A GRAZING BARRIER

6.5.1 INTRODUCTION

Elachista infestation of *Fucus* is often seen in the host above the *Elachista* fouled area of the thallus. Observations in the field had led to the belief that *Elachista* is not grazed by littorinids and as the younger areas appeared to undergo less grazing, the possibility existed that the snails were being physically deterred from traversing the *Elachista*.

It has been observed that *Littorina* are certainly capable of crawling over *Elachista*. If, however, the travelling time for crossing from one patch of hairs to another is raised by *Elachista* infestaiton of the thallus, then theoretical considerations of optimal foraging would suggest that behavioural changes may occur. Such changes could result in the snail avoiding crossing the *Elachista* to reach the apical section of the *Fucus*. It was considered that *F. vesiculosus* would derive the most benefit from such avoidance behaviour since its vesicles would help to keep the plant upright when submerged. Any snails climbing from below would therefore have to cross any *Elachista* occurring on the mid-thallus region to reach the 'clean', hair bearing, apical branches. The suggestion being that the *Elachista* may be indirectly protecting the *Fucus* from grazing. To examine this possibility, use was made of the observed negative geotaxis exihibited by littorinids when they are initially placed in a tank in the laboratory.

6.5.2 METHOD

Areas of the mid-thallus region of *Fucus vesiculosus*, which were infested with dense aggregations of *Elachista* were excised from whole plants. These portions of the thallus were then pieced to gether in an interlocking fashion and glued, using Loctite Superglue Xtra, around the inside of a glass aquarium, 5cm from the lip of the tank. When fixed to the tank they presented a continuous barrier to snali movement such that the snails would have to crawl across the *Elachista* to reach the top of the tank. Pieces of *F. vesiculosus*,

without any attached *Elachista*, were also cut from the mid-thallus region and glued to a second glass tank. A third tank was smeared with glue only and a fourth had strips of Vileda sponge glued to the sides to act as an artificial barrier which was a similar height to the *Fucus* barriers but would be traversed without the snails foraging upon it. A fifth tank, without any barrier whatsoever but with a line drawn on the outside of the tank at the same height as the experimental barriers, was used as a control. The glass tanks were then left for 24 hours at room temperature to allow the glue to cure. At the end of this period they were filled with sea-water to above the level of the algae, sponge and glue. The tanks then stood for a further 6 hours to leach potential toxins from both the Vileda sponge and the superglue. The sea-water was then changed five times and the tanks allowed to stand for another 6 hour period.

After the water had been changed a further five times, twenty five *Littorina obtusata* were placed around the bottom of each tank. *Littorina* will travel whilst clinging to the back of other individuals, therefore, only those snails which actually crawled across the barrier themselves were recorded in the results. The number of snails which had crossed each barrier was noted at ten minute intervals.

6.5.3 RESULTS

Figure 6.1 and Table 6.3, reveal that the *Fucus vesiculosus* with *Elachista, F. vesiculosus* without *Elachista* and the artificial Vileda barrier, will all significantly impair the progress of *L. obtusata*. There was no significant reduction in the time taken for the snails to cross the glue relative to the control tank. The artificial Vileda sponge was equally effective, in slowing the progress of the snails, as both the *Fucus vesiculosus* and the *F. vesiculosus* covered with *Elachista*.

6.5.4 DISCUSSION

Placing a barrier in the path of a browsing snails is seen to impede its progress to a significant extent. The results obtained here suggest that the presence of *Elachista*, however, does not significantly hamper the snails any more than crawling over the surface of *Fucus vesiculosus*. It would appear, therefore, that the energy cost of travelling between patches of hairs would be equal with of without the presence of *Elachista* and the snail would not be influenced to the extent where the host *Fucus* would be afforded any extra protection.

6.6 LITTORINA GRAZING AND ELACHISTA

6.6.1 INTRODUCTION

The thick covering of *Elachista* observed on many fucoid plants in the field and the settlement of zoospores around the cryptostomata suggests that the adult *Elachista* may be blocking the normal entry route for the *Littorina* into the thallus of the *Fucus* preventing cellular grazing. It was decided to study this possibility with *Elachista* fouled fucoids in the laboratory.

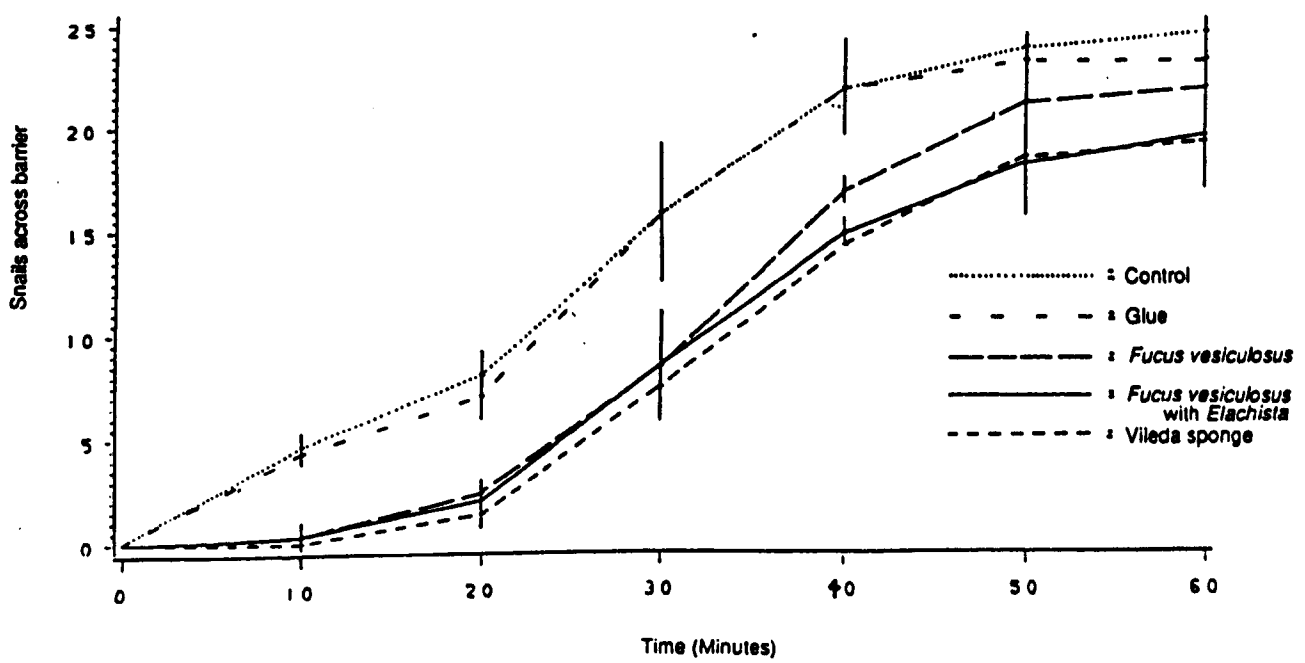
6.6.2 METHODS

Whole, glabrous *F. vesiculosus* and *F. serratus* plants of approximately equal size, and with varying degrees of *Elachista* epihytization, were placed in six large glass aquaria. The degree of infestation was estimatd subjectively and the 'host' algae were arranged such that each tank contained either five *F. vesiculosus* or five *F. serratus* plants with 0, 5, 10, 20 and 30% *Elachista* cover. The tanks were placed in a controlled temperature room at 10° C ± 2° under a 16L:8D light regime and twenty five adult *L. obtusata* were placed in the bottom of each tank. The plants were examined every 24 hours for grazing damage to either the *Fucus* or the *Elachista* and

Table 6.3: Chisquare analysis on the effects of differing barriers to the climbing behaviour of Littorina obtusata (**=0.001<P<0.01, *=0.01<P<0.05, N/S=Not Significant).

	Barriers To Movement				
	Control	Glue	F.vesiculosus with Elachista	F.vesiculosus	Vileda
Control	-	0.17 N/S	13.63 *	20.62 **	16.29 **
Glue		-	13.39 *	19.93 **	15.94 **
F.vesiculosus with Elachista			-	2.27 N/S	0.23 N/S
F.vesiculosus				-	2.02 N/S

Figure 6.1: The time taken for *Littorina obtusata* to cross natural and artificial barriers to movement.



removed from the water for the period of desiccation detailed in Chapter Four. The experiment was carried out for fifteen days after which the algae were removed and an estimate made of total grazing damage. The *Elachista* were then carefully removed with a scalpel and the area of *Fucus* underneath examined for any further evidence of grazing.

6.6.2 RESULTS

The results in Table 6.4, show a significant inverse relationship between the amount of *Elachista* epiphytisation and the degree of grazing damage for both species of *Fucus* (Kruskall-Wallis, $H=12.47$, $0.001<P<0.01$ (*F. serratus*) and $H=11.72$, $0.001<P<0.01$ (*F. vesiculosus*)). When the *Elachista* was scraped from the plants it was found that no grazing had taken place beneath its fronds.

6.6.3 DISCUSSION

The inverse relationship between grazing damage and levels of *Elachista* infestation suggest strongly that the epiphyte is affording some degree of protection to the underlying alga. This is further emphasised by the apparent inability of the littorinids to graze the area beneath the *Elachista* cover. At no time were the littorinids observed to consume the *Elachista*.

Table 6.4: The grazing by *L.obtusata* of *Fucus serratus* and *Fucus vesiculosus* with differing loads of epiphytic *Elachista fucicola* (Percentage thallus area grazed, Mean and Standard Deviation).

Fucus serratus					
	% Epiphytisation				
	0	5	10	20	30
% Grazed	55.0	45.0	30.0	28.3	11.7
Std. Dev.	13.2	5.0	5.0	2.9	5.8
Fucus vesiculosus					
	% Epiphytisation				
	0	5	10	20	30
% Grazed	56.6	35.0	23.3	25.0	8.3
Std. Dev.	12.6	10.0	2.9	5.0	2.9

The effects of epiphytes upon their hosts are wide ranging and are usually considered detrimental to the underlying algae. The growth rates of hosts have been reduced by epiphytes (Brawley and Adey, 1981; D'Antonio, 1985) and increased physical drag, induced by epiphyte loads, is suggested as a cause of decreased survivorship (Lubchenco-Menge, 1975; Knight and Parke, 1931; Friedlander and Lipkin, 1982). A pilot survey carried out in a water flume during the course of this work, also suggests that *Elachista* will increase the drag on *Fucus*.

Epiphytes are, therefore, generally considered to be detrimental to their host algae. The suggestion that *Elachista* may be of benefit to *Fucus*, by reducing cellular grazing, is unusual in that it contradicts the generally negative view of epiphyte-host interactions outlined above. The work of Gutterstam et al (1978), introduced a further factor in the relationship between *Fucus* and *Elachista* in their work on primary production. They found that a heavily *Elachista* infested mid-thallus region on *F. vesiculosus* would almost double the productivity, in terms of dry weight, of that part of the host. The increase in dry weight took place even if the epiphyte did not comprise a major part of the weight. The increase in primary production for *Elachista* coated *F. vesiculosus* was confirmed by Wallentinus (1978), who suggested that the increased meiofauna inhabiting the *Elachista* fronds may be increasing the nutrient supply to the *Fucus*. The grazing protection and the possibility of increased growth rates in the host *Fucus* suggests that further work

on the full ecological implications of its relationships with its epiphytes is necessary.

6.7 GENERAL DISCUSSION

As suggested by Russell and Veltkamp (1984), and confirmed in this study, *Elachisa* settlement on *Fucus* is clearly concentrated upon the cryptostomata. The increased settlement on *Fucus vesiculosus* found in the laboratory was reflected in the field results. The closing of the cryptostomata in *F. serratus* is therefore proposed as a defensive mechanism against *Elachista* infestation.

Defence systems used by algae against epiphytes and epizooites have been proposed by a number of authors. The potential of phenol secretion to deter settlement has been suggested for the Fucales (Craigie and McLachlan, 1964; Ragan and Craigie, 1976) and *Laminaria* (Davis et al, 1973; Al-Ogily and Knight-Jones, 1977). The work of Hornsey and Hide (1974), showed the presence of antibiotic activity in extracts from several species in the Fucales, again suggested as a potential deterrent to settlement by epibionts.

In addition to these chemical defences, physical shedding of the epidermis by *Ascophyllum* has been proposed by Filion-Myklebust and Norton (1981), as a means of controlling epiphytes. Moss(1982), has suggested that it is the outermost layers of the meristoderm cells which are shed and not the whole epidermis. She further states that

skin shedding has been observed in *Ascophyllum, Himenthalia* and *Halidrys siliquosa* (L.) Lyngb, and may be of general occurrence in the Fucales. Plates 6.13 and 6.14 show shedding of meristoderm in *Ascophyllum* and the clean cells underneath. Skin shedding in *Fucus vesiculosus* and *F. serratus* has been observed in this study but only in plants which were physiologically stressed.

If skin shedding is common in the Fucales, as proposed by Moss(1982), then the presence of *Elachista* within the cryptostomata may be a means of avoiding rejection by the 'host' (Russell and Veltkamp, 1984). The closing of the cryptostomata in *F. serratus* might be an adaptation by the fucoid to the settlement of the epiphyte. Whether or not the cryptostoma will close when the epiphyte is already resident within it is unknown but considered unlikely.

If an epiphyte is to be successful in colonising its host, there must be some synchronicity between the growth and reproduction of the two plants (Russell, 1983). It would appear, therefore, that the existence of hairs in summer, potentially sealing the cryptostomata against spore settlement, coupled with the observed skin shedding suggests that successful colonisers of the *Fucus* thallus would produce spores in winter and utilise the cryptostomata, as does *Elachisa*.

It has been proposed that the role of the closing cryptostomata is a defence mechanism against *Elachista* zoospore settlement and also cellular grazing by *Littorina obtusata*. It would be incorrect

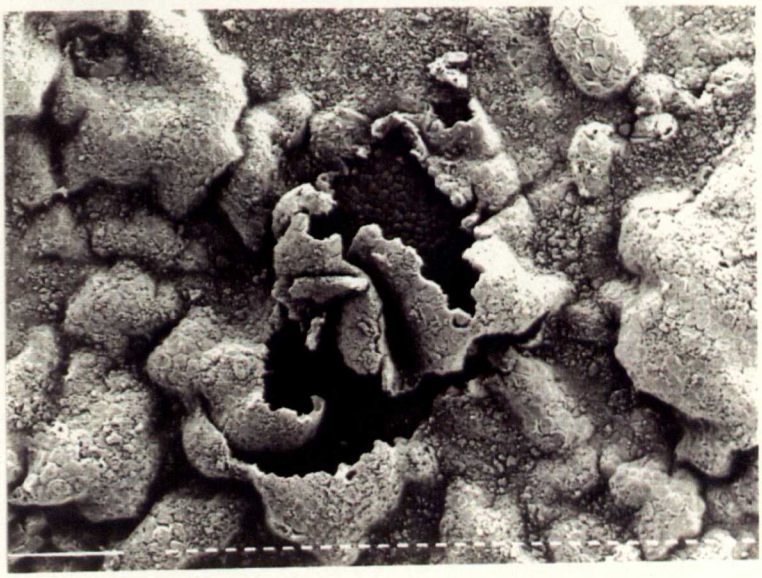


Plate 6.13: Meristoderm shedding by Ascophyllum (scale bars=10μm).

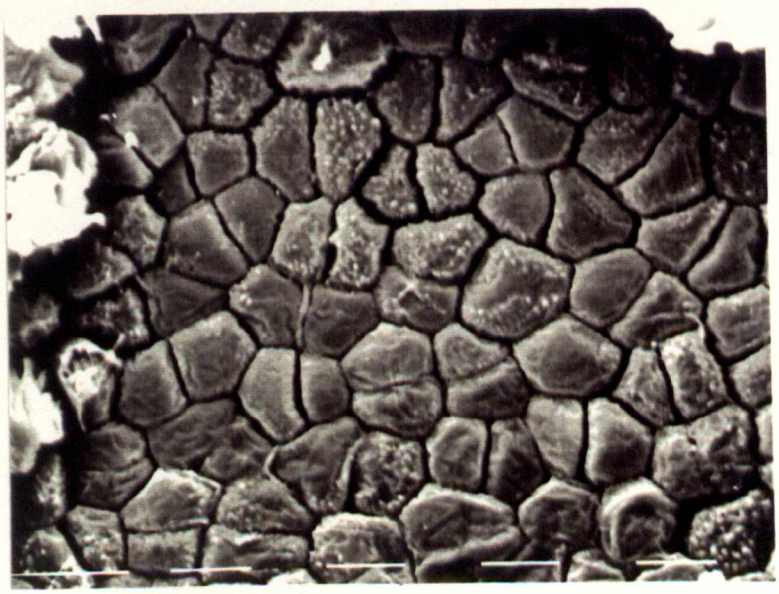


Plate 6.14: Increased magnification showing the clear cells under the shedding skin (10μm).

however, to suggest that these two species are the only ones affected by the sealing of the cryptostomata. Other factors not examined in this work, such as bacterial or fungal infestation, may also be significant and further study, upon the full ecological effects of cryptostomata closing, is recommended.

CHAPTER SEVEN - GENERAL DISCUSSION

The intertidal *Fucus* of St Michael's Island show a clear seasonal cycle of hair production and subsequent attrition. The seasonality of hair formation is clearly linked with nutrient availability and laboratory experiments have suggested that concentrations of phosphate and nitrate are the main factors determining the timing of hair production. Light levels will also alter the amounts of nutrient available to the algae. Lapointe and Tenore (1981), have pointed out that the interactive effects of light and nitrogen on algal growth are common in natural situations. As a general point they stress that nitrogen limitation is not as important under low light as it is during high light conditions. The formation of hairs in spring therefore, could be a direct response to increasing light levels allied to a decrease in the available ambient nutrient concentrations.

The function of the hyaline hairs once they are produced, seems far more complex than the assimilatory function attributed to them by a number of authors (e.g. Sinclair and Whitton, 1977; Whitton and Harding, 1978; DeBoer and Whoriskey, 1983; Wallentinus, 1984). That the hairs do increase the uptake of phosphates and nitrates is clear. What is perhaps of greater interest is the less obvious role hairs play in the ecology of *Fucus*

The preferential grazing of the hairs by *Littorina obtusata* and their subsequent regrowth reveals an interesting "trade-off" between

the plants and their grazers. It is clear that grazing of hairs will cause less immediate damage to the plants than cellular grazing. Damage to the thallus tissue by grazers can cause extensive problems for the plant, potentially causing its destruction (Lobban et al, 1985), but hair grazing may, limit the effects to a temporary depression in growth rates. In areas of higher grazing pressure however, continual removal of hairs by littorinids might reduce long-term growth and ultimately reproductive potential. The long-term effects of hair grazing on individual plants and at the population level should be given greater consideration in the future.

The ability of hairs to prevent settlement of spores within the cryptostomata during the summer months may have a major effect on the epiphytic flora which is able to colonise *Fucus*. The idea that hairs may act in a defence role against epiphytes, is inferred from the settlement of *Elachista* which makes use of the ostiole as a means of ingress when the hairs are not present in the winter. Intuitively, it would seem that colonisation, via the ostiole of the cryptostomata, should be possible for a wide range of epibionts or micro-orgainisms (Rugg and Norton, 1987).

The role of hairs in preventing settlement may also be linked with the closing of the cryptostomata in *Fucus serratus*. The production of hairs ceases in low shore *F. serratus* before it does in *Fucus* plants growing further up the intertidal zone. The time when hairs are 'protecting' the cryptostomata from ingress by spores or other

infective organisms, is therefore shorter for *F. serratus*. The sealing of the cryptostomata could possibly be a response to the evolutionary pressure of increased settlement.

The hyaline hairs found on a variety of algal species are usually formed in nutrient deficient waters or those with little wave action. It would be wrong however, to assume that this will also be the case for other algae. Ginsburg-Ardre (1966), working on *Peyssonelia harveyana* Crouan, found that hyaline hairs are found on this species in sites which are exposed or very exposed. In addition, this alga is found intertidally on very shaded rocks. Both of these conditions, heavy wave action and low light, would reduce the expectation that *Fucus* would bear hairs unless the ambient nutrient levels were low. Ginsburg-Ardre (1966), gives no data on nutrient availability but this example indicates the difficulties in generalising about the conditions in which hairs would be produced by algae.

As this study has shown, the role of hairs is not only a simple assimilatory one. The complexity of the function of hairs in *Fucus*, however, was further emphasised by the observation of Fries (1985). Leaving a unialgal culture of *Fucus vesiculosus* for some months without replenishing the media, she found that hairs shed by the *Fucus* were developing into new individuals.

The role of hairs in the ecology of *Fucus* is believed to be profound and open to a number of further studies. It is felt that this work,

in common with so many biological studies, has not achieved a final conclusion with regard to its intended aims. It is hoped, however, that enough information has been supplied to stimulate further research into the hyaline hairs of *Fucus* and other algal genera.

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Appendix 1: Composition of seawater Medium

Glass distilled water	100 ml
$NaNO_3$	350 mg
Na-glycerophosphate	50 mg
Fe (as EDTA; 1:1M)*	250 mg
P II Trace metals **	25 ml
Vitamin B	10 g
Thiamine	0.5 mg
Biotin	5 µg
Tris Buffer	500 mg

Adjust pH to 7.8

add 2 ml of the above to 100 ml of filtered seawater.

***Fe as EDTA 1:1 M** = Dissolve 351mg Fe $(NH4)_2$ $(SO_4)_2$. $6H_2O$ and 300 mg Na-EDTA in 500 ml H_2O. 1 ml of this solution is equal to 0.1 mg Fe.

****P II Trace Metals.**
Add the following to 100 ml of H_2O.

H_3Bo_3	(114 mg)
$FeCl_3$. $6H_2O$	(4.9 mg)
$MnSO_4$. $4H_2O$	(16.4 mg)
$ZnSO_4$. $7H_2O$	(2.2 mg)
$CoSO_4$. $7H_2O$	(0.48 mg)
Na-EDTA	(100 mg)