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Seaweed Palatability and Selective Grazing by Littoral
Gastropods

by

David Cunningham Watson, B.Sc.

A thesis submitted for the degree of Ph.D.

University of Glasgow
Department of Botany
December 1983

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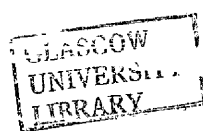
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FRONTISPIECE - Common winkles feeding on the sea-lettuce, *Ulva lactuca*.

To my parents

CONTENTS

	<u>Page</u>
Acknowledgements	I
Summary	II
<u>Chapter 1 : General Introduction</u>	1
<u>Chapter 2 : An Introduction to the Herbivores</u>	4
2.1 General biology, ecology and taxonomy.	5
2.1.1 The archaeogastropod limpets.	6
2.1.2 The mesogastropod littorinids.	10
2.1.3 The prosobranch alimentary system.	16
2.2 Intertidal zonation	19
2.2.1 Methods.	20
2.2.2 Results.	20
2.2.3 Discussion.	22
2.3 Microdistribution of littorinids.	25
2.3.1 Methods.	25
2.3.2 Results.	26
2.3.3 Discussion.	27
<u>Chapter 3 : Behavioural Studies</u>	28
3.1 Methods.	31
3.1.1 Laboratory studies.	31
3.1.2 Field studies.	33
3.2 Results.	34
3.2.1 Laboratory studies.	34
3.2.2 Field studies.	35
3.3 Discussion.	36

	<u>Page</u>
<u>Chapter 4 : Feeding</u>	39
4.1 Methods.	40
4.2 Structure of the feeding apparatus.	42
4.2.1 Radular structure.	43
4.2.2 Denticular hardness.	46
4.3 The feeding cycle.	47
4.3.1 <i>Patella vulgata</i> .	49
4.3.2 <i>Littorina littorea</i> .	50
4.4 Effects of grazing.	52
4.4.1 Analysis of grazing marks.	53
4.4.2 Grazing damage.	57
4.5 Discussion.	60
<u>Chapter 5 : Food Preferences</u>	66
5.1 Macroalgal preferences (laboratory).	68
5.1.1 Edibility.	69
5.1.2 Edibility of fucoid reproductive tissue.	78
5.1.3 Attractiveness.	80
5.1.4 Effect of starvation on preference.	84
5.1.5 Effect of habituation on preference.	87
5.2 Preference studies with algal germlings.	88
5.2.1 Germling edibility.	89
5.2.2 Direct comparison of adult and juvenile edibility.	95
5.2.3 Dislodgement of germlings during foraging.	97
5.3 Selective grazing in the field.	98
5.3.1 Microdistribution of common and flat winkles on the shore.	99
5.3.2 Microdistribution of high-shore common winkles at Portencross, Firth of Clyde.	102
5.3.3 Grazing of mature fucoids by the common winkle.	103
5.4 Discussion.	105

	<u>Page</u>
<u>Chapter 6 : Structural and Chemical Plant Defences</u>	113
6.1 Influence of thallus form on the edibility of mature <i>Enteromorpha</i> .	114
6.2 Thallus toughness.	116
6.2.1 Puncture strength.	117
6.2.2 Resistance to abrasion.	119
6.3 Digestion of algal cell walls.	122
6.3.1 Digestion of total cell wall polysaccharides by <i>Littorina littorea</i> .	123
6.3.2 Digestion of cellulose by <i>Littorina littorea</i> and <i>Littorina obtusata</i> .	124
6.3.3 Cellulase activity in <i>Littorina littorea</i> .	127
6.4 Chemical defences.	128
6.4.1 Palatability of algal extracts for <i>Littorina littorea</i> .	131
6.4.2 Effect of algal exudates on consumption by <i>Littorina littorea</i> .	134
6.4.3 Determination of total phenols in aqueous extracts.	135
6.4.4 Components of the phenolic fraction.	138
6.5 Chemoreception.	141
6.6 Discussion.	144
6.6.1 Structural defences.	144
6.6.2 Chemical defences.	148
 <u>Chapter 7 : General Discussion</u>	 153
<u>Appendices</u>	
Appendix A - Composition of enriched seawater medium (Boney's medium).	160
Appendix B - Carbohydrate assay.	161
Appendix C - Extraction of non-cellulosic carbohydrates.	162
Appendix D - Total phenol estimation.	163
Appendix E - Hydrolysis of aqueous extracts.	164
<u>References</u>	165

LIST OF TABLES

<u>Table</u>	<u>Title</u>	<u>Page</u>
1	Mean shell height of adult flat winkles at two sites on the Isle of Cumbrae.	13
2	Flat winkle abundance at two sites on the Isle of Cumbrae.	15
3	Colour polymorphism in flat winkles from Butter Lump, Isle of Cumbrae.	16
4	Microdistribution of <i>Littorina littorea</i> at low tide.	26
5	Microdistribution of flat winkles at low tide.	26
6	Microdistribution of littorinids following submersion.	26
7	Response of common winkle to immersion by rising tide.	35
8	Response of flat winkle to simulated rising tide.	36
9	Response of common winkle to emersion by falling tide.	36
10	Winkle activity at low tide.	36
11	Minerals used to assess radular hardness.	41
12	The docoglossan radula.	44a
13	Features of the taenioglossan radula.	45a
14	Denticular hardness.	48
15	Effect of light regime on algal growth rate.	73
16	Effect of light regime on common winkle feeding rates.	73
17	Macroalgal food preferences of <i>Littorina littorea</i> - Edibility.	75
18	Macroalgal food preferences of <i>Littorina obtusata</i> - Edibility.	76
19	Macroalgal food preferences of <i>Littorina mariae</i> - Edibility.	77
20	Single alga feeding experiments with various intertidal gastropods.	78
21	Attractiveness of macroalgae for <i>Littorina littorea</i> .	82
22	Attractiveness of macroalgae for <i>Littorina obtusata</i> .	83

<u>Table</u>	<u>Title</u>	<u>Page</u>
23	Attractiveness of macroalgae for <i>Littorina mariae</i> .	83
24	Influence of nutritional state on feeding rate of <i>Littorina littorea</i> .	86
25	Effect of starvation of <i>Littorina littorea</i> on algal edibility.	86
26	Effect of starvation of <i>Littorina littorea</i> on algal attractiveness.	86
27	Effect of <i>Fucus</i> habituation on algal attractiveness to <i>Littorina littorea</i> .	88
28	Relationship between density and dry weight of germling lawns : Linear regression equations.	94
29	Germling choice experiments.	95
30	Relationship between wet and dry weight of adult tissue : Linear regression equations.	96
31	Adult versus germling choice experiments.	97
32	Microdistribution of foraging <i>Littorina littorea</i> in the <i>Ascophyllum/Fucus serratus</i> zone.	101
33	Microdistribution of foraging <i>Littorina littorea</i> in the upper <i>Ascophyllum</i> zone.	101
34	Microdistribution of foraging flat winkles in the <i>Fucus serratus</i> zone.	101
35	Microdistribution of foraging flat winkles in a mixed furoid stand.	102
36	Herbivore inclusion/exclusion experiments : Treatment of experimental plots.	105
37	Puncture strength of macroalgal thallus : durometer readings.	118
38	Spearman rank correlation coefficients for algal edibility and puncture strength.	120
39	Resistance of macroalgal thallus to abrasion on the abrasive wheel.	121
40	Palatability of aqueous extracts for <i>Littorina littorea</i> .	133
41	Palatability of algal exudates for <i>Littorina littorea</i> .	136

<u>Table</u>	<u>Title</u>	<u>Page</u>
42	Phenolic content of aqueous extracts.	138
43	Thin layer chromatography : <i>Ascophyllum</i> extract.	140
44	Thin layer chromatography : <i>Fucus serratus</i> extract.	140
45	Chemoreceptive response of littorinids to <i>Ascophyllum</i> exudate.	143

LIST OF FIGURES

<u>Fig.</u>	<u>Title</u>	<u>Page</u>
1	Dense common winkle population at Portencross, Firth of Clyde.	10
2	Collecting sites on the Isle of Cumbrae.	13
3	Vertical distribution of herbivores and dominant macroalgae at Butter Lump, Isle of Cumbrae - spring.	20
4	Vertical distribution of herbivores and dominant macroalgae at Butter Lump, Isle of Cumbrae - autumn.	20
5	Common winkles foraging in fucoid canopy on rising tide.	27
6	IR-sensitive video camera recording snail movement in behavioural experiments.	32
7	Video tape recorder and television monitor.	32
8	Response to immersion (light).	34
9	Response to immersion (dark).	34
10	Response to emersion (light).	34
11	Response to emersion (dark).	34
12	Response to increasing light intensity (submerged)	34
13	Response to increasing light intensity (emersed).	34
14	Response to decreasing light intensity (submerged).	34
15	Response to decreasing light intensity (emersed).	34
16	Radular morphology.	43
17	Patellid tricuspid teeth.	44a
18	Morphological variation in flat winkle radulae.	45a
19	Chemical hardening of acmaeid denticles.	46
20	Pigmentation of denticles.	47
21	Head and mouthparts of <i>Patella vulgata</i> .	49
22	Grazing stroke of <i>Patella vulgata</i> .	49
23	Grazing stroke of <i>Littorina littorea</i> .	50
24	Effect of cartilage groove on orientation of teeth in <i>Littorina littorea</i> .	51

<u>Fig.</u>	<u>Title</u>	<u>Page</u>
25	Pendulum grazing traces.	54
26	Solitary groups of acmaeid grazing prints.	54
27	Grazing prints.	55
28	Grazing print of <i>Littorina littorea</i> , showing sideways twist in response to movement of the muzzle.	56
29	<i>Patella vulgata</i> grazing damage on <i>Ascophyllum</i> frond.	58
30	Lawn of germling <i>Ulva</i> showing signs of grazing by <i>Littorina littorea</i> .	59
31	Progressive stages of common wrinkle grazing damage to <i>Fucus serratus</i> thallus.	59
32	Common wrinkle grazing damage to mature <i>Ulva</i> .	59
33	Common wrinkle grazing prints on an <i>Ascophyllum</i> frond.	59
34	<i>Littorina littorea</i> grazing damage to fertile <i>Fucus serratus</i> .	59
35	Evidence of common wrinkle grazing on <i>Pelvetia</i> .	59
36	Destruction of furoid thallus by <i>Littorina obtusata</i> .	59
37	<i>Littorina obtusata</i> grazing damage to <i>Ascophyllum</i> .	59
38	Plan of aquarium used in determination of attractiveness.	80
39	<i>Littorina littorea</i> feeding on unattached drift <i>Ulva</i> .	102
40	<i>Littorina littorea</i> feeding on unattached drift <i>Fucus serratus</i> .	103
41	<i>Fucus</i> plots in <i>Littorina littorea</i> inclusion/exclusion experiments.	104
42	Effect of dense common wrinkle population on <i>Fucus serratus</i> in inclusion/exclusion experiments.	105
43	Effect of dense common wrinkle population on <i>Ascophyllum</i> in inclusion/exclusion experiments.	105
44	Abrasive wheel used in the assessment of thallus toughness.	119
45	Carbohydrate level in faecal material of <i>Littorina littorea</i> .	124
46	Cellulose level in faecal material of <i>Littorina littorea</i> .	126
47	Cellulose level in faecal material of <i>Littorina obtusata</i> .	126
48	System of perspex runways used to test chemosensory response to <i>Ascophyllum</i> exudate.	141

DECLARATION

I hereby declare that this thesis is composed of work carried out by myself unless otherwise cited or acknowledged and that none of the work has been presented previously - in whole or in part - for any other degree. The research was carried out during the period October, 1980 - November, 1983.

Signed David C. Watson.

David C. Watson

Date: 14 December 1983

Acknowledgements

First and foremost, I would like to extend my grateful thanks to Prof. Trevor A. Norton, without whose careful supervision and boundless enthusiasm this work would not have been possible. I would also like to acknowledge Prof. Malcolm B. Wilkins and the staff of the Botany Department for making available the necessary facilities, together with the staff and students of the Universities Marine Station at Millport, whose assistance during my frequent visits was greatly appreciated.

For the preparation of photographic material - particularly the time-consuming work on gastropod feeding apparatus - I am very much indebted to the professional expertise and enormous patience of Mr. T. Norman Tait. I would also like to extend my thanks to Dr. C.T. Brett for his invaluable assistance and advice regarding biochemical techniques and to Miss Muriel G. Cuthill for her excellent typing services. Finally I wish to convey my special appreciation to the following, whose helpful discussions, encouragement and, in some cases, non-academic diversions, have contributed a great deal to the completion of this work: Dr. A.M.M. Berrie; Prof. A.D. Boney; Miss Alison M. Bray; Prof. Vera Fretter (University of Reading); Dr. S.J. Hawkins (University of Manchester); Dr. C. Hollingworth (University College of North Wales); Dr. R. Huang (University of Taiwan); Miss R. Wendy Loveless (Portsmouth Polytechnic); Mr. D.A. Rugg (University of Liverpool); Dr. C.T. Wheeler; Mr. C.P.L. Young (University of Liverpool).

SUMMARY

The vertical zonation and microdistribution of the dominant gastropod herbivores was examined at a sheltered site in the Firth of Clyde. The flat winkles *Littorina obtusata* and *Littorina mariae*, constitute potential competitors on the low-shore. On the mid-shore, the limpet *Patella vulgata*, may compete with the common winkle, *Littorina littorea*, but *Patella* is not abundant under a dense furoid canopy - conditions which favour the common winkle.

The response of *L. littorea* to various stimuli was assessed in a series of controlled laboratory experiments. Changes in light intensity and the wetting/drying effects of immersion/emersion, prompted changes in the level of littorinid activity. Such behaviour is paralleled in the field, where immersion/emersion by the tide generally illicits a rapid response.

A high degree of niche separation is evident between common and flat winkles. This is reflected in food preferences and adaptations to the feeding apparatus. The two sibling species of flat winkle are inhabitants of the algal canopy and prefer to feed on the perennial thalli. Subtle adaptations to the radular teeth permit the flat winkles to excavate the furoid lamina efficiently. In contrast, the common winkle possesses denticles ideally suited to feeding on the adult foliose thallus and consequently, the ephemeral algae may constitute an important seasonal food source on some shores. Both the flat winkle and the common winkle graze algal germlings efficiently and *Littorina littorea* displays a high degree of selectivity in laboratory choice experiments.

On the shore, the microdistribution of the littorinids in relation to the availability of potential food sources, is closely related to laboratory-determined preferences. The flat winkles are confined largely to the algal canopy, while the common winkle forages predominantly on the rock surface and amongst the ephemeral understory species, only occasionally venturing into

the canopy. Where macroalgal foods and benthic diatoms/microalgae are scarce, the opportunist common winkle can adapt and utilise unattached, "drift" material deposited by the tide.

The anti-herbivore defences of the macrophytic algae are based on the contrasting strategies of escape in time and space (r-selected species) and the utilisation of metabolically expensive structural and chemical defences (K-selected species). The latter confer varying degrees of immunity to excessive grazing damage. Thallus form and toughness both represent potential structural grazing inhibitors and consequently influence grazer preferences markedly. The nature of the algal cell wall may affect the digestibility of the plant, as the cell wall must be breached to allow the animal's digestive enzymes access to the cell contents. Both *Littorina littorea* and *Littorina obtusata* digest a relatively high proportion of ingested cellulose when feeding on preferred foods.

Laboratory experiments with aqueous algal extracts demonstrate the important role of "taste" in food selection. The presence of noxious or attractive allelochemicals is probably the prime motivation behind selective grazing at the germling phase of the algal life cycle. The phenolic content of the furoid extracts has been examined closely and discussed in the light of current theories concerning the digestibility-reducing activities of the intracellular phenolics.

Polyphenols and other plant secondary chemicals are often exuded into the surrounding seawater. "Long-distance" detection of exudates may influence both the microdistribution and the vertical zonation of littorinids on the shore. The chemosensory capabilities of the littorinids were tested in a series of controlled laboratory experiments. Both *Littorina littorea* and *Littorina obtusata* responded to the presence of *Ascophyllum* exudate. While *L. littorea* was repelled, *L. obtusata* was strongly attracted to the source of exudation.

CHAPTER 1 : GENERAL INTRODUCTION

*"Where should an animal feed to get the most food,
and what items of food should it pursue?"*

MacArthur (1972)

Herbivory is potentially a major determinant of marine benthic community structure. Invertebrate grazing may exert a profound influence on the appearance of the shore and shallow sublittoral, dramatically affecting plant distribution, abundance and diversity. Indeed, as Paine and Vadas (1969a) point out, experimental studies indicate that many marine herbivores, unlike their terrestrial counterparts, consistently overgraze and at times almost entirely eliminate their food source. Such behaviour is particularly well documented for various species of echinoderm. Breen and Mann (1976) recorded the destruction of 70 per cent of Nova Scotia kelp beds by *Strongylocentrotus droebachiensis*, while numerous other workers have observed similar large scale destruction of both kelp and seagrasses (e.g. North, 1965; Leighton *et al.*, 1966; Camp *et al.*, 1973; Ogden *et al.*, 1973). In the tropics, comparable devastation may be wrought by teleost fishes (e.g. Bakus, 1969; Earle, 1972; Vine, 1974; Wonders, 1977).

In North Atlantic waters, sea urchins do not normally penetrate the intertidal and the gastropod molluscs assume the role of principal littoral grazers. While molluscan herbivores are relatively sluggish animals and seldom exert immediate or dramatic effects on the algal flora, many species are nonetheless known to control or influence algal abundance and diversity on a long-term basis. The abundance and limited mobility of the common limpet, *Patella vulgata*, make this species well suited to *in situ* experiments on the shore and consequently the foraging activities of *Patella* have been observed by a number of workers (e.g. Jones, 1946, 1948; Lodge, 1948; Aitken, 1962; Southward, 1962). In North-temperate regions, where the mid-littoral is characterised by dense belts of furoid algae, *Patella*

participates in a cyclic relationship with the canopy-forming brown seaweeds (Southward, 1962), temporary stabilisation of which determines the dominant biota and hence the appearance of the shore.

Although work on other herbivorous gastropods is less common, Lubchenco (1978) has successfully demonstrated the controlling influence of the mesogastropod *Littorina littorea*, on the abundance and type of algae inhabiting high intertidal pools on New England shores. As the preferred food is competitively dominant in tide pools, the maximum species diversity occurs at intermediate littorinid densities. Moderate grazing permits inferior algal competitors to persist, while intensive grazing eliminates most individuals and species. On open rock where the preferred food is competitively inferior, increasing herbivory correlates with a decline in algal diversity. Further work by Sze (1980) has since confirmed Lubchenco's observations for tide pools sheltered from intense wave action. On the same shores, Bertness *et al.* (1983) have elucidated the relationship between the common winkle and the encrusting alga, *Ralfsia verrucosa*. The wider implications of littorinid grazing were illustrated eloquently by the indirect relationship between littorinid foraging and barnacle settlement which has apparently arisen as a consequence of the mediating influence of *Littorina* on *Ralfsia* growth.

Despite the widespread occurrence and high population densities which littorinid molluscs achieve on British shores, the feeding ecology of these species has received scant attention. In many ways this is symptomatic of the relative sparsity of information regarding marine plant/herbivore interactions - particularly in this country. Herbivory has been studied widely in terrestrial ecosystems, where various vertebrate grazers as well as numerous herbivorous insects, have been shown to exert dramatic effects on the biomass, species composition and diversity of plant communities (e.g. Harper, 1969; Janzen, 1973; Root, 1973). In the marine context however, "...almost any idea is fair game for thought" (Carefoot, 1977). Indeed Hughes (1980), commenting on the rapid expansion of marine-oriented Optimal

foraging theory, stressed the shortage of relevant data against which to test new theories.

Undoubtedly, the key to a better understanding of the effects of herbivory lies in a greater knowledge of herbivore food preferences (Lubchenco, 1978). Further data concerning the relationship between food preferences and algal competitive abilities may be particularly valuable in elucidating the underlying reasons behind the divergent influence of herbivory on local species diversity patterns. In different circumstances herbivores appear either to increase plant diversity, decrease plant diversity, or both (e.g. Vadas, 1968; Harper, 1969; Paine and Vadas, 1969a; Dayton, 1975; Lubchenco, 1978).

Plant palatability is perhaps most usefully regarded not as a plant characteristic, but rather as a function of plant and animal attributes under a particular set of environmental conditions (Tribe, 1950). Structure, physiology and behaviour combine to determine the grazing capabilities of the herbivore and, indeed, probably evolve in partial response to plant defence characteristics.

While specific factors involved in the determination of food preferences have been identified and documented, little is known of the manner in which these factors interact to determine preference rankings. Where strict selection operates, competition for preferred foods may influence both the vertical distribution of the herbivores (Underwood, 1979) and the corresponding algal zonation patterns (e.g. Burrows and Lodge, 1950; Schonbeck and Norton, 1980).

Clearly, grazing selectivity underlies most of the influence which herbivores exert on population and community phenomena. This study aims principally at establishing the food preferences of the abundant and ubiquitous littorinid grazers and shedding light on the ecological and evolutionary relationships which exist between these species and their foods.

CHAPTER 2 : AN INTRODUCTION TO THE HERBIVORES

The gastropods undoubtedly constitute the most numerous, diversified and ubiquitous molluscan group, accounting for approximately 80% of all living molluscs - close to 40,000 species (Yonge and Thompson, 1976). Nowhere is their diversity of form and habitat more apparent than in the marine environment. Indeed it may well be the gastropod molluscs which prompted the Devonshire squire and naturalist Col. George Montagu (1753-1815) to refer to every marine creature "as if one like it, yet different from it, would be washed up by the waves next tide" (Yonge and Thompson, 1976).

In many parts of the world, the sheer diversity of littoral herbivores complicates the study of alga-grazer interactions immeasurably. In temperate waters, however, diversity is relatively low and as a result it becomes much easier to separate the effects of co-existing species.

Around the coasts of Britain the most important littoral herbivores fall into three categories - the limpets, the top-shells and the winkles. It is with the winkles and to a lesser extent the limpets, that this study is concerned.

The role of the common winkle, *Littorina littorea*, in the determination of intertidal community structure has been closely studied on the eastern coast of America (see Menge, 1975; Lubchenco, 1980; Sze, 1980; Lubchenco and Gaines, 1981). However, little attention has been paid to the ecological implications of littorinid grazing on British shores, despite the exceptionally dense winkle populations which occur locally in many areas. Perhaps because the effects of limpet grazing are often visually striking, field studies have focussed largely on this group (e.g. Jones, 1946, 1948; Burrows and Lodge, 1950; Southward, 1962; Hawkins, 1981).

This chapter introduces the gastropods prominent in this study and endeavours to highlight the biological and ecological facets which may influence their foraging behaviour and feeding preferences.

2.1. General biology, ecology and taxonomy

The evolution of the gastropods tells a complicated story of major structural changes. Undoubtedly the most significant single event - one which prompted the tremendous adaptive radiation apparent in the class today - concerns torsion of the entire visceral mass together with the covering mantle and shell. This caused a migration of the mantle cavity and gills from a posterior position to an anterior position opening above the head and enabled the gills to inhale clean water free from sediment raised by the foot. Consequently, it became possible to develop and employ chemoreceptive means to test the environment ahead.

More recently, in evolutionary terms, coiling of the shell became asymmetrical and indeed disappeared altogether in some cases. At the same time the right gill was lost and the anus moved forward to open near the margin on the extreme right of the mantle cavity.

Against this background of extreme change, three major sub-classes have developed: the pulmonates (terrestrial species in which the mantle cavity becomes a primitive lung); the opisthobranchs (largely sea-slugs characterised by the loss or reduction of the shell and mantle cavity); and the prosobranchs. It is the last sub-class, distinguished by an obviously asymmetrical body plan and by the anteriorly placed mantle cavity and gills, which contains the majority of marine snails.

In the most primitive modern-day prosobranchs, members of the archaeogastropod group, the elaborate, elongated gill is situated in a position which subjects the mantle cavity to the dangerous possibility of clogging when the sediment load is high. Consequently, these species are found only on hard surfaces and it is, in general, left to the more advanced mesogastropods to develop a simplified gill and mantle circulation which permits the exploitation of sand and mud substrata.

2.1.1. The archaeogastropod limpets

No sub-division of the archaeogastropod group has met with greater success than the Patellacea. In all members of the super-family, the body whorl becomes disproportionately large during early development, accommodating the entire viscera in the adult form. The broad, round or oval foot, together with the complete absence of an operculum, completes the external appearance typical of the "limpet".

Within the group there is considerable variation in the structure and functioning of the respiratory apparatus. Beneath the shell an overgrowth of the mantle edge encloses a channel termed the pallial groove, which encircles the foot, opening anteriorly into the pallial (or nuchal) cavity. The direction of the water current flowing through the pallial cavity and groove differs from genus to genus.

In this study we are concerned with four species pertaining to the two major families of the Patellacea, the Acmaeidae and the Patellidae. The acmaeids are characterised by a solitary, elongated ctenidium situated in the pallial cavity, but frequently protruding beneath the edge of the shell. In the patellids, this somewhat primitive structure is replaced by a series of secondary gills close to the outer edge of the pallial groove, between the foot and the mantle margin. Periodic sharp contractions of the powerful shell muscle expel accumulated particulate matter from the right pallial groove. It is widely held that the efficiency of this waste-disposal mechanism is decisive in enabling many patellids to live where the sediment load is relatively high - conditions which do not generally favour the limpet's mode of existence.

Limpet reproduction is planktotrophic. For various morphological reasons the archaeogastropods cannot easily produce complex egg-capsules or undertake internal fertilisation (Yonge, 1947). Eggs are shed singly and possess no protective covering apart from a thin membrane and an albumen layer which is rapidly lost (Morton, 1958). The larval phase develops in the surface-waters

before settlement occurs on a suitable substratum.

a) *Acmaea*

Acmaea tessulata (Müller) is characterised by a flattened shell of rather delicate appearance which can reach a length of around 2.5 cm. It is readily distinguished from the smaller *Acmaea virginea* by the irregular, reddish-brown markings which usually decorate the shell, replacing the pink or brown rays typical of *A. virginea*. The shell apex is also located rather closer to the animal's posterior.

Acmaea is known to feed predominantly on encrusting algal growth and diatoms. Indeed Castenholz (1961) has demonstrated the capacity of various acmaeids to control the distribution of littoral diatoms under certain conditions.

The breeding season extends from April - July (Fretter and Graham, 1962). Following the deposition of eggs on the substratum, free-swimming trochophore larvae emerge to develop in the plankton.

b) *Patella*

The larger and more robust patellids are better adapted to survival in the extreme littoral environment and are consequently the dominant limpets on North Atlantic shores. *Patella vulgata* L., the common limpet, is the most ubiquitous patellid found around the British coast, occurring abundantly on most rocky and stony shores at both exposed and sheltered locations. The black-footed limpet, *Patella depressa* Pennant (= *P. intermedia* Jeffreys) and the china limpet, *Patella aspera* Lamarck, also occur, though less commonly. The former is restricted to exposed, rocky shores along the S. and S.W. coasts of England (Fretter and Graham, 1962), while the latter, though also restricted largely to exposed shores, has a wider geographic range and does occur (locally) in the Firth of Clyde. It is generally found low on the shore or in the sublittoral fringe where the growth of furoid algae is sparse or absent (pers. obs.).

In vivo, *Patella aspera* from the Isle of Cumbrae can usually be identified

by the distinct light, cream-orange foot which presents a marked contrast to the olive-green of *Patella vulgata* and the very dark foot of *P. depressa*. Previous studies, however, suggest that foot colour is not always a reliable character for systematic purposes (see Ebling *et al.*, 1962). The inner surface of the *P. aspera* shell has a porcelanous texture and the head scar appears orange-yellow. In *P. vulgata* the head scar is silvery or, alternatively, an opaque white-grey colour, while in *P. depressa* the shell has an unmistakeably dark interior with distinctive dark rays. *Patella vulgata* can also be recognised by the transparent marginal tentacles which contrast with the opaque, cream-white tentacles of *P. aspera* and *P. depressa*. Further differences in radula ratio (radula length : shell length) and mean radula length are reported by Fischer-Piette (1935), Evans (1947a) and Ebling *et al.* (1962), and are summarised by Fretter and Graham (1962).

Juvenile limpets are recruited from spat settling with maximum abundance near LWNT (Jones, 1948) and in pools and moist crevices at higher levels (Orton, 1929). *P. vulgata* breeds during the winter months (predominantly October-December, Yonge and Thompson, 1976), while the reproductive season of *Patella depressa* and *P. aspera* falls largely during the summer (Fretter and Graham, 1962). In an investigation of annual fluctuations in recruitment of *P. vulgata* at Robin Hood's Bay in N. Yorkshire, Bowman and Lewis (1977) concluded that short periods of low air temperatures during the first few weeks after settlement (late autumn), exerted the greatest influence on recruitment levels. Furthermore, variations of up to seven weeks in gonad ripening and the occurrence of spawning were recorded in response to fluctuating summer temperatures and rough seas. Regional data from the north of Britain, including the Isle of Cumbrae, appeared to accord with the frost-control hypothesis.

Following settlement, the survival of juvenile *Patella* may be affected by a number of different factors (see Underwood, 1979) including the degree of exposure of the shore. Clearly desiccation is a greater threat on sheltered shores where wave action is less intense. Biological variables such as the presence or absence of barnacles and the population density of adult limpets,

may also be of importance.

The characteristic homing behaviour of the intertidal limpets is well documented. Many species have restricted microhabitats such that each individual returns to precisely the same spot after feeding excursions (Underwood, 1979). *Patella vulgata*, for example, may develop a home scar which affects growth to such an extent that the margin of the shell comes to fit the contours of the substratum precisely.

Although the mechanism enabling *Patella* to develop such precise orientation of movement is not fully understood, numerous theories have been volunteered. Hewat (1940) maintained that *Patella vulgata* returned "home" by dint of a chemical trail laid on the outward journey. This hypothesis is perhaps the most acceptable and was strongly supported by the observations of Cook *et al.* (1969), which eliminated the possibility of topographic and kinaesthetic memory and the use of celestial cues. Further details of homing in a number of patellid and acmaeid limpets are recorded by Branch (1981).

As a high-shore animal exposed at low tide to environmental conditions essentially the same as those of a terrestrial environment, *Patella vulgata* is liable to encounter the full effects of desiccation. The animal's body temperature will be influenced by a number of factors, including the degree of shade provided by the microtopography of the habitat and also the nature of the substratum and orientation of the specimen to incident sunlight. Spencer Davies (1970) recorded a maximum body temperature of 35°C in a survey of upper shore limpets but felt that the magnitude of difference in body temperature between high and low shore groups, when integrated over 24 hours or longer, was not sufficient to explain the observed differences in metabolic rate on the basis of temperature acclimation alone.

A number of workers have reported a downshore migration of *Patella vulgata* coincident with falling temperatures in the autumn (e.g. Jones, 1948; Williams and Ellis, 1975; Underwood, 1979). A corresponding upward migration occurred during the spring.

2.1.2. The mesogastropod littorinids

The littorinidae have a world-wide distribution in the littoral zone and are probably the most primitive existing mesogastropods. The original Anglo-Saxon name - pinewinclan or winewinclan - referred only to the largest of the European littorinids, *Littorina littorea*. However today, the term periwinkle (or winkle) applies equally to a number of related species. The biology, ecology and distribution of British and Danish littorinids is summarised by Fretter and Graham (1980).

Around British coasts, littorinids occur on almost all shores, from exposed to sheltered and from mud to rock. Predation by shore birds and crabs, and at high tide by bottom-feeding fish (Reimchen, 1974), appears to have little effect on winkle populations.

This study is concerned with the common winkle, *Littorina littorea*, a species known to be a herbivore of considerable ecological importance (e.g. Menge, 1975; Lein, 1980; Sze, 1980) and the sibling flat winkles, *Littorina obtusata* and *Littorina mariae*, which, unlike other British littorinids, actually inhabit the macrophytic fronds on which they feed.

a) *Littorina littorea* (L.)

Littorina littorea (L.) is one of the most abundant intertidal gastropods. Population densities of up to 8,000-10,000 live specimens per m² (pers. obs.) are not uncommon in the Firth of Clyde (see Fig. 1). The species thrives under widely varying conditions, often occurring in relatively polluted estuaries (Moore, 1937) where it is tolerant of salinities in the order of ten parts per thousand (Fischer, 1948). It can colonise a variety of different surfaces, ranging from rock and small stones to mud and sand but generally avoids shifting substrata and is uncommon on chalk/limestone (Fretter and Graham, 1962) and on exposed shores (pers. obs.).

The common winkle is characterised by a sharply-pointed, coiled shell (maximum height approximately 35-38 mm) which is ridged in young specimens but



Fig. 1. Dense common winkle population at Portencross,
Firth of Clyde.

smooth in older individuals. The shell colour ranges from grey-black to reddish-brown. Small specimens may be confused with members of the *Littorina saxatilis* complex but, on close examination, can be distinguished fairly readily by the dark transverse bands which appear on the tentacles and by the angle formed between the upper lip of the shell aperture and the axis of the spire. In *L. saxatilis* the junction between spire and lip very nearly constitutes a right angle. By contrast, in the common winkle, the axis of the spire extends almost parallel to the upper lip.

A high degree of tolerance towards the drying effects of exposure at low tide, is a prerequisite to life on the upper-shore. The relatively immobile common winkle has successfully evolved behavioural and physiological adaptations to withstand desiccation and survive in this extreme environment. When the substratum dries, a secretion from the anterior pedal gland sticks the lip of the shell to the rock surface and, as the secretion hardens, the foot can be withdrawn into the shell. In this state *Littorina* is able to survive constant emersion for long periods. Indeed Perkins (1967) recorded a 50% mortality (LE_{50}) for common winkles exposed to air, of 22-40 days. Under cover of algae, survival increased. Adult animals have also been shown to survive relatively high air temperatures. Tolerance of temperatures in excess of 40°C has been recorded under both laboratory and field conditions by Evans (1948) and by Fraenkel (1960).

The littorinids exhibit a particularly wide variety of reproductive modes, ranging from brooding of young, through laying of benthic egg masses to the production of planktonic larvae. Recent investigations of population dynamics have identified the selective forces probably responsible for many of these life history adaptations (e.g. Raffaelli and Hughes, 1978; Hughes, 1980; Roberts and Hughes, 1980).

The common winkle is planktotrophic, eggs being laid in lens-shaped capsules (one to three eggs per capsule), from which ciliated veligers emerge after several days. At Millport on the Isle of Cumbrae, Elmhirst (1923) noted that the spawning season extended from January to July and, occasionally, into August. The larvae normally remain in water masses close to the shore

(Underwood, 1979) before eventually settling low down in the littoral zone. Under certain circumstances, developing embryos can be transported considerable distances before hatching and this is thought to be a major factor in the southward spread of the species on the east coast of America (Wells, 1965). Mileikovsky (1975) observed that complete pelagic development of this nature was most common in littorinids inhabiting the upper shore.

Hughes and Roberts (1980) investigated the reproductive effort of a variety of winkles and found a general trend towards an increased reproductive effort with increasing age. Three indices were employed, *L. littorea* achieving the greatest reproductive effort when ranked on a time axis standardised for growth rate.

The analysis of littorinid gastropods has been used by several workers to assess heavy-metal contamination in estuaries and coastal areas (e.g. Portmann, 1979). The value of the common winkle as an indicator species was investigated by Bryan *et al.* (1983) who concluded that, whilst potentially useful as an indicator for Cd and several other metals, it was not a perfect indicator organism. Heavy-metal concentrations were found to be generally higher in the flat winkle.

b) *Littorina obtusata* (L.) and *Littorina mariae* Sacchi and Rastelli

The taxonomy of the British littorinids has frequently been in dispute, especially with regard to the highly polymorphic "species" commonly known as *Littorina saxatilis* (L.) (= *rudis*) and *Littorina obtusata* (L.) (= *littoralis*). The "*L. obtusata*" complex is readily identified by the depressed spire of the shell, from which the common name, flat winkle, is derived.

The flat winkle reproduces by means of benthic egg capsules laid between March and October on the fronds of the furoid algae (Barkman, 1955). Reproduction does not at any stage involve dispersal larvae and consequently genetic variability among populations may contribute significantly to flat winkle ecology (Underwood, 1979).

In the course of a detailed investigation of polymorphism and its relation to the ecology of "*Littorina obtusata*", Sacchi (1961a,b; 1963; 1964; 1966a,b) recognised a size dimorphism in which he termed the morphs, "dwarf" and "normal". Subsequently, a number of other morphological, physiological and ecological differences were revealed, prompting Sacchi and Rastelli (1966) to separate "*L. obtusata*" into two sympatric units, describing the dwarf form as a new species, *Littorina mariaae*. Interspecific differences have since been elaborated by Sacchi (1967; 1969a,b; 1972), Reimchen (1974) and Goodwin and Fish (1977).

The mean shell height of adult specimens collected at two sites of contrasting exposure on the Isle of Cumbrae is listed in Table 1. Butter Lump (O.S. Map ref. NS182556) is a relatively sheltered east-facing location, while Farland Point (O.S. Map ref. NS172544) is a more exposed south-facing site (Fig. 2). The classic bimodality of adult shell size recognised by Sacchi (1961a,b) was evident at both locations. However the magnitude of variation in size was less at the more exposed Farland Point (cf. Reimchen, 1974; Goodwin and Fish, 1977). Reimchen (1974) considered that much of the difference in shell size, both within and between species, could be attributed to the degree of exposure to wave action and to the effects of predation by crabs, especially *Carcinus maenus*.

Table 1. Mean shell height of adult flat winkles at two sites on the Isle of Cumbrae.

	Mean shell height (cm) \pm S.E.	
	Butter Lump	Farland Point
<i>L. obtusata</i>	1.20 \pm 0.037 (N=74)	1.00 \pm 0.003 (N=88)
<i>L. mariaae</i>	0.94 \pm 0.019 (N=74)	0.95 \pm 0.041 (N=69)



A



B

Fig. 2. Collecting sites on the Isle of Cumbrae.

A. Butter Lump.

B. Farland Point.

The abundance of *Littorina obtusata* and *Littorina mariae* varies greatly both at sheltered and exposed sites (pers. obs.). Nevertheless, Reimchen (1974) and Sacchi (1969a) found that *L. obtusata* was generally more common at sheltered locations where furoid cover was greatest. *L. mariae*, predominantly an inhabitant of the *Fucus serratus* canopy, tended to form a greater proportion of the total flat winkle population at exposed sites, where the quantity of *Fucus serratus* increased relative to other furoids. The abundance of each species at the sampling sites on the Isle of Cumbrae is shown in Table 2. Abundance of *L. mariae* (relative to the amount of furoid cover) remained fairly constant, suggesting that exposure did not affect abundance directly. Populations of *L. obtusata* appeared to vary quite dramatically between the two sites and between the two algal zones, showing no discernible relation to exposure.

A number of features have been employed to separate *L. obtusata* and *L. mariae* for research purposes. The morphology of the shell (male and female specimens) and of the reproductive organs (males) are particularly convenient features in distinguishing live specimens. All mature male flat winkles display one of the two characteristic types of penis described by Sacchi and Rastelli (1966) and Reimchen (1974). The penis of the larger *Littorina obtusata* is muscular and stout, the distal tubule constituting less than 10% of the total length. More than 20 adhesive glands occur at the base. By contrast, in *L. mariae* the organ is relatively long and slender with less than 12 basal adhesive glands and the distal tubule can account for up to 50% of the entire length (Reimchen, 1974; pers. obs.).

Although the basic shape of the shell is similar in most respects, subtle differences do occur in the thickness of the lip relative to the diameter of the aperture. In *Littorina mariae*, the lip is generally thicker and the aperture smaller than in *L. obtusata*. When the author became familiar with the shell form of mature specimens from the collecting sites, it proved possible to separate the two species, both in the laboratory and in the field, on this basis alone. Successful identification of male and female specimens was achieved with a

consistency in the region of 94%. This technique cannot, however, be used to separate juveniles.

The specific status of the two sibling species is further confirmed by differences in radular morphology (see Chapter 4), by biochemical characteristics and by the pigmentation of the ovipositor in females.

Table 2. Flat wrinkle abundance at two sites on the Isle of Cumbrae.

	Abundance (number per 100g alga)			
	Butter Lump		Farland Point	
	<i>Ascophyllum</i>	<i>Fucus serratus</i>	<i>Ascophyllum</i>	<i>Fucus serratus</i>
<i>L. obtusata</i>	2.68	0.98	0.74	2.08
<i>L. mariae</i>	0.19	2.10	-	2.41
Juveniles	2.39	2.39	3.32	7.89

Perhaps the most noticeable attribute of the flat wrinkle is the high degree of variability in shell colour. In each species the four principle morphs - citrina, olivacea, dark reticulata and light reticulata - occur in different proportions. Table 3 records the frequency of these colour morphs at Butter Lump, Isle of Cumbrae. The figures are in general agreement with the observations made by Sacchi (1967; 1969b) and by Reimchen (1974), showing the dominant colour morph of *L. obtusata* to be olivacea (followed by dark reticulata), while the dark reticulate form (followed by citrina and light reticulata) was most common in *L. mariae*.

Colour polymorphism is known to be genetically controlled (Reimchen, 1974). For both species, phenotypic frequencies vary with exposure, converging on exposed shores and diverging on sheltered shores. Reimchen found these changes to reflect similar changes in the background on which each phenotype was cryptic. In *Littorina obtusata*, frequencies correlated with the species of alga inhabited, while in *L. mariae*, the important variable appeared to be the microdistribution

of juvenile individuals within plants of the same species.

Table 3. Colour polymorphism in flat winkles from Butter Lump,
Isle of Cumbrae.

Colour morph	<i>Littorina obtusata</i> (N=183)	<i>Littorina mariae</i> (N=164)
Olivacea	73%	-
Citrina	6%	24%
Dark reticulata	21%	52%
Light reticulata	0.5%	23%

2.1.3. The prosobranch alimentary system

Clearly the efficiency of the grazer's digestive system is likely to influence the composition of the diet. However the basic features of the alimentary tract vary little between the herbivores of the archaeogastropod and mesogastropod orders. The intention of this section is to summarise the major structural and functional characteristics of the prosobranch digestive system. It is drawn primarily from the reviews of Fretter and Graham (1962) and Morton (1958) but is supported by personal observations made during the dissection of dead animals.

The alimentary canal is basically U-shaped, rising from the mouth to the stomach in the visceral hump and then falling again to the point where the anus discharges into the mantle cavity. The mouth leads into the oral tube, a short passage which itself expands rapidly into the buccal cavity. When the mollusc is not actively feeding, the oral tube is separated from the buccal cavity by the inner lips.

The mechanics of food-gathering and ingestion will be discussed in detail in a later chapter but rely heavily on a muscular, tongue-like structure, the

odontophore, which is attached posteriorly to the floor of the buccal cavity. The other prominent feature of the buccal cavity is a pair of longitudinally directed folds lined with ciliated epithelium which carry ingested food particles backwards into the oesophagus. Mucus secreted by the salivary glands aids this process, acting as a lubricant and as an adhesive for the food particles. In most prosobranchs the saliva does not, in fact, appear to contain digestive enzymes, although Jenkins (1955) did detect amylase in the salivary glands of *Littorina littorea*.

The oesophagus, which connects the buccal cavity to the stomach, is divided morphologically and histologically into three sections. Along the dorsal wall of the anterior oesophagus and the mid-oesophagus, the dorsal folds form a deep channel termed the dorsal food channel, which carries food back towards the stomach. In *Patella* there is a further double fold running along the mid-ventral line.

The lateral and ventral walls of the mid-oesophagus are lined with a glandular epithelium responsible for the secretion of digestive enzymes and causing a considerable swelling in this section of the alimentary tract. The direction of the ciliary beat in the glandular areas channels the secretion from the oesophageal glands towards the food channel. The dorsal folds and the associated glandular epithelium disappear in the posterior oesophagus, where the principal function is simply to deliver food and digestive enzymes to the stomach.

The stomach itself lies in the visceral hump and can generally be divided into a globular posterior portion and a narrower anterior style sac. In *Littorina* the oesophagus enters the stomach half-way between the posterior and anterior ends and the intestine emerges from the anterior end of the style sac. The digestive diverticula (ducts leading to the digestive gland) open close to the oesophageal aperture.

Inside the stomach, a mixture of food and enzymes secreted by the digestive glands, is acted upon by ciliary currents and by muscular contraction of the stomach wall. A cuticular structure, termed the gastric shield, protects the

stomach wall from abrasion.

Following a period of digestion in the posterior stomach, a solution of the digested food material is forced into the ducts and tubules of the digestive gland to be absorbed by the active digestive cells. In the patellid and acmaeid limpets, phagocytosis and intracellular digestion of minute particulate matter may also occur. Indigestible material is held in "residual vesicles" until discharged into the lumen by fragmentation of the tips of the absorbing cells.

The two lobes of the digestive gland invariably occupy a large proportion of the visceral hump, although the actual quantity of reserve food (glycogen) stored in the connective tissue is dependent both on the nutritive and the sexual state of the animal.

The indigestible material remaining in the posterior chamber of the stomach is transported to the style sac where it is supplemented by material excreted from the digestive gland. This comprises a mixture of particulate food originating in the posterior stomach, excretory matter extracted from the blood, and the indigestible residue of food undergoing intracellular digestion (in species where such digestion occurs).

In the patellids, the posterior portion of the stomach is almost entirely lost and the oesophagus and style sac are particularly well developed. As Fretter and Graham (1962) acknowledge, it is difficult to ascribe a convincing reason to this evolutionary anomaly which parallels, in many ways, development in the carnivorous higher prosobranchs.

The major function of the style sac is the formation of faeces. The remaining indigestible material is rotated and cemented to form a rod held together by mucus. This then passes into the intestine where further secretion from intestinal glands consolidates the process of adhesion and compaction. In *Littorina*, segmentation of the faecal rod into faecal pellets also occurs. Hence the primary function of the intestine is not to absorb the products of digestion, but rather to produce faeces which will not disintegrate within the mantle cavity and threaten the efficient operation of the gills.

2.2. Intertidal zonation

Biological and ecological differences are reflected in intertidal distribution patterns. The vertical zonation of macrophytic algae is a well known and abundantly documented phenomenon. The abundance of the major canopy-forming furoid species varies in response to the degree of exposure and the nature of the substratum, but the basic pattern of down-shore succession remains fundamentally the same.

Determination of the factors limiting furoid distribution has prompted much scientific research. As an ecological problem involving the interaction of each component in a frequently complex community, together with the numerous physical factors which influence shore life, it has proven difficult to separate unequivocally the ultimate distributional determinants. It is easy to fall into the trap of making unwarranted generalisations in the light of observations on only one species or at just one location. This tendency is exemplified by the frequently quoted theory stating that upper limits of algal distribution are controlled exclusively by physical factors (e.g. Colman, 1939; Lewis, 1964; Schonbeck and Norton, 1978), while lower limits are determined by biological factors, including grazing (e.g. Burrows and Lodge, 1950; Schonbeck and Norton, 1980) and competition (e.g. Schonbeck and Norton, 1980). While this maxim may be applicable in many instances, it is by no means infallible (see Schonbeck and Norton, 1978).

Zonation of littoral invertebrate populations also occurs, often in response to algal distribution. Seaweed cover provides shelter for the inhabitants of a potentially extreme environment, as well as constituting, at all stages of development, an abundant source of food for herbivorous species.

It is widely believed that upper limits of invertebrate distribution coincide with limits of physiological tolerance to physical factors during low tide, although the evidence to support this theory is minimal (Underwood, 1979). Alternatively, upper limits may be determined by interspecific competition for food. Underwood (1979) suggested that lower limits might depend on predation

at high tide or behavioural responses to light, gravity and food, while Wolcott (1973) felt that selection pressures would favour range expansion to the limits of physiological tolerances only where the range bordered on unexploited resources. If this is true, then physical factors will seldom determine the range limits of invertebrate populations.

Whatever the determinants of distribution, zonation is often sufficiently flexible to permit seasonal fluctuations, especially where the more mobile species are concerned. Vertical migration of adults may occur in response to seasonal climatic changes, while the preferential settlement of juveniles - where there is a planktonic phase of development - has the potential to alter distribution dramatically for short periods, often on an annual basis.

In this section, the zonation of the test molluscs in relation to the dominant canopy and understory algae is examined at Butter Lump. The intention is not only to look at community structure but also to define the potential macroalgal food sources available to each grazer.

2.2.1. Methods

Abundance of algae and snails was recorded in adjacent 0.5m. x 0.5m. quadrats along a 16m. transect line stretching down the shore from the uppermost limit of fucoid distribution (3.2m. above chart datum). The quadrats were divided into 10cm. x 10 cm. grids to facilitate ready assessment of percentage cover for each algal species.

Identification of adult flat winkles was based on shell morphology and was carried out in the field. No identification of juvenile specimens was attempted.

Distribution along the transect was examined initially during September, 1982 and was re-assessed in April, 1983.

2.2.2. Results

The results are summarised in Figs. 3 and 4. Only those algae achieving a maximum cover in excess of ten percent are recorded.

Fig. 3. Vertical distribution of herbivores and dominant macroalgae
at Butter Lump, Isle of Cumbrae - spring.

Dark bars denote canopy forming algae.

White bars denote understorey species.

Stippled bars denote herbivores.

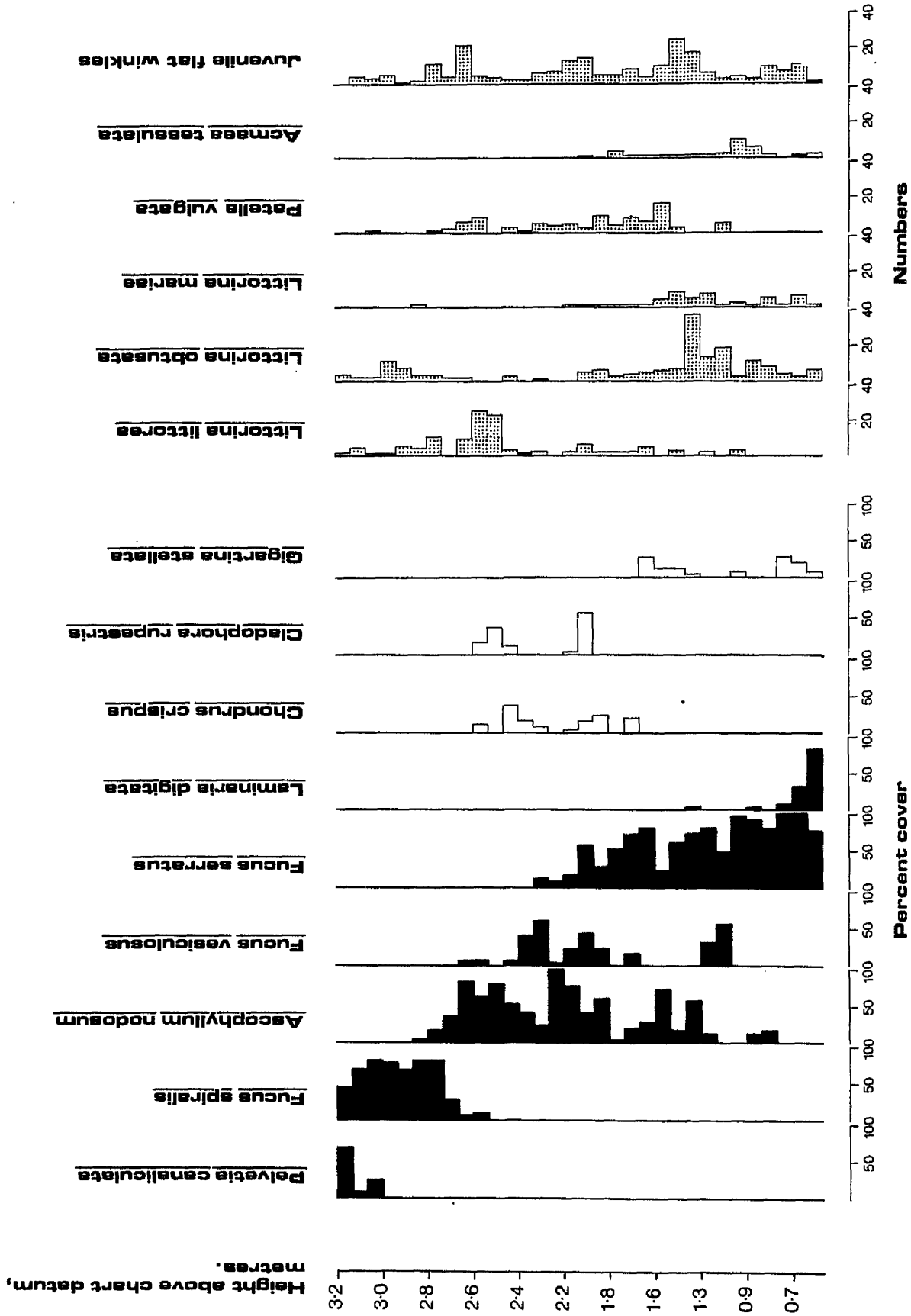


Fig. 4. Vertical distribution of herbivores and dominant macroalgae at
Butter Lump, Isle of Cumbrae - autumn.

Dark bars denote canopy forming algae.
White bars denote understorey species.
Stippled bars denote herbivores.

Height above chart datum,
metres.

3.2
3.0
2.8
2.6
2.4
2.2
1.8
1.6
1.3
0.9
0.7

Pelvetia canaliculata

Fucus spiralis

Ascophyllum nodosum

Fucus vesiculosus

Fucus serratus

Laminaria digitata

Cladophora rupestris

Gigartina stellata

Littorina littorea

Littorina obtusata

Littorina mariae

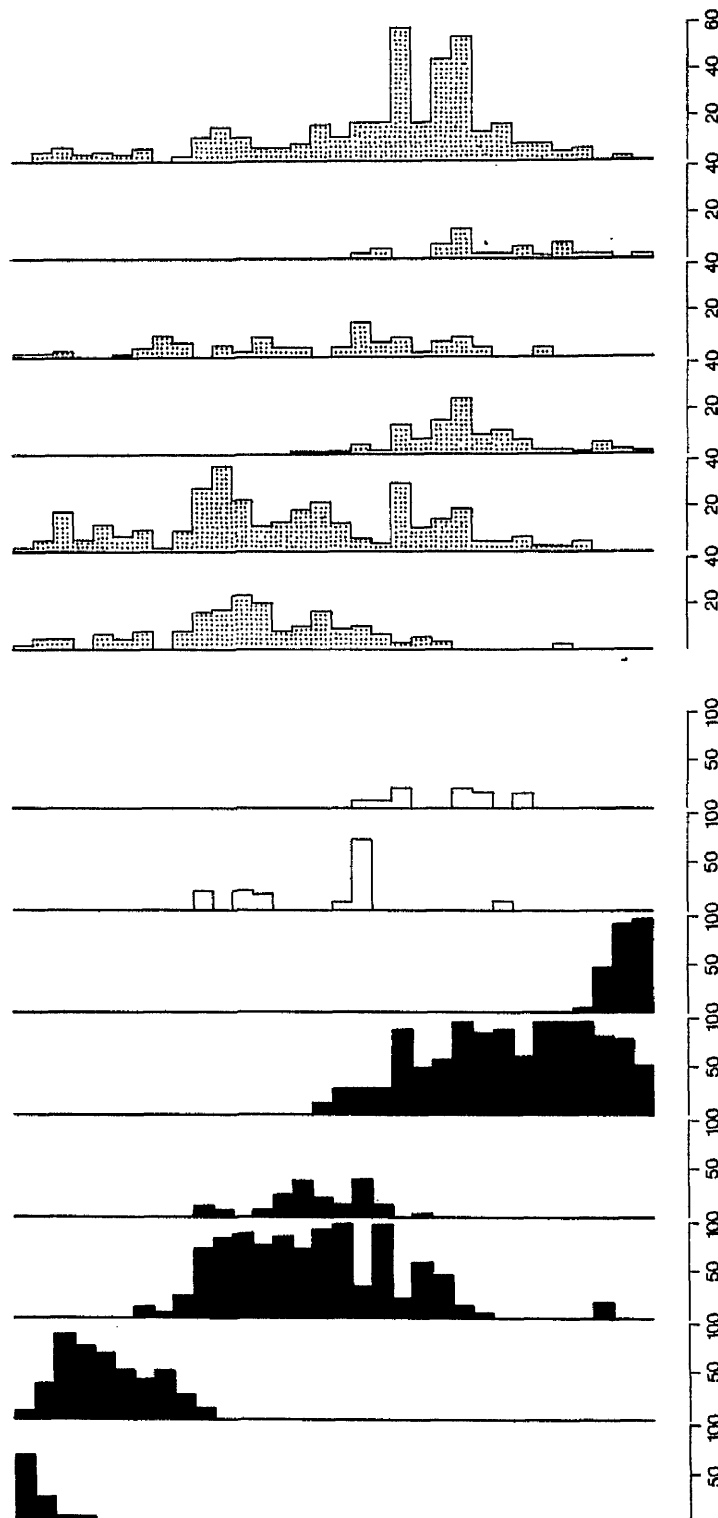
Patella vulgata

Acmaea tessulata

Juvenile flat winkles

Numbers

Percent cover



The succession of fucoids from the high-shore *Pelvetia* and *Fucus spiralis* to the low-shore *Fucus serratus* and littoral fringe *Laminaria digitata*, is typical of most sheltered, rocky shores in Britain (e.g. Colman, 1939; Lewis, 1964; Schonbeck and Norton, 1978). The dense furoid canopy effectively stifles the development of understorey species, only *Chondrus crispus*, *Cladophora rupestris* and *Gigartina stellata* occurring in any great abundance. *Chondrus* and *Cladophora* were associated predominantly with the brown macrophyte *Ascophyllum*, while *Gigartina* tended to colonise (sparsely) the lower shore, under a canopy of *Fucus serratus*. The red alga, *Chondrus*, was absent later in the year. Contrary to expectations, the foliose ephemeral species *Ulva* and *Enteromorpha* were recorded only in the autumn. The dense furoid canopy ensured that both species achieved only a sparse cover in the sample quadrats (always less than ten percent).

Of the study molluscs, *Littorina obtusata* achieved the widest vertical distribution, extending from the narrow *Pelvetia* zone, 3.2m above chart datum, to the upper limits of the *Laminaria* zone at the bottom of the shore. Optimum population densities during the spring, occurred in the *Fucus serratus* canopy. In the autumn, peak abundance shifted slightly higher, to the *Ascophyllum* zone. Reimchen (1974) recorded optimum densities in the *Ascophyllum* zone throughout the year. This was the only evidence of a seasonal alteration to the distribution pattern of any of the test species.

As anticipated (see Reimchen, 1974), *Littorina mariae* was associated predominantly with the furoid alga *Fucus serratus*, only the occasional specimen appearing amongst other species - usually *F. vesiculosus*. *L. mariae* was found only rarely on *Ascophyllum* fronds even where those were in close association with inhabited plants.

Juvenile flat winkles were abundant along the entire length of the transect in both spring and autumn. Overall densities were markedly higher in the autumn, reaching a distinct peak at 1.6-1.8m. above chart datum (just below MTL) (cf. Colman, 1939).

The distribution of the common winkle, *Littorina littorea*, corresponded closely to that of *L. obtusata* but was curtailed downshore, very few specimens occurring below the lower limit of *Ascophyllum* distribution. Optimum abundance was achieved in the upper *Ascophyllum* zone, around 2.4-2.7m. above chart datum (just below MHWN).

Neither *Patella* nor *Acmaea* achieved high population densities at Butter Lump. *Patella vulgata*, however, was characterised by a fairly wide vertical distribution, ranging from the *Pelvetia* zone down to MLWN. *Acmaea* displayed the typical distribution of a low shore and sub-littoral species, extending up to approximately 1.8m. above chart datum (just below MTL), but only under dense furoid cover. *Patella aspera* was not recorded at Butter Lump.

2.2.3. Discussion

Figs. 3 and 4 illustrate the classical succession of algal species from upper to lower shore. The zonation of each species falls within the limits recorded by Clokie and Boney (1979) and corresponds closely to the observations made by Schonbeck and Norton (1978) on similar shores on the Isle of Cumbrae.

Zonation is probably more clearly delineated in the littorinids than in any other group of intertidal gastropods. The common winkle is generally considered to occupy the broadest zone (e.g. Newell, 1958a). Fretter and Graham (1962) recorded a distribution ranging from HWNT to ELWST, while Moore (1937), Newell (1958a) and Allen (1962) described an intertidal range extending down to the *Laminaria* zone and into the sub-littoral. At Butter Lump, distribution was curtailed downshore, remarkably few specimens appearing in the *Fucus serratus* zone below 1.6m. There was no obvious change in substratum or the availability of suitable microhabitats to explain the sparsity of common winkles at this level.

It is interesting to note that no seasonal change in distribution was recorded at this site and that there was no evidence to suggest an influx of juvenile common winkles low down the shore in April, despite Elmhirst's (1923) assertion that young appeared abundantly during this month. Elmhirst's

observations, though made at Millport, were later disputed by Moore (1937).

As regards the flat winkles, Reimchen (1974) states that the two sibling species occur at essentially different levels on the shore but with a zone of overlap. This study suggests that, rather than occupying distinct but overlapping bands, the distribution of *Littorina mariae* is simply more restricted than that of *L. obtusata*. At least in the spring, the latter species occurred everywhere that *L. mariae* was found, extending well below the lower limits of distribution recorded by Reimchen and by Sacchi (1969a). *Littorina obtusata* also extended up the shore to the top of the *Pelvetia* zone - well beyond MHWN, which Reimchen considered to be the upper limit of the species. Colman (1939) and Bakker (1959) also found that the *Pelvetia* zone was beyond the flat winkle range. This unusual extension of the range at Butter Lump occurs in spite of the sheltered nature of the site which precludes the possibility of a wide splash zone.

The distribution of *Littorina mariae* concurs with the distribution recorded by Reimchen (1974) on sheltered shores. In a broader context, the close link between *L. mariae* and *Fucus serratus* suggests the possibility that intertidal invertebrates may be confined to particular levels on the shore, not by their degree of tolerance towards physical extremes but rather by behavioural adaptations in response to the presence of a favoured microhabitat or preferred food (see Underwood, 1979).

The restricted distribution of *Acmaea tessulata* confirms the poor adaptation of the species to life in the intertidal. High on the shore, the delicate ctenidium would undoubtedly suffer severe damage from the effects of desiccation.

The relative sparsity of the patellid population at Butter Lump, accords with the observations of Fischer-Piette (1948) and Jones (1948) who maintained that abundance declined as algal cover increased. Fischer-Piette felt that the fucoid canopy probably provided a mechanical barrier to the settlement of spat.

While not present in large numbers, *Patella vulgata* does achieve a relatively wide distribution. However no specimens were recorded below MLWN, despite Fretter and Graham's (1962) records of dense populations down to MLWS and Jones'

(1948) assertion that maximum population densities occurred at LWN on the Isle of Man (see also Southward, 1953). As Butter Lump is a well sheltered site, *Patella* is also uncommon above MHWN (see Evans, 1947b).

Observations on the distribution of herbivores at low tide do not necessarily give any indication of food preferences. Active foraging at low tide is relatively rare (see Chapter 3) and it is possible that the micro-distribution of resting and feeding snails may not correspond (see Section 2.3). Nevertheless, gastropod grazers are relatively immobile and distribution at low tide will give some indication of the range of potential macroalgal foods available.

The flat winkles are known to forage predominantly on the fronds of the fucoid algae (e.g. Van Dongen, 1956; Bakker, 1959; Fretter and Graham, 1962; Reimchen, 1974). The restricted distribution of *Littorina mariae* is such that the species will encounter only two canopy-forming algae - *Fucus serratus* and *Ascophyllum* - with any degree of regularity. In contrast, both *Littorina obtusata* and *L. littorea*, can be expected to encounter the entire range of intertidal fucoids and might therefore display a more catholic diet.

If food is a limiting factor and if the feeding preferences of the three littorinids correspond, *L. littorea* and *L. obtusata* will compete on the mid- and upper-shore, while *L. obtusata* and *L. mariae* would compete lower down the shore. However niche separation between *Littorina littorea* and *L. obtusata* is likely to reduce any potential competition (see Section 2.3). The ecological significance of competition between *L. obtusata* and *L. mariae* is probably minimal, as algal biomass in the more stable environment of the lower shore is especially high.

Although *Chondrus* seasonally, and *Cladophora* and *Gigartina*, were the only abundant understorey macrophytes recorded, it is possible that a bloom of ephemeral green algae (*Ulva* and *Enteromorpha*) may appear in the early summer and provide a potential food source for several months. *Ulva* also occurs within the fucoid canopy as an epiphyte and may be available to those species foraging within, rather than under, the canopy. However the growth of ephemeral green

algae is patchy on many shores and these species may only be available very locally as a food source.

Both species of limpet forage predominantly on the rock surface and consequently macroalgae will be of secondary importance as a food source, to the diatoms, microalgae and algal germlings which comprise the microflora. Interspecific competition may occur between the two limpets on the low shore and between *Patella* and the common wrinkle on the mid-shore.

2.3. Microdistribution of littorinids

The investigation of vertical zonation has shown the common wrinkle to co-exist with the flat wrinkle over its entire intertidal range. To avoid competition for space and food between two abundant and voracious herbivores, some form of niche separation may be anticipated.

In this section the microdistribution of the flat wrinkle and the common wrinkle at Butter Lump is examined and compared.

2.3.1. Methods

Specimens sampled at low tide were classified according to activity and position. Snails occurring on the algal thallus and visible without prior disturbance of the fronds were allocated to the category "upper thallus". Specimens resting or crawling on the thallus, but visible only after disturbance of the upper fronds, were classified "lower thallus". The level of activity on hidden fronds may be underestimated to some extent, as a degree of disturbance was necessary to observe covered individuals and this almost certainly prompted a cessation of activity and withdrawal of head and tentacles by some specimens. No attempt was made to distinguish between the two species of flat wrinkle.

Microdistribution was also assessed after 10-15 minutes submersion by a rising tide. Under these conditions, however, it was possible only to record accurately, the position of the snails.

2.3.2. Results

The results are summarised in Tables 4-6. At low tide the majority of common winkles - both active and inactive specimens - were found on the rock surface. A tendency to aggregate in damp, sheltered crevices was prevalent, particularly where algal cover was sparse. Very few individuals were found amongst the algal fronds. This trend was reversed for the flat winkle,

Table 4. Microdistribution of *Littorina littorea* at low tide.

	Distribution on available substrata (%)			N
	Upper thallus	Lower thallus	Rock	
Active specimens	-	-	100	7
Inactive specimens	4	3	93	200

Table 5. Microdistribution of flat winkles at low tide.

	Distribution on available substrata (%)			N
	Upper thallus	Lower thallus	Rock	
Active specimens	31	67	2	54
Inactive specimens	18	80	2	311

Table 6. Microdistribution of littorinids following submersion.

	Distribution (%)		N
	Seaweed	Rock	
<i>Littorina littorea</i>	28	72	114
<i>L. obtusata/L. mariae</i>	97	3	200

most specimens inhabiting the algal thallus itself. During emersion, a high proportion of flat winkles took advantage of the shelter offered by the seaweed cover, crawling or resting in the interior of the algal clumps and avoiding the exposed upper fronds (cf. Wieser, 1952).

Following submersion, the distribution of both species remained fundamentally the same, although the proportion of common winkles within the canopy showed an increase (see also Fig. 5).

2.3.3. Discussion

The results indicate a high degree of niche separation between flat winkle and common winkle. The flat winkle is predominantly an inhabitant of the algal canopy, while both active (= foraging) and inactive common winkles occur most abundantly on the rock surface (cf. Hagerman, 1966). This suggests that niche separation where the species co-inhabit the same site, may involve differences in foraging strategy as well as differences in microhabitat preference. Nevertheless, the results also indicate a limited migration of common winkles from substratum to algal thallus coincident with immersion and it therefore seems likely that at least some common winkles will forage side-by-side with *Littorina obtusata* and occasionally *L. mariae*, at high tide.

A marked seasonal change in the microdistribution of the two flat winkles occurred at all sites investigated (both sheltered and exposed). During the winter months (October/November - late March) a migration from the canopy to the substratum takes place, potentially bringing the flat winkles into direct competition with the rock-dwelling common winkle for a limited period of time. However, temperate-water littorinids are thought to become largely inactive and to feed little - if at all - during the winter (Newell, 1958b; Fretter and Graham, 1962; Hawkins and Hartnoll, 1983). Working with the trochid herbivore, *Melagraphia aethiops*, Zeldis and Boyden (1979) found a 60% decrease in radular rasping rate in winter-acclimatised specimens and a reduction of 71% in field crawling rates. Hence if competition does occur between *L. littorea* and the flat winkles, it will be predominantly for shelter and not for food.



Fig. 5. Common winkles foraging in fucoid canopy on rising tide.

CHAPTER 3 : BEHAVIOURAL STUDIES

The distinctive zonation of shore-dwelling organisms has intrigued ecologists for many years. Several attempts have been made to explain intertidal distribution patterns on the basis of simple behavioural responses to physical environmental factors. Fraenkel's (1927) description of responses to light and gravity by *Littorina neritoides* is an early example. More recently, Newell (1958 b,c) accounted for the observed distribution patterns of the common winkle in terms of geo- and photo-tactic responses.

The role of geotaxis as a behavioural cue is widely recognised. Under certain circumstances the response to gravity may override any directional response to light by intertidal gastropods (Underwood, 1979). British species of periwinkle are known to display negative geotaxis and this is thought to permit the re-establishment of zonation amongst displaced specimens. There is, however, considerable confusion over the precise roles of both geo- and photo-taxis in the determination of zonation patterns.

Responses to current and wave directions have also been proposed as behavioural mechanisms permitting the maintenance of set distribution patterns. However, as Underwood (1979) recognises, there is no convincing evidence to suggest that any of the simplified patterns of response which have been described can adequately account for the observed zonation of a species, or indeed its subsequent maintenance. Newell's (1958b) "light-compass" reaction for example, though widely quoted in the literature, can be dismissed as it is quite clearly based on a number of false premises (see Underwood, 1979).

Nevertheless, it is likely that vertical distribution patterns are maintained largely by behavioural responses - however complex - to environmental variables. Additionally, orientation by means of mucus trails may make an important contribution to the maintenance of zonation. For locomotory purposes it is probably obligatory for snails to deposit mucus (Townsend, 1974) and consequently the information present in the trail may be of only secondary

importance. Nevertheless, where population densities are high, foraging individuals will frequently encounter mucus trails during feeding excursions. Townsend has shown that the freshwater pulmonate, *Biomphalaria glabrata*, turns towards mucus trails after making contact and thereafter spends more time in contact with the trail than expected by chance. These results are supported by the observations of D.P. Cheney (unpubl. data) working with *Littorina littorea*. Consequently, mucus trail following may play a major role in the formation of aggregates in gregarious species. Perhaps more important, however, it may contribute to the maintenance of zonation patterns by continually drawing foraging snails back towards the centre of population density.

Inevitably, studies of gastropod movement in relation to zonation are concerned primarily with responsiveness to directional stimuli. Similarly, optimal foraging theory places the major emphasis on the location of potential food items and hence aims to predict the direction taken during feeding excursions. However, foraging (and consequently feeding) occupies only a small part of day-to-day existence in the intertidal environment. Much time is spent resting or sheltering from environmental extremes and feeding excursions may occur only infrequently. As a result, some form of preliminary stimulus is required to initiate activity before directional stimuli can take effect.

Many workers consider gastropod activity to be restricted largely to periods of submergence at high tide. Kitting (1979, 1980) states that the plate limpet, *Aeolaea scutum* feeds only when submerged, stopping feeding while awash, even during the brief periods between successive waves. Similarly, Branch (1976) found that *Patella longicosta* and *P. oculus* remained inactive at low tide, commencing feeding on the incoming tide. Various other species of limpet, however, will continue to feed at low tide if the substratum retains a film of moisture (Kitting, 1979; Hawkins and Hartnoll, 1983).

Littorinid foraging is thought to be restricted predominantly to periods of submersion (Underwood, 1979). Wieser (1952) suggested that flat winkle

movements were synchronous with the rise and fall of the tide, while Newell *et al.* (1971) and Lubchenco (1978) observed a similar rhythm in common winkle activity. Foraging under suitable conditions at low tide has been recorded occasionally by Newell *et al.* (1971) and with greater frequency by Thandrup (1935). Newell (1958b) actually considered the receding tide to activate resting winkles.

Diurnal cycles in feeding activity coincident with changes in light intensity have also been observed. Evans (1965) reported that light was of major importance as a behavioural cue to *Littorina neritoides*, *L. saxatilis* and "*L. littoralis*". This was confirmed for the two sibling flat winkles by Reimchen (1974) who found that the majority of specimens were active at night, regardless of tidal level. Despite the largely sub-littoral range of the limpet *Acmaea virginea*, Clokie and Norton (1974) detected a very marked diurnal behaviour in this species, too, with foraging once again occurring predominantly at night. Surprisingly, the closely-related *Acmaea tessulata* - a species more common in the intertidal - did not exhibit diurnal behaviour.

A number of workers have suggested that rhythmical activity may be endogenous and not, in fact, induced directly by environmental factors (e.g. Fretter and Graham, 1967; Newell *et al.*, 1971). Thain (1971) found that *Littorina littorea* and *Gibbula* spp. retained the activity rhythm displayed on the shore, when transferred to laboratory aquaria under constant illumination. Similarly Sandeen *et al.* (1954) noted that the diurnal and tidal rhythms of *L. littorea* and *Urosalpinx cinerea* were persistent under artificial conditions.

A comprehensive survey of observations on the timing of gastropod foraging is presented in tabular form by Hawkins and Hartnoll (1983).

In this chapter littorinid response to the potential environmental stimuli of immersion/emersion and changing light intensity are examined in the field and under controlled conditions in the laboratory.

3.1. Methods

3.1.1. Laboratory studies

All experiments were carried out in a simple "simulated tide tank". This comprised a shallow perspex tank approximately 37 cm. long by 26 cm. wide, with a depth of 4 cm. An inlet pipe at one end was connected by a length of tubing to a carboy containing seawater. A tap on the carboy permitted control over the rate of inflow, while an outlet in the base of the tank enabled water to be drained as required. The lid was raised by approximately 4 mm. to allow adequate air circulation and was marked with a numbered grid of cm. squares.

The grid was used to assess movement of specimens throughout the experiments, avoiding the time-consuming and tedious procedure of recording exact paths. In ecological applications of this nature the grid method is frequently used, as it is often feasible to count the number of squares traversed but not to record precise movements.

In all experiments, snails were used within 1-2 days of collection on the shore.

a) Response to immersion/emersion

The response to immersion/emersion under "light" and "dark" regimes was tested in the simulated tide tank. The former experiments were carried out in a growth cabinet at a temperature of c. 10°C and a light intensity of c. 1.9 W.m⁻². All experiments involving a dark phase, were performed at room temperature in a dark room.

To examine response to immersion under illumination, the floor of the tank was moistened with seawater and ten specimens (numbered one to ten with waterproof white paint) were placed randomly in the tank and allowed to settle for 90 minutes. Subsequently, the position of each snail relative to the numbered grid (viewed from directly above) was recorded at five minute intervals for a period of 60 minutes. The tank was then flooded and movement was traced

for a further 80 minutes. The trial was repeated with a fresh batch of snails.

Response to emersion was tested in a similar manner. After settling for 90 minutes in the submerged state the positions of the snails were noted over a period of 60 minutes. The tank was then drained until empty and movement was traced for a further 80 minutes.

Assessment of activity under a "dark" regime was fundamentally the same. To permit movement to be observed and recorded in the dark, it was necessary to illuminate the tank periodically with infra-red light (I.R.) and to record the position of the snails using an IR-sensitive video camera (Fig. 6) connected to a video tape recorder (Fig. 7). Tapes were later played back on a television monitor and snail movement was measured on a grid covering the screen.

The camera, tape recorder and IR sources, were controlled by an automatic timer, operating for eight seconds at five minute intervals. The two IR light sources comprised 100W tungsten filament bulbs with plastic-based cinemoid filters (three green, three blue, three red). The short duration of illumination served to minimise any undesirable effect on behaviour, although there appeared to be no response to IR irradiation during preliminary trials.

b) Response to changing light intensity

The response to changes in light intensity was assessed for both submerged and emersed specimens. The incident light intensity was altered using a variac (variable transformer) operated manually. Each trial comprised three distinct phases: an initial light or dark phase, a phase of decreasing or increasing light intensity, and a subsequent dark or light phase. In all experiments the maximum light intensity during the light phase was approximately 6.0 W.m^{-2} .

The simulated tide tank was illuminated with infra-red light at five minute intervals during the dark phase of each trial and snail movements were recorded for analysis on the video tape recorder (as above). The IR light sources were

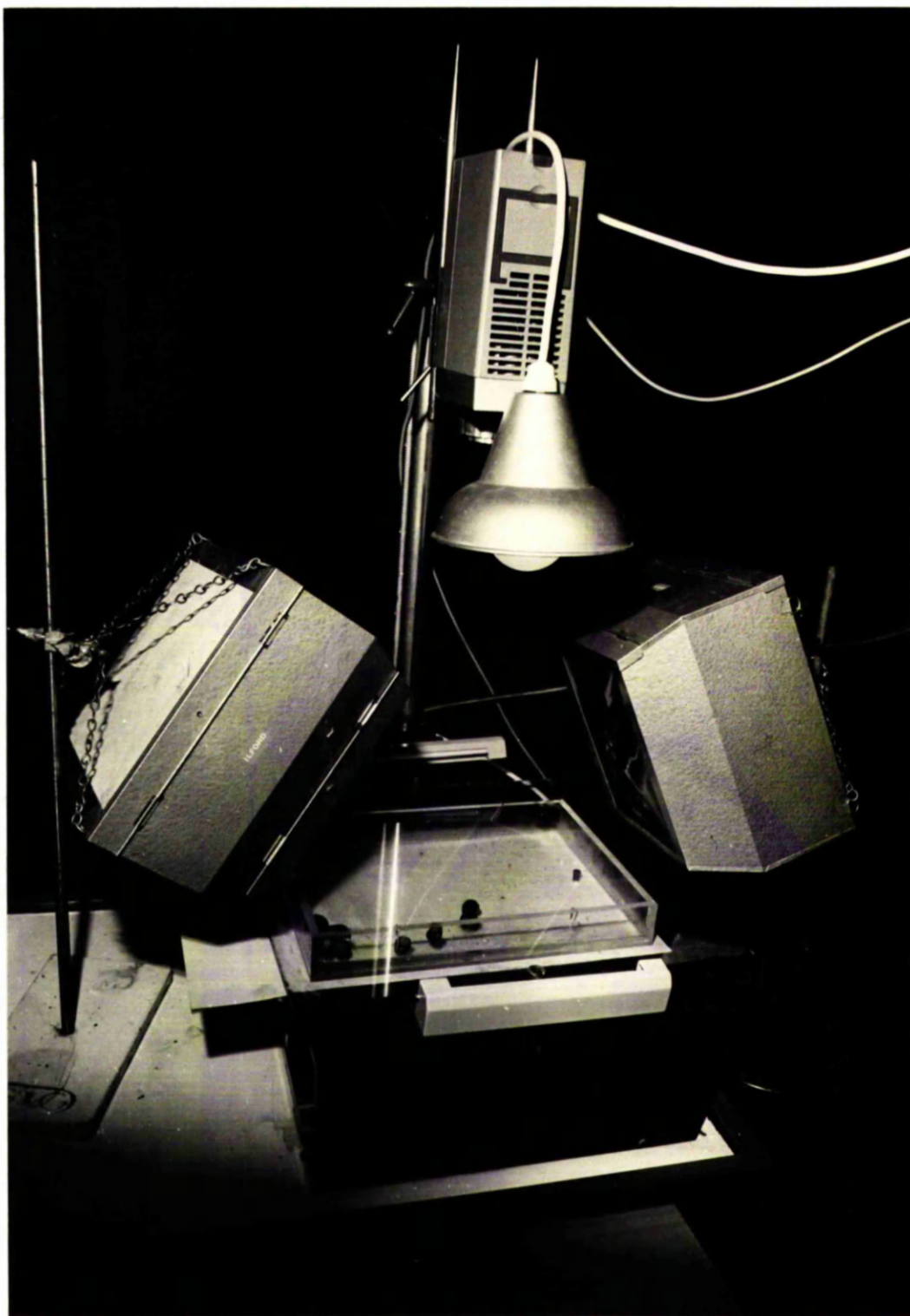


Fig. 6. IR-sensitive video camera recording snail movement in behavioural experiments. Picture also shows IR and visible light sources.



Fig. 7. Video tape recorder and television monitor.

disconnected during the light phase.

Prior to each experiment the trial specimens were permitted to settle for 90 minutes. Activity was then recorded during the initial light/dark phase for 60 minutes. Recording continued as the light intensity decreased gradually to nil or alternatively increased to maximum illumination. Finally, activity was assessed over a further 80 minutes in the dark/light phase. Each experiment comprised two trials, with two batches of ten snails.

3.1.2. Field studies

Response to immersion/emersion was assessed in the field for comparison with laboratory results. The level of littorinid activity at low tide was also examined. All observations were made at Butter Lump, Isle of Cumbrae.

a) Response to immersion/emersion

To investigate the response of the common winkle to submergence, several 0.5 m x 0.5 m quadrats were positioned in the upper *Ascophyllum* zone at low tide. Activity within each quadrat was recorded immediately before and after flooding by the rising tide. Observations were repeated on a receding tide to gauge response to emersion.

Initially it was intended to repeat these experiments with the flat winkle. However the small size and canopy-dwelling habit, rendered direct observation of submerged specimens virtually impossible. Consequently, a simple controlled experiment was devised to assess response to immersion in the field. A number of algal fronds with attached flat winkles, were gently severed from the parent plants at low tide and transferred to submerged sites. The level of activity was recorded after five minutes. Control flat winkles were transferred from one exposed site to another.

Results p(34)

) Dramatic increase
in activity
following immersion

Upsurge with inc.
light.

Dedine at maximum.

) No response to
decreasing light.

b) Activity at low tide

The level of activity in flat winkle and common winkle populations was examined at low tide. Observations were made approximately half an hour and three hours after exposure by the receding tide. Conditions were mild and dry, but overcast.

3.2. Results

3.2.1. Laboratory studies

The results of the controlled environment experiments carried out in the simulated tide tank are presented in Figs. 8-15. Mean movement and % activity are shown for each experiment.

a) Response to immersion/emersion (Figs. 8-11)

The littorinid response to immersion was marked under both light and dark regimes. There was invariably a dramatic increase in activity either during or immediately following immersion and this was reflected in both mean movement and % activity. There was also some indication of a subsequent decline in activity 45-50 minutes after immersion.

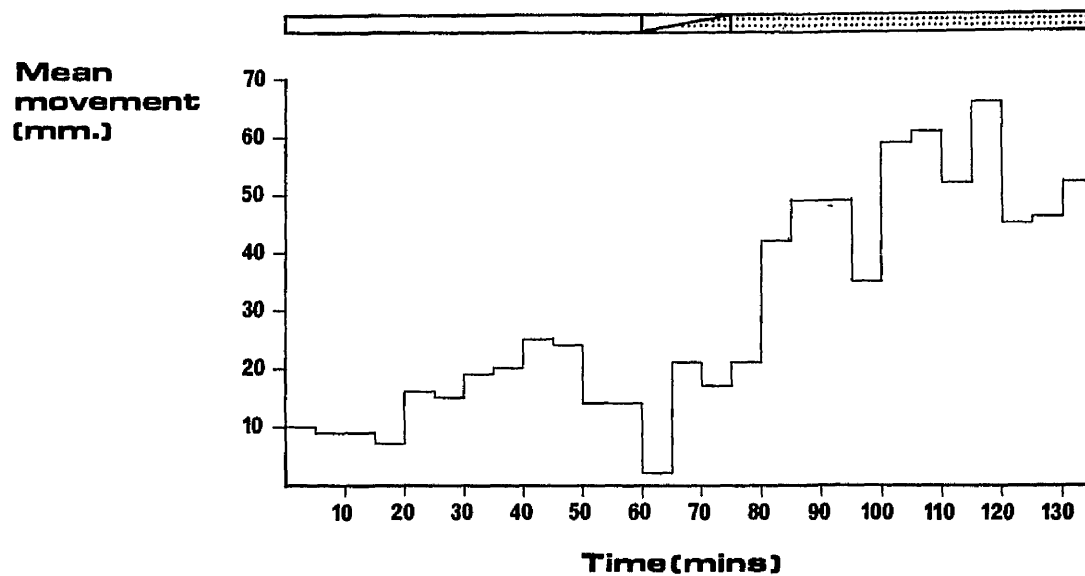
The response to emersion was even more marked, activity falling rapidly in both experiments following drainage of the tank.

b) Response to changing light intensity (Figs. 12-15)

Increasing the incident light intensity provoked two characteristic responses: an upsurge in activity coincident with increasing intensity; and a very marked decline in activity at the maximum light intensity (6.0 W.m^{-2}). The former trend was clearcut when the specimens were submerged (Fig. 12) confirming Newell's (1958c) observations but was obscured to a certain extent with emersed specimens (Fig. 13), as the overall level of activity was much lower.

There was no clearly discernible pattern of response to decreasing light

a.



b.

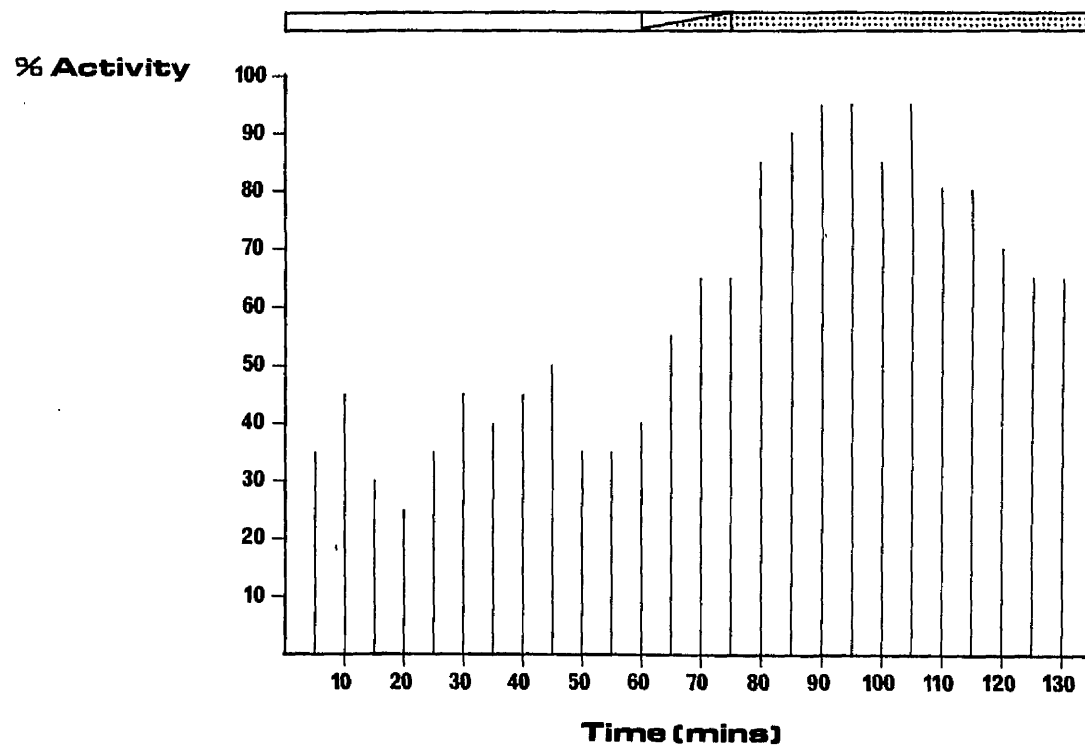


Fig. 8 Response to immersion (light)

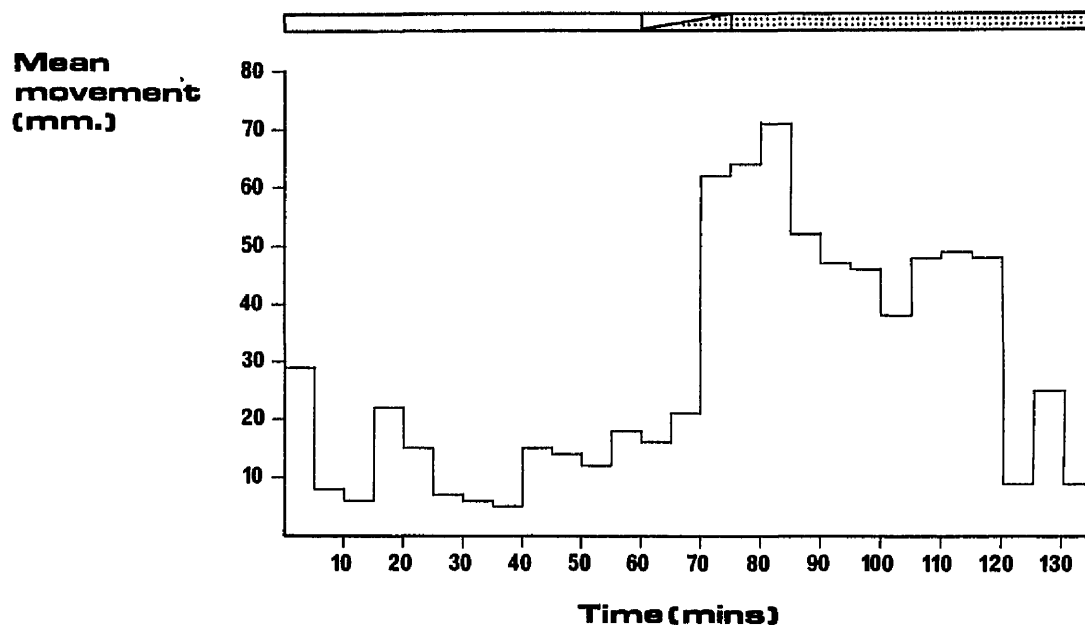
Stippled bar represents immersion, white bar represents emersion

a) Mean movement

b) % activity

Kruskal-Wallis one way analysis of variance reveals significant variation in mean movement ($P < 0.001$) and in % activity ($P < 0.001$) over the three phases of the experiment.

a.



b.

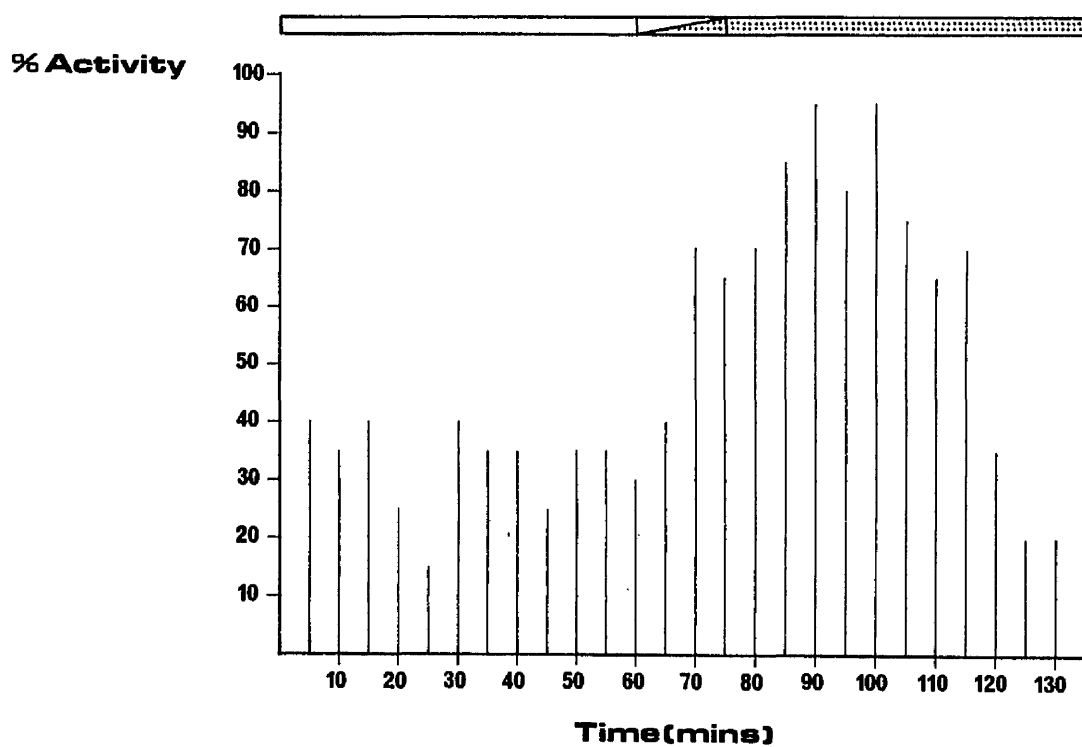


Fig. 9 Response to immersion (dark)

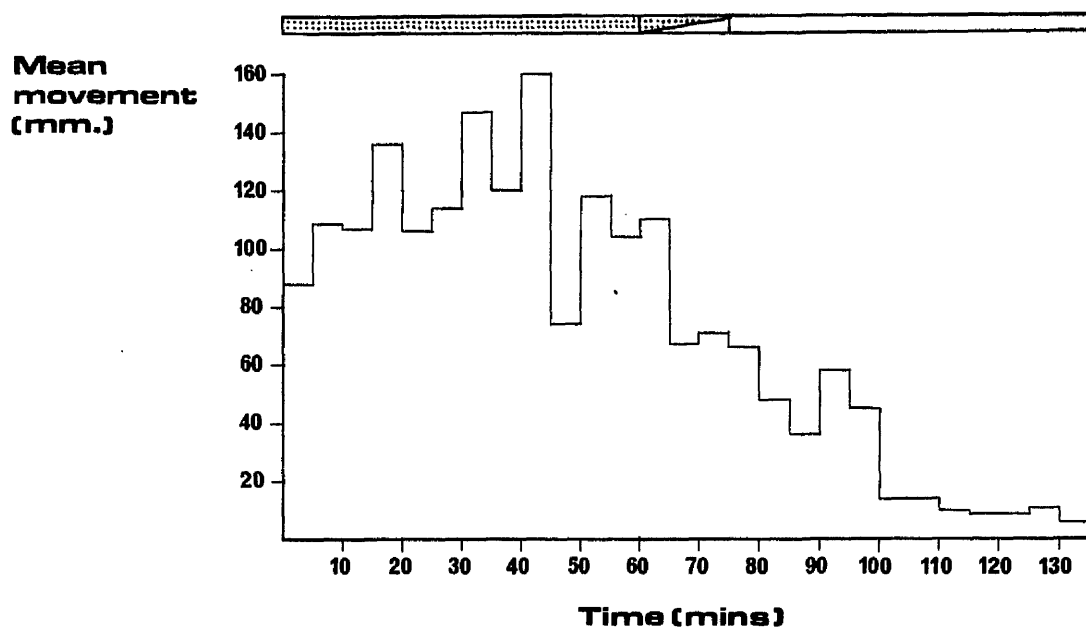
Stippled bar represents immersion, white bar represents emersion

a) Mean movement

b) % activity

Kruskal-Wallis one-way analysis of variance reveals significant variation in mean movement ($0.001 < P < 0.01$) and in % activity ($0.01 < P < 0.025$) over the three phases of the experiment.

a.



b.

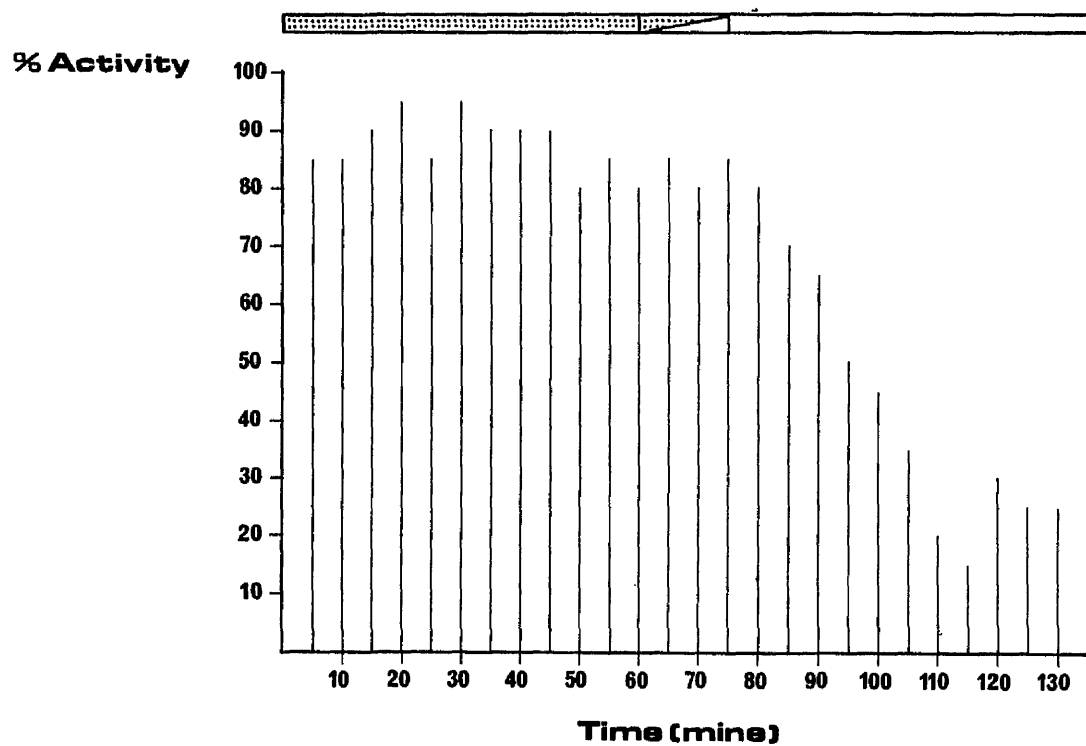


Fig. 10 Response to emersion (light)

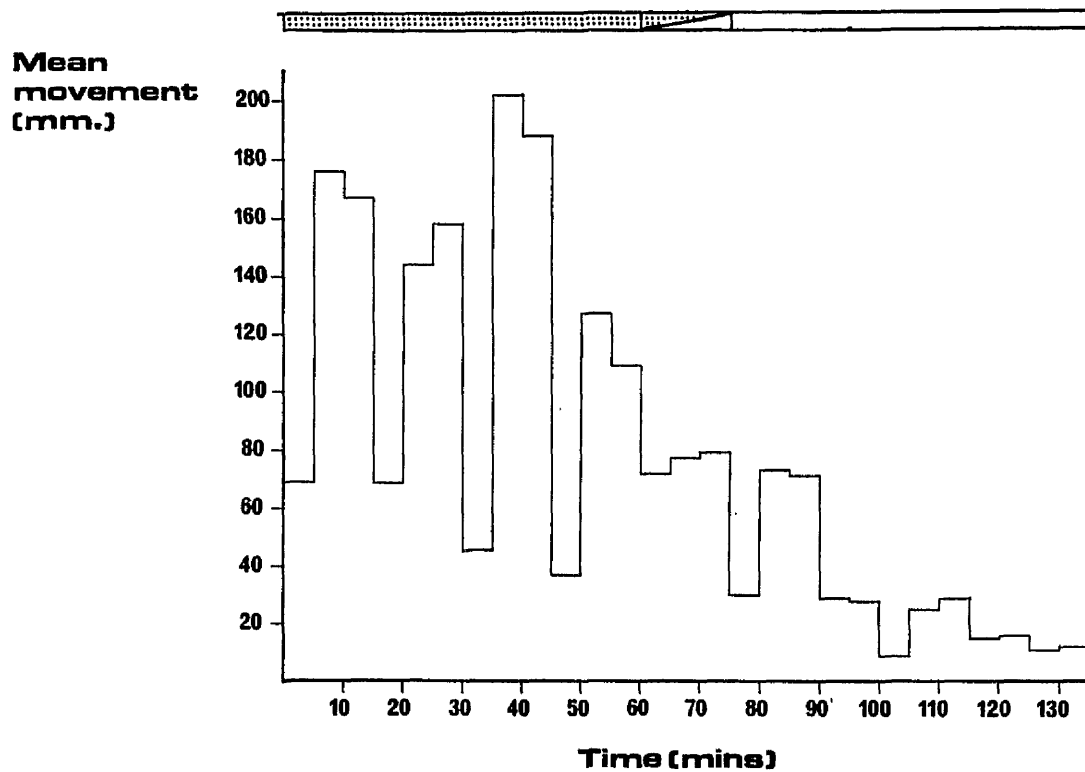
Stippled bar represents immersion, white bar represents emersion

a) Mean movement

b) % activity

Kruskal-Wallis one way analysis of variance reveals significant variation in mean movement ($P < 0.001$) and in % activity ($P < 0.001$) over the three phases of the experiment.

a.



b.

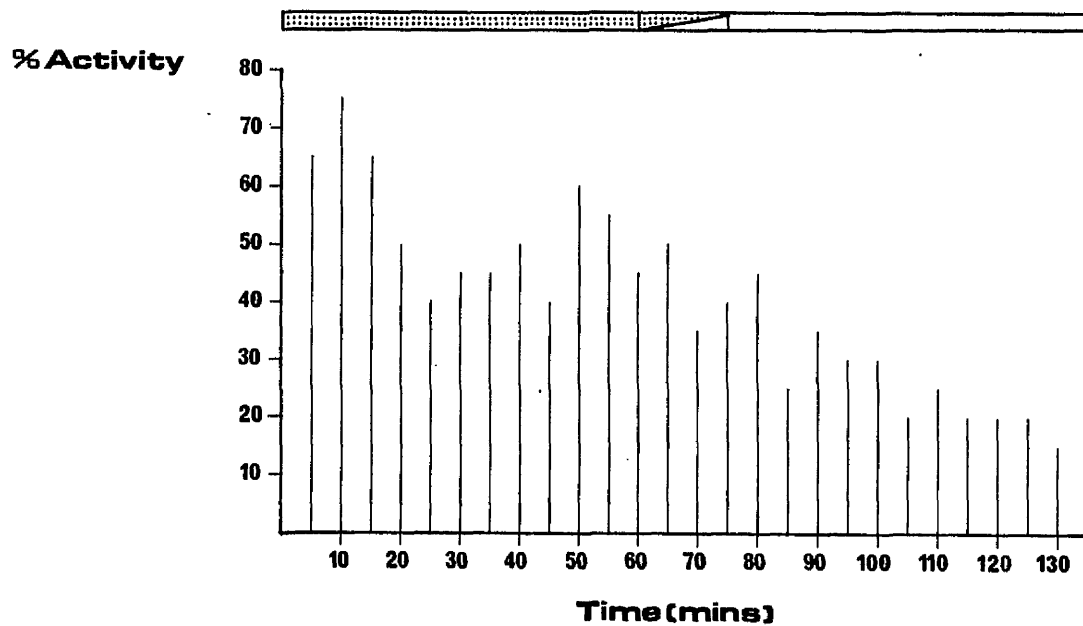


Fig. 11 Response to emersion (dark)

Stippled bar represents immersion, white bar represents emersion

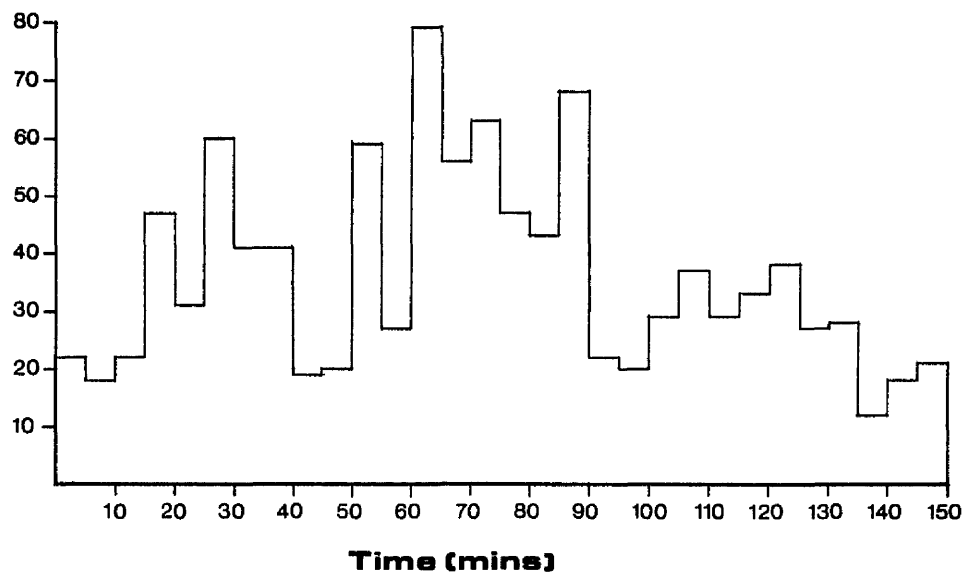
a) Mean movement.

b) % activity

Kruskal-Wallis one way analysis of variance reveals significant variation in mean movement ($P < 0.001$) and in % activity ($P < 0.001$) over the three phases of the experiment.

a.

**Mean
movement
(mm.)**



b.

% Activity

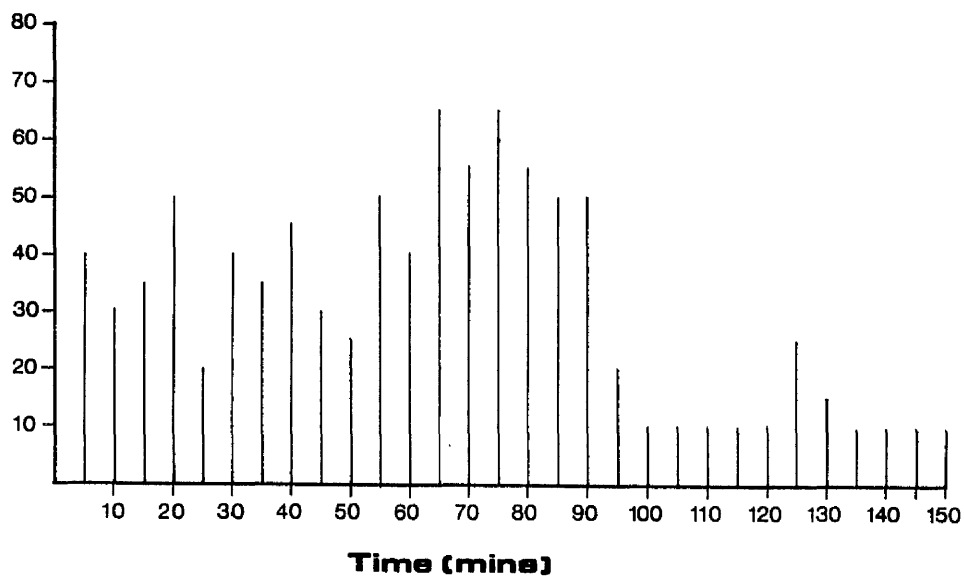


Fig. 12 Response to increasing light intensity (submerged)

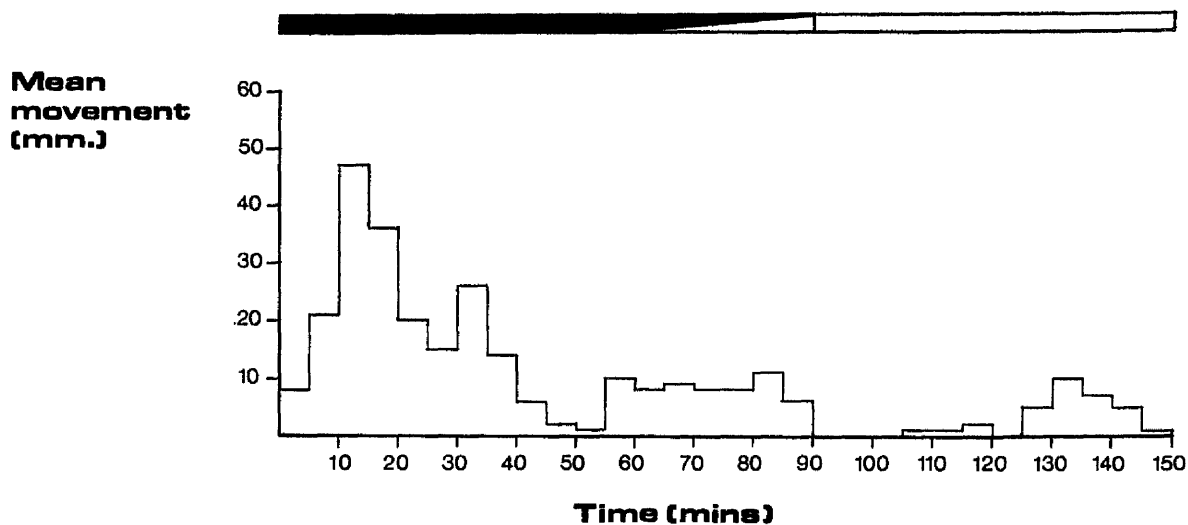
Black bar represents dark phase, white bar represents light phase.

a) Mean movement

b) % activity

Kruskal-Wallis one way analysis of variance reveals significant variation in mean movement ($0.001 < P < 0.01$) and in % activity ($P < 0.001$) over the three phases of the experiment.

a.



b.

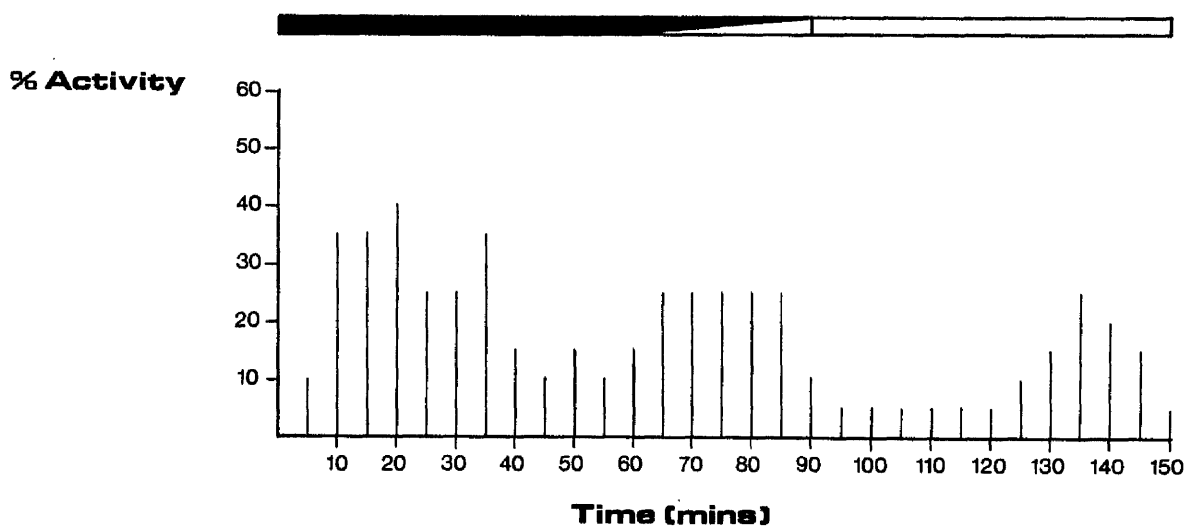


Fig. 13 Response to increasing light intensity (emersed)

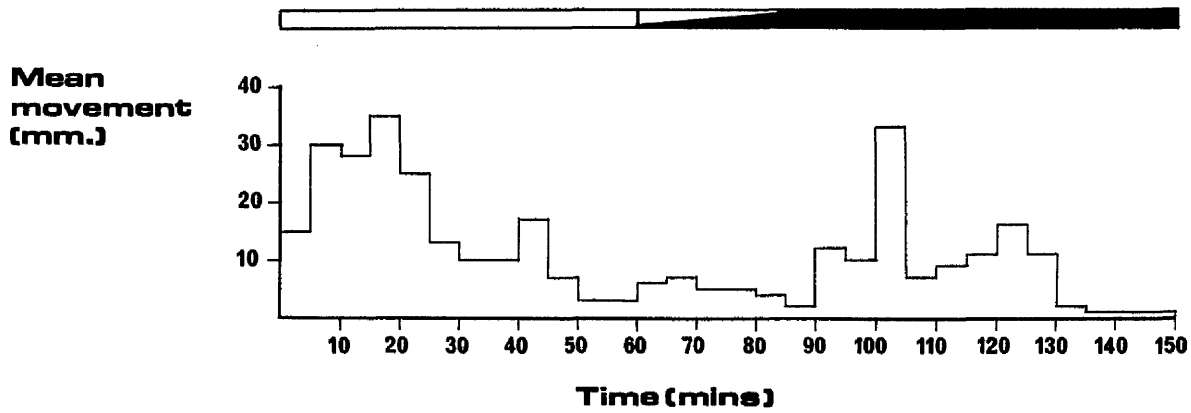
Black bar represents dark phase, white bar represents light phase.

a) Mean movement

b) % activity

Kruskal-Wallis one way analysis of variance reveals significant variation in mean movement ($P < 0.001$) and in % activity ($0.001 < P < 0.01$) over the three phases of the experiment.

a.



b.

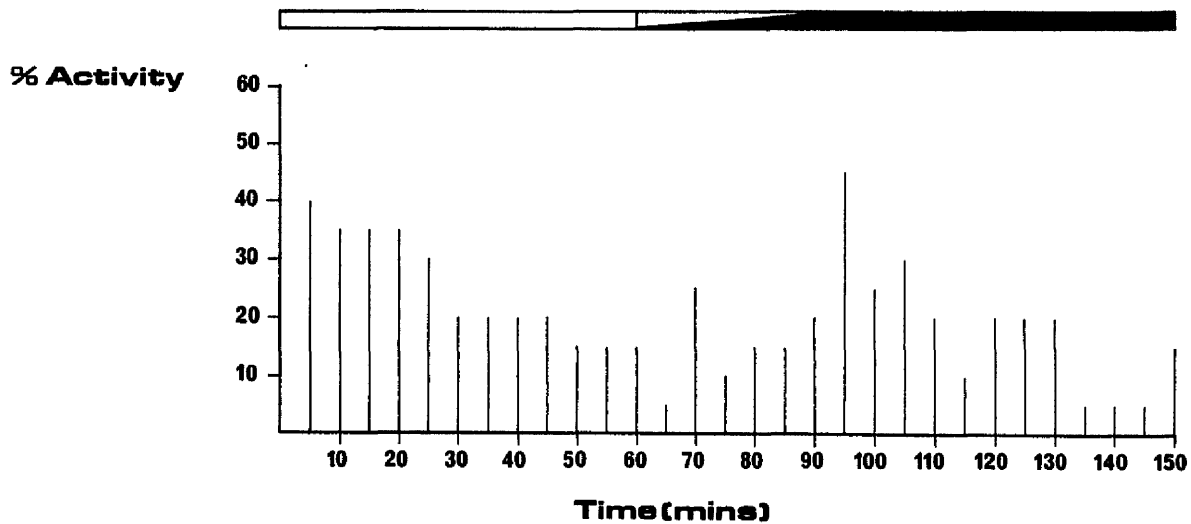


Fig. 14 Response to decreasing light intensity (submerged)

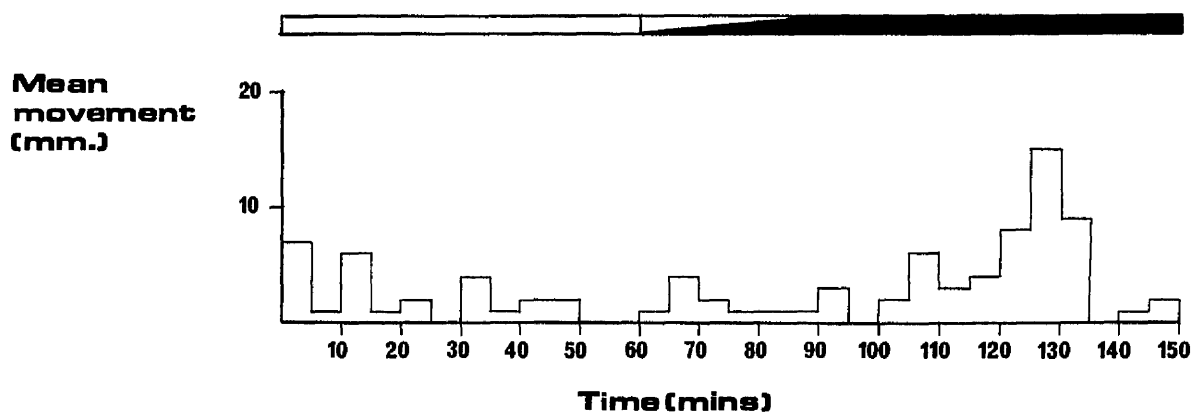
Black bar represents dark phase, white bar represents light phase.

a) Mean movement

b) % activity

Kruskal-Wallis one way analysis of variance reveals significant variation in mean movement ($0.025 < p < 0.05$) over the three phases of the experiment. % activity did not vary significantly.

a.



b.

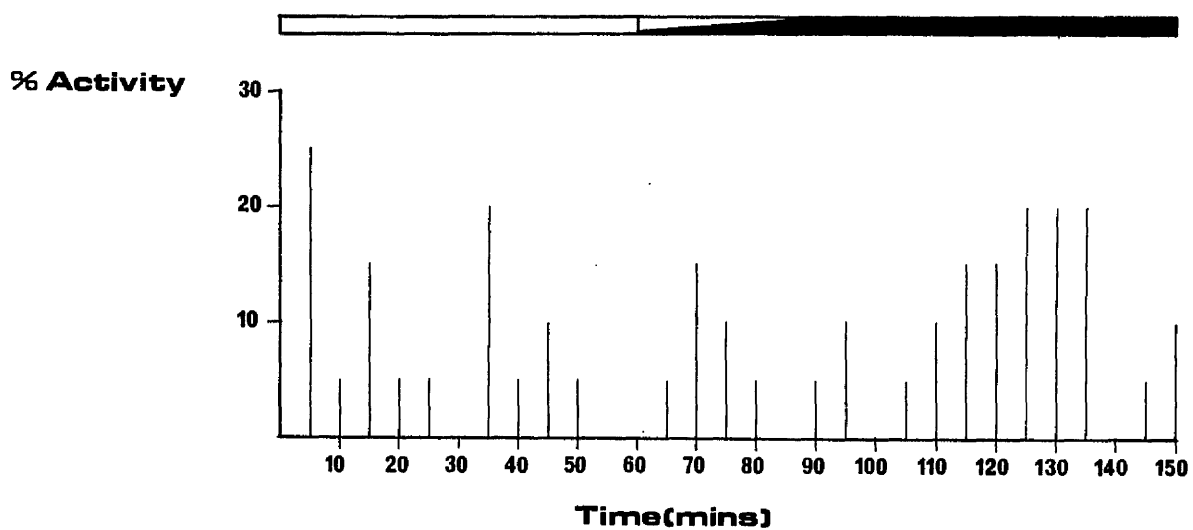


Fig. 15 Response to decreasing light intensity (emersed)

Black bar represents dark phase, white bar represents light phase.

a) Mean movement.

b) % activity.

Kruskal-Wallis one way analysis of variance reveals no significant variation in mean movement or % activity over the three phases of the experiment.

intensity, although some indication of an increase in activity at the onset of the dark phase, was apparent (Figs. 14, 15).

3.2.2. Field studies

The results are summarised in Tables 7-10.

a) Response to immersion/emersion (Tables 7-9)

Immersion illicited a dramatic response from both common and flat winkles. Common winkle activity increased from 1% to 62% within ten minutes of submergence (Table 7). Similarly, flat winkles transferred from dry to submerged sites (Table 8) achieved a high level of activity (83%).

This trend was reversed as the tide receded, common winkle activity dropping from 57% (submerged) to 16% (emersed) over a 15-20 minute time interval (Table 9).

b) Activity at low tide (Table 10)

Activity dropped rapidly following exposure by the receding tide, eventually reaching 2% (common winkle) and 15% (flat winkle) after three hours emersion. Flat winkle activity was generally higher than activity amongst common winkles.

Table 7. Response of common winkle to immersion by rising tide.

<u>State of quadrat</u>	<u>N</u>	<u>% Activity</u>
Uncovered	180	1
Submerged (0-5 mins)	171	44
Submerged (5-10 mins)	169	62

Table 8. Response of flat winkle to simulated rising tide.

	N	% activity
Control specimens	200	19
Experimental specimens	200	83

Table 9. Response of common winkle to emersion by falling tide.

State of quadrat	N	% activity
Covered	176	57
Uncovered (0-5 mins)	179	21
Uncovered (5-10 mins)	184	16

Table 10. Winkle activity at low tide.

Duration of exposure	% Activity	
	Common winkle	Flat winkle
25-35 mins	8 (N=393)	29 (N=389)
3 hours	2 (N=279)	15 (N=365)

3.3. Discussion

The results of work in the laboratory and in the field permit several conclusions to be drawn regarding littorinid behavioural responses:

- 1) Under controlled conditions, immersion and increasing light intensity stimulate activity. There is a corresponding increase in activity coincident with immersion on the shore.

- 2) Emersion in the laboratory and in the field, is followed by a rapid decline in activity. There was no evidence to support Newell's (1958b) contention that emersion acts as a stimulus to crawling.
- 3) Activity remains low during exposure at low tide but does not cease altogether.
- 4) Activity is depressed at high light intensities.

Whether behaviour on the shore is governed primarily by an endogenous rhythm or by environmental stimuli, the common winkle will respond directly to changes in environmental parameters. Under artificially controlled conditions in the laboratory, behavioural responses to increases in light intensity and to the wetting/drying effects of immersion/emersion are incontrovertible.

Diurnal activity has been observed in many species of intertidal prosobranchs, active foraging occurring predominantly during the hours of darkness (e.g. Clokie and Norton, 1974; Reimchen, 1974; Connor, 1975; Branch, 1981). Consequently it is somewhat surprising to find no clearcut behavioural response to decreasing light intensity in *Littorina littorea*. If common winkle activity does increase at night, then the snails would appear to be responding to an endogenous stimulant, or to temperature or humidity changes and not primarily to light intensity as is often assumed.

Littorinid activity undoubtedly occurs predominantly when submerged. In controlled experiments, specimens responded directly to immersion and to emersion in the simulated tide tank. It is possible, however, that environmental stimuli are reinforced in the field (as Sandeen *et al.* (1954) and Thain (1971) have suggested) by an endogenous activity rhythm corresponding to the rise and fall of the tide. During laboratory experiments, specimens were frequently observed to undertake excursions without being subjected to any form of outside stimulus. Furthermore, there was a considerable degree of variability in the overall level of activity recorded in separate trials carried out at different times.

Although foraging is most intensive at high tide, activity is unlikely to be

continuous throughout submergence (see Barkman, 1955; Newell, 1958b, 1958c). Newell (1958b) concluded that common winkles probably undertook feeding excursions only at relatively infrequent intervals, while Kitting (1980) found that *Acmaea scutum* fed most intensively during the hour immediately following submergence. In this study there was a distinct decline in common winkle activity 45-50 minutes after immersion in the simulated tide tank.

Nevertheless, the numerous attempts to explain the survival of upper-shore prosobranchs in terms of elevated radular rasping rates are undoubtedly prompted by the assumption that foraging is continuous during submergence and that upper-shore animals consequently have less "feeding time" available during each tidal cycle than their counterparts lower down the shore (e.g. Newell *et al.*, 1971). However Zeldis and Boyden (1979) found that differences in submergence time were balanced not by variations in rasping rate but by more intensive and consistent activity on the upper shore. The radular rasping rate of the trochid, *Melagraphia aethiops*, varied little over the entire vertical range of the species. However high shore specimens fed fairly consistently throughout immersion, while low-shore *Melagraphia* fed discontinuously, periods of rhythmical rasping alternating with periods of quiescence.

Although foraging does occur predominantly during high tide, field observations indicate some degree of activity during emersion. The precise level of activity may depend largely on the prevailing climatic conditions and the degree of shelter provided locally by the microhabitat. Both Thandrup (1935) and Lubchenco (1978) observed common winkles foraging during cool, humid low tides, while Kitting (1979) found *Acmaea limatula* feeding in damp, shaded crevices when exposed to the air.

The level of activity at low tide was invariably highest amongst flat winkles. *Littorina obtusata* and *L. mariae* both tend to migrate as the tide recedes, towards the centre of the dense fucoid clumps in which they are habitually found (pers. obs.; Wieser, 1952). In the interior of the clumps the algal thallus maintains a surface film of moisture for a longer period of time, permitting foraging to continue after the tide has receded.

CHAPTER 4 : FEEDING

Not all algae are potential food for all herbivorous molluscs. What can and cannot be consumed will be determined ultimately by the capabilities of the feeding apparatus. Within these limitations, preference will be given to those species and age - classes which can be ingested readily. Consequently, careful consideration of the structure and functioning of the feeding apparatus is essential if molluscan food preferences are to be understood fully.

The remarkable adaptive radiation of the Gastropoda, which allows the class to utilise a tremendous variety of ecological niches - both aquatic and terrestrial - is based largely on adaptations to the feeding apparatus. Although the buccal mass and the radula are invariably retained, the form and function of the radula and associated musculature is highly plastic (Purchon, 1968). This facilitates feeding on a wide variety of food substrates and involves numerous very different feeding strategies, ranging from herbivory and plankton feeding, to scavenging and carnivory.

Consequently, the composition and structure of the radula and buccal mass, is directly related to the type of food which the snails consume. This applies equally well, though on a different scale, between two species of herbivore as between herbivore and carnivore. Hence the study and comparison of radular structure and functioning may yield valuable information regarding grazing capabilities and limitations.

Herbivorous molluscs feed in two fundamentally different fashions (Steneck and Watling, 1982). Feeding may involve the ingestion of the entire food plant or intact portions of the plant, or alternatively, the evacuation of the cell contents with a radula modified for sucking. Here, we are concerned only with species using the radula to ingest algal tissue of cell size or larger. The chapter will review radular structure and functioning with particular emphasis on those features influencing foraging techniques and the allocation of food resources between species living in close proximity on the shore. Comparison

will be made between the mesogastropod littorinids and the contrasting archaeogastropod limpets, *Patella* and *Acmaea*. Existing knowledge is analysed critically and fresh work presented.

4.1. Methods

Radulae were prepared for microscopic examination by rinsing in a dilute solution of sodium hypochlorite to remove debris. Specimens for viewing under the scanning electron microscope were subsequently dried in a graded acetone series (30%, 50%, 70%, 90%) and stub-mounted. The Fischer-Piette (1935) technique was used to mount the patellid radulae for viewing under the light microscope. *Patella depressa* radulae were collected at Plymouth by S.J. Hawkins (Manchester University).

A series of sample minerals with known values on Mohs' scale of (empirical) hardness were ground down to provide smooth surfaces and set in epoxy resin. Table 11 lists the minerals and their corresponding hardness values. Radular hardness was assessed by drawing specimen radulae across the surface of each mineral in turn and subsequently examining all samples under a binocular microscope using both reflected and incident light. The hardness of each radula equalled or exceeded that of any mineral scratched by the denticles.

Observations on the feeding cycle of *Patella* and *Littorina* are based on sequential photographs of the radular "stroke" taken through the side of a perspex tank, and on dissection of the dead animals. Though feeding could be observed more clearly through glass, the grazing stroke was inhibited as the teeth were unable to grip the smooth surface. The photographs were taken using a Nikon camera with motor drive, in conjunction with a Sunpak 3400 Thyristor Flash (1:16 power ratio). The lens was a Nikon 35 mm wide angle lens used in reverse on an M ring extension tube. The auto-diaphragm was function operated by a cable release. Three pictures were taken per second. Consequently, to construct a detailed sequence of the entire grazing stroke, it was necessary to use photographs taken during a number of successive strokes.

TABLE 11. Minerals used to assess radular hardness

<u>Mineral</u>	<u>Hardness (Mohs' Scale)</u>
Gypsum	2.0
Halite	2.0-2.5
Biotite	2.5-3.0
Calcite	3.0
Barytes	3.0-3.5
Fluorite	4.0
Hemimorphite	4.5-5.0
Horneblende	5.0-6.0
Orthoclase	6.0

Limpet grazing traces were obtained readily on perspex plates. It proved more difficult, however, to find a medium on which littorinid grazing could be induced, and clear traces obtained. Trials with paraffin wax, agar (after Eigenbrodt, 1941) and animal fat (after Ziegler, 1910) were relatively unsuccessful. Eventually, satisfactory prints were obtained on glass microscope slides covered with a fine layer of soot. To enable the slide to be immersed in seawater, the soot was covered with a thin protective film of wax.

4.2. Structure of the feeding apparatus

The molluscan radula is one of the major hallmarks of the phylum. It is situated in the gastropod buccal cavity, a musculo-cutaneous tube with an epithelial and cuticular lining, and extends forwards across the mid-dorsal surface of the odontophore or buccal mass. The basal membrane of the radula is fused to the sub-radular membrane which covers the surface of the odontophore (Fretter and Graham, 1962).

In the archaeogastropod limpets, *Patella* and *Aemaea*, the buccal mass is a remarkably robust and powerful structure, strengthened by five pairs of cartilage (Fretter and Graham, 1962) and manipulated by muscles inserted into the body wall of the head (Purchon, 1968). A hypertrophy of the muscles enables the radula to be pressed against the substratum with considerable force (Fretter and Graham, 1962). The anterior cartilages are arranged to provide a shock absorber, damping the powerful vibrations of the radula which inevitably arise from contact with the substratum and possibly also the jaw (Nisbet, 1953). In contrast, the odontophore of the mesogastropod, *Littorina* is a much less elaborate structure and is controlled by two pairs of cartilage (Fretter and Graham, 1962).

In the roof of the buccal cavity anteriorly, *Patella* possesses a single, protruding, crescentic jaw (Fig. 21C). Structurally this comprises a cartilaginous thickening of the buccal wall, the epithelium of which is cuticularised and appears to be chitinous (Fretter and Graham, 1962). In the

archaeogastropod *Monodonta lineata*, Nisbet (1953) found that the material forming the jaws resembled that of the buccal cartilages, though the cells of the jaw skeleton were smaller and less vacuolated. The patellid jaw has a straight edge with no serrations. No jaw is present in either the acmaeids or the littorinids.

The structure actively involved in food gathering is the radula. During grazing, the radula functions rather like a conveyor belt. It consists of a ribbon with a continuous cuticular base of chitin and protein, bearing successive transverse and longitudinal rows of teeth. These are formed at the inner end of the radular sac and gradually move forward onto the dorsal surface of the odontophore as the teeth at the front of the radula become worn and broken. The radular sac itself comprises a blind diverticulum which lies coiled in a spiral dorsal to the oesophagus, on the right hand side of the body. Specialised cells at the innermost end, termed odontoblasts, secrete the teeth, while the cells forming the epithelium of the dorsal wall impregnate the denticles with inorganic salts, particularly iron and silicon, and are ultimately responsible for the chemical constitution and physical consistency of the functional teeth (Prenant, 1924). Though the precise nature of these salts varies between species, the basic composition of the radula probably remains substantially the same (Fretter and Graham, 1976).

4.2.1. Radular Structure

For any given species, each transverse row of denticles is normally identical with respect to both the number and the shape of teeth. The single, central tooth, the rachidian, is flanked on either side by a series of laterals which typically diminish in size from the mid-line laterally, forming two distinct groups: intermediates nearest to the rachidian, and marginals towards the outside of the radula. The most prominent lateral tooth is termed the dominant. As radular structure is a feature of considerable taxonomic importance, for most genera gross structure has been described thoroughly.

Fig. 16. Radular morphology.
(Scanning electron microscope)

- A. *Patella vulgata* x704
- B. *Acmaea tessulata* x763
- C. *Littorina littorea* x704
- D. *Littorina obtusata* x763

r = rachidian tooth.
i = intermediate lateral tooth.
m = marginal lateral tooth.
im = inner marginal tooth.
om = outer marginal tooth.
d = dominant lateral tooth.



However, less apparent intra-generic differences in radular form have received little attention despite their possible significance in the partitioning of food resources between sympatric species.

The gastropod molluscs are frequently sub-divided according to the structure of the radula. The rhipidoglossan Zeugobranchia, Trochacea and Neritacea almost certainly possess the most primitive radula. Structurally, the rhipidoglossan radula is the most complex of all prosobranch radulae, possessing a distinct rachidian tooth, on either side of which lies a fan of smaller teeth composed of five intermediates and a vast array of needle-like marginals. The docoglossan radula of the limpets and the taenioglossan radula of the littorinids are often considered to be derived from the rhipidoglossan form (e.g. Fretter and Graham, 1962).

a) The docoglossan radula (see Table 12)

The archaeogastropod limpets *Patella* and *Acmaea*, are characterised by radulae of docoglossan structure (Fig. 16 A,B). In the patellids, Fretter and Graham (1962) state that a rachidian tooth is present but diminished. However my own scanning electron microscope studies of *Patella vulgata*, *P. aspera* and *P. depressa* (see Jones *et al.*, in press) show no evidence of a rachidian tooth, however vestigial. The apparent tooth visible by light microscopy would appear to be a shadow or refraction artefact.

Each transverse row is composed of four well-developed intermediate teeth flanked on either side by a large, dominant pluricuspid marginal tooth. On the periphery of the radula, three inconspicuous and much reduced outer marginal teeth occur.

The intermediate and dominant marginal teeth all possess an unusually broad, non-linear base of attachment to the radular membrane (pers. obs.). This effectively restricts the movement of the teeth and influences the mechanics of docoglossan feeding profoundly. In all other prosobranch molluscs the base of attachment is always approximately linear (pers. obs.; Steneck and Watling, 1982).

TABLE 12. The Docoglossan Radula





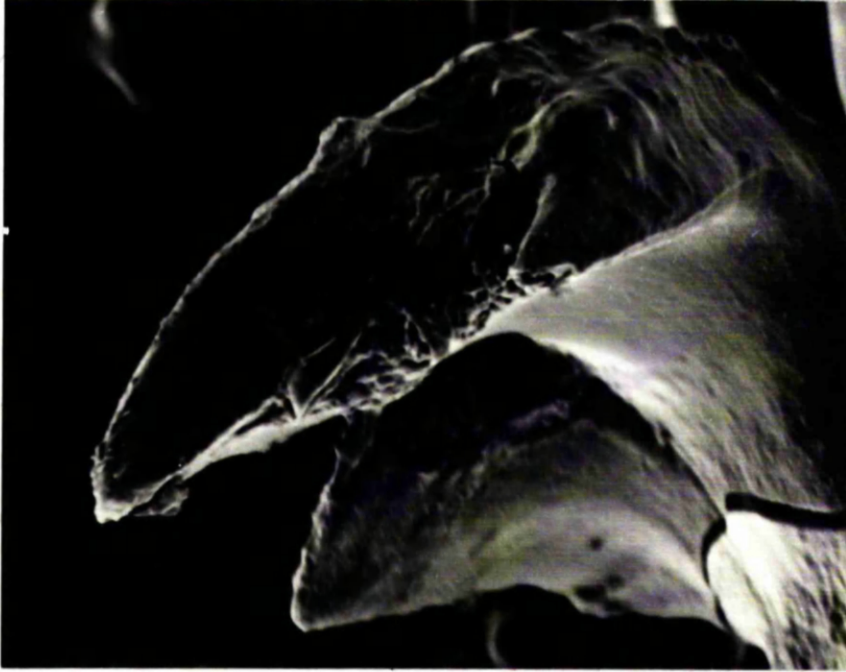
Species	Number of teeth per transverse row			Outer marginal	Diagrammatic representation of dominant marginal tooth
	Intermediate	Inner marginal	Outer marginal		
<i>Patella aspera</i>	4	2	6 (Possibly non-functional)	outer cusp	
<i>Patella depressa</i>	4	2	6 (Possibly non-functional)	outer cusp	
<i>Patella vulgata</i>	4	2	6 (Possibly non-functional)	outer cusp	
<i>Acmaea tessulata</i>	2	2	0	outer cusp	

Fig. 17. Patellid tricuspid teeth.
(Scanning electron microscope)

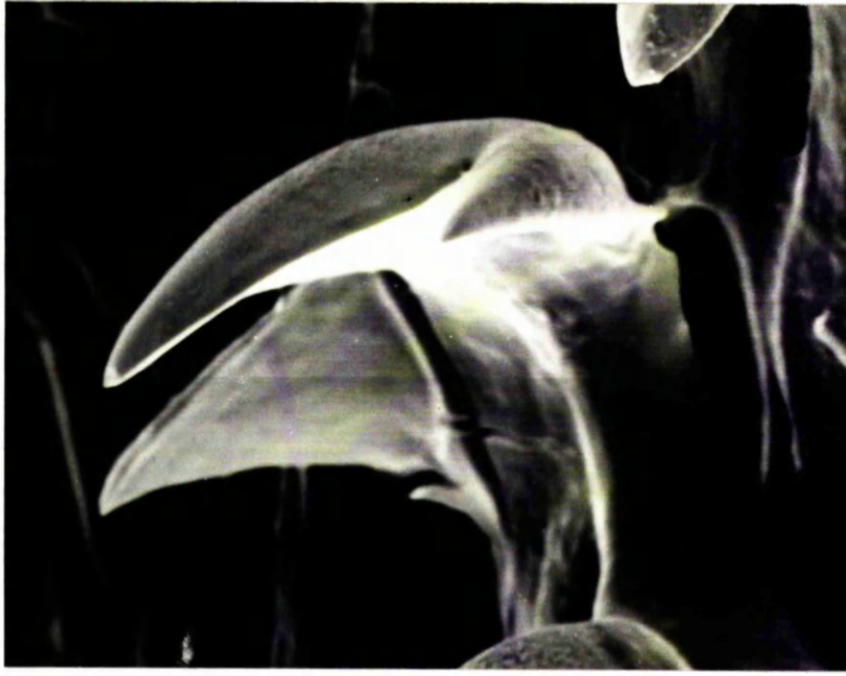
- A. *Patella aspera* x 1680
- B. *Patella depressa* x 3360
- C. *Patella vulgata* x 1240



A.



B.



C.

Close examination of the tricuspid marginal teeth of *Patella vulgata* and the sibling species *P. aspera* and *P. depressa* reveals several distinct differences (Fig. 17 and Table 12; see also Fischer-Piette, 1935). In all three species the inner cusp is small. The two outer cusps of *P. vulgata* are slender and straight-sided while those of *P. aspera* and *P. depressa* are broad with distinctly convex sides. In *P. vulgata* and *P. depressa* the outer cusp is shorter than the middle cusp. This trend is reversed in *P. aspera*. Despite chemical hardening of the denticles in all species, grazing on the rock surface frequently leads to tooth breakage (pers. obs.).




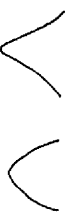








The radula of the acmaeids (Fig. 16B) is, in appearance, very different. The outer marginal teeth have disappeared completely and the number of intermediate teeth in each transverse row is reduced to two, the dominant marginal being bicuspid with a large inner cusp and a small outer cusp. All the teeth are aligned perpendicularly to the substratum and are distinctly blunt and shovel-like compared with the slender pointed denticles of *Patella* (pers. obs.).

Fretter and Graham (1962) suggest that the general format of the docoglossan radula may be derived, either by reduction or loss of the marginal and rachidian teeth, from the less specialised polyodont rhipidoglossate type. The pluricuspid dominant tooth may arise from the fusion of several marginals.

b) The taenioglossan radula (see Table 13)

In contrast to the docoglossans, the rachidian tooth on the taenioglossan littorinid radula is both prominent and functional and is flanked on either side, by a single intermediate tooth and two marginals (Fig. 16 C,D). Although this configuration may also be derived from the rhipidoglossan form through fusion or loss of the marginal teeth, the basic structure and mechanics of the taenioglossan radula are very different from those of the docoglossans. The wide basal plates which reinforce the basal membrane of the limpet radula do not occur, and as a result, the basal membrane and the individual teeth attain a high degree of mobility (pers. obs.).

TABLE 13. Features of the taenioglossan radula

Species	Rachidian Tooth		Intermediate Tooth		Inner marginal tooth		Outer marginal tooth	
	Shape of tooth		Shape of dominant cusps		Shape of dominant cusps		Shape of dominant cusps	
<i>Littorina littorea</i>								
<i>Littorina obtusata</i>								
<i>Littorina mariae</i>								

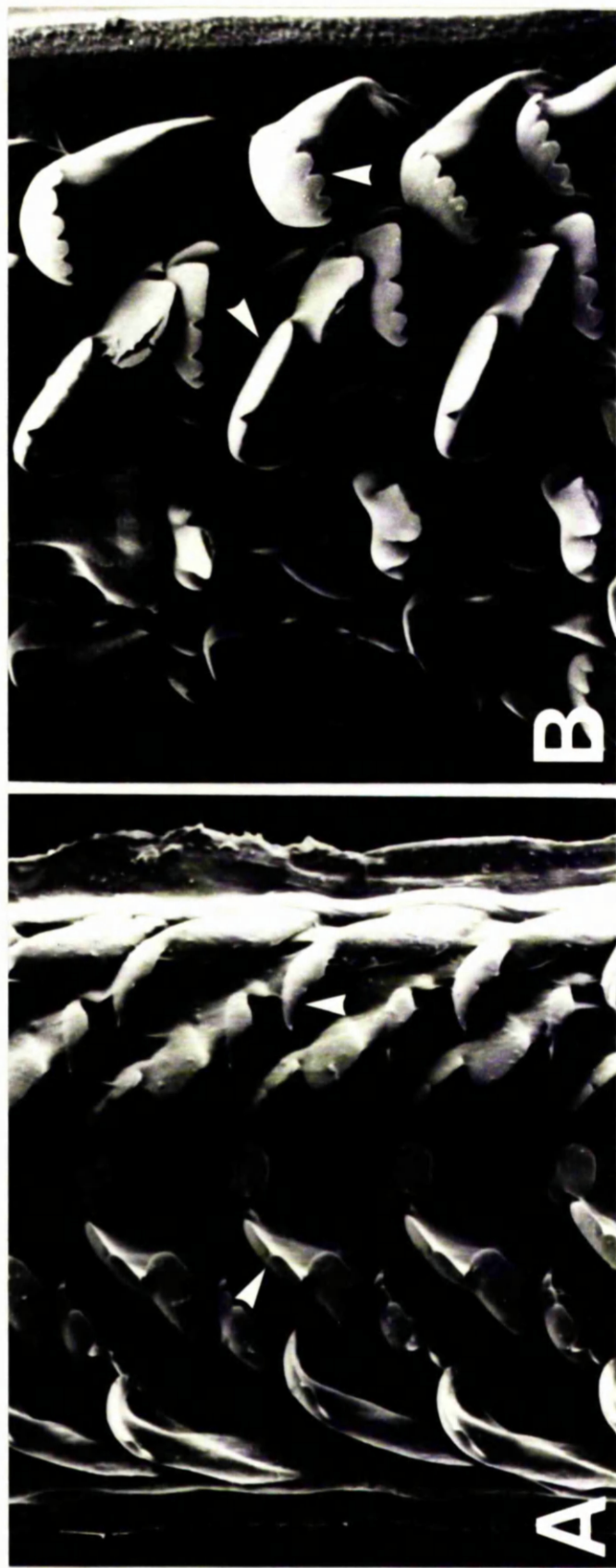


Fig. 18. Morphological variation in flat winkle radulae.
(Scanning electron microscope)

A. *Littorina obtusata* x960.

B. *Littorina mariae* x1920.

Pointers indicate differences in shape and number of cusps on intermediate and outer marginal teeth.

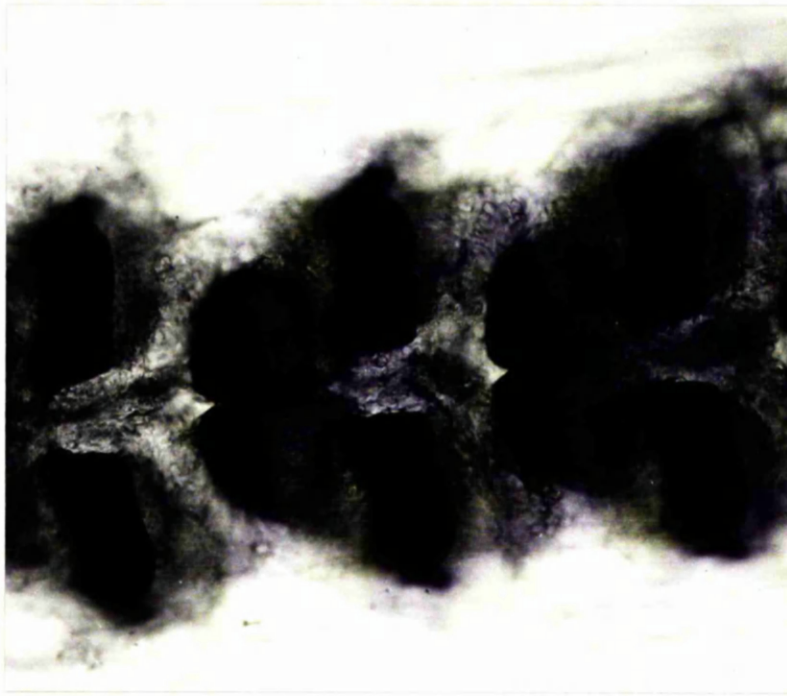
The rachidian tooth of the common winkle is composed of three pointed cusps, the central cusp being largest. The intermediate tooth also has a sharp, dominant cusp, but this is flanked on either side by two or three shorter cusps. The inner marginal tooth is smaller and possesses several sharp cusps of varying length. The outer marginal is quite distinct, the cusps being of similar length to one another, but shorter than those of the inner marginal.

In comparison, the radular teeth of the flat winkle have much broader, blunter cusps. This applies in particular, to the rachidian and the dominant intermediate teeth. One of the features used by Reimchen (1974) to confirm the specific status of the two sibling flat winkles, *Littorina obtusata* and *L. mariae*, was the morphology of the radula. Firth of Clyde specimens examined and identified by the author show similar traits. Fig. 18 and Table 13 illustrate the major differences highlighted by Reimchen. In *L. obtusata* the outer marginal tooth possesses numerous rather indistinct, angular cusps. In *L. mariae* the same tooth has very distinct, lobate cusps. The intermediate teeth also differ markedly. In *L. obtusata* the dominant has three large cusps, flanked on the inside and on the outside by a small, pointed cusp. While both small cusps are retained in *L. mariae* only two large cusps are apparent in the middle of the tooth. Reimchen found that these characters, along with differences in shell morphology and sculpturing, reliably distinguished all but the very smallest individuals (<2 mm in shell length).

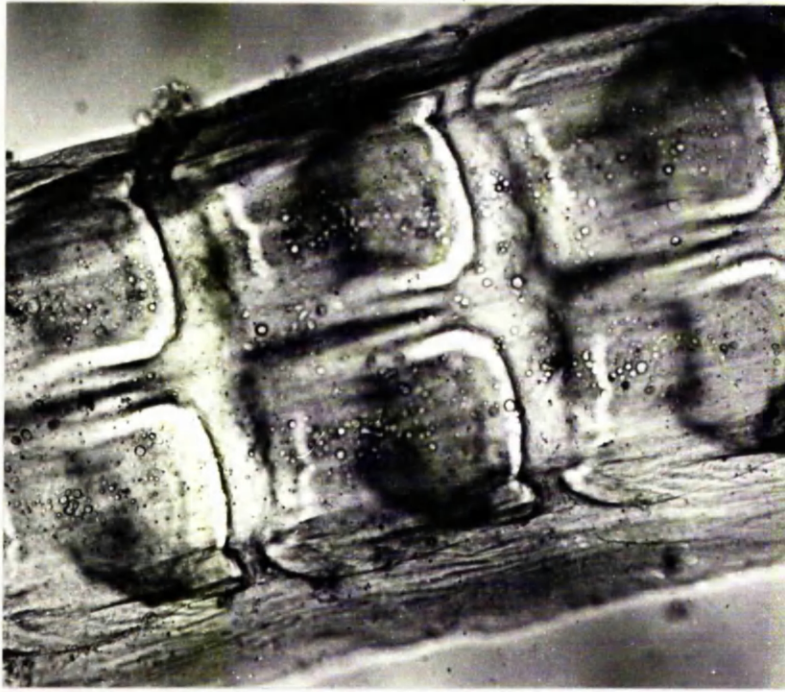
4.2.2. Denticular hardness

The occurrence of hardening agents on the distal portions of the intermediate and dominant marginal teeth causes a characteristic black-brown to yellow-brown opaque pigmentation of the docoglossan denticles (Figs. 19,20). Impregnation with hardening agents occurs as the teeth are formed in the radular sac. Fig. 19 compares teeth from the inner end of the sac (non-functional) with teeth from the operational portion of the radula. Only the latter are fully pigmented.

Detailed chemical analysis of the patellid radula led Jones *et al.* (1935) to



A



B

Fig. 19. Chemical hardening of acmaeid denticles.
(Light microscope)

A. *A. tessulata* functional teeth x 400.

B. *A. tessulata* non-functional teeth x 400.

conclude that it was composed largely of protein and polysaccharide strengthened by salts of iron and silicon, and that the ferrous constituent probably occurred in the form of the oxide, haematite (Fe_2O_3). Despite Lowenstam's (1962b) reference to goethite ($\alpha \text{Fe}_2\text{O}_3 \cdot \text{H}_2\text{O}$) in *Patella* and *Acmaea*, it is now generally accepted that the incorporation of iron in the docoglossan radula does occur in the form, Fe_2O_3 (Carefoot, 1977).

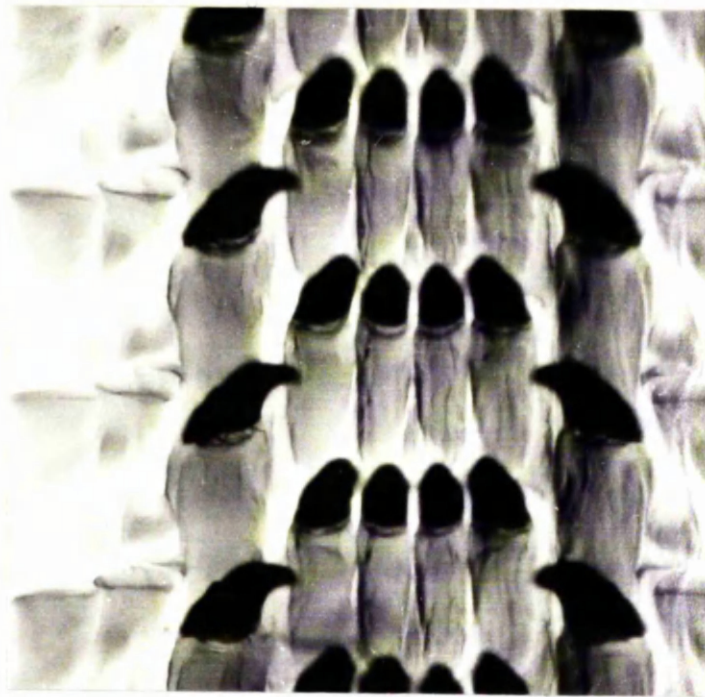
Strengthening with ferrous salts has also been identified in the chitons, Lowenstam (1962a) finding a 65% dry-weight concentration of magnetite (Fe_3O_4) in the radular teeth. Littorinid radulae lack comparable hardening and when viewed under the light microscope, the denticles appear uniformly clear (Fig. 20B).

In this study, Mohs' scale of hardness was used to compare the hardness of both limpet and littorinid radulae. The results are summarised in Table 14. The apparent difference in hardness of the acmaeid and patellid denticles may be an artefact arising from the difference in size of the two radulae. Not surprisingly, the larger radula of *Patella* left larger scratch marks on the test minerals. These were more readily identified against the inevitable background scratches present on all mineral samples.

Clearly, incorporation of iron in the docoglossan radula hardens the denticles considerably. On Mohs' scale, *Patella* and *Acmaea* both have values comparable to that for human teeth. In contrast, the littorinid denticles are little harder than a fingernail (Whitten and Brooks, 1972).

4.3. The feeding cycle

Variations in radular structure are often connected with subtle differences in the mechanics of feeding. The complex feeding movements associated with a variety of intertidal gastropods have been described previously by Ankel (1937, 1938), Eigenbrodt (1941), Nisbet (1953) and Graham (1973) and reviewed by Fretter and Graham (1962, 1976), Newell (1970) and Purchon (1968). The more detailed descriptions of the feeding cycle, however, tend to adopt an anatomical approach which obscures the relevance of the subject to gastropod feeding ecology, while



A.



B.

Fig. 20. Pigmentation of denticles.
(Light microscope)

A. *Patella vulgata* x 500.

B. *Littorina littorea* x 400.

TABLE 14. Denticular hardness

<u>Material</u>	<u>Hardness (Mohs' Scale)</u>
*Human fingernail	2.0
<i>Littorina littorea</i> denticles	2.0-2.5
<i>Littorina mariae</i> denticles	2.0-2.5
<i>Littorina obtusata</i> denticles	2.0-2.5
<i>Acmaea tessulata</i> denticles	4.0-4.5
<i>Patella aspera</i> denticles	4.5-5.0
<i>Patella depressa</i> denticles	4.5-5.0
<i>Patella vulgata</i> denticles	4.5-5.0
*Human teeth	5.0
*Window glass	6.0

*From Whitten and Brooks (1972)

many of the less detailed accounts are both incomplete and confusing. Here, the feeding mechanism of the docoglossan herbivore *Patella* and the taenioglossan grazer *Littorina*, are examined closely and compared, with the emphasis on those features influencing foraging strategy and food preferences.

4.3.1. *Patella vulgata*

The mouth of *Patella* comprises a vertical opening covered by "inner lips" of a yellowish colour (Fig. 21B). When the animal is not actively feeding, the skin of the head folds over the mouth to form lateral "outer lips" (Fig. 21A). At the commencement of feeding, the inner lips part and the buccal mass performs a rhythmical succession of forward strokes, being withdrawn under cover between each. A series of strokes are made side by side as the head describes a slow arc in front of the foot. At the end of each series, the position of the foot is altered slightly and another sweep begins in the reverse direction.

Prior to each stroke, the buccal mass is in the retracted position and the subradular membrane lies in a longitudinal groove between two pairs of cartilage. As the inner lips open from the anterior (Fig. 22 A,B), the odontophore is moved forward within the buccal cavity and the groove is pulled open to leave the radula lying flat on the surface of the cartilage. The protracting buccal mass strikes the substratum near the ventral lip, applying several rows of teeth to the surface (Fig. 22D). The entire structure is then pulled forwards to the region of the dorsal lip (Fig. 22 E,F). The radula is subsequently withdrawn around the anterior extremity of the odontophore as the subradular membrane is pulled into the buccal cavity close to the dorsal lips and jaw (Fig. 22F). Simultaneously the odontophore is lifted and withdrawn (Fig. 22 G,H), the inner lips closing from the posterior.

Hence protraction of the odontophore constitutes the most important phase of the feeding cycle. As the highly developed protractor muscles drive the buccal mass across the substratum the hardened tips of the intermediate teeth and the dominant marginal teeth rasp across the surface.

Throughout the feeding stroke, the wide basal plates ensure that the position

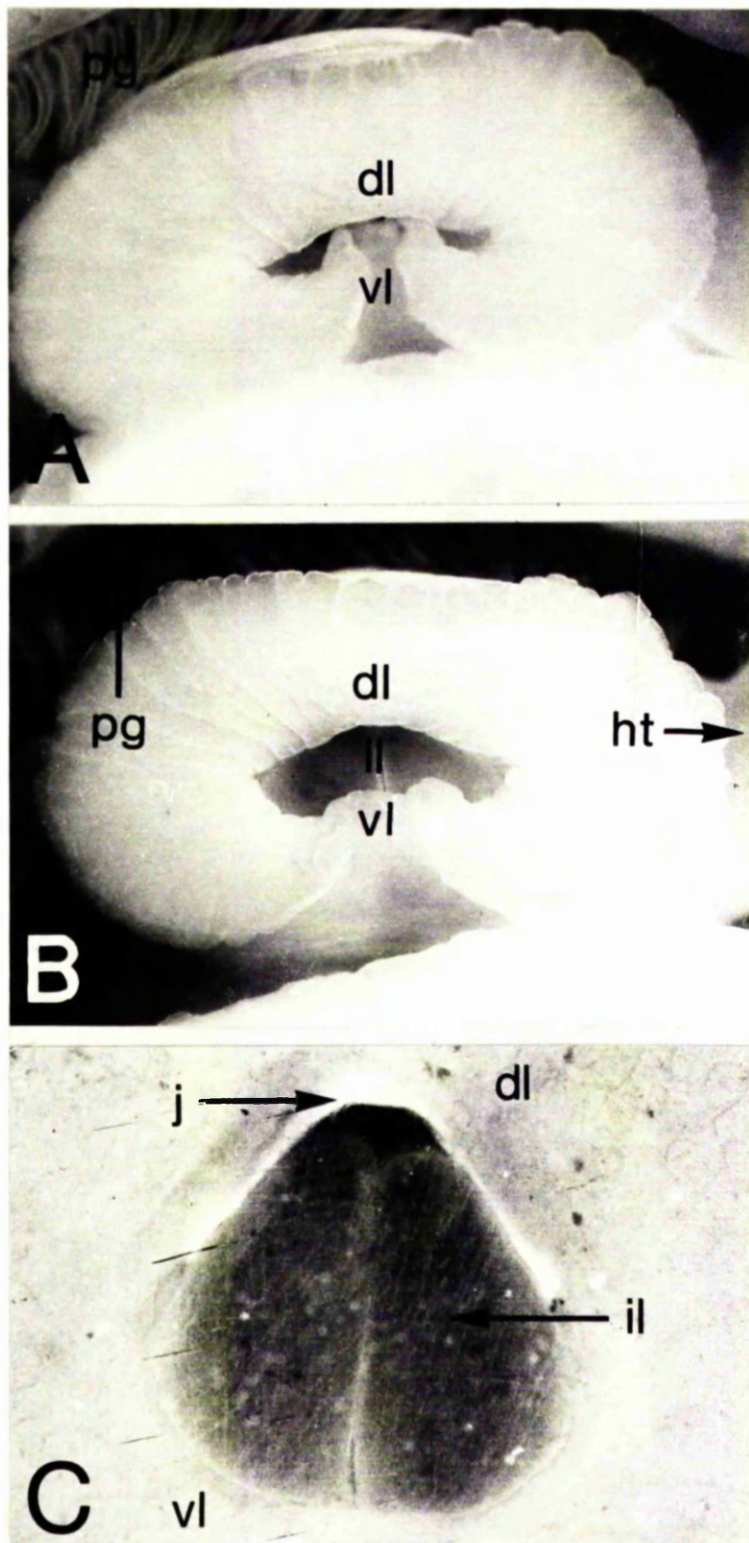


Fig. 21. Head and mouthparts of *Patella vulgata*.

A,B x15

C x23

pg = pallial gills
ht = head tentacles
dl = dorsal lip
vl = ventral lip
il = inner lips
j = jaw

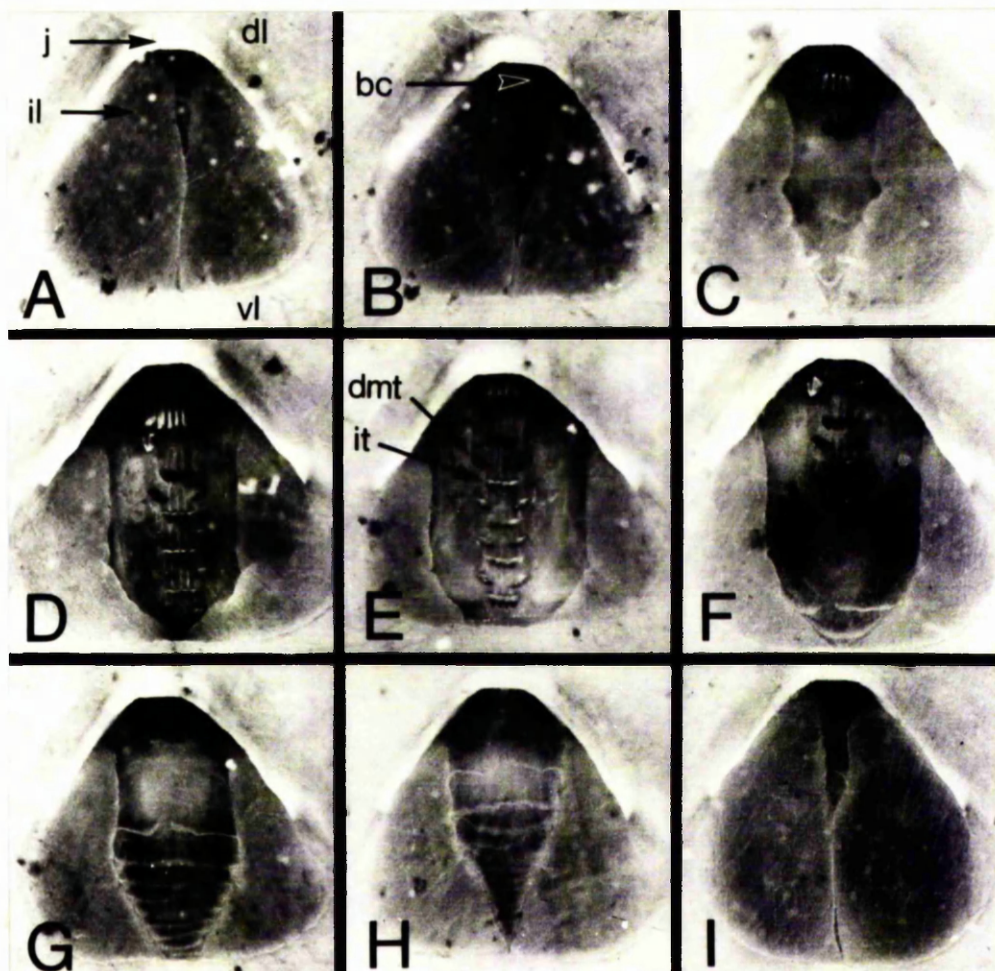


Fig. 22. Grazing stroke of *Patella vulgata* (x16).

dl = dorsal lip
 vl = ventral lip
 il = inner lips
 j = jaw
 bc = buccal cavity
 dmt = dominant marginal tooth
 it = intermediate teeth

Entire sequence represents approximately 1.1 seconds.

of the pigmented intermediate teeth on the basal membrane, remains unchanged. Runham and Thornton (1967), however, suggested that some erection of the dominant marginal teeth may occur. Close examination of the sequential photographs confirms this theory, revealing a degree of movement of the pluricuspid marginals at the apex of the buccal mass. As the buccal mass moves forward across the substratum the pluricuspid teeth rise slightly and swing toward the centre of the radula so that, in each transverse row, opposite pluricuspids lie closer together immediately prior to being lifted from the substratum.

In contrast to the littorinids, *Patella* possesses anteriorly, a single protruding jaw against which pieces of food may be pressed by the odontophore. As the radula is pulled back into the buccal cavity at the end of each grazing stroke, the teeth automatically brush against the jaw. The primary function of the jaw, as Nisbet (1953) and Fretter and Graham (1962) point out, is undoubtedly to prevent food escaping from the buccal cavity rather than to facilitate biting or tearing of macroscopic food items (but see Section 4.4.2 below).

4.3.2. *Littorina littorea*

In *Littorina*, the movements of the head and foot are similar to those of *Patella*. However, the "splay-teeth" taenioglossan radula differs basically from the patellid radula, lacking the rigidity which prevents movement of teeth on the basal membrane during grazing and the well developed musculature concerned with protraction of the patellid buccal mass. As a result, the taenioglossan radula functions in a rather different manner, relying heavily on changes in the configuration of the marginal and intermediate teeth as each successive row contacts the substratum.

At rest, the tip of the snout forms a circular surface crossed by a vertical cleft (the closed mouth) (Fig. 23A). As the mouth opens, the lateral buccal protractors pull the buccal mass forward within the buccal cavity until the tip of the odontophore projects through the mouth, the radula moving dorso-ventrally across the oral opening close to the ventral lips (Fig. 23C).

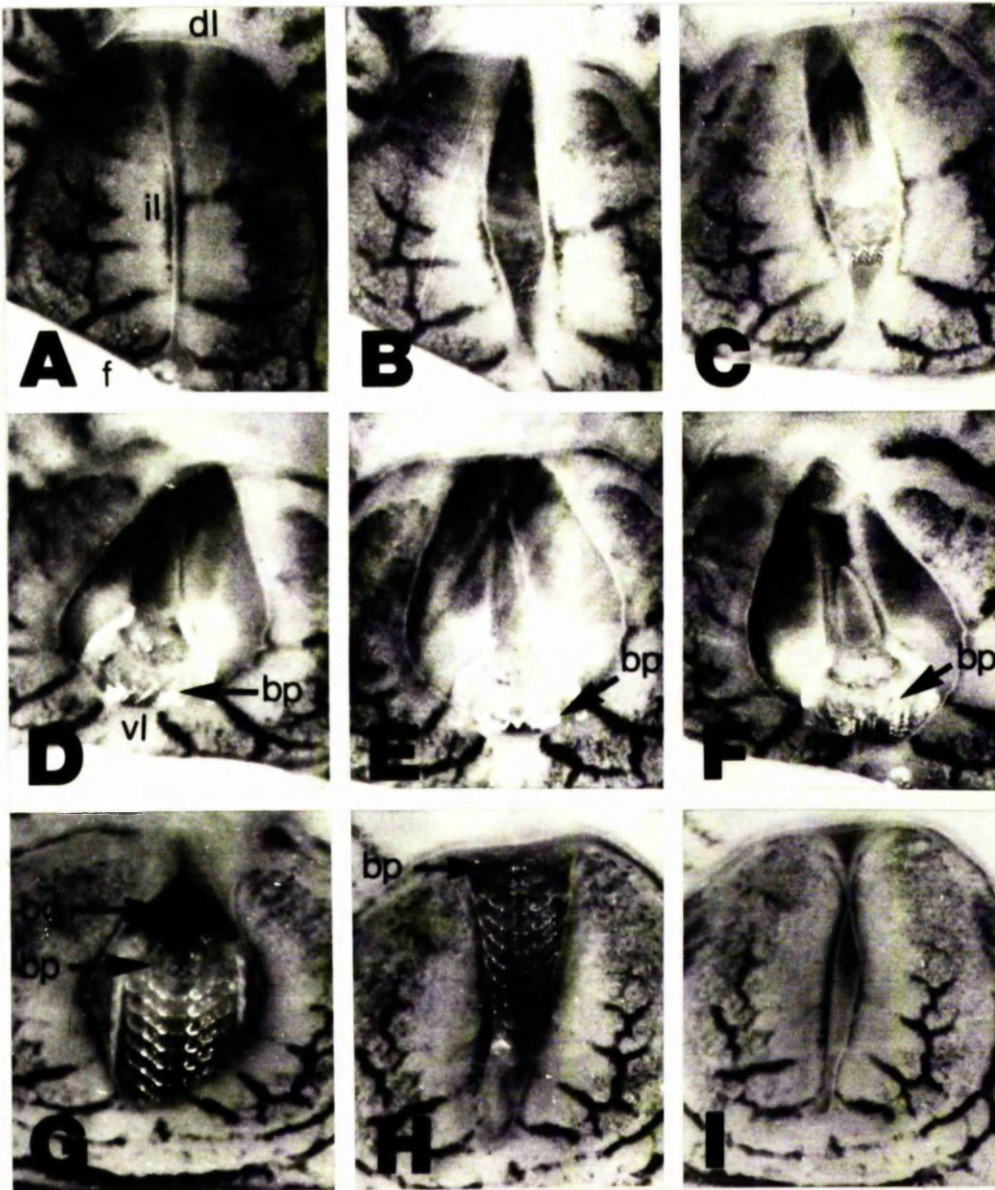


Fig. 23. Grazing stroke of *Littorina littorea* (x25).

dl = dorsal lip
 vl = ventral lip
 il = inner lips
 f = foot
 bp = bending plane
 bc = buccal cavity

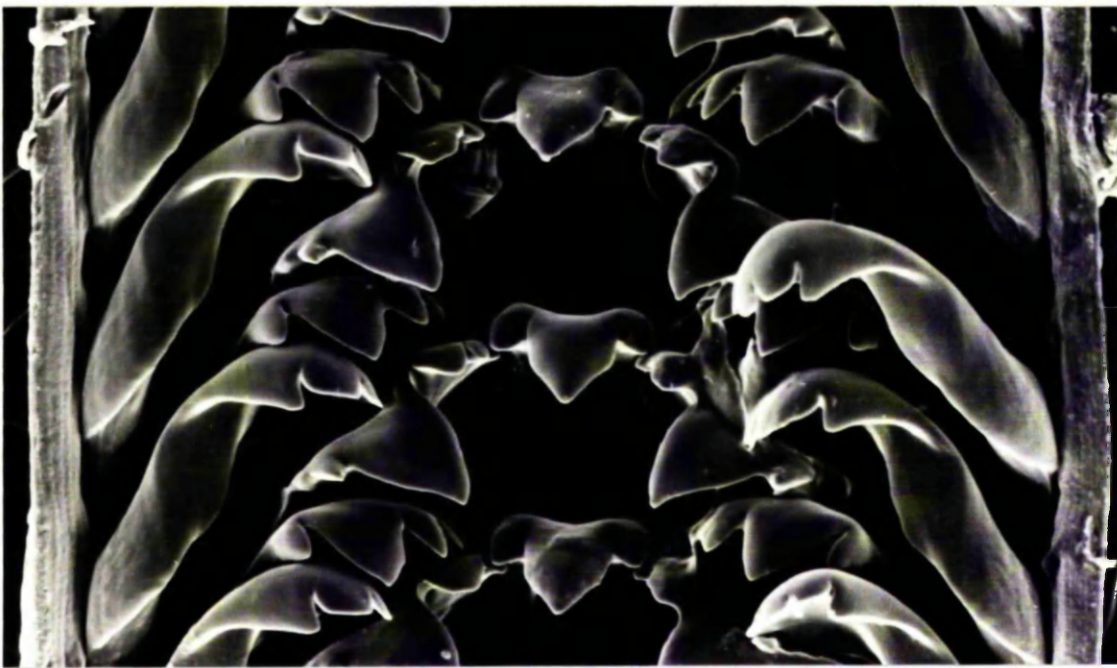
Entire sequence represents approximately 0.9 seconds.

The anterior horns of the cartilages of the buccal mass form a sharp edge facing the oral orifice. At this point the shape of the radula undergoes a basic change during grazing. Ankel (1937) named the cartilage edge the "Knickkante", a term which may be translated as the "bending plane". Within the buccal cavity, on the dorsal surface of the buccal mass, the teeth lie flat on the basal membrane and the laterals remain folded in towards the mid-line, overlapping each other so that each transverse row forms an arc. The radula is folded in the cartilage groove to protect the buccal tissues from inadvertent damage (Fig. 24). As protraction of the odontophore occurs at the beginning of each stroke, the subradular membrane moves out of the buccal cavity and over the bending plane of the odontophore cartilages in a laterally stretched state. The effect of the bend on the radular teeth constitutes the major difference between the grazing strokes of *Littorina* and *Patella*.

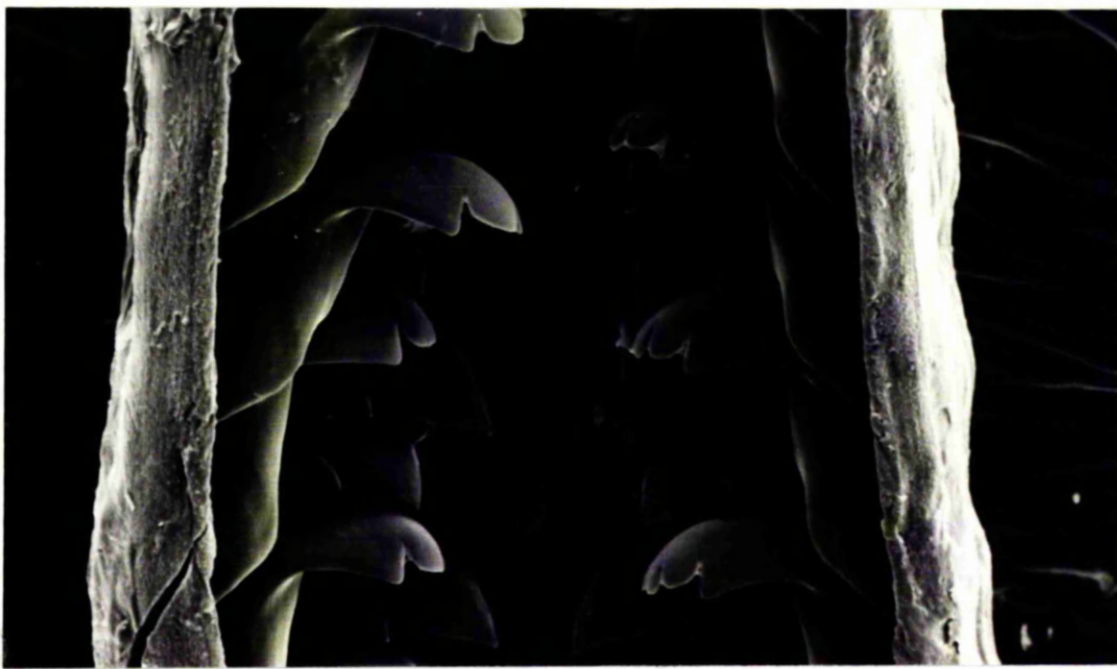
In *Littorina*, it is the position of the radula relative to the cartilage edge, and hence the tensions experienced by the basal membrane, which ultimately determines the configuration of the radular teeth. At the point where the radula passes over the cartilages a change occurs in the position of the teeth relative to the surface of the basal membrane. At the bend, the outer marginal teeth are raised and rotate outwards while the inner marginal teeth and intermediate teeth are raised and spread apart (Fig. 23E). The middle rachidian tooth does not change its position on the basal membrane. In contrast to *Patella*, only the teeth at the cartilage edge and immediately behind it contact the substratum.

Opening of the radula and erection of the radular teeth occurs primarily because the subradular membrane is pulled forwards over the bending plane by muscular action. In each grazing stroke, the free end of the radula approaches the bend and is pulled across the edge of the cartilage. Further rows of teeth are drawn across the bend in turn.

Following protrusion of the odontophore and the simultaneous opening of the radula, the buccal mass is applied to the substratum close to the ventral lip (Fig. 23D). Immediately, a movement of the bending plane from an antero-dorsal to a postero-ventral position causes several rows of teeth to cross back to the



A



B

Fig. 24. Effect of cartilage groove on orientation of teeth in *Littorina littorea* (x960, scanning electron microscope).

- A. Basal membrane of radula flat.
- B. Basal membrane folded, as in cartilage groove.

dorsal surface of the odontophore and return to their original closed position (Fig. 23 D,E). During this brief phase, the portion of the radula lying ventral to the bending plane remains stationary.

As the final phase of the feeding cycle commences, the radula and the odontophore both move dorsally, the former faster than the latter, and further rows of teeth are pulled back across the cartilage edge, disappearing into the buccal cavity. This results in the collapse of each erect tooth. As the movement advances, the radula is lifted (Fig. 23G) and withdrawn into the buccal cavity (Fig. 23H).

It is therefore the progress of the erect teeth back across the bending plane into the buccal cavity that constitutes the essential food-gathering phase of the feeding cycle. This is brought about by a combination of two distinct movements:

- a) A ventral movement of the bending plane; radula stationary
- b) Withdrawal of the radula across the bending plane while the entire odontophore moves dorsally; bending plane stationary relative to the radula.

Retraction of the radula (movement b) constitutes the basis of taenioglossan feeding (Fretter and Graham, 1962). This is in stark contrast with the docoglossan feeding stroke where the dorsal movement of the buccal mass during the protraction phase assumes the food-gathering role. This subsequently merges into the beginnings of retraction when the subradular membrane is pulled into the buccal cavity.

4.4. Effects of grazing

Having analysed the structure of the radula and the manner in which the radula and associated musculature operates, it should now be possible to look objectively at the effects of feeding on the food plant.

Invertebrate grazing may influence the fitness and longevity of the algal victim quite profoundly. The nature of the predator/prey relationship depends largely on the form of damage which the alga sustains. Numerous reports, for

example, document the complete destruction of mature kelp beds by various species of sea urchin (e.g. Leighton, 1966; Breen and Mann, 1976). This is brought about by grazing low down on the stipe which severs the frond close to the holdfast.

However, alga/herbivore interactions may also be self-sustaining. Steneck (1982) described an association between the coralline alga *Clathromorphum circumscriptum* and the limpet *Acmaea tessulata*. *Clathromorphum* possesses a uniquely thick layer of calcareous protective tissue over the region of growth. Acmaeid grazing seldom penetrates this layer to reach the reproductive structures buried beneath the surface. It does, however, remove epiphytes (which Steneck considers to be potentially lethal) along with cells from the 'host' plant's protective layers.

4.4.1. Analysis of grazing marks

The earliest attempts to analyse gastropod grazing traces are probably those of Sterki (1895) and Ziegler (1910) (cited by Ankel, 1938). More detailed analyses of the grazing prints of *Littorina littorea* were carried out by Ankel (1937, 1938) and by Eigenbrodt (1941). In recent years the value of the grazing trace as a means of studying and comparing radulae of different types has been largely overlooked. However, close examination of feeding marks undoubtedly yields much useful information concerning the structure of the radula, the mechanics of grazing and most significantly, the effects of grazing on the food surface.

Before examining the grazing marks of the test species in detail, it is essential to define clearly the terms that will be used. Hereafter, the term "grazing trace" will refer to the track left on the substratum by successive strokes of the radula. The trace is composed of a series of "prints", each of which corresponds to a single radular stroke. Each print consists of several "scratch" or "gouge" marks left by individual teeth or cusps.

a) The "pendulum" grazing trace

The grazing trace of the littorinids and the limpets, *Patella* and *Acmaea*, takes

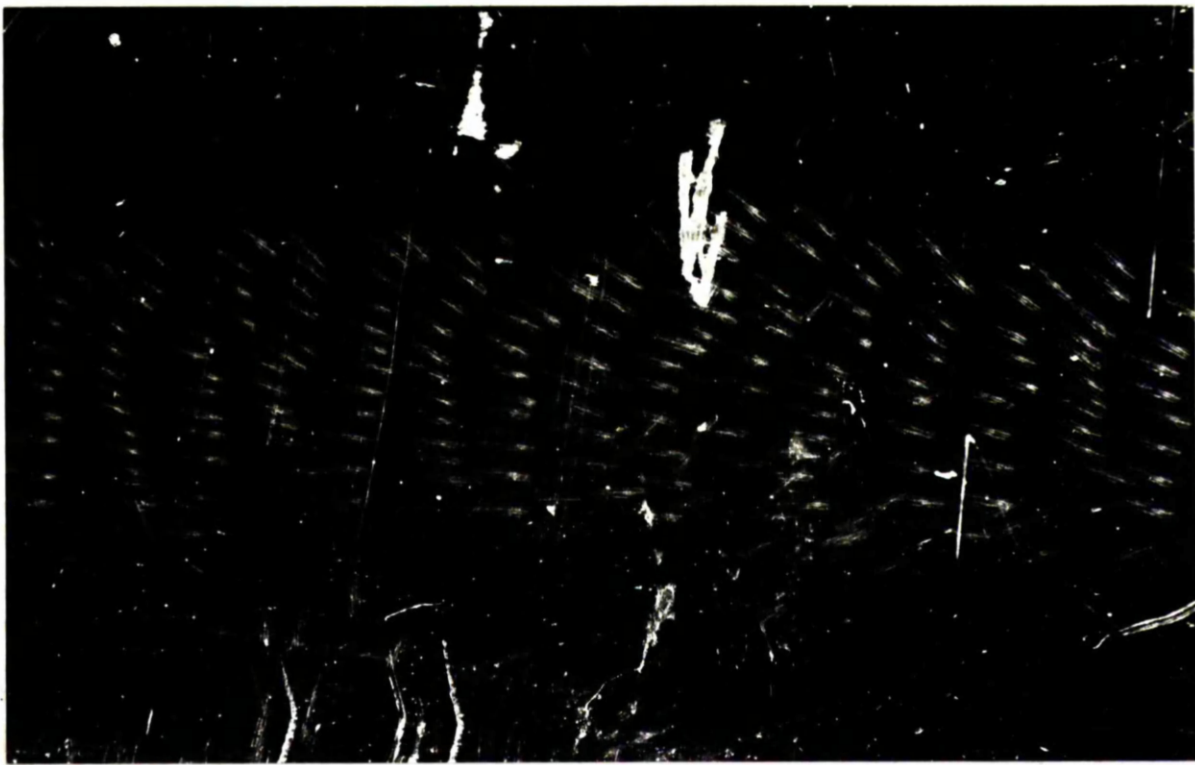
the form of a zigzag due to the characteristic side-to-side movement of the head during foraging. Ankel (1937) termed this "pendulum grazing" (Fig. 25).

In this study the pattern of the pendulum trace has been examined closely using *Acmaea tessulata*. Each section of the trace is curved, the concave side facing towards the foraging animal. During the formation of each section the animal remains in the same position, with only the head moving. The number of prints in each section, the distance between successive prints and the length of successive sections, vary during the normal course of grazing. Consequently, the characteristic zigzag pattern is often ill-defined.

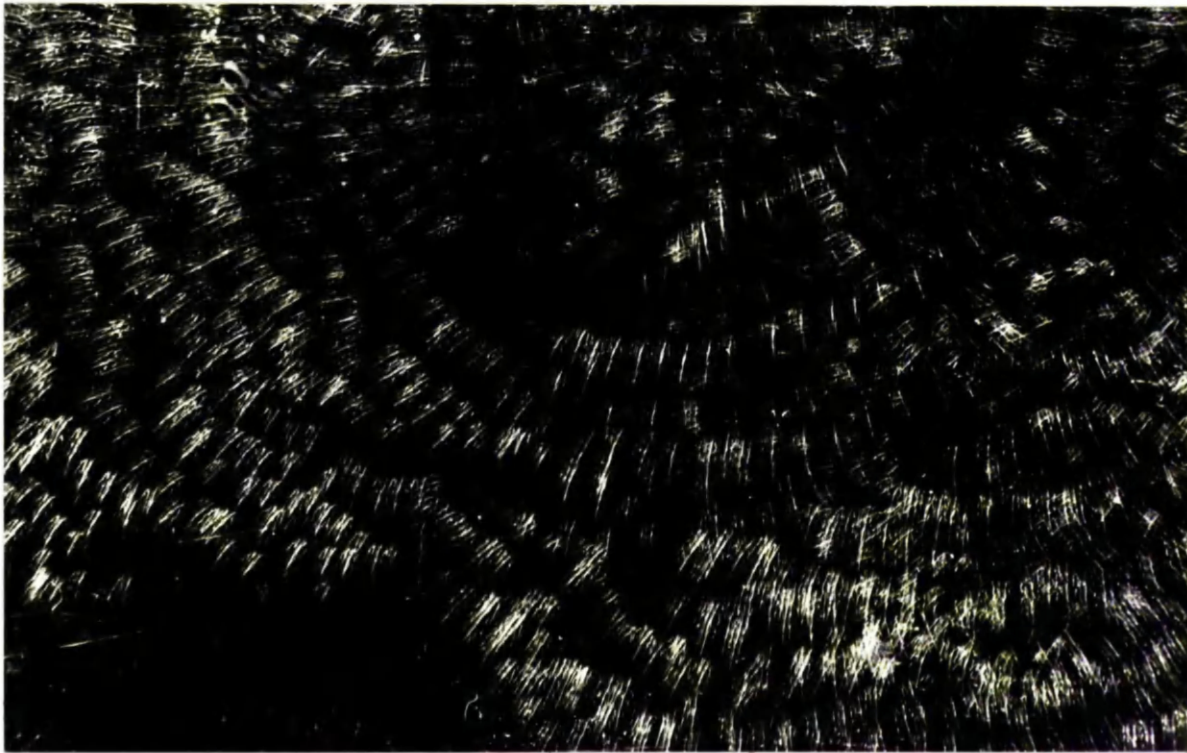
The angle between successive sections of the trace is influenced strongly by the abundance of food on the substratum. For *Acmaea tessulata* "grazing" on clean perspex, the angle between successive sections ranged from 20° to 25°. On perspex supporting a dense population of diatoms, the corresponding angle fell to 10°-15°. Hence abundant food sources are utilised efficiently while areas supporting little food, are traversed rapidly.

During foraging, the snail may use the radula to test the substratum for the presence or absence of food. On clean perspex, both *Acmaea* (pers. obs.) and *Patella* (S.J. Hawkins, pers. comm.) frequently leave solitary groups of two or three radula prints, no coherent trace being visible (Fig. 26). Similarly, regular grazing movements are generally preceded by a few random strokes. Nevertheless, protrusion of the radula itself, is not the most important means of food detection. Grazing traces on a perspex plate, half of which was clean while the other half was covered with a dense film of diatoms, were confined entirely to the "fouled" area, halting abruptly at the dividing line. While Ankel (1938) assumed that the well developed tactile sense of the paired tentacles was instrumental in food detection, Bovbjerg (1968) found that feeding behaviour in lymnaeid snails was elicited by contact stimulus of chemoreceptors in the foot and head.

Although Ankel (1937) maintained that the recognition of mucus trails caused some foraging gastropods to change course and avoid previously grazed areas, overlapping traces were observed repeatedly in this study with both *Acmaea* (on perspex)



A



B

Fig. 25. Pendulum grazing traces.

A. *Acmaea tessulata* x20.

B. *Patella vulgata* x4.



Fig. 26. Solitary groups of acmaeid grazing prints (x16).

and *Littorina littorea* (on germling lawns). Indeed my own observations and those of D.P. Cheney (pers. comm.) indicate that *Littorina* actively follows mucus trails encountered while foraging. Hence, patchy grazing is common even when food is uniformly distributed on the substratum.

b) Patellid grazing print

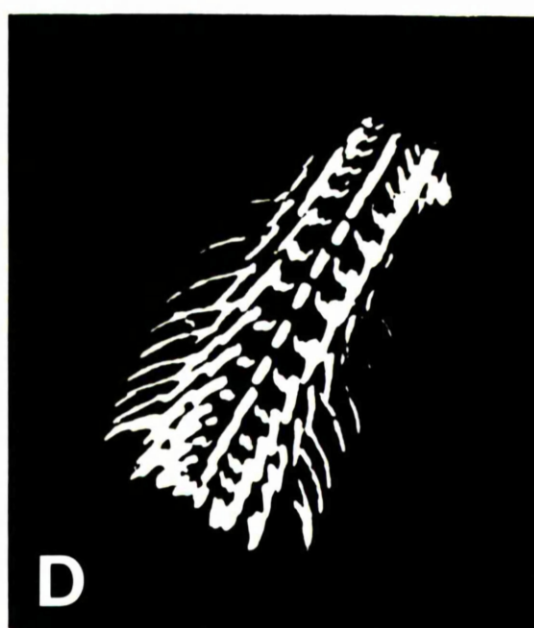
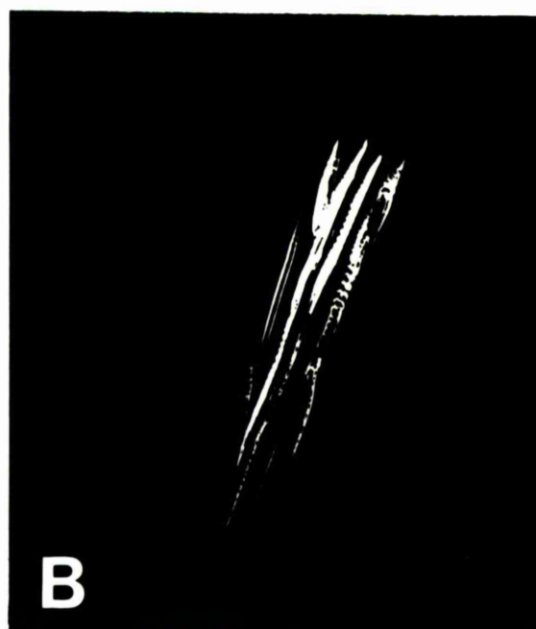
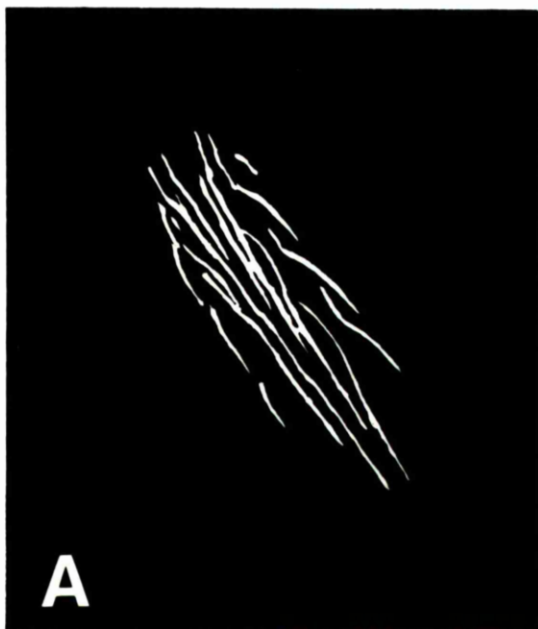
Each individual print in the patellid trace, comprises a series of grooves corresponding to the intermediate teeth and the dominant pluricuspid marginals (Fig. 27A). As the radula is pushed across the substratum four approximately parallel lines are left by the intermediate teeth. These are flanked on either side by a series of diagonal grooves which curve gently inwards and may be attributed to the large central cusps of the dominant marginal teeth. Four such lines normally appear on either side of the trace. Hence only four transverse rows of teeth are applied to the substratum and are active during each stroke of the radula. The inward slant of the grooves provides further confirmation of the observed movement of the pluricuspid marginals at the apex of the buccal mass - a feature which Eigenbrodt (1941) failed to recognise, leading him to conclude that the feeding apparatus of *Patella* functioned in a similar manner to that of *Patina pellucida*, leaving a series of six parallel grooves on the substratum. A similar assumption was made by Steneck (1982).

c) Acmaeid grazing print

The blunt, shovel-like teeth of the acmaeid, *Acmaea tessulata* give rise to somewhat broader gouge marks with relatively sharp, narrow interstitial ridges (Fig. 27B). The scoops also appear deeper than those of *Patella* (see Steneck, 1982). Movement of the marginal teeth during grazing does not occur, hence the grazing print simply comprises four parallel grooves corresponding to the two intermediate teeth and the two marginals. Movement of the head frequently obscures this pattern, often resulting in a series of broken lines which appear to be made by a much greater number of teeth.

Fig. 27. Grazing prints.

- A. *Patella vulgata* x37.
- B. *Acmaea tessulata* x185.
- C. *Littorina littorea* x42.
- D. *Littorina obtusata* x74.



d) Littorinid grazing print

In the littorinids, movement of the teeth on the basal membrane gives rise to a much more complex grazing print. The movement of each tooth is the result of a combination of two factors: movement of the teeth (in relation to the basal membrane) and movement of the feeding apparatus (in relation to the substratum). The latter is composed of movement of the radula, odontophore (see Section 4.3.2) and muzzle. When the central, rachidian tooth reaches the cartilage edge it is pulled across the substratum leaving a short scratch mark. The central line of the grazing print consists of the traces left by successive rachidian teeth. Initially these marks produce a continuous line. This corresponds to the brief ventral movement of the bending plane described in Section 4.3.2. At this stage, only the lifting of the muzzle moves the teeth in relation to the substratum. As the entire odontophore begins to retract, however, the scratches become longer and the gaps between scratches become more distinct (Fig. 27 C,D). During each grazing stroke, 13-14 transverse rows of teeth are applied to the substratum. Occasionally, the entire littorinid print curves to the side as a result of a sideways movement of the muzzle during the grazing stroke (Fig. 28).

The pattern made by the marginal and intermediate teeth is more elaborate as a result of the rotation and folding which occurs as the teeth cross the bending plane. Each lateral tooth is splayed to the maximum degree when the cusps contact the substratum. As the teeth cross the bending plane they begin to close, folding in towards the mid-line. Hence all the lateral teeth leave diagonal or curving traces.

In each transverse row the outer marginal tooth is first to touch the substratum and has furthest to move to return to the folded, resting position. The tooth rotates as it folds inwards, drawing a long curve which runs towards the mid-line. The inner marginal teeth and the intermediate teeth do not rotate in the same manner. They do, however, fold in towards the rachidian tooth as they cross the edge of the cartilage. The resulting marks on the substratum consist of diagonal lines of varying length.

The cusps of the intermediate teeth leave one or more lines on either side of



Fig. 28. Grazing print of *Littorina littorea*, showing sideways twist in response to movement of the muzzle (x45).

the mid-line, the longest (outer) scratch being attributable to the largest cusp. The inner marginal teeth leave a similar diagonal trace which consists of a solitary scratch mark in the flat winkle (Fig. 27D) but may comprise two parallel lines corresponding to the two largest cusps in the common winkle (Fig. 27C). This is apparently the sole justification for Steneck and Watling's (1982) assertion that the radula of *L. littorea* has a greater number of functional points touching the substratum than *L. obtusata* and *L. mariae*. In one respect the prints of the flat winkle and the common winkle differ quite markedly. The broad, blunt cusps of the flat winkle leave much wider and more distinct scratch marks. The rachidian tooth and the intermediate tooth in particular, leave very well-defined marks. The two species of flat winkle produce identical prints.

4.4.2. Grazing damage

As the examination of radular structure and functioning predicts, the feeding trace differs markedly amongst the test species. As a result, it is likely that different forms of damage may characterise grazing by each mollusc. Furthermore, differences in the efficiency of grazing may be anticipated on a variety of potential foods.

a) Patellid damage

The diet of the patellid limpets is composed predominantly of algal germlings, unicellular algae and diatoms (Graham, 1932; Jones, 1946; Purchon, 1968). Germling lawns cultured during the germling choice experiments reported in Chapter 5, were used to assess the damage incurred following grazing by *Patella vulgata*. *Enteromorpha* and *Gigartina* germlings were grown to a mean (erect) thallus length of approximately 1mm on glass microscope slides. Grazing by both species resulted in the removal of the entire thallus, including the characteristic basal discs of the *Gigartina* germlings. The lawns were grazed methodically, *Patella* usually working from one end of the slide to the other.

Despite the apparent preponderance of small items in the diet, grazing of

macroalgae does occur. Orton (1914) observed *Patella* eating food-paths through a sward of green algae, while Jones (1948) confirmed Fischer-Piette's observations of patellid grazing of the adult furoids *Ascophyllum*, *Fucus vesiculosus* and *F. spiralis*. Further evidence of macroalgal grazing has been observed in the Firth of Clyde by the author and was recorded by Davis and Fleure (1903) (cited by Jones, 1948) and by Conway (1946) and Southward (1962).

In the laboratory, specimens of *P. vulgata* kept in clean, aerated sea-water tanks, will consume most species of macroalga fairly readily. Fig. 29 illustrates typical patellid grazing damage on an *Ascophyllum* frond. Two forms of damage are apparent:

- 1) Pale areas in the centre of the frond formed by scraping the thallus. Here, the foot pins the thallus down while the radula performs a series of strokes, functioning in the manner employed during normal grazing on the rock substratum.
- 2) Erosion along the edge of the thallus. Damage of this nature has been reported in the field by Southward (1962) and is probably the result of a "biting" motion of the radula involving the anteriorly placed, cuticular jaw. Here the jaw may function as a cutting edge against which the buccal mass presses the algal thallus, causing chunks of material to be sheared off by successive rows of teeth. No evidence of a comparable mechanism was apparent in the docoglossan acmaeids, while grazing of *Ascophyllum* by the littorinids (Figs. 33,37) was restricted to scratch marks on the surface of the frond. Neither *Acmaea* nor *Littorina* possess a jaw.

b) Littorinid damage

Grazing of juvenile *Enteromorpha*, *Ulva* and *Fucus serratus* by both *Littorina littorea* and *L. obtusata*, was similar to that reported for *Patella*. The entire thallus was removed, even on slides retained until the average thallus length reached 2-3 mm. *Ascophyllum* and *Fucus* germlings were also frequently dislodged by non-feeding snails simply crawling across the slides. With juvenile *Gigartina*, a red alga, both the erect frond and the basal disc were generally destroyed, although occasionally the discs were left undamaged while the fronds



Fig. 29. *Patella vulgata* grazing damage on *Ascophyllum* frond.

were broken off. This was more often caused by the foot of a foraging snail than by the radula. Foraging on germling lawns tended to be less methodical than observed for *Patella*. Grazing traces frequently wandered obliquely across the slides leaving bands of undamaged germlings (Fig. 30).

Macroalgal grazing by *Littorina littorea* simply consists of scraping the thallus with successive rows of teeth. The rachidian and lateral teeth scrape across the surface of the alga and the characteristic inward folding of the laterals at the bending plane, ensures that loose material is brushed toward the centre of the radula and carried into the buccal cavity. When feeding on the delicate, foliose species, the central rachidian tooth tears the frond very effectively.

When the wrinkle actually occupies the lamina on which it is feeding, abrasion by the radular teeth initially causes damage to the centre of the thallus. Fig. 31 illustrates progressive stages of grazing on *Fucus serratus* and Fig. 32 shows damage of the same type sustained by *Ulva lactuca*.

The common wrinkle only rarely feeds on *Ascophyllum*. The frond pictured in Fig. 33 was not grazed until the snails had starved for 40-50 days. Individual radula marks can be distinguished within the grazed area. In this instance, no additional damage was recorded ten days after the initial appearance of scrape marks.

Where the thallus is thin (permitting rapid excavation) or narrow (preventing the entire foot from resting on the frond) grazing damage may appear to take a similar form to the bites described previously for *Patella* (Figs. 34,35). However, such damage is caused either by gradual abrasion of the thallus from the edge inwards or by tearing the frond and not by the biting motion observed in the patellids.

As the flat wrinkles, *Littorina obtusata* and *L. mariae*, utilise the seaweed lamina both as a food source and as a habitat, grazing is seldom initiated at the margin of the frond. Indeed feeding on *Fucus* spp. often leaves a characteristic "rib" of ungrazed material along the thallus edge (Fig. 36). Trimming of the borders (as in Fig. 37) only occurs on exceptionally narrow fronds which are in

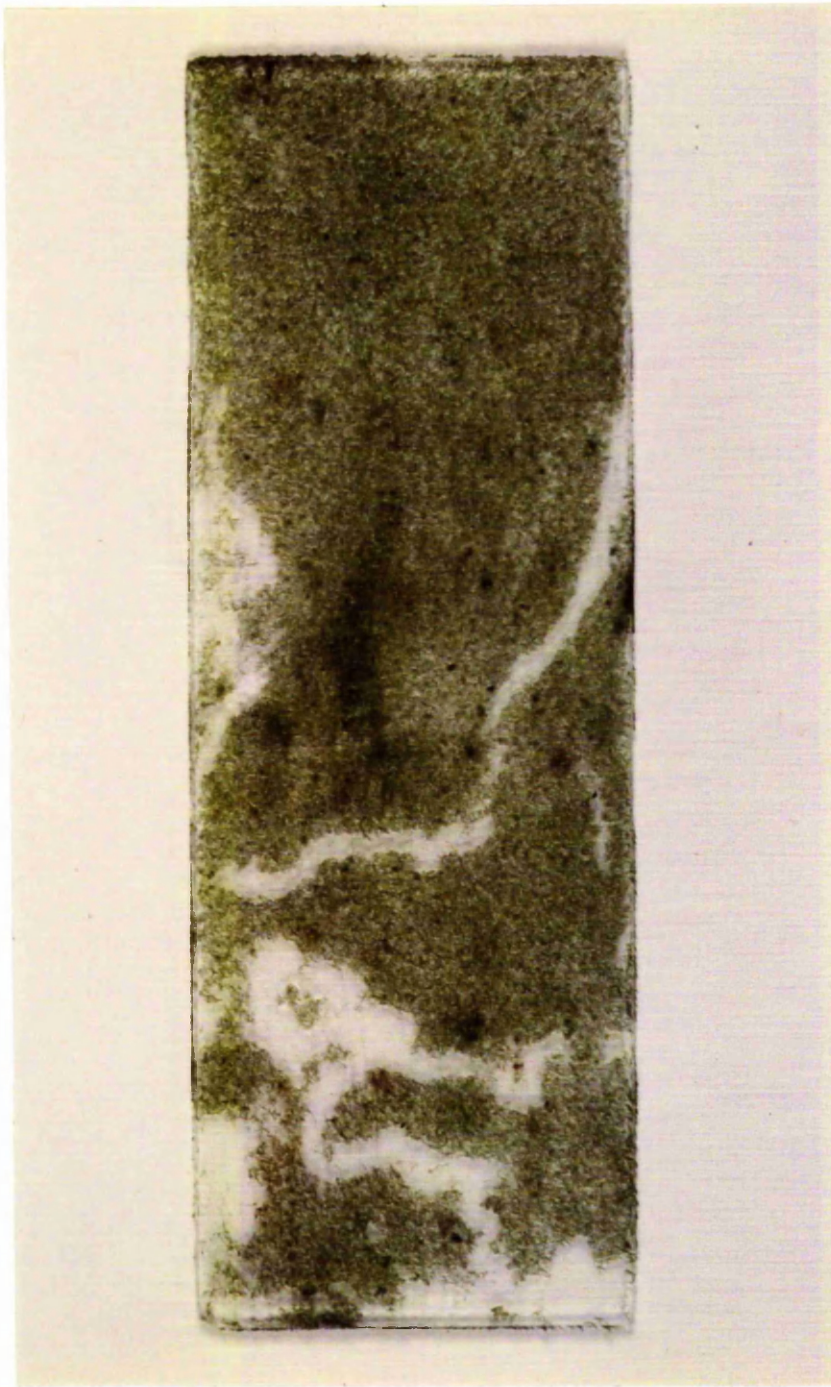


Fig. 30. Lawn of germling *Ulva* showing signs of grazing by *Littorina littorea*.

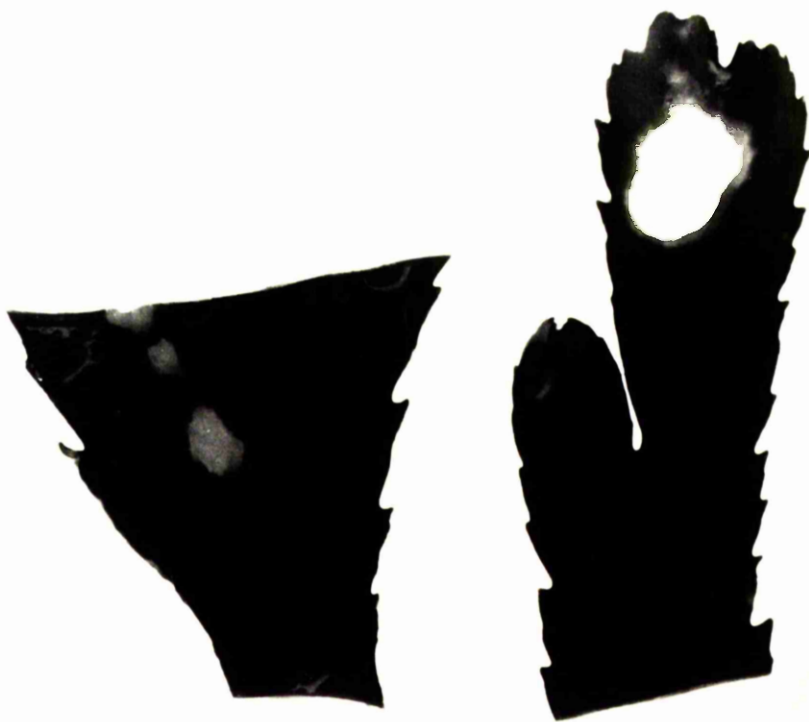


Fig. 31. Progressive stages of common winkle grazing damage to *Fucus serratus* thallus.



Fig. 32. Common winkle grazing damage to mature *Ulva*.



Fig. 33. Common winkle grazing prints on an *Ascophyllum* frond.



Fig. 34. *Littorina littorea* grazing damage to fertile *Fucus serratus*.



Fig. 35. Evidence of common winkle grazing on *Pelvetia*.



Fig. 36. Destruction of furoid thallus by *Littorina obtusata*.



A.



B.

Fig. 37. *Littorina obtusata* grazing damage to *Ascophyllum*.

A. Radula marks on central thallus.

B. Grazing initiated at thallus edge.

any case generally ignored unless no alternative food source is available.

The blunt teeth permit rapid excavation of the softer internal tissue of the fucoid thalli. Although the tougher outer epithelial layer must be breached to allow access to the inner tissues it is, thereafter, rarely consumed (Fig. 36).

4.5. Discussion

The grouping of gastropods according to the functioning of the radula is common in the literature. Ankel (1936, 1938) and Eigenbrodt (1941) first described the "rigid teeth radula" of the docoglossan limpets and the "splay teeth radula" of the littorinids, while Purchon (1968) classifies both *Littorina* and *Patella* as "raspers" of algae. More recently, Steneck and Watling (1982) divided the gastropod herbivores into three groups corresponding to the rhipidoglossa, taenioglossa and docoglossa. The radulae were described respectively as "brooms", "rakes" and "shovels".

Divisions of this nature are useful generalisations which provide a graphic description of the radula and an intuitive insight into foraging habits and food preferences. Nevertheless, the limitations of such rigid classification must be recognised. The taenioglossan radula of *Littorina*, for example, is a highly versatile structure able to utilise efficiently a wide variety of food resources. While the mechanics of the radular stroke remain the same, the function and importance of each tooth changes according to the nature of the food. As Purchon (1968) surmises, the winkle is able to feed on many of the perennial macroalgae simply by rasping the surface of the thallus with the radula. The blunt shovel-like teeth of *Littorina obtusata* and *L. mariae* are particularly adept at gouging material from the leathery fucoid thalli and can excavate the lamina with remarkable rapidity. The flat winkles are, however, less accomplished feeders on foliose algae, which require the thallus to be torn by sharp cusps. Nevertheless, the general body plan of the various littorinids is probably as important in determining foraging strategy as the structure of the radula itself. The large size of the foot in relation to the shell, enables the flat winkle to occupy the

thallus and maintain this position when the alga is submerged and subject to disturbance by water movement. The common winkle, on the other hand, is readily dislodged by the slightest perturbation - whether artificial or natural - and consequently, is confined largely to the rock substratum when submerged and active.

To permit effective grazing of the foliose, ephemeral algae, the foot must hold the lamina down while the teeth tear through the thin thallus. The stationary rachidian tooth seems well suited to perform the latter function. In *Littorina littorea* it has a very sharp and distinct central cusp which tears the thallus with great efficiency.

The characteristic collapse and rotation of the littorinid lateral teeth as successive rows approach the apex of the buccal mass, is a relic of the more primitive rhipidoglossan radula. The inward sweep of the lateral teeth can be used both to scrape and to brush the substratum. Whether *Littorina* is able to "scrape" and "brush" discriminately, however, is open to question.

During grazing on juvenile algae in the laboratory, clumps of germlings are swept towards the central rachidian tooth and trapped in the radular groove as the radula is withdrawn into the buccal cavity. It is this sweeping motion which enables the flat winkles to remove epiphytes from the furoid thalli. Indeed where diatoms and epiphytic microalgae are abundant, these may constitute the major part of the diet and ingestion of macroalgal material may be coincidental.

Sweeping of the stone substratum provides the common winkle with a mixed diet of microalgae, germlings, diatoms and detritus. The brushing action of the lateral teeth gathers loose detritus very efficiently and this may form a much more important component of the diet than previously recognised. Certainly the common winkle is unusual in its ability to survive and indeed often to thrive, on mudflats (pers. obs.; Ankel, 1937) where particulate detritus is the major source of food. In the Firth of Clyde, many shores comprise predominantly, vast expanses of mud flat broken by occasional boulders. In these circumstances, the boulders form refuges where winkles congregate when the tide recedes, foraging

excursions onto the surface of the mud occurring as the tide rises. The gut contents of these specimens appears to be identical in composition to the surface mud itself (pers. obs.).

The absence of chemical hardening in the denticles is a further indication of the relative importance of the brushing action of the radula. Forceful scraping of stone substrata would probably cause excessive wear to the taenioglossan radula, though this may be counteracted to some extent by the length of the radula and the rate of replacement of the teeth. Pelseneer (1935) (cited by Fretter and Graham, 1962) recorded a mean radular length to shell length ratio of 2.00 for the common winkle. This compares with 1.80 for *Patella vulgata*.

Comparison of the littorinid feeding apparatus with the more primitive rhipidoglossan radula suggests that two distinct factors are instrumental in the increased versatility of the taenioglossans:

- i) A reduction in the number of marginal teeth.
- ii) A loss of ancillary muscles used to adjust radular tension and position, shifting the emphasis from positional adjustments of the radula to the force with which the radula is applied to the substratum (Steneck and Watling, 1982).

Nisbet (1953), commenting on the inability of *Monodonta lineata* to consume adult *Ulva* or *Enteromorpha*, concludes that the cutting edges of the rachidian and intermediate teeth are rendered obsolete by the vast array of small marginal teeth designed to sweep loose debris towards the mid-line. The marginal teeth are not attached directly to the basal membrane but are instead, attached to a separate chitinous strip which is inserted into the radula. Consequently, when *Monodonta* attempts to feed on thin foliose thalli, the lamina "may be dimpled into the mouth as many as six times at the same spot on the frond, without causing any visible damage" (Nisbet, 1953).

In contrast to the versatile taenioglossan radula, the docoglossan radula of the archaeogastropod limpets is a highly specialised structure designed primarily to scrape microalgae and diatoms from the substratum. Each pigmented functional tooth is large and heavily built and only the dominant marginal retains

any vestige of the mobility manifest in rhipidoglossan and taenioglossan laterals. The working place lies morphologically anterior to the bending plane (Fretter and Graham, 1962) and a large part of the underside of the protracting buccal mass is applied to the substratum during each grazing stroke. Consequently, the musculature associated with the radula is particularly well developed, five pairs of cartilage replacing the two pairs found in most other prosobranchs (Fretter and Graham, 1962). The superior rasping capability of the limpet radula is further augmented by the occurrence of hardening agents on the distal portions of the teeth.

The advantages of specialisation are offset to some extent by the inevitable predator/prey dependency which results. The abundance of microalgae and diatoms is seasonal and subject to substantial fluctuation (see Castenholz, 1961; Steneck, 1982) and consequently it is important that the limpet should be able to utilise alternative food sources when necessary. Reports of limpet grazing damage on the shore testify to the periodic consumption of macroalgal foods. The body plan and size of the adult patellid, however, are not well suited to foraging on macrophytic thalli and grazing of this nature probably occurs only when other food sources are scarce.

The taenioglossan and docoglossan radulae both dislodge algal germlings with consummate ease. The limpet, however, tends to graze germling lawns with superior efficiency. *Patella* actively avoids previously grazed areas whereas littorinid grazing tracks frequently overlap and follow each other closely. It is possible that, on encountering existing mucus trails, the territorial *Patella* (see Stimson, 1970) reacts in a different manner to the more gregarious *Littorina* (see Ankel, 1937).

Although the common winkle is a much more versatile feeder, the basic components of the diet are, more often than not, very similar to those of *Patella*. In many ways, the feeding apparatus and mode of grazing of the two species provide a good example of how an evolutionary problem may be solved satisfactorily in a number of different ways. Despite having very different radulae, both species graze algal germlings, diatoms and microalgae efficiently, though the hardened

denticles of the docoglossan radula may enable *Patella* to exploit the microflora on harder rock substrata where the littorinid radula would incur excessive wear.

Successful foraging on the rock surface, relies heavily on the efficient gathering of relatively small food particles. The inward sweep of the lateral teeth on the taenioglossan radula ensures that dislodged food does not escape and is transported rapidly towards the buccal cavity. In *Patella*, however, the pigmented functional teeth do not move substantially on the basal membrane. It has been suggested that the unpigmented outer marginal teeth may fulfil the collecting function of the taenioglossan laterals, but examination of the sequential photographs presented in Section 4.3.1., Fig. 22 suggests that these teeth do not, in fact, touch the substratum during grazing and are, consequently, non-functional.

The patellid radula then, must employ an alternative means of ensuring that food particles loosened by the teeth are ensnared and delivered safely to the buccal cavity. In this context the hardening of the denticles may be particularly important. The functional teeth are both immobile and widely spaced and grazing prints show clearly the interstitial ridges between individual scratches. Hence filamentous microalgae and germlings may be expected to slip between the teeth. However, on all but the hardest substrata, the strengthened denticles actually gouge material from the rock surface while foraging, effectively "uprooting" the attached microflora together with chunks of the mineral substratum. In this way, *Patella* increases the size of particles ingested and minimises the possibility of food slipping between the teeth.

Basic differences in the body plan of the two species may also be of some significance. In contrast to *Littorina*, the head of *Patella* is surrounded by the shell both at rest and when grazing. This probably reduces the likelihood of loose particles being washed out of reach of the radula.

In the acmaeids, the docoglossan radula becomes still more highly specialised. The dentition is reduced and the radula has fewer points of contact with the substratum. Each tooth is short and blunt and is aligned perpendicular to the substratum. All teeth remain stationary on the basal membrane. These features

allow *Acmaea* to excavate coralline cells efficiently, but may also reduce the limpet's ability to ingest filamentous and unicellular algae since the area of contact is small and large gaps exist between teeth (Steneck, 1982).

Such a high degree of specialisation is permitted by the abundance and dependency of the crustose algae on which *Acmaea* characteristically feeds. In a detailed survey of the existing literature, Steneck and Watling (1982) found no recent reference to macrophytic grazing by *A. tessulata*. My own observations in the laboratory, suggest that macroalgae are not grazed and this is further supported by the experiments of Steneck (1982). The acoustic techniques used by Kitting (1979) indicate that microscopic epiphytes are seldom consumed as a distinct food item, but are ingested coincidentally with pieces of encrusting alga. Kitting worked predominantly with *Acmaea scutum*.

It would appear, therefore, that *Acmaea* is quite different from both *Littorina* and *Patella*. It is, as Steneck (1982) concludes, a trophic specialist "adapted to graze low quality, abundant and temporally predictable foods in preference to other less abundant or unpredictable foods of higher quality".

It is no coincidence that *Acmaea*, the specialist, should occur low down the shore and in the sublittoral, where physical conditions are less exacting and less changeable. The common winkle, *Littorina littorea*, has a much wider intertidal range and encounters a greater variety of physical conditions which fluctuate both diurnally and seasonally. The feeding apparatus of *Littorina* is consequently better equipped to utilise a wide variety of food sources, relying on a policy of opportunism as opposed to specialisation.

CHAPTER 5 : FOOD PREFERENCES

The composition of an animal's diet is of fundamental biological and ecological significance. As most organisms choose their prey from a broad spectrum of potential food sources, there is often considerable scope for selectivity. The literature concerning preferential grazing in the intertidal is particularly extensive. Much of this, however, is anecdotal and is derived from casual observations rather than controlled experiments. Nevertheless, diet has been assessed competently on the basis of a number of criteria including gut contents (e.g. Jones, 1948; Kitching and Ebling, 1961; Kitting, 1979; Vadas *et al.*, 1982), composition of faecal material (e.g. Jones, 1948), structure of mouthparts (e.g. Steneck and Watling, 1982) and response to laboratory choice experiments involving diverse techniques (e.g. Bakker, 1959; Lowe, 1974; Vadas, 1977; Lubchenco, 1978; Nicotri, 1980).

More sophisticated methods of determining food preferences have also been developed and applied, both in the laboratory and in the field. Kitting (1979, 1980) employed micro-acoustic and time-lapse photographic techniques to analyse the feeding behaviour of various limpet species, an approach which may prove particularly valuable in its ability to distinguish grazing on micro-epiphytes from consumption of the host plant. Grazing by microphagous species has been investigated using scanning electron microscopy (Nicotri, 1977; Underwood and Jernakoff, 1981), while the examination of food chains with radionuclide tracers has been attempted by Marples (1966) and by Estep and Dabrowski (1980).

Herbivore food preferences undoubtedly play a major part in determining the quantity and quality of food ingested and consequently the theoretical and practical importance of grazing selectivity should not be underestimated. Grazing preferences influence both the fitness and the physiological condition of the grazer (Hsiao and Fraenkel, 1968) and interact with plant competitive abilities, life histories and physical tolerances to determine the impact of the herbivore on the plant community (Lubchenco, 1978; Nicotri, 1980).

In a marine context, experimental studies of selective grazing have

concentrated primarily on the echinoderms, perhaps because of the relatively large size and tremendous destructive capabilities of these abundant and ubiquitous invertebrates. Echinoderm preferences are particularly well documented for species occurring in American waters (e.g. Leighton, 1966; Vadas, 1977; Larson *et al.*, 1980; Vadas *et al.*, 1982).

Definitive studies of gastropod preferences are less common and are concerned predominantly with the archaeogastropod limpets (e.g. Branch, 1975, 1976; Nicotri, 1977; Kitting, 1979, 1980; Steneck, 1982). Dietary preferences of the British limpets have been studied by a number of workers, including Jones (1948) (*Patella vulgata*) and Clokie and Norton (1974) (*Acmaea* spp.).

In recent years, the role of the common winkle as a determinant of community structure on northeastern American shores, has been scrutinised closely (e.g. Lubchenco, 1978; Sze, 1980; Bertness *et al.*, 1983; Cheney, unpub. data). This work prompted the oft-quoted experimental examination of grazing preferences by Lubchenco (1978) and the continuing studies by D.P. Cheney (pers. comm.) at Northeastern University and R.H. Seeley at Yale University.

Prior to changes in the taxonomy of "*Littorina obtusata*", the macroalgal feeding preferences of the flat winkle were assessed in controlled experiments by Barkmann (1955), Van Dongen (1956) and Bakker (1959). In light of the ecological differences between *L. obtusata sensu stricto* and *L. mariae*, and in particular the distinctive intertidal zonation, the value of such studies is now open to question.

Gastropod diets and food preferences have been reviewed by Clark (1958), Fretter and Graham (1962, 1977, 1980) and Hawkins and Hartnoll (1983). A comprehensive list of relevant references is supplied by Steneck and Watling (1982) in appendix form.

In this chapter, littorinid preferences are examined and compared. Where possible, the results of laboratory choice experiments are tested in controlled experiments on the shore.

5.1. Macroalgal preferences (laboratory)

Two distinct components of food preference can be recognised: one relating to the selection of a potential prey item (attractiveness) and the other concerning the rate at which that prey is ingested (edibility) (Nicotri, 1980). Edibility will reflect both the speed with which a given food item satisfies the physiological needs of the herbivore and the ease with which that item can be manipulated and ingested. Attractiveness will embrace a number of additional factors, including the capacity of the herbivore to detect plant odours and the non-nutritive characteristics which determine the value of the plant as a habitat (e.g. suitability as a spawning substrate; degree of shelter provided).

Techniques designed to measure attractiveness necessarily involve a choice between two or more potential food items. The choice may be made at close range on the basis of tactile and chemical sampling (e.g. Van Dongen, 1956; Bakker, 1959; Himmelman and Carefoot, 1975; Lawrence, 1975; Lubchenco, 1978; Nicotri, 1980; Jensen, 1983) or over some distance by chemoreception (e.g. Van Dongen, 1956; Vadas, 1977; Larson *et al.*, 1980; Bonsdorff and Vahl, 1982). Measurement of edibility on the other hand, entails active feeding and assumes that preferred foods are consumed faster, given equivalent hunger conditions (Leighton and Boolootian, 1963; Carefoot, 1967). The importance of incorporating an element of choice in gustatory experiments was strongly emphasised by Lawrence (1975) and by Vadas (1977). Both Lowe (1974) and Vadas (1977) working with sea-urchins, and also Nicotri (1980) working with isopods and amphipods, found considerable discrepancies between mean grazing rates when algae were offered singly and in paired combinations.

There has inevitably been much discussion and disagreement regarding the "best" method of assessing food preference. Larson *et al.* (1980) considered gustation to provide the most realistic measure of preference because of the complex behavioural mechanisms called into play. However I feel that there can be no single, "best" technique, as attractiveness and edibility clearly

measure quite different aspects of choice. Consequently, experiments have been designed to investigate both components of preference in this study. The selection of test algae was based on a number of criteria. All are species commonly encountered in the littoral zone in the Firth of Clyde but each was chosen carefully to ensure that a wide variety of growth forms, physiological types and life histories are represented. For practical purposes, crustose algae have been excluded.

5.1.1. Edibility

a) Methods

To determine the relative edibility of the test algae, specimens were presented with a series of choices between each test alga and a standard reference material. The necessity for gauging consumption against a standard was emphasised in preliminary experiments which revealed a high level of variability in feeding potential - both in time and between individual snails - regardless of previous diet (cf. Grime *et al.*, 1970).

Initially, trials were conducted with a number of artificial reference materials, including agar blocks and filter paper. None, however, proved sufficiently palatable. Consequently the approach advocated by Cates and Orians (1975) was adopted and a highly palatable food plant was selected as reference material. This technique is inherently less satisfactory, as inevitable differences between individual plants and between plant parts - particularly with respect to laminar toughness - may lead to unwanted variation in the edibility of the reference material. However a series of tests using a durometer (shore dial type) to measure toughness (see Section 6.2.1) showed that the flat, membranous lamina of the palatable green alga, *Ulva lactuca* was of relatively uniform toughness. Lowe and Lawrence (1976) also found that *Ulva* survived a seven day maintenance period in the laboratory with no significant loss of total organic material (ash analysis). Consequently, *Ulva* was used throughout gustatory experiments as a standard reference material. As a final

"quality check", durometer tests were carried out on all samples prior to choice experiments, thus ensuring that a realistic and consistent standard was applied.

In experiments involving gustation, the accurate measurement of consumption can constitute a major practical problem. In studies of terrestrial herbivory, where the plant material comprises leaves of comparable thickness, consumption may be measured and compared in terms of leaf area (e.g. Grime *et al.*, 1970; Cates and Oriens, 1975). Owing to the highly variable form of the seaweed thallus, wet weight is more commonly applicable as a measure of the consumption of algae, although difficulties arise where comparisons of feeding rates on calcareous and non-calcareous species are made.

Considerable time and effort was devoted to the standardisation of weighing techniques in an attempt to minimise error. Before weighing, algal samples were blotted methodically to remove surface moisture (cf. Leighton, 1966; Paine and Vadas, 1969a; Ayling, 1978). The time allocated to this procedure was standardised to ensure that prolonged exposure to the air did not occur.

Weighed samples (0.4-0.8 g) of the test alga and the reference material were placed in one compartment of a partitioned petri-dish set in the bottom of a shallow pyrex crystallising dish (diameter 9 cm). Filtered seawater was added to the level of the petri-dish rim. This arrangement ensured that crawling snails initially contacted both algal samples simultaneously.

To avoid unpredictable weight changes associated with the release of reproductive propagules, reproductive tissue was, wherever possible, removed from samples. The tough, cylindrical stipe of the fucoid algae was also discarded, samples being procured from the softer frond tissue.

As decaying tissue may bring undesired additional factors into play (Bakker, 1959), only freshly collected plants (3-10 days) in good physiological condition were used. Seasonal variation in plant quality was minimised by restricting trials to the period of April-July.

Macro-epiphytes were removed from all algal material prior to choice

experiments. Additionally, each sample was washed thoroughly in filtered seawater, restoring cell turgidity after storage and further cleaning the surface of the thallus.

Grime *et al.* (1968, 1970) acknowledged that the preparation of samples may, in itself, influence edibility, causing a breach in the plant's mechanical protection or facilitating the release of cell contents potentially repellent or attractive to the grazing mollusc. In this study it is hoped that the cleansing process effectively removed any inter- or intra-cellular products released from damaged regions of the thallus.

Snails were maintained until required for experimental purposes, in aerated seawater tanks (8-10°C) containing a selection of the test algae. To obtain uniformity of response and to minimise dietary preconditioning, the requisite number of snails was removed from the storage tanks and starved for six days prior to the initiation of feeding trials.

The trials were replicated ten times with each test alga using fresh batches of three adult common winkles or 10-15 adult flat winkles, in each replicate. At the end of the feeding period all remaining algal fragments were removed from the dishes, blotted and reweighed. Any replicate in which total consumption after five days failed to exceed 30 mg. (for common winkles) or 20 mg. (for flat winkles) was discarded and subsequently repeated. An additional series of ten ungrazed controls was monitored for *Ulva* and for each test alga. The controls contained samples of alga prepared as described, but lacked grazers and provided an estimate of weight changes resulting from growth, decomposition or imbibition. All experiments were carried out under a 16L : 8D photoregime (temperature 10-15°C).

Consumption was corrected for weight changes recorded in ungrazed controls and the ratio of test alga consumed to reference material consumed (wet weight), was calculated, giving a palatability index (P.I.) similar to that of Carefoot (1967) and Grime *et al.* (1970), so that:

$$\text{P.I.} = \text{mg. test alga consumed} / \text{mg. reference material consumed.}$$

Where the calculated P.I. was close to zero, it was necessary to establish with

greater certainty whether consumption of the test alga had in fact occurred. Initially, the possibility of labelling algal material was investigated using various dyes. Only the fluorescent dye, Ultraphor GPB PDR (BASF U.K. Ltd.), was found to stain algal material efficiently and to appear subsequently in the faeces. However trials showed that faecal pellets from *Littorina* fed on an unlabelled diet, also frequently fluoresced at the same wavelengths, albeit to a lesser extent.

Consequently it was decided simply to repeat the "two-way choice experiments" for all species with a calculated P.I. less than 0.1 (three replicates), using carefully selected, unblemished fragments of test alga. Where no evidence of grazing (macroscopic or microscopic) was apparent in any replicate after four days, the P.I. was amended to zero.

At some point, survival presumably dictates that feeding be invoked regardless of preference. Consequently, test algae with an amended P.I. equal to zero, were presented to *Littorina* in a series of "single alga feeding experiments". Evidence of grazing was monitored over a period of fifty days. Faecal material was removed daily and algal samples were replaced at ten day intervals with fresh material. Each experiment was replicated three times. A similar series of single alga feeding experiments was carried out using a small selection of the test algae and a variety of different intertidal gastropods.

b) Results

Although growth of algal samples during feeding trials could be discouraged by conducting the experiments in complete darkness (Table 15), a light/dark regime clearly stimulates considerably higher feeding rates (Table 16). Preliminary behavioural observations have shown that winkles placed in a tank containing seawater with light excluded, generally settle on the sides of the tank just above water level, following an initial spell of activity (cf. Dahl, 1964). Subsequently, disturbance of the water or illumination of the tank,

generally stimulates crawling (pers. obs.). Excursions from the position of settlement - whether in the laboratory or on the shore - may be regarded as feeding migrations (pers. obs.; Newell, 1958c). As high feeding rates were desirable, all gustatory experiments were conducted under a 16L : 8D photoregime.

Table 15. Effect of light regime on algal growth rate

Values represent the mean (\pm S.E.) of ten discrete four-day experiments.

Species	Mean growth rate	
	(mg wet weight . g initial weight ⁻¹ .day ⁻¹)	
	16L : 8D	24D
<i>Pelvetia canaliculata</i>	6.18 \pm 2.201	0.49 \pm 0.453
<i>Enteromorpha intestinalis</i>	1.95 \pm 2.393	-1.89 \pm 2.481*
<i>Ulva lactuca</i>	0.34 \pm 3.364	-0.12 \pm 2.967

* Release of spores prompted by change in light regime.

Table 16. Effect of light regime on common wrinkle feeding rates

Values represent the mean (\pm S.E.) of ten discrete four-day experiments, in which consumption of *Ulva lactuca* by four common wrinkle (20-25 mm. shell height) was calculated after correction of weight loss for growth of *Ulva*.

Light regime	Mean feeding rates
	(mg wet weight.snail ⁻¹ .day ⁻¹)
16L : 8D	7.19 \pm 1.243
24D	2.52 \pm 0.518

The results of feeding experiments with the common wrinkle *Littorina littorea*, are summarised in Table 17. To assess the reliability of the palatability index

as a means of comparing edibility, the common winkle was presented with a choice of two species (*Porphyra* and *Laurencia*) with similar palatability indices. The alga with the higher P.I. (*Porphyra*) was preferred in all replicates ($0.01 < P < 0.05$, Wilcoxon signed-rank test).

Common winkle grazing was highly selective in favour of the foliose ephemeral algae - particularly the greens, *Ulva* and *Enteromorpha*. The fucoid *Ascophyllum nodosum* and the calcified red alga *Corallina officinalis*, were rejected even under conditions of considerable nutritional duress. Grazing of *Ascophyllum* was eventually recorded after 42-49 days. However this consisted only of exploratory rasps inflicting superficial damage to the surface of the lamina (see Section 4.4.2b) and did not constitute concerted feeding.

Results of trials involving *Littorina obtusata* and *Littorina mariae* are presented in Tables 18 and 19. Preference rankings are virtually identical for both species. The fucoid algae are generally preferred, with the upper shore alga *Fucus spiralis*, heading the rankings for both species. *Ascophyllum nodosum* constituted the lowest ranked fucoid, with a mean P.I. of less than one for *L. mariae*. As with *Littorina littorea*, a series of trials were carried out with *L. obtusata* in which the edibility of test species was compared directly. Preference rankings were as follows:

Fucus spiralis > *F. serratus*; *F. vesiculosus* > *Ascophyllum*.

This corresponds closely to rankings determined on the basis of palatability indices (Table 18).

Single alga feeding experiments (Table 20) confirm that *Aemaea* is not primarily a grazer of erect macroalgal thalli, although radula marks were recorded on the thallus of *Fucus serratus* in each replicate. *Corallina* was consistently rejected by all species and *Ascophyllum* was only grazed readily by the two flat winkles.

Table 17. Macroalgal food preferences of *Littorina littorea* - Edibility.

Asterisks denote significance of difference between consumption of test and reference material (Wilcoxon signed-rank test).

Species	Choice experiments (Mean P.I. \pm S.E.)	Single alga feeding experiments (Time lapse before grazing, days)
<i>Ulva lactuca</i>	1	-
<i>Enteromorpha intestinalis</i>	0.54 \pm 0.241*	-
<i>Porphyra umbilicalis</i>	0.19 \pm 0.088**	-
<i>Laurencia pinnatifida</i>	0.11 \pm 0.045***	-
<i>Pelvetia canaliculata</i>	0.04 \pm 0.019***	-
<i>Fucus serratus</i>	0	3-6
<i>Polysiphonia lanosa</i>	0	6-7
<i>Cladophora rupestris</i>	0	20-24
<i>Gigartina stellata</i>	0	20-25
<i>Ascophyllum nodosum</i>	0	42-49
<i>Corallina officinalis</i>	0	No grazing

* 0.01 < P < 0.05

** 0.001 < P < 0.01

*** P < 0.001

Table 18. Macroalgal food preferences of *Littorina obtusata* - Edibility.

Asterisks denote significance of difference between consumption of test and reference material (Wilcoxon signed-rank test).

Species	Palatability Index (\pm S.E.)
<i>Fucus spiralis</i>	9.67 \pm 1.433**
<i>Fucus vesiculosus</i>	3.72 \pm 0.671**
<i>Fucus serratus</i>	3.63 \pm 1.016*
<i>Pelvetia canaliculata</i>	3.51 \pm 0.998**
<i>Ascophyllum nodosum</i>	2.05 \pm 0.796 n.s.
<i>Ulva lactuca</i>	1
<i>Cladophora rupestris</i>	0
<i>Polysiphonia lanosa</i>	0

n.s. not significant

* 0.01 < P < 0.05

** 0.001 < P < 0.01

*** P < 0.001

Table 19. Macroalgal food preferences of *Littorina mariae* - Edibility.

Asterisks denote significance of difference between consumption of test and reference material (Wilcoxon signed-rank test).

Species	Palatability Index (\pm S.E.)
<i>Fucus spiralis</i>	5.36 \pm 1.273**
<i>Fucus vesiculosus</i>	4.04 \pm 1.593 n.s.
<i>Fucus serratus</i>	3.10 \pm 0.532**
<i>Pelvetia canaliculata</i>	2.51 \pm 0.357*
<i>Ulva lactuca</i>	1
<i>Ascophyllum nodosum</i>	0.94 \pm 0.171 n.s.
<i>Cladophora rupestris</i>	0
<i>Polysiphonia lanosa</i>	0

n.s. not significant

* 0.01 < P < 0.05

** 0.001 < P < 0.01

*** P < 0.001

Table 20. Single alga feeding experiments with various intertidal gastropods.

Herbivore	Time lapse before grazing (days)			
	<i>Ulva lactuca</i>	<i>Ascophyllum nodosum</i>	<i>Fucus serratus</i>	<i>Corallina officinalis</i>
<i>Amaea tessulata</i>	-	-	6-12	-
<i>Littorina littorea</i>	1-2	42-49	3-6	-
<i>Littorina mariae</i>	1-2	2-4	1-2	-
<i>Littorina obtusata</i>	1-2	2-4	1-3	-
<i>Patella vulgata</i>	6-9	10-14	5-7	-

5.1.2. Edibility of furoid reproductive tissue

Investigations of marine plant-herbivore interactions tend to consider the algae as homogeneous entities. Yet Optimal Defence Theory predicts that plant defences will be allocated in direct proportion to the risk of each specific tissue and to the value of that tissue in terms of fitness loss resulting from attack by grazers (Rhoades, 1979).

There is also some evidence to suggest that different plant parts may rank differently in food preferences (Leighton, 1971; Faller-Fritsch and Emson, 1972; Janzen, 1973; Rhoades and Cates, 1976; Moore, 1977) and have different caloric compositions (Paine and Vadas, 1969b; Himmelman and Carefoot, 1975). Moore (1977) found that the amphipod, *Hyale nilssoni* preferred fertile *Pelvetia* frond tips to vegetative tips, while Himmelman and Carefoot (1975) felt that reproductive tissues (particularly female) may have a higher calorific value than non-fertile tissue. The caloric content of *Hedophyllum sessile*, *Iridaea cordata* and *Lessoniopsis littoralis* was shown to be maximal during reproductive periods. In view of these considerations, the relative palatability of female

Fucus serratus reproductive tissue, was tested for the littorinid snails, *Littorina littorea* and *L. obtusata*.

a) Methods

The edibility of the reproductive tissue was investigated in a series of two-way choice experiments. Each replicate (ten per species) compared consumption of reproductive and vegetative samples. As the weight of fertile reproductive tissue inevitably fluctuates in response to the release of gametes, wet weight was discarded in favour of surface area as a means of measuring consumption. (The apical portion of the thallus of *F. serratus* is of relatively uniform thickness.)

Surface area measurements were made using a portable area meter (LAMBDA Instruments Corporation Model L1-3000). The mean of 20 readings was calculated for each algal sample.

The toughness of vegetative and reproductive tissue was also compared using the Shore durometer. 50 readings were procured for each tissue.

b) Results

In grazing experiments with *Littorina littorea* (monitored over a four day period), reproductive tissue was grazed exclusively in nine replicates ($0.001 < P < 0.01$, Wilcoxon signed-rank test). Similarly, consumption of reproductive tissue by *Littorina obtusata* exceeded consumption of vegetative tissue in nine replicates ($0.01 < P < 0.05$, Wilcoxon signed-rank test).

Preferential grazing of the reproductive portion of the thallus (see also Fig. 34) did not appear to be related to thallus toughness. Durometer readings for female reproductive tissue (34.5 ± 1.3) did not differ significantly (Student's *t* test) from readings for vegetative tissue (32.4 ± 0.7).

5.1.3. Attractiveness

a) Methods

To investigate the attractiveness of the test algae a series of experiments were conducted similar to those of Lubchenco (1978). The principle was straightforward: pairs of test algae were placed in an aquarium to which snails were subsequently introduced and the position of the snails relative to the algae was recorded after a pre-determined period of time. Lawrence (1975) considered this to be the most efficient method of studying food preferences.

The base of a glass tank (20 cm. x 30 cm. x 20 cm. deep) was divided into three zones (Fig. 38), each of which was clearly delineated using a china-graph pencil. After filling the tank with filtered seawater to a depth of five to six centimetres, two test algae were selected and each was allocated to one of the peripheral zones (A and B, Fig. 38). The algae were held in position with plasticine. Wherever possible, entire undamaged fronds were used.

Following preparation of the tank, a batch of littorinids was introduced to the central area (Zone C, Fig. 38). As light is known to influence the direction of littorinid movement (e.g. Van Dongen, 1956; Newell, 1958b, 1958c; Evans, 1965; Underwood, 1979) the tank was covered with black polythene. 30 minutes after the initial introduction of specimens, snails climbing the walls of the tank were returned to the central area. After a further 30 minutes, exploratory behaviour had declined markedly and the number of snails on each alga was recorded. The experiment was repeated with every possible combination of test algae, using a fresh batch of 40 adult common winkles or 60 adult flat winkles in each trial.

All experiments were conducted at room temperature, to which specimens were acclimatised for 60-90 minutes prior to commencement of trials. Macro-epiphytes were removed from the algae (excepting *Ascophyllum* fouled with *Polysiphonia lanosa*) and all samples were cleansed in filtered seawater.

The major difference between the experimental design employed in this study

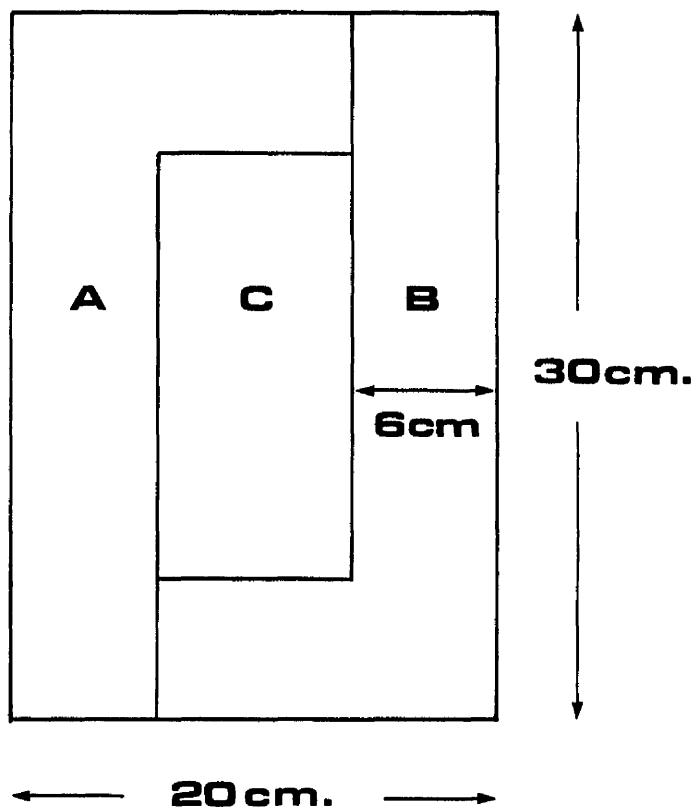


Fig.38 Plan of aquarium used in determination of attractiveness.

and that of Lubchenco (1978), concerns the size of the aquarium and the number of snails used in each trial. Here, the much smaller aquarium and the relatively larger sample size will abrogate mucus-trail following, a factor overlooked by Lubchenco and one which represents a serious flaw, effectively reducing her sample size greatly.

b) Results

The experiments enforced Lubchenco's (1978) behavioural observations. When a crawling snail contacted a piece of alga it responded either positively, by commencing feeding or stopping to rest, or negatively by continuing to crawl. Tactile-chemical means of food detection appeared to be more important than distance chemoreception.

The results are summarised in Tables 21-23. In all trials the number of snails on each alga was compared by Chi-squared analysis and each species was allocated a preference ranking based on the results. Consequently all members of Group I were significantly preferred to each member of Group II, and so on.

Experiments with *Littorina littorea* (Table 21) indicate three distinct categories of preference. Once again the three foliose ephemeral species were strongly preferred while *Corallina* was emphatically avoided. The remaining species were inseparable on the basis of Chi-squared analysis.

Both species of flat winkle were attracted primarily to the furoid algae (Tables 22 and 23). The preferences of the two species corresponded closely, although *Fucus serratus*, the phaeophyte on which *Littorina mariae* occurs almost exclusively in the field, ranks higher in the preferences of this species. Surprisingly, *Ascophyllum* is also highly ranked for *L. mariae* despite the apparent avoidance of *Ascophyllum* fronds in the field (see Chapter 2). *Ascophyllum* fouled with the epiphyte *Polysiphonia lanosa*, is consistently ranked lower than "clean" *Ascophyllum*, while the branched, filamentous alga *Cladophora*, is avoided by both species.

Table 21. Attractiveness of macroalgae for *Littorina littorea*.

Preference ranking (Chi-squared analysis)	Algae	Percentage of snails choosing each alga (in all comparisons)
I	<i>Enteromorpha intestinalis</i>	63
	<i>Porphyra umbilicalis</i>	68
	<i>Ulva lactuca</i>	61
II	<i>Ascophyllum nodosum</i>	49
	<i>Ascophyllum</i> + <i>Polysiphonia lanosa</i>	44
	<i>Cladophora rupestris</i>	43
	<i>Fucus serratus</i>	50
	<i>Gigartina stellata</i>	48
	<i>Laurencia pinnatifida</i>	51
	<i>Pelvetia canaliculata</i>	50
III	<i>Corallina officinalis</i>	21

Table 22. Attractiveness of macroalgae for *Littorina obtusata*.

Preference ranking (Chi-squared analysis)	Algae	Percentage of snails choosing each alga (in all comparisons)
I	<i>Fucus spiralis</i>	61
	<i>Fucus vesiculosus</i>	67
II	<i>Ascophyllum nodosum</i>	64
	<i>Fucus serratus</i>	50
	<i>Pelvetia canaliculata</i>	49
III	<i>Ascophyllum</i> + <i>Polysiphonia lanosa</i>	45
	<i>Ulva lactuca</i>	36
IV	<i>Cladophora rupestris</i>	24

Table 23. Attractiveness of macroalgae for *Littorina mariae*.

Preference ranking (Chi-squared analysis)	Algae	Percentage of snails choosing each alga (in all comparisons)
I	<i>Ascophyllum nodosum</i>	52
	<i>Fucus serratus</i>	57
	<i>Fucus spiralis</i>	60
	<i>Fucus vesiculosus</i>	53
II	<i>Pelvetia canaliculata</i>	54
III	<i>Ascophyllum</i> + <i>Polysiphonia lanosa</i>	45
IV	<i>Ulva lactuca</i>	43
V	<i>Cladophora rupestris</i>	33

5.1.4. Effect of starvation on preference

The effects of past feeding history on the diets and preferences of marine herbivores are largely unknown. However, theoretical studies of generalised herbivory (Emlen, 1966; MacArthur and Pianka, 1966) and empirical studies with arthropods and vertebrates (Young, 1945; Bernays and Chapman, 1970; Werner and Hall, 1974) strongly suggest that animals feed less discriminately after a period of starvation or when food is scarce. This theory was tested with the common winkle.

a) Methods

The winkles used in edibility trials with *Enteromorpha* and *Pelvetia* (Section 5.1.1) were subsequently isolated in aerated seawater tanks (16L : 8D photo-regime; 10-15°C) without food. Faecal material was removed daily and the tanks were cleaned regularly. After starvation for 50 days, the edibility of *Enteromorpha* and *Pelvetia* was re-assessed following the procedure outlined in Section 5.1.1a.

The influence of starvation on attractiveness was also examined. The attractiveness of two pairs of algae, *Ulva* and *Fucus serratus*, and *Enteromorpha* and *Pelvetia*, was assessed in four trials (40 specimens per trial). Following the trials the two batches of molluscs were placed in separate tanks and maintained for 50 days under the regime outlined above. The preference trials were then repeated.

b) Results

Following starvation, feeding rates were elevated considerably (Table 24). Mean palatability indices, however, did not differ significantly (Table 25) before and after starvation (Student's *t* test).

Application of Chi-squared analysis to the results of attractiveness experiments (Table 26) revealed no significant change in the relative attractiveness of *Ulva* and *Fucus*. Following starvation, however, the attractiveness of *Pelvetia* (relative to *Enteromorpha*) did increase significantly ($P < 0.05$).

It may be concluded that edibility remains unaffected by starvation (at least over this period of time). However, there is some evidence to suggest that differences in attractiveness of algae are less marked for specimens under nutritional duress.

During the course of these experiments, trial specimens continued to defaecate despite prolonged starvation. A number of fresh snails were subsequently maintained for over three months without cessation of faecal production. Preparations of faecal material excreted after 8-12 weeks' starvation were invariably found to comprise:

- 1) Large quantities of amorphous granules (often with a greenish-yellow pigmentation).
- 2) Smooth spherules similar in appearance to oil droplets but which did not stain with Sudan III.
- 3) Mucus.

The extract (in 90% Acetone) from samples of this material had an absorption spectrum with major peaks of activity at 660-670 nm and at 410 nm. An extract from the digestive gland of a starved specimen had an absorption spectrum which corresponded closely.

Table 24. Influence of nutritional state on feeding rate of *Littorina littorea*.

Diet	Mean feeding rate \pm S.E. (mg wet weight.snail ⁻¹ .day ⁻¹)	
	Pre-starvation	Post-starvation
<i>Ulva, Enteromorpha</i>	6.4 \pm 1.47	16.4 \pm 2.01
<i>Ulva, Pelvetia</i>	2.0 \pm 0.52	11.8 \pm 2.21

Table 25. Effect of starvation of *Littorina littorea* on algal edibility.

Alga	Mean P.I. \pm S.E.	
	Pre-starvation	Post-starvation
<i>Enteromorpha</i>	0.54 \pm 0.241	0.55 \pm 0.089
<i>Pelvetia</i>	0.04 \pm 0.019	0.07 \pm 0.030

Table 26. Effect of starvation of *Littorina littorea* on algal attractiveness.

Algal combinations	Distribution of snails		Significance level (Chi-squared analysis)
	Pre-starvation trials	Post-starvation trials	
<i>Ulva</i>	72	76	not significant
<i>Fucus serratus</i>	66	56	
<i>Enteromorpha</i>	70	61	0.01 < P < 0.05
<i>Pelvetia</i>	42	59	

5.1.5. Effect of habituation on preference

Elton (1927) and more recently Emlen (1966) and Kitting (1980), predicted that in situations where one food type achieves high levels of abundance, this food may be consumed with greater frequency relative to its abundance than another, even if the second food is richer and more efficiently exploited. Richter (1942) and Young (1945), working with vertebrates, and Wood (1968), Murdoch (1969) and Bernays and Chapman (1970) working with terrestrial molluscs, confirmed Elton's prediction, demonstrating experimentally that "preconditioning" or "habituation" can influence food preferences.

To test the applicability of this theory to marine organisms, the effect of prolonged exposure to an alga of medium-low preference ranking was assessed in the laboratory with *Littorina littorea*.

a) Methods

The specimens involved in gustatory preference experiments with *Fucus serratus* (Section 5.1.1) were subsequently transferred to an aerated seawater tank containing excised *Fucus* fronds (16L : 8D photoregime; 10-15°C). Faecal material was removed daily and fresh *Fucus* was added at regular intervals. 100 days after completion of the initial experiments, edibility trials were repeated in a second series of two-way choice experiments.

To investigate the effect of habituation on attractiveness, four trials (40 animals per trial) were performed with two pairs of test algae, *Fucus*/*Pelvetia* and *Fucus*/*Enteromorpha*. Specimens were subsequently preconditioned to *Fucus* as described above and the preference experiments were then repeated using the same algal pairs.

b) Results

Following enforced habituation, no change was apparent in the edibility of *Fucus*. In all replicates the reference material alone was consumed. The results of experiments on attractiveness, are summarised in Table 27. Following

enforced exposure to *Fucus*, the brown alga proved more attractive in both comparisons. The observed deviation from scores recorded in pre-habituations trials was highly significant ($P < 0.001$, Chi-squared test) for the *Fucus/Enteromorpha* combination but was not significant for the other pairing.

Clearly, recent dietary experience does not influence algal edibility. The attractiveness of non-preferred species may, however, be increased by prolonged exposure to that species.

Table 27. Effect of *Fucus* habituation on algal attractiveness to *Littorina littorea*.

Algal combinations	Distribution of snails		Significance level (Chi-squared analysis)
	Pre-habituations trials	Post-habituations trials	
<i>Fucus serratus</i>	45	56	not significant
<i>Pelvetia</i>	61	57	
<i>Fucus serratus</i>	34	55	$P < 0.001$
<i>Enteromorpha</i>	73	54	

5.2. Preference studies with algal germlings

The major influence of herbivory on the composition of littoral algal communities undoubtedly stems from intensive grazing of the early germling stages of the life history (see Geisselman, 1980; Lubchenco and Gaines, 1981; Jernakoff, in press). Assuming young plants to be most susceptible to herbivory, the length of time spent at the germling stage and the level of grazer activity during that period, will be of fundamental importance to the outcome of alga/grazer interactions. Both Jones (1946, 1948) and Lodge (1948) conclude that the mechanism enabling dense patellid populations to maintain large areas of rock completely devoid of macroalgal cover, is the efficient destruction of germlings soon after establishment. In central California, Dahl (1964) reported the occurrence of very young thalli in littorinid stomachs

and related this to the frequent appearance of completely bare rock, while in New England the persistence of *Chondrus crispus* in certain high tide pools has been attributed to the dense common winkle populations which eliminate the competitively dominant ephemeral species by feeding on the germling stages (Lubchenco, 1980).

The slower growing perennial algae have recourse to a size-related escape whereby sporelings either become immune to, or are able to withstand, gastropod depredation. This phenomenon was first recorded by Jones (1946) and has since been confirmed by Knight and Parke (1950) and by Menge (1975). Menge noted that *Fucus* germlings achieved relative immunity to snail grazing on reaching a thallus length of three to five centimetres.

Despite the potentially crucial nature of grazing during the early phase of algal development, very few attempts have been made to elucidate the possible influence of selective herbivory at this level. Cheney (unpub. data) attributed differences in the distribution of *Chondrus crispus* and *Fucus distichus* to anatomical adaptations to grazing exhibited by juvenile *Chondrus* but lacking in *Fucus*. The unpublished work of McPhail and Norton also points to strong selectivity at the germling level.

5.2.1. Germling edibility

Littorinid preferences were examined in two-way choice experiments using lawns of germlings grown on glass microscope slides. Consumption of test species was gauged, where possible, against consumption of *Ulva lactuca* which was highly palatable in both its adult and juvenile phases.

a) Methods

Culture of juvenile Chlorophyceae

Release of zoospores (and occasionally gametes) from freshly collected *Ulva* and *Enteromorpha* was achieved readily in the laboratory during spring and early summer. Fertile fronds of both species were recognised by the presence of

yellowish or brownish areas along the margins of the blade. In contrast to the observations reported by Sawada (1971), Sawada and Watanabe (1974) and Okuda (1975), optimum swarmer release from *Ulva* was achieved from plants collected at spring tides.

To secure release of spores from *Ulva lactuca*, fertile fronds were removed from several different plants and washed alternately in distilled water and sterile seawater. Fronds were then placed in square petri-dishes containing enriched seawater (see Appendix A) and clean microscope slides. The dishes were illuminated by bench lamps and examined periodically for release of swarmers. When sporulation was observed, the fronds were removed and the spores were permitted to settle overnight. If insufficient spores were present, the process was repeated with fresh fronds. Once adequate spore settlement was achieved, the enriched seawater medium was changed.

A similar procedure was adopted with *Enteromorpha*. Where release of swarmers from fertile tissue was poor, the fronds were refrigerated overnight on damp blotting paper. Re-immersion frequently stimulated heavy release of spores.

The rapid, dense growth of *Ulva* and *Enteromorpha* lawns generally suppressed the development of contaminating microflora. However large numbers of protozoans occasionally invaded the cultures and multiplied rapidly, feeding on the algal spores. Latterly, protozoan infestation was largely precluded by dipping the fertile fronds into one percent sodium hypochlorite solution followed by ice-cold enriched seawater.

Culture of juvenile Phaeophyceae

Fertile *Fucus serratus* was abundant on the shore from October/November to February/March. Following collection, excised fronds were stored overnight at 5°C. The following day, fertile receptacles were washed in cool tap water and wiped with paper towelling to eliminate gross contaminants and remove discharged gametes. Subsequently the fronds were placed on blotting paper and allowed to dry until slightly dehydrated (30-40 minutes at room temperature). Male and

female receptacles were separated and placed in beakers of cold, sterile seawater under an irradiance of approximately 12W.m^{-2} from warm white fluorescent tubes. Oospheres and antherozoids were usually released after several hours.

Insemination was accomplished by mixing the reproductive cells thoroughly and leaving the mixture to stand for approximately 30 minutes at room temperature. Subsequently the seawater supernatant was decanted, the fertilised eggs were re-suspended in cold, sterile seawater and the zygotes were permitted to settle.

On occasion, improved release of antheridia was stimulated by placing excised male receptacles on moistened blotting paper and refrigerating overnight. Re-immersion in cold seawater was invariably followed by copious discharge (after McLachlan *et al.*, 1971).

To achieve an even distribution of *Fucus* zygotes on the substratum, the microscope slides were placed in a container holding sterile seawater to a depth of five to six centimetres. The zygotes were dropped gently onto the water surface and permitted to settle undisturbed. Firm attachment was achieved after several hours and the slides were then transferred to standard square petri-dishes containing enriched seawater medium.

Sodium hypochlorite was initially used to eliminate surface contaminants from the excised thalli. However this was found to cause high gamete mortality and was consequently discontinued. Contamination of *Fucus* lawns was not generally a major problem.

Juvenile *Ascophyllum* was cultured in a similar manner, fertile fronds being collected from February to May/June. Excised male and female conceptacles were washed in tap water and placed in separate beakers of cold sterile seawater under an irradiance of approximately 12W.m^{-2} . Release of gametes from fertile conceptacles usually occurred within two to three hours. Following release, insemination was achieved as described above and zygotes were settled and transferred to square petri-dishes containing enriched seawater. The simple technique described by McLachlan *et al.* (1971) and discussed above, was frequently employed to stimulate release of gametes from "stubborn" conceptacles.

Culture of juvenile Rhodophyceae

Fertile *Gigartina stellata* was present on the shore from October/November to January/February. In the laboratory, papillae bearing fertile carposporangia were picked from the fronds and placed in vials containing sterile seawater. To reduce contamination, the vials were agitated with a flask shaker at a moderate speed for approximately two minutes. After shaking, the seawater was decanted and replaced with fresh, sterile seawater. This procedure was repeated six times. The papillae were then placed individually in drops of enriched seawater and left undisturbed in a growth cabinet overnight (culture conditions as below). Released carpospores were transferred by sterile Pasteur pipette to square petri-dishes containing enriched seawater and clean microscope slides.

Despite the elaborate cleansing technique, the slow growth of *Gigartina* discs permitted contaminating diatoms, green and blue-green algae and red algae of the genus *Audouinella*, to flourish. Consequently a large percentage of slides were necessarily discarded. Prior to the appearance of erect fronds, it was possible to restrict certain forms of contamination - notably diatoms - by periodic brushing of infested slides with a light paint brush. Attempts to minimise contamination by washing excised papillae in an ultrasonic cleaning bath (see Polne *et al.*, 1980) and by dipping fertile fronds in alcohol (75-80%) did not reduce contamination noticeably. Addition of germanium dioxide ($1-5 \text{ mg.l}^{-1}$) to the enriched medium for short periods, inhibited growth of diatoms but did not prevent contamination by other organisms.

Culture conditions

Prior to choice experiments, all germlings were cultured in a growth cabinet in enriched seawater medium (see Appendix A) under a 16L : 8D photo-regime, at an irradiance of approximately 12 W.m^{-2} and a temperature of $15 \pm 1^\circ\text{C}$. The enriched medium was changed on a weekly basis.

The germling lawns were used in choice experiments on reaching a thallus length of 0.85-1.10 mm. Under these conditions, the time taken for this length

to be attained, varied as follows: *Enteromorpha* and *Ulva*, 19-22 days depending on the density of the lawn; *Fucus*, 19-27 days; *Ascophyllum*, 28-34 days; *Gigartina*, 80-90 days.

Choice experiments

Preference experiments compared consumption (dry weight) of two test species. The initial density of each lawn was assessed using a portable area meter (LAMBDA Instruments Corporation Model LI-3000). To ensure consistency, 20 readings were procured for each slide and an average value was calculated. Excess moisture was drained from all lawns before the readings were taken and a clean microscope slide was drawn across the photocell periodically to ensure that the readings were genuine.

For each test species, a linear regression equation was computed between germling density (independent x variable) and dry weight (dependent y variable) based on a sample of 15 lawns (see Table 28). This enabled meter readings to be converted to estimates of dry weight.

Each comparison of test species was replicated ten times. In each replicate three common winkles (or ten flat winkles) were placed in a crystallising dish containing filtered seawater and a paired combination of germling lawns. Lawns of comparable dry weight, mean thallus length and density were selected.

Grazing was permitted to proceed under a 16L : 8D photoregime and at a temperature of 10-15°C for one to three days, depending on the voracity of the snails. Each experiment was terminated before consumption of all the germlings had occurred. Subsequently the remnants of each lawn were dried overnight in an oven and the dry weight of the surviving germlings was determined.

b) Results

Results of germling choice experiments are recorded in Table 29. All results confirmed visual assessments of grazing preferences. The common winkle

significantly preferred *Ulva* to all other species, excepting *Enteromorpha*. In paired combination with *Ulva* and *Fucus*, *Ascophyllum* germlings were not grazed. In a supplementary series of single-alga feeding experiments (five replicates), *Ascophyllum* lawns were initially avoided by the common winkle for five to six days. Subsequently a considerable quantity of germlings was dislodged by crawling snails and it proved impossible to assess accurately the quantity actually ingested.

The red alga *Gigartina*, also remained ungrazed during two-way choice experiments but in single-alga feeding trials (five replicates), grazing of both discs and erect fronds was invariably recorded within four days.

Foraging snails were occasionally observed to break the erect *Gigartina* fronds while crawling across the lawns. To assess the ability of *Gigartina* to replace broken fronds, a sample of fifty healthy sporelings was selected and a blunt scalpel was used to snap the upright fronds at the base. The average diameter of the discs was approximately 2.5 mm. and the average length of the fronds 1.6 mm. After 98 days, all discs showed clear signs of decay and no re-growth of erect fronds was apparent.

The flat winkle *Littorina obtusata*, was offered a choice between two contrasting species: the furoid, *Ascophyllum* and the ephemeral green alga, *Enteromorpha*. No significant preference was shown towards either alga and both were grazed enthusiastically.

Table 28. Relationship between density and dry weight of germling lawns:
Linear regression equations (x = density; y = dry weight; $y = a + bx$).

Alga	Y-intercept, a	Regression coefficient, b	Correlation coefficient, r
<i>Ascophyllum nodosum</i>	0.0153	0.0028	0.898***
<i>Enteromorpha intestinalis</i>	0.0058	0.0184	0.962***
<i>Fucus serratus</i>	0.0060	0.0296	0.889***
<i>Ulva lactuca</i>	0.0066	0.0190	0.969***

*** $P < 0.001$

Table 29. Germling choice experiments.

Species	Germling combination	Preferred alga (Wilcoxon signed-rank test)
<i>Littorina littorea</i>	<i>Ascophyllum</i> ; <i>Ulva</i>	<i>Ulva</i> ***
<i>Littorina littorea</i>	<i>Enteromorpha</i> ; <i>Ulva</i>	-
<i>Littorina littorea</i>	<i>Fucus serratus</i> ; <i>Ulva</i>	<i>Ulva</i> *
<i>Littorina littorea</i>	<i>Gigartina</i> ; <i>Ulva</i>	<i>Ulva</i> ***
<i>Littorina littorea</i>	<i>Ascophyllum</i> ; <i>Fucus</i>	<i>Fucus</i> **
<i>Littorina obtusata</i>	<i>Ascophyllum</i> ; <i>Enteromorpha</i>	-

* $0.01 < P < 0.05$

** $0.001 < P < 0.01$

*** $P < 0.001$

5.2.2. Direct comparison of adult and juvenile edibility

There has apparently been no attempt to compare experimentally, preferential grazing of the adult and juvenile stages of an alga. Field observations of littorinid microdistribution suggest that the common wrinkle may be, by preference, a grazer of the germling stages, while the flat wrinkle selects the adult thallus. This section compares grazing on the adult and juvenile stages of taxonomically and structurally contrasting algae.

a) Methods

Feeding on germling lawns and excised adult tissue was compared in a series of two-way choice experiments. The basic experimental procedure was identical to that described above for germling versus germling trials. To estimate the initial dry weight of the adult material, linear regression equations of wet

weight (independent x variable) against dry weight (dependent y variable) were computed for adult tissue (see Table 30). The regression equations were based on wet weight/dry weight data for 30 samples.

Table 30. Relationship between wet and dry weight of adult tissue: Linear regression equations. (x = wet weight; y = dry weight; $y = a + bx$)

Alga	Y-intercept, a	Regression coefficient, b	Correlation coefficient, \bar{r}
<i>Fucus serratus</i>	-0.0130	0.2510	0.930***
<i>Ulva lactuca</i>	0.0305	0.0439	0.973***

*** P < 0.001

b) Results

The results are summarised in Table 31. Once again, all results confirm visual assessment of grazing preferences. Adult *Ulva* and juvenile *Fucus* were significantly preferred by the common wrinkle. *Littorina obtusata* revealed contrasting preferences, selecting for germling *Ulva* and adult *Fucus*.

Table 31. Adult versus germling choice experiments.

Littorinid species	Preferred stage (Wilcoxon signed-rank test)	
	<i>Fucus serratus</i>	<i>Ulva lactuca</i>
<i>Littorina littorea</i>	Germling**	Adult*
<i>Littorina obtusata</i>	Adult*	Germling*

* $0.01 < P < 0.05$

** $0.001 < P < 0.01$

5.2.3. Dislodgement of germlings during foraging

Germling choice experiments show that *Ascophyllum* is seldom grazed at the juvenile phase, even under conditions of moderate nutritional duress. Yet grazing of *Ascophyllum* germlings has been blamed for low survivorship of juvenile *Ascophyllum* on the shore (Keser *et al.*, 1981; S.L. Miller and R.L. Vadas, pers. comm.). A factor which is frequently overlooked but which was very evident in laboratory choice experiments, is the accidental dislodgement of germlings by crawling (not feeding) snails. Dislodgement of germlings by non-feeding *Littorina littorea* has been assessed in a series of simple laboratory experiments.

a) Methods

Three glass microscope slides supporting *Ascophyllum* lawns of approximately equal density, were fitted into the base of an 8cm. square plastic dish. The dish (2cm. deep) was filled with filtered seawater and two common winkles (21-22 mm. shell height) were introduced. The snails were permitted to crawl for 30 minutes, during which time any specimen becoming inactive was immediately

replaced. Throughout the experiment the snails were physically prevented from climbing the walls of the dish.

The initial germling density was estimated by counting the germlings in ten randomly selected fields (x5, binocular microscope) on each slide. 30 minutes later the snails were removed and the total number of dislodged germlings was recorded.

To provide the germlings with an ideal surface on which to attach firmly, the glass slides were covered with a layer of fine sand held in place by a thin film of Aquaseal aquarium sealant. Germlings were grown to a mean thallus length of 0.85-1.10 mm. The experiment was replicated five times and five control dishes (without snails) were also monitored.

b) Results

The average percentage of germlings dislodged in trial and control runs was calculated. Even over the short duration of the experiment, almost five times as many germlings were dislodged in trials (0.76%) as in controls (0.16%). Application of the Student's *t* test to transformed data showed a significant difference at the 99.9% probability level.

5.3. Selective grazing in the field

Ayling (1978) stressed the need for extreme caution when predicting diets in the field, from the results of laboratory choice experiments. Nevertheless, it is undoubtedly simplest to separate the interactions controlling dietary selection, in the laboratory. If such an approach is adopted, it is essential that the applicability of laboratory preference rankings to foraging strategy on the shore, be adequately tested.

In the past, herbivore field diets have been assessed by the analysis of gut contents and/or faecal pellets (e.g. Jones, 1948; Grime *et al.*, 1970; Kitching and Ebling, 1961; Kitting, 1979; Vadas *et al.*, 1982). However, algae have few skeletal or hard structures and differential digestion or

decomposition, rather than preference, may account for the presence, absence or abundance of prey in the gut (Vadas, 1977). Indeed indigestible and hence readily identifiable species, may be poorly utilised metabolically and possibly consumed only in relatively small quantities.

Here, macroalgal grazing on the shore is assessed instead, by examining the microdistribution of foraging specimens. As the gastropod grazers are not highly mobile, this approach is likely to give a good indication of field diets.

5.3.1. Microdistribution of common and flat winkles on the shore

a) Methods

The distribution of foraging winkles with respect to a variety of potential food resources was examined at Butter Lump on the Isle of Cumbrae. To coincide with a period of high littorinid activity, all samples were collected on a rising tide 10-15 minutes after submersion.

Sampling on the mid-shore was carried out in an area with a relatively dense common winkle population under a mixed canopy of *Ascophyllum* and *Fucus serratus*. In this area the dominant understorey alga was the ephemeral green *Ulva lactuca*. Two quadrats (0.5m x 0.5m) were placed on the substratum and each plant attached within the quadrats was carefully excised at the holdfast and placed in a polythene bag for subsequent examination in the laboratory. Particular care was taken not to dislodge snails from the algae during removal. It was often possible to minimise disturbance by engulfing the entire plant in a fine mesh bag prior to breaking the holdfast.

In the laboratory, the total surface area of each plant was determined using a portable surface area meter. Inevitably, surface area measurements based on planimeter readings, fail to take into account the additional surface area of the elipsoid *Ascophyllum* bladders. To make allowance for this potential source of error, the surface area of a randomly collected sample of *Ascophyllum* was determined using the surface area meter. The diameter and length of each

bladder was measured using Vernier calipers and the total surface area of the bladders was estimated, assuming each bladder to approximate to a pair of cones placed base to base. Finally, the bladders were excised from the plants and the surface area of the remaining thallus material was determined using the surface area meter. This enabled a correction factor to be computed and applied to all *Ascophyllum* surface area measurements. The discrepancy between "actual" surface area and planimeter surface area was approximately 29 cm^2 per 1000 cm^2 planimeter surface area. For each quadrat the surface area of the stone substratum was assumed to be 0.25 m^2 , though this is undoubtedly a slight underestimate.

At Butter Lump the common wrinkle reaches its highest density in the upper *Ascophyllum* zone, a part of the shore on which loose "drift" algal material is regularly deposited by the receding tide. A second area of high littorinid density was selected within this zone and microdistribution was recorded in two $0.5\text{m} \times 0.5\text{m}$ quadrats, following the procedure outlined above.

The distribution of *Littorina obtusata* and *Littorina mariae* was assessed in a similar manner. Two areas were selected for sampling: a *Fucus serratus* stand with an understorey of *Ulva lactuca*; and a mixed stand of *Ascophyllum*, *Fucus serratus* and *Fucus vesiculosus*. Juvenile flat wrinkles (immature specimens, shell length generally $< 0.7 \text{ cm}$) were recorded separately. No allowance was made for the additional surface area of *Fucus vesiculosus* bladders. Bladders of this species are less abundant, smaller and flatter than those of *Ascophyllum*.

b) Results

The results are summarised in Tables 32-35. All figures represent wrinkle abundance per m^2 of stone substratum or algal lamina. On the mid-shore, common wrinkle foraging is confined predominantly to the stone substratum and to attached *Ulva*. Very few specimens were found on either of the perennial fucoids - particularly the inedible *Ascophyllum*. Further up the shore, where littorinid populations are generally denser, but more patchily distributed, loose "drift" algal material attracts foraging individuals at high densities.

Table 32. Microdistribution of foraging *Littorina littorea* in the *Ascophyllum/Fucus serratus* zone.

Substratum	Abundance (Numbers.m ⁻²)
<i>Ascophyllum nodosum</i>	0.92
<i>Fucus serratus</i>	3.34
<i>Ulva lactuca</i> (attached)	18.68
Rock	30.00

Table 33. Microdistribution of foraging *Littorina littorea* in the upper *Ascophyllum* zone.

Substratum	Abundance (Numbers.m ⁻²)
<i>Ascophyllum nodosum</i>	11.08
<i>Porphyra umbilicalis</i> (unattached drift)	60.72
<i>Ulva lactuca</i> (unattached drift)	107.68
Rock	134.00

Table 34. Microdistribution of foraging flat winkles in the *Fucus serratus* zone.

Substratum	Abundance (Numbers.m ⁻²)		
	<i>Littorina obtusata</i>	<i>Littorina mariae</i>	Juveniles
<i>Fucus serratus</i>	3.51	6.09	37.04
<i>Ulva lactuca</i> (attached)	-	1.23	73.53
Rock	-	-	14.00

Table 35. Microdistribution of foraging flat winkles in a mixed furoid stand.

Substratum	Abundance (Numbers.m ⁻²)		
	<i>Littorina obtusata</i>	<i>Littorina mariae</i>	Juveniles
<i>Ascophyllum nodosum</i>	7.70	2.70	20.31
<i>Fucus serratus</i>	5.43	6.07	11.82
<i>Fucus vesiculosus</i>	12.85	3.10	13.73

In the *Fucus serratus* zone, adult flat winkles of both species are confined almost exclusively to the lamina of the canopy species. Juvenile specimens, however, occurred at high densities in the "wrinkles" of attached *Ulva* fronds and in crevices on the rock surface - both of which provided suitably sheltered and protected microhabitats.

Amongst mixed furoids, *Littorina obtusata* was most abundant on *Fucus vesiculosus*, a species which ranked very highly in both edibility and attractiveness trials. *L. mariae* was most common on *Fucus serratus* but notably rare on *Ascophyllum*. Juvenile specimens were common on all furoids but achieved maximum densities on *Ascophyllum nodosum*. Damaged *Ascophyllum* bladders appeared to provide ideal sanctuary for immature specimens.

5.3.2. Microdistribution of high-shore common winkles at Portencross,

Firth of Clyde

The common winkle populations occurring at sheltered locations in the Portencross area (O.S. Map Ref.: NS176489) are unusually dense. On the upper shore, the mean population density generally falls between 200 and 1200 specimens per m². Locally, however, populations of up to 8,000-10,000 per m² were recorded (see Fig. 1). The substratum at these sites is composed largely of loose stones and small boulders. Attached macroalgae are completely absent.



Fig. 39. *Littorina littorea* feeding on unattached drift *Ulva*.

Casual observations at Portencross suggest that loose, "drift" algal material deposited on the upper shore - particularly *Ulva lactuca* - may fulfil a potentially critical role in the diet of upper shore populations (see Figs. 39 and 40).

a) Methods

Ten 0.5m. x 0.5m. quadrats were sampled in areas with abundant drift *Ulva*. In each quadrat, common wrinkle distribution was recorded relative to the available surface area (rocky substratum and ulvoid thallus). For five of the quadrats, the number of active snails on each surface was also recorded. All *Ulva* appeared to be in good condition and durometer tests did not reveal any significant difference in toughness from fresh, attached *Ulva* collected lower down the shore.

b) Results

The mean density of snails on unattached *Ulva* was $0.062.\text{cm}^{-2} \pm 0.008$ and on the rock, $0.050.\text{cm}^{-2} \pm 0.008$. The Wilcoxon signed-rank test revealed a significant difference at the 95% probability level. 88% of specimens on *Ulva* compared with 39% on the rock, were active at the time of sampling.

5.3.3. Grazing of mature furoids by the common wrinkle

Laboratory preference experiments indicate that *Ascophyllum* is peculiarly distasteful to *Littorina littorea*. Despite the occurrence of locally dense littorinid populations in the upper *Ascophyllum* zone, there is no visual evidence of intensive common wrinkle grazing damage on *Ascophyllum* fronds in the field. To investigate the short-term impact of dense common wrinkle populations on a stand of *Ascophyllum*, a series of herbivore inclusion/exclusion experiments were designed.



Fig. 40. *Littorina littorea* feeding on unattached drift
Fucus serratus.

a) Methods

Four sheltered plots (0.6m.x 0.5m) were selected on open rock at Butter Lump: two in the *Ascophyllum* zone and two in a pure stand of *Fucus serratus*. All attached algae were cleared from the rock surface immediately surrounding the plots and a band of TBT anti-fouling paint 0.1-0.2m.wide, was applied to the substratum to prevent immigration of limpets, restrict movement of littorinids and mark the location of the plots (see Fig. 41). Despite Hawkins and Hartnoll's (1983) concern over the effects of toxic run-off from anti-fouling paints, neither the snails nor the algae appeared to suffer adverse effects over the two-week experiment. All flat winkles, limpets and predatory *Thais lapillus* were removed from the plots at the outset. Table 36 outlines the treatments applied to each plot.

The adjusted littorinid populations in plots A2 and B2 were maintained at a density of $500-670.m^{-2}$. Although the experiment coincided with a period of unusually calm weather, snails were frequently washed from the trial plots and it was necessary to visit the site daily to replace dislodged specimens. The experiment was terminated after two weeks, when heavy seas dislodged all the snails and severely damaged *Fucus* and *Ascophyllum* plants within the plots.

b) Results

The initial absence of *Littorina littorea* from all plots was characteristic of "open rock" at every site examined in the Firth of Clyde in the course of this study, regardless of exposure and the corresponding presence/absence of furoid cover.

Figs. 42 and 43 illustrate the condition of algae in the trial plots before the introduction of winkles and two weeks later, at the end of the experiment. *Fucus* suffered extensive grazing damage - particularly at the frond tips, but no damage was apparent on *Ascophyllum* fronds despite the dense winkle population. Algae in both control plots remained in excellent condition throughout the experiment.

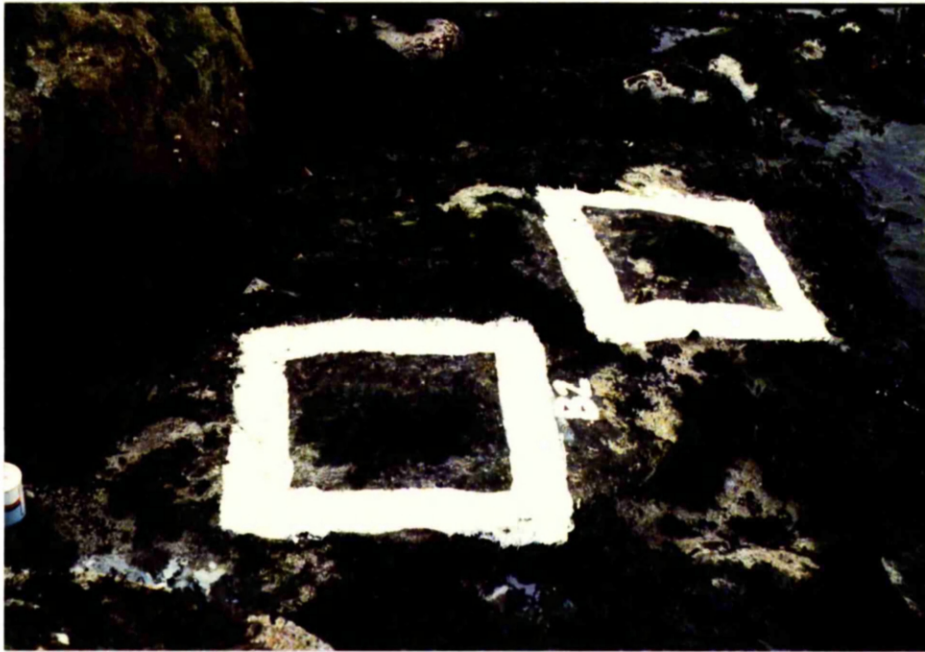


Fig. 41. *Fucus* plots in *Littorina littorea* inclusion/exclusion experiments.

Table 36. Herbivore inclusion/exclusion experiments : Treatment of experimental plots.

Plot Number	Alga	<i>Littorina littorea</i> population density (.m ⁻²)	
		Original population	Adjusted population
A1 (control)	<i>Ascophyllum</i>	0	0
A2	<i>Ascophyllum</i>	0	500-670
B1 (control)	<i>Fucus serratus</i>	0	0
B2	<i>Fucus serratus</i>	0	500-670

5.4. Discussion

Laboratory choice experiments indicate strong food preferences. For the common winkle these correspond closely to the preferences described previously by Lubchenco (1978) working with specimens collected in the New England inter-tidal.

Attractiveness and edibility rankings are similar for both common and flat winkles. Being slow moving herbivores, the littorinids are unable to deal separately with food requirements and habitat requirements, and consequently the snails must balance these two - often conflicting - sets of selective pressures in arriving at a clearly defined series of preferences. For the common winkle, which is primarily an inhabitant of the rock surface, gustatory requirements will constitute the predominant factor in determining this balance. The high ranking of the foliose ephemeral algae indicates that these species are nutritionally valuable and/or easily consumed.

In contrast, the flat winkle relies on the macroalgal thallus to provide both food and a secure and safe habitat. As a result, it is not surprising to find the perennial canopy-forming algae outranking the ephemeral understory



A.



B.

Fig. 42. Effect of dense common winkle population on *Fucus serratus* in inclusion/exclusion experiments.

A. Typical fronds prior to introduction of *Littorina*.

B. The same fronds two weeks after introduction.



A.



B.

Fig. 43. Effect of dense common wrinkle population on *Ascophyllum* in inclusion/exclusion experiments.

A. Typical fronds prior to introduction of *Littorina*.

B. The same fronds two weeks after introduction.

species in attractiveness trials. That the same species also rank highest in gustatory experiments suggests that the flat winkles have evolved the additional capacity to utilise the thalli efficiently as food sources. This is borne out by differences in the shape of the radular teeth (Chapter 4) which permit *L. obtusata* and *L. mariae* to excavate the furoid thalli with particular dexterity.

Single-alga feeding experiments with the common winkle demonstrate a capacity to graze algae representing a wide variety of contrasting taxonomic and morphological types. Nevertheless, many of these species are grazed only after a lengthy period of starvation. A premise of Optimal Foraging Theory is that foraging behaviour is designed to maximise energy assimilation per unit time (Hughes, 1980). It is probable that those algae rejected under normal circumstances, are either difficult to consume or low in energy content. Hence the effort of ingestion and assimilation is unwarranted in the presence of alternative higher-ranking food items.

Grazing of the erect, coralline alga *Corallina*, is precluded by the nature of the alga's structural defences. The articulated joints between calcareous segments constitute the only potential "Achilles Heel". As the gastropod radula is invariably wider than the uncalcified joints, the alga's defences are, to all intents and purposes, impregnable to all species (see Goss-Custard *et al.*, 1979). Erect, calcareous plants also rank very low in urchin preferences (Vadas *et al.*, 1982; N.T. Hagen, pers. comm.) and are seldom grazed by herbivorous fish (Vermeij, 1978), perhaps because structural calcium carbonate reduces the alga's calorific value (Hawkins and Hartnoll, 1983). As a result, members of this functional group are largely immune to grazing both in the intertidal and in the subtidal (see Paine, 1979).

The effect of recent feeding history on the subsequent diets and preferences of marine herbivores is largely unknown. The results of starvation experiments carried out in this study underlined the importance of distinguishing between attractiveness and edibility. Edibility is a function of the mechanical capabilities of the feeding apparatus together with the nutritional value of the potential food item. Consequently, edibility is not influenced by the

nutritional state of the grazer. Attractiveness, however, balances not only the gustatory aspects of the food substrate, but also those attributes which determine its value as a habitat. Under conditions of nutritional duress a shift of emphasis away from habitat value and towards food value may be anticipated. Certainly *Ulva* attracted a higher proportion of snails in post-starvation trials, although this difference was not statistically significant. With respect to the *Enteromorpha/Pelvetia* comparison, foraging actually became significantly less discriminate following starvation, with more specimens attracted to the less edible species, *Pelvetia*. Unless there is a particularly wide gulf in edibility between the two species (e.g. *Ulva/Fucus serratus*) the tendency for foraging snails simply to settle and feed on the first alga encountered (regardless of the position of that species in normal preference rankings) will probably grow as the snails become hungrier, resulting in less discriminate grazing.

The components of faecal material excreted by starved specimens were not identified positively. However, J.A. Allen (pers. comm.) made comparable observations on the excreted products of starved bivalves and considered the faecal material to comprise primarily the products of the continuing activity of the vacuolated cells of the digestive gland. In starved bivalves, these cells continue to cut off spherules into the lumen of the digestive diverticulum. Fretter and Graham (1962) refer to a similar mechanism in certain gastropods, "large yellowish concretions, usually spherical but frequently quite irregular in shape" appearing in the faeces. Though the true nature of the spherules is unknown, the concretions appear to originate in the crypts of the digestive gland and probably comprise truly excretory material rather than faecal matter derived from the indigestible residue of particulate food (Fretter and Graham, 1962).

Previous feeding experience, like nutritional status, affects attractiveness and not edibility. In trials with *Littorina littorea*, edibility remained unchanged following prolonged exposure to *Fucus serratus*. The furoid, however, attracted significantly more snails in post-habitation trials. The underlying

mechanics of the pre-conditioning process are uncertain. On an evolutionary time-scale, the feeding apparatus of each species is undoubtedly refined to permit feeding on those food sources with which the animal comes into contact most frequently. Over a short period of time, individual specimens may be pre-conditioned to respond strongly to some facet of an abundant alga - perhaps the exudate - simply because this is associated with the presence of food, irrespective of whether or not that food is highly preferred.

Temporary habituation to a specific food item should be distinguished from the form of habituation demonstrated by Hirsch (1951) and by Humphrey (1930), who examined the termination of responses to artificial stimuli such as mechanical agitation. The former, based on a strengthened response, may be termed positive habituation and the latter, involving a loss of response, negative habituation.

The elevated edibility of fucoid reproductive tissue has been noted previously by Faller-Fritsch and Emson (1972), working with sea urchins, and is a phenomenon which may influence algal fitness markedly - especially where the canopy-dwelling flat winkle is abundant. Greater edibility may result from a higher nutritional value or, alternatively, from a reduction in anti-herbivore defences. With regard to structural defences, fucoid receptacles are not significantly softer than vegetative tissue (durometer tests). However, Rhoades and Cates (1976) suggest that different tissues may vary in their content of defensive compounds and/or digestibility reducers. The phenolics most commonly associated with the fucoid algae, act as quantitative, generalist barriers to consumption and Levin (1976) considered the within-plant distribution of such compounds to vary according to the age, but not the physiological state, of the tissue. Hence it is improbable that the level of phenolics causes the high edibility of the *Fucus* receptacles. It is more likely to be a reflection of their elevated nutritional or caloric value (see Paine and Vadas, 1969b; Geiselman, 1980). It should be borne in mind, however, that the within-plant distribution of secondary plant metabolites is still largely unexplored.

On the mid-shore, the microdistribution of common winkles confirms the

macroalgal preference rankings derived in laboratory experiments. A high proportion of foraging specimens were found on the rocky substratum and this is probably indicative of the important role played by diatoms and microalgae in the diet. A very definite avoidance of *Ascophyllum*, both as a food plant and as a habitat, was manifest throughout, irrespective of the epiphyte load.

In the upper *Ascophyllum* zone, where snail densities are generally higher, changes in the availability of food resources prompt a corresponding change in foraging strategy. Here, the only potential macroalgal food occurring in any abundance is the non-preferred *Ascophyllum* and even this may be virtually absent at more exposed sites such as Portencross. However each high tide deposits a quantity of loose, drift algal material on this part of the shore which provides an ideal food source for the opportunist grazer. At sites supporting dense winkle populations, unattached macroalgal material may be of vital importance, as intensive grazing and crawling maintains a "clean" rock surface virtually devoid of edible detritus and microflora. Normal macroalgal preferences also appear to operate with respect to the consumption of drift material. Drift green algae are rapidly consumed and the loose material which accumulates in greatest quantities is invariably derived from *Ascophyllum* and the tough laminarians (pers. obs.).

Utilisation of drift algae by the common winkle is not unique (see Hawkins and Hartnoll, 1983). D.W. Keats (pers. comm.) also noted the importance of drift algae to sea urchins in an urchin/coralline dominated subtidal community in Newfoundland. The dietary role of drift material was confirmed by Vadas (1977), who stressed its value when food is scarce and the location of preferred food items incurs an abnormally high energy expenditure.

Grazer inclusion/exclusion experiments reinforced the observed distaste for *Ascophyllum*, confirming that, in spite of its abundance, this furoid cannot be considered to constitute a potential food source. Feeding on *Fucus serratus*, also a non-preferred item in laboratory experiments, did occur however, indicating that specialisation has not evolved to a point where the occurrence of preferred prey items is essential to survival.

The flat winkle is characterised by a rather higher degree of specialisation. On all parts of the shore the distribution of foraging flat winkles emphasises the strong preference for perennial canopy-forming algae, both as a habitat and as a food source. For *Littorina obtusata*, the high ranking of *Fucus vesiculosus* in both attractiveness and edibility trials is paralleled by a strong preference for this species in the field. *L. mariae* was strongly attracted in the laboratory to *Fucus serratus* and this, too, is reflected in the microdistribution of the species on the shore. The low edibility of *Ascophyllum* is also borne out in the field, as *L. mariae* occurs only rarely on *Ascophyllum*, even where this species is closely associated with inhabited *Fucus serratus*. However it remains something of an anomaly that *Ascophyllum* should at the same time rank highly in attractiveness experiments.

It has been suggested (e.g. Reimchen, 1974) that the flat winkle's main source of food may be the microepiphytic flora inhabiting the surface of the fucoid "host" and that the ingestion of fucoid tissue during grazing is inadvertent. However the quantity of thallus material ingested, both in laboratory experiments (see Figs. 36, 37) and in the field (pers. obs.), is such that this appears highly improbable. Temporal fluctuations in epiphyte populations, particularly on the epidermis-shedding *Ascophyllum nodosum* (see Filion-Myklebust and Norton, 1981), render this an unreliable food source even where flat winkle population densities are low. Laboratory observations, however, do suggest that the epiflora may constitute a much more important dietary component for juvenile specimens.

Attractiveness trials involving *Ascophyllum* epiphytised by the red alga, *Polysiphonia lanosa*, show that heavy fouling with an inedible macroscopic species reduces attractiveness, presumably hindering both crawling and grazing on the fucoid thallus.

Grazing of germling algae by the common winkle was also highly selective, with the ephemeral species being preferred to the perennial fucoids. In choice experiments, juvenile *Ulva* and *Enteromorpha* were consumed with equal relish. The difference in edibility between adult *Ulva* (P.I. = 1) and adult *Enteromorpha*

(P.I. = 0.54) must therefore arise from some property developing as the algae mature.

At the delicate germling stage, thallus toughness does not inhibit consumption of the furoid species. Instead, selection at this level must be based on taste (presence/absence of secondary plant metabolites), digestibility or nutritive value. It may be ecologically significant that the preferred furoid, *Fucus serratus*, grows faster than *Ascophyllum*. A compromise may exist between growth rate and palatability, each of which offers an alternative escape from grazing. It is particularly interesting to note that whatever inhibits common winkle grazing of juvenile *Ascophyllum*, does not have the same effect on the flat winkle.

The slow-growing red alga *Gigartina*, is the only species which appears to be protected at the juvenile phase by defences of a structural nature. Considerable effort is required to scrape the holdfast from the substratum, while the upright frond is cartilagenous and highly flexible and hence not readily severed from the disc by radular action (pers. obs.). The fronds are unable to regenerate from the disc, as has been reported for *Chondrus crispus* (D.P. Cheney, pers. comm.) and consequently frond breakage is lethal. In such a slow growing species, effective structural or chemical protection is therefore exceedingly important.

Clearly a high level of selectivity operates at the germling phase and this may be of some considerable ecological significance. Preferential grazing of the juvenile stages has the potential to influence community structure and algal distribution in a way that selectivity at the adult phase cannot do, unless grazing is abnormally intensive. For this reason alone, it is surprising that germling preferences have not previously been examined in any depth.

Adult versus germling choice experiments do not demonstrate a consistent clear-cut preference for either stage of growth. As the common winkle grazes furoid germlings more efficiently than the adult thallus (see Chapter 4), it is not surprising that this species should prefer juvenile to adult *Fucus*. The

green alga *Ulva*, however, is preferred in its adult form where the characteristic sharp cusps on the radular teeth can tear the thallus efficiently, enabling more food to be ingested per unit time than could be achieved on a diet of germlings. In contrast, the feeding apparatus and body plan of the flat winkle are well adapted to the consumption of adult furoid material but cannot cope effectively with mature *Ulva* (see Chapter 4). Distinct preferences for different developmental stages, serve to strengthen the partitioning of resources already observed in adult versus adult choice experiments.

In the laboratory, firmly attached *Ascophyllum* germlings are dislodged by crawling, non-feeding snails. Where gastropod population densities are high, foraging in the field may thus reduce the relative abundance of germlings considerably, without any active feeding occurring. This offers an alternative explanation for the low survivorship of juvenile *Ascophyllum* recorded on American shores and normally attributed to active consumption by the abundant common winkle (Keser *et al.*, 1981; S.L. Miller and R.L. Vadas, pers. comm.). It may also help to explain why such a fecund and unpalatable plant often fails to recolonise European shores following the experimental removal of adult plants (see Schonbeck and Norton, 1980).

CHAPTER 6 : STRUCTURAL AND CHEMICAL PLANT DEFENCES

Although browsing animals feed on sedentary organisms which are by nature, unable to flee, a wide range of obstacles to grazing may still be encountered. Anti-herbivore defence mechanisms fall into two distinct categories enabling the plant to escape damage by:

- a) avoiding direct interaction with the herbivore (a so-called non-coexistence escape).
- b) preventing or reducing to a tolerable level, the quantity of plant material consumed. (Here structural or chemical defences confer a coexistence escape, rendering the plant inedible or of low preference.)

Plants adopting non-coexistence escapes are ecological "fugitives" (Hutchinson, 1947) characterised by annual or short life-cycles and high reproductive capacities. These species rely on escaping predation by avoiding predators in time and space.

Perennial plants, however, coexist with browsing herbivores on a long-term basis and must adopt a policy of defence rather than avoidance. Chemical and structural characteristics may render such organisms unsuitable as food items for most predators. Consequently, diet width amongst browsers may be expected to depend on the level of defences displayed by the prey organisms (Hughes, 1980).

The simpler form and physiology of the seaweeds does not permit the same potential for elaborate and complex defence structures that is apparent in terrestrial seed-bearing plants. Nevertheless, the vulnerability of the macroalgae to herbivory is known to vary according to the physical nature of the tissues and possibly also the presence or absence of chemical repellents. In higher plants there are many data and much circumstantial evidence to suggest that secondary compounds in both vegetative and reproductive tissue contribute to defence against herbivores and microbes (Janzen, 1973). Compounds similar to the secondary plant substances have been found in various species of marine alga (e.g. Fenical, 1975; Ragan and Jensen, 1978;

Glombitza, 1981; Zavodnik and Jensen, 1981; Geiselman and McConnell, 1981) and here too are thought to affect palatability.

Optimal defence theory predicts that the evolution and allocation of defences should maximise individual inclusive fitness (Rhoades, 1979). However, defence necessitates the diversion of energy and nutrients from other needs and may prove costly in terms of fitness. Hence investment in defence may impose metabolic costs which are themselves, a source of compromise to the effectiveness of plant defence systems (McKey, 1979). Consequently, evolution must strive to balance "expenditure" on defence and on other factors important to plant fitness and survival. "Trade-offs" between competitive ability and anti-herbivore defence have been recognised by Lubchenco and Gaines (1981) and provide a plausible evolutionary explanation for the strong correlation between herbivore feeding preferences and algal competitive abilities.

Empirical and theoretical studies of feeding preferences are based on the assumption that animals experience a strong selective pressure to eat those foods which yield the maximum "value" per unit time (Emlen, 1973). In this context "value" is composed of several different components, including the quality and quantity of chemical nutrients in the food, its energy content and availability, and perhaps most important of all, the capacity of the animal to obtain the required benefits from it. The last will depend on the ability of the grazer to manipulate and ingest the food, and to digest, absorb and assimilate the nutrients. Each of these steps constitutes a potential obstacle to the grazer and provides scope for the development of effective anti-herbivore defences by the plant. The presence of distinct gustatory preferences (Chapter 5) testifies to the role which these defences play in deterring the gastropod grazers.

6.1 Influence of thallus form on the edibility of mature *Enteromorpha*

Dahl (1964) was quick to acknowledge the potential importance of algal "stability" in the determination of gastropod feeding preferences. *Littorina*

Littoreia carries a heavy shell and is consequently better adapted to foraging on stone substratum than the relatively unstable surface proffered by the algal lamina. Slight agitation is all that is required to dislodge both active and resting common winkles from the algal canopy (pers. obs.). In contrast, the flat winkles have a much smaller shell height : foot width ratio and, as a result, adhere more firmly to the thallus. The necessity for a secure surface to grip was further underlined by Nicotri's (1980) preference experiments with *Ampithoë* and *Idotea*, and those of Norton and Benson (1983) with *Caprella*.

Lawrence (1975) noted that foraging urchins may be attracted preferentially to one food source, yet feed more rapidly on another. This suggests that the rate of ingestion is determined ultimately by the ability of the herbivore to manipulate the food item. Working with a variety of asco-glossan herbivores, Jensen (1983) found a correlation between foot width and the filament diameter of the food plants. In the present study, thallus form and interspecific variation in the structure of the feeding apparatus have been shown to be largely responsible for differences in the efficiency of littorinid feeding on the foliose ephemeral thalli (see Chapters 4, 5).

Further examples of the relationship between thallus form and herbivore diet are furnished by Schiel (1981), Larson *et al.* (1980), Slocum (1980), Steneck (1981) and Steneck and Watling (1982). The last derived functional groups based on algal physiology and evaluated the dietary preferences of a variety of molluscs on this basis. Molluscan body plan and feeding apparatus were shown to restrict diet width.

a) Methods

Observations made during feeding trials suggest that the common winkle experienced considerable difficulty manipulating the narrow, ribbon-like thallus of *Enteromorpha*. To assess the role that growth form plays in determining the snail's ability to handle and ingest foliose thalli, a second series of two-way choice experiments was initiated. The thalli were cut

into small pieces and presented to the test snails in fragments of similar size. Each fragment was washed carefully in filtered sea water to remove potentially attractive or repellent chemicals from damaged cells. The experiment was replicated ten times and a series of ten ungrazed controls was also monitored for each species.

b) Results

Consumption of *Enteromorpha* exceeded consumption of *Ulva* in nine replicates (mean P.I. = 2.82 ± 0.623). *Enteromorpha* was preferred at the 95% probability level (Wilcoxon signed-rank test), indicating a reversal of common winkle preference rankings.

6.2. Thallus toughness

A tough epithelial layer provides the first barrier to consumption of a potential food item by the grazer and must be penetrated before any nutritive benefit can be derived from the alga. As a result, laminar toughness constitutes a potentially effective defence against herbivory. Many herbivorous invertebrates appear to prefer softer textured seaweeds (Ravanko, 1969) or tackle the tougher parts of the thallus only after consumption of the more tender portions (Saitô and Nakamura, 1961).

A number of terms have been used more or less interchangeably, to describe a plant's resistance to mechanical abrasion. Some, such as "texture", are undoubtedly misleading and open to mis-interpretation. The term adopted in this study, "toughness", describes the relevant attribute of the algal thallus without ambiguity. A "tough" alga has a lamina highly resistant to mechanical abrasion and considerable effort is required to penetrate the epidermal layer.

Though the literature abounds with vague references to the relative toughness of algal thalli, quantitative assessments are hard to find - particularly ones which relate to herbivory. Lubchenco (pers. comm.) has

developed a fine-toothed "hacksaw device" which mimics (albeit crudely) the action of the gastropod radula. However, there are major practical problems in the application of this technique - particularly when comparing laminae (such as *Ulva*) with filaments (such as *Cladophora*). Working at Plymouth, W. Farnham and S. Morrell (pers. comm.) designed a primitive abrasive wheel to test the resistance of the seaweed thallus to mechanical abrasion, while Bertness *et al.* (1983) assessed the "scratch strength" of algal crusts by recording the pressure required for a standardised 45° steel point to scratch the epithelial surface as it passed underneath.

This section assesses two quite different facets of macroalgal toughness and examines the results in the light of littorinid food preferences.

6.2.1. Puncture strength

a) Methods

A durometer (Shore Instrument and M.F.G. Co. Ltd., Jamaica, New York 11436, U.S.A. - Hardness type A-2.) was used to measure the point force required to penetrate the thallus of each of the test plants. This technique is similar in principal to that employed by Feeny (1970) and by Bertness *et al.* (1983). A set of 50 durometer readings was procured for each alga. For most plants, measurements were taken randomly from all parts of the thallus, but for the finely branched filamentous species such as *Cladophora* and *Polysiphonia*, measurements were confined to the thicker main axis of each branch. The penetrometer needle was too broad to fit between the calcareous segments of *Corallina*, so for this species only the segments themselves were tested.

b) Results

The results are summarised in Table 37. For *Pelvetia* and *Ascophyllum* the large standard errors attached to the mean, reflect steep gradients of toughness between tender frond tips and tough frond bases. For each species a separate series of readings (n=20) was taken for tip and basal regions (distal 2cm. for *Pelvetia* and 6cm. for *Ascophyllum*).

The foliose ephemeral species and the finely branched *Cladophora* and *Polysiphonia*, all had relatively low durometer readings. The furoid species recorded much higher values, with *Ascophyllum* proving particularly tough, while the calcified portions of *Corallina* failed to register on the durometer scale at all.

Table 37. Puncture strength of macroalgal thallus : durometer readings.

Species	Mean Durometer Reading \pm S.E. (Arbitrary units)
<i>Enteromorpha</i>	2.0 \pm 0.18
<i>Cladophora rupestris</i>	1.0 - 7.0*
<i>Polysiphonia lanosa</i>	3.0 - 6.0*
<i>Porphyra umbilicalis</i>	7.7 \pm 0.44
<i>Ulva lactuca</i>	15.6 \pm 0.63
<i>Laurencia pinnatifida</i>	15.8 \pm 1.00
<i>Fucus spiralis</i>	18.5 \pm 0.81
<i>Fucus vesiculosus</i>	18.6 \pm 0.41
<i>Pelvetia canaliculata</i>	29.7 \pm 1.83
Frond tip	7.2 \pm 0.43
Frond base	48.4 \pm 0.61
<i>Fucus serratus</i>	32.4 \pm 0.79
<i>Gigartina stellata</i>	38.5 \pm 0.70
<i>Ascophyllum nodosum</i>	61.6 \pm 1.31
Frond tip	50.1 \pm 0.56
Frond base	74.6 \pm 0.56
<i>Corallina officinalis</i>	**

* Non-random set of readings.

** Did not register on durometer scale.

For the common winkle, the edibility of macroalgal foods is closely correlated with their puncture strengths (Table 38). However, no significant correlation is evident with the food preference rankings exhibited by either species of flat winkle. *Cladophora*, *Corallina* and *Polysiphonia* were necessarily omitted from these comparisons, due to the non-random nature of durometer readings.

6.2.2. Resistance to abrasion

The durometer assesses physical toughness in terms of the pressure required to pierce the outer surface of the algal lamina - the "puncture strength" (Bertness *et al.*, 1983). However, this may not constitute a realistic measure of resistance to radular penetration, particularly for those species using the radula to gouge rather than tear. Consequently, an alternative technique was designed to assess toughness in terms of susceptibility to abrasion.

a) Methods

An abrasive wheel was developed (see Fig. 44), similar in basic design to that described by W. Farnham (pers. comm.). The wheel was driven by a variable speed motor at a speed (free-running) of 960 r.p.m. The algal samples were taped to a hinged arm held firmly in place on the wheel by a standard 44g. weight. The weight was suspended by a spring, 4cm. from the hinge. A strip of emery paper (Tufbak Durite T423) was attached to the wheel and replaced at regular intervals.

Each trial run lasted 60 seconds and the loss of algal tissue (wet weight) was measured over this period and subsequently corrected for weight losses recorded in control samples (ten per species) exposed to the air for two minutes. The area of abraded lamina was measured using a portable area meter and the tissue loss was computed in $\text{mg.cm}^{-2}.\text{s}^{-1}$. For each species 20 samples were tested. The emery paper was replaced after each series of five trials.

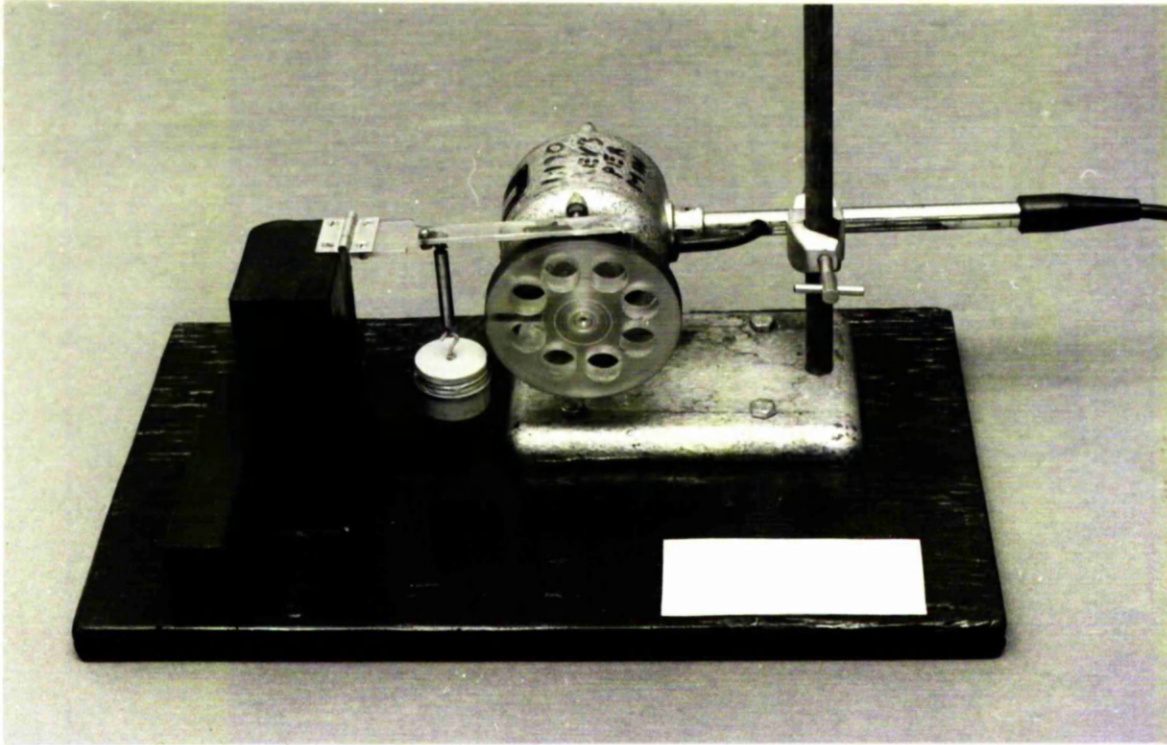


Fig. 44. Abrasive wheel used in the assessment of thallus toughness.

Table 38. Spearman rank correlation coefficients for algal edibility and puncture strength.

Species	Spearman correlation coefficient rs
<i>Littorina littorea</i>	0.929**
<i>Littorina mariae</i>	0.371 n.s.
<i>Littorina obtusata</i>	-0.029 n.s.
n.s. not significant.	
** P < 0.01	

The foliose algae *Porphyra* and *Ulva*, were attached to the apparatus in compact "wads" of tissue. Unfortunately, due to the diversity of thallus form, it was impossible to obtain comparable readings for *Cladophora*, *Corallina*, *Enteromorpha*, *Laurencia* and *Polysiphonia*. To permit comparison with durometer data, *Ascophyllum* and *Pelvetia* tip and basal regions were tested separately.

b) Results

Toughness rankings derived from abrasion tests (Table 39) differ markedly from durometer readings. Although *Fucus* spp. suffered high tissue losses, *Ascophyllum* and *Pelvetia* again proved relatively tough. The corticated red alga *Gigartina*, was also highly resistant to abrasion. However, the clearcut division between foliose and coarsely-branched species apparent in durometer tests, was not evident. In terms of susceptibility to abrasion, *Ulva* ranked lower than *Fucus serratus*, *F. spiralis* and *F. vesiculosus*, while *Porphyra* ranked alongside *Gigartina* as the most resistant species. That apical tissue from both *Ascophyllum* and *Pelvetia* should prove more abrasion-resistant than basal tissue is also worthy of note.

Algal edibility and resistance to abrasion are not compared statistically

Table 39. Resistance of macroalgal thallus to abrasion on the abrasive wheel.

Species	Mean tissue loss \pm S.E. (mg.cm ⁻² .s ⁻¹)
<i>Fucus vesiculosus</i>	6.78 \pm 0.356
<i>Fucus spiralis</i>	6.18 \pm 0.818
<i>Fucus serratus</i>	4.90 \pm 0.385
<i>Ulva lactuca</i>	2.98 \pm 0.604
<i>Ascophyllum nodosum</i>	1.33 \pm 0.055
Frond tip	1.34 \pm 0.100
Frond base	1.49 \pm 0.075
<i>Pelvetia canaliculata</i>	1.19 \pm 0.174
Frond tip	1.07 \pm 0.133
Frond base	1.26 \pm 0.101
<i>Gigartina stellata</i>	0.88 \pm 0.108
<i>Porphyra umbilicalis</i>	0.88 \pm 0.048

owing to the small sample sizes. However, tissue edibility, as exhibited by *Littorina littorea* and *L. obtusata*, clearly bears no relation to abrasion figures. Edibility rankings for *L. mariae*, however, correspond fairly closely to the susceptibility of the species to damage by abrasion.

6.3. Digestion of algal cell walls

Since the pioneer work of Coupin (1900) (cited by Seiderer *et al.*, 1982) and Yonge (1923) established the presence of amylase (1,4- α -D-glucan glucanohydrolase) in the crystalline style of the bivalves, there have been a number of further studies on molluscan digestive enzymes. Laminarases, in particular, have been examined in some detail (e.g. Sova *et al.*, 1970), while carbohydrases have been isolated from both the crystalline style (e.g. Hara *et al.*, 1979) and the digestive gland (Santoro and Dain, 1981). Recently, studies on alginate lyase (Muramatsu and Egawa, 1980), cellulase (Mirza and Serban, 1981) and amylase (Newell *et al.*, 1980) have demonstrated the occurrence of several enzyme systems, although their precise role is as yet unknown (Seiderer *et al.*, 1982).

This work has, however, centred almost exclusively on the bivalves, as the concentration of enzymes from the crystalline style of these animals is relatively straightforward. With regard to the gastropod molluscs, Hirsch (1915) and Brock (1936) investigated the digestive enzymes of a variety of carnivorous species, while Dodgson and Spencer (1954) reported the occurrence of sulphatases in a number of herbivorous prosobranchs (including *Patella* and *Littorina littorea*).

The algal foods with which we are concerned in this study consist of cells entirely surrounded by cell walls, in many respects similar to those of the higher plants. In order to reach the contents of the algal cells and derive nutritional benefit from them, it is necessary first for the grazer to breach the cell wall. This may be achieved either by mechanical means (maceration) or by digestion, permitting the additional assimilation and metabolic utilisation of the cell wall constituents themselves. Where

chunks of alga greater than cell size are ingested during the grazing process, the ability to digest structural carbohydrates will largely determine the accessibility of the cell contents to the digestive enzymes. The object of this section is to assess the capacity of the littorinids to breach the cell wall by chemical means.

6.3.1. Digestion of total cell wall polysaccharides by *Littorina littorea*

a) Methods

The extent of cell wall digestion by *Littorina littorea* was estimated by assessing the digestion of total plant carbohydrates in the palatable green alga, *Ulva lactuca*. The storage polysaccharides of the Chlorophyta (i.e. starch) take the form of amylose, a linear chain of α -1,4-linked glucose residues, and amylopectin, which consists of α -1,4-linked glucose with branches of α -1,6-glycoside (McCandless, 1981). Such polysaccharides can be eliminated by digestion with amylase, so that only the cell wall polysaccharides remain. Unfortunately, the furoid species characteristically grazed by *Littorina obtusata* and *L. mariae* utilise laminaran as a storage glucan, and consequently the analysis of cell wall carbohydrate digestion has not been attempted for these species.

Prior to experiments, sample snails of a standard 24-26mm. shell height were starved for seven days. Three specimens were then placed in a Pyrex crystallising dish with a weighed portion of ulvoid thallus. A small piece of *Ulva* from the same thallus was retained and frozen for subsequent carbohydrate analysis.

Faecal material was collected over a seven day period by Pasteur pipette. Each sample was frozen immediately after collection. Uneaten *Ulva* was removed from the dish after two days' feeding and re-weighed.

The basic experimental procedure was replicated three times and faecal material was also collected from three control vessels containing unfed *Littorina*. Enrichment of faecal material by bacteria or other micro- and

meiofauna may occur in the crystallising dish and/or the gut. However, this does not represent a potential source of error, as faecal material collected from both control and trial dishes will presumably suffer the same fate.

The analysis of samples used the carbohydrate assay described by Dubois *et al.* (1956) (see Appendix B). Samples of faecal material were first centrifuged and resuspended in distilled water to remove salt. Faeces and algal samples were then treated with salivary amylase to digest intracellular amyloses (see Olaitan and Northcote, 1962). The spectrophotometer was calibrated using glucose solutions of known concentration.

b) Results

A mean cell wall polysaccharide content of 23.0% wet weight, was calculated for *Ulva*. The percentage of ingested cell wall carbohydrate subsequently appearing in the faecal material, ranged from 2.5% to 7.7%, giving a mean digestion efficiency of 94.8%.

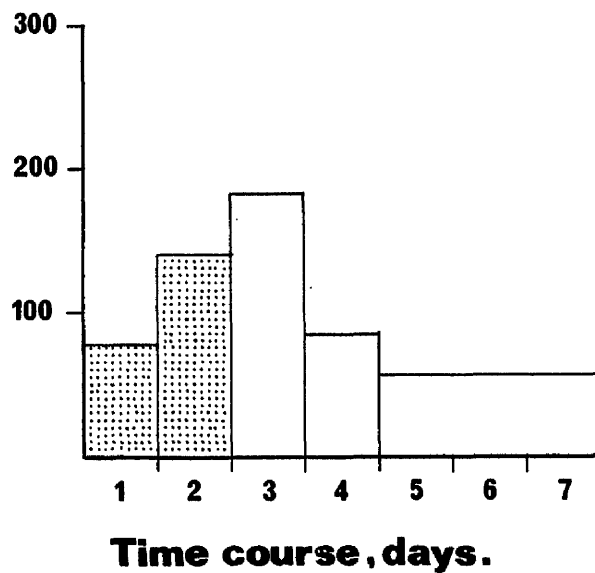
Fig. 45 shows the carbohydrate level in faecal material over the course of the experiment. Faecal material from days 5-7 was assayed together. In fed specimens, the carbohydrate content dropped to the "basal level" displayed by starved *Littorina*, two to five days after the termination of feeding. The standard error of the mean for the replicates (both trial and control) generally fell within 25% of the mean.

6.3.2. Digestion of cellulose by *Littorina littorea* and *Littorina obtusata*

Cellulose, a polymer of 1,4 linked β -D-glucose, is undoubtedly the best known and most common skeletal component of algal cell walls. However, the abundance of the fibrillar cellulose component varies considerably, and it may even be replaced, in some species, by fibrils comprising polymers of 1,4 linked β -D-mannose or 1,3 linked β -D-xylose.

In all brown algae investigated to date, the cell walls are strengthened

A.
µg. carbohydrate
in faeces.



B.
µg. carbohydrate
in faeces.

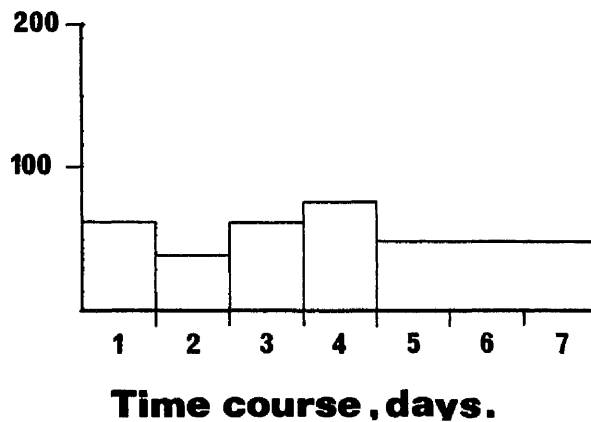


Fig. 45 Carbohydrate level in faecal material of *Littorina littorea*.

A. Trial specimens fed on *Ulva*.
 (Stippled bars represent feeding period)

B. Unfed control specimens.

All figures represent the mean of three replicates.

primarily by a framework of cellulose microfibrils. However, the cellulose component may constitute only a small percentage of the entire wall. Thus, in *Pelvetia* cellulose comprises just 1.5% of the wall, and in *Ascophyllum* 7% (Cronshaw *et al.*, 1958).

Although the cell wall structure of the Rhodophyta has been studied less extensively, the Florideophyceae are known to contain randomly oriented cellulose microfibrils in the inner layer of the wall (Brawley and Wetherbee, 1981). This group contains the study species *Corallina*, *Gigartina*, *Laurencia* and *Polysiphonia*. In contrast, the Bangiophycidae, including the foliose alga *Porphyra*, replace the cellulosic component with β 1-3 linked xylan.

In the filamentous green alga *Cladophora*, cellulose constitutes a relatively high proportion of the cell wall - up to 29% in *Cladophora rupestris* (Preston, 1974). Cellulose is also present in the foliose species *Ulva* and *Enteromorpha*, although the organisation of cellulose fibrils is less distinct and there is much amorphous material.

Cellulose levels may be of some significance to grazing herbivores, as cellulose has long been recognised as the least digestible structural carbohydrate. Huang and Giese (1958), for example, found that stomach extracts from the giant chiton, *Cryptochiton stelleri*, were totally ineffective on a cellulose substrate. However, there are several recent reports which suggest that certain molluscs can, and do, digest cellulose (e.g. Mirza and Serban, 1981; Seiderer *et al.*, 1982). Furthermore, symbiotic gut bacteria have been implicated in the utilisation of cellulose by sea urchins (Fong and Mann, 1980).

In this section the ability of *Littorina littorea* and *Littorina obtusata* to digest the structural cellulose in favoured food items is examined experimentally. Boolootian and Lasker (1964) present evidence to suggest that marine herbivores in general eat algal foods which are compatible with the enzymic and other equipment of digestion. Hence differences in the relative ability of the two littorinids to digest cellulose may be expected to contribute to differences in feeding preferences.

a) Methods

Samples for cellulose analysis were obtained using the experimental procedure described in section 6.3.1a) above. *Littorina littorea* was fed on *Ulva* and *Littorina obtusata*, on *Fucus serratus*. Three trials and three controls were monitored for each species. Ten flat winkles (12-14mm. shell height) were used in each replicate.

Subsequently, non-cellulosic carbohydrates were removed from all samples using Updegraff's (1969) extraction procedure (Appendix C). This is purported to remove all lipid, protein, lignin and non-cellulosic polysaccharides, leaving the cellulose fibres intact (Sloneker, 1971), and has been widely used as an assay of the cellulose content of plant tissues (e.g. Freeze and Loomis, 1977, 1978). The remaining carbohydrate content (cellulose) was established using the Dubois *et al.* (1956) method, as above (see also Appendix B).

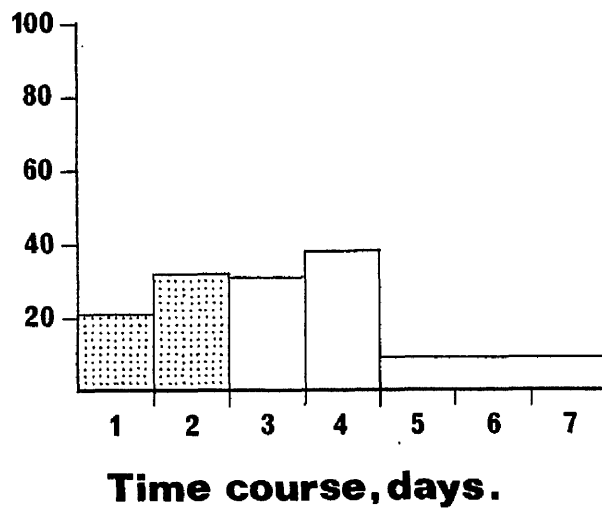
b) Results

On the basis of these experiments, a mean cellulose content of 1.3% wet weight was computed for *Ulva*. This compared with the slightly lower figure of 1.0% for *Fucus*. 18.2-37.9% of ingested cellulose subsequently appeared in the faecal material of *Littorina littorea*, giving a mean cellulose digestion efficiency of 72.8% for this species. For *Littorina obtusata* the digestion efficiency was considerably lower. 50.0-80.2% of ingested cellulose appeared in the faeces, corresponding to a mean digestion efficiency of 34.9%.

Figs. 46 and 47 show the fluctuating cellulose level in faecal material during the course of the experiments. Faecal material from days 5-7 was assayed together. In fed specimens of both species, the cellulose content fell to the "basal level" recorded in the faeces of starved snails, three to five days after the termination of feeding. The standard error of the mean for the replicates (both trial and control) generally fell within 25% of the mean.

A.

**µg. cellulose
in faeces.**



B.

**µg. cellulose
in faeces.**

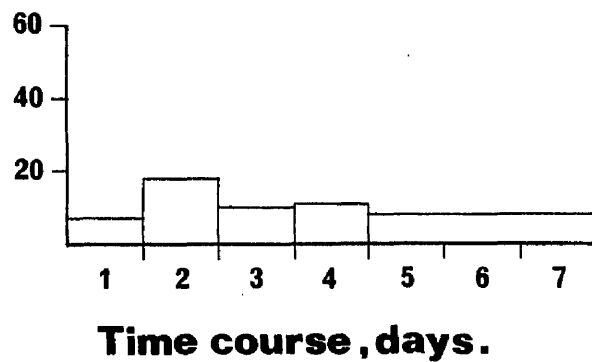


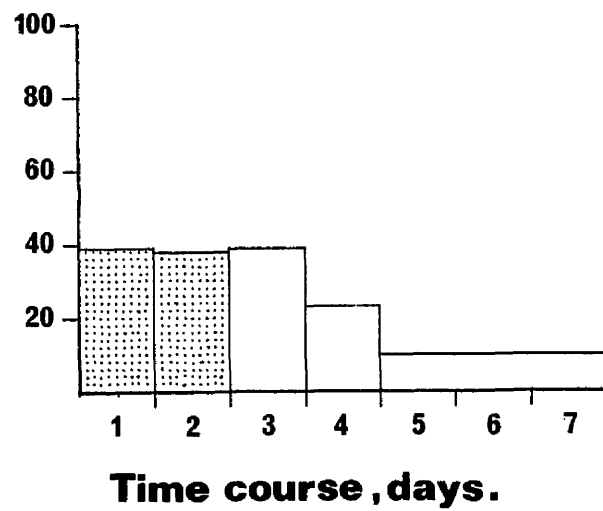
Fig. 46 Cellulose level in faecal material of *Littorina littorea*.

- A. Trial specimens fed on *Ulva*.
(Stippled bars represent feeding period)
- B. Unfed control specimens.

All figures represent the mean of three replicates.

A.

**µg. cellulose
in faeces.**



B.

**µg. cellulose
in faeces.**

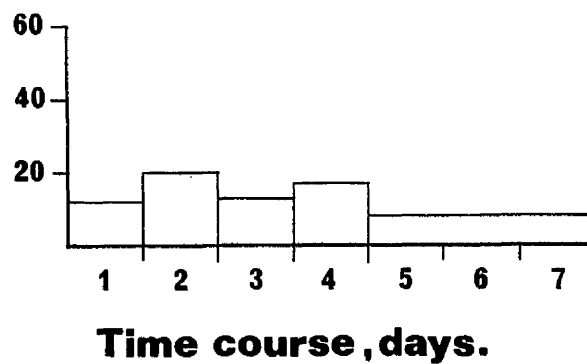


Fig. 47 Cellulose level in faecal material of *Littorina obtusata*.

- A. Trial specimens fed on *Fucus serratus*.
(Stippled bars represent feeding period)
- B. Unfed control specimens.

All figures represent the mean of three replicates.

6.3.3. Cellulase activity in *Littorina littorea*

The relative efficiency of cellulose digestion indicates a high level of cellulase activity in the littorinid gut. This section assesses the cellulase content of the faecal material and its likely origin.

a) Methods

Faecal samples were obtained by feeding six starved common winkles on *Ulva* for three days. Faecal material was collected daily for five days and frozen until analysis. The cellulase activity was assessed by measuring the reduction in viscosity of carboxymethylcellulose. Viscosities were measured using a Cannon-Fenske semi-microviscometer (see Swenson, 1963) at a temperature of 25°C. 1ml. of the sample solution was added to 2ml. of 0.1% carboxymethylcellulose and the change in viscosity was recorded during incubation in a water bath. One unit of cellulase activity was defined arbitrarily as the amount of cellulase required to decrease the viscosity at an initial rate of one second per minute. This corresponds to 0.8 units of Sigma cellulase, catalogue number C-7377, from *Aspergillus niger*.

The total cellulase content of the faecal material was assessed in two steps. As diffusion of cellulase from the faeces into solution probably occurred during feeding, the cellulase activity of the sea water was first tested. The faecal material itself was then homogenised with 1.0 ml. cold 0.1M Tris-hydrochloric acid buffer (pH 7.5) and the homogenate was centrifuged at 10,000g. for 30 seconds. The cellulase in the supernatant was then assayed.

The cellulase content of the food was estimated as follows: 2g. *Ulva* (wet weight) was homogenised with 8ml. 0.3M Tris-hydrochloric acid buffer (pH 7.5) at 0°C. The homogenate was centrifuged at 10,000g. for 60 seconds and the cellulase in the supernatant was assayed.

b) Results

A total cellulase activity of 4.4 units was recorded for the faecal material. The estimated cellulase activity of the *Ulva* consumed during feeding was 3.9 units (1.39 units per gram wet weight).

6.4. Chemical defences

Defence against pathogens and herbivores may be secured by many different mechanisms. Much of the literature concerning the food preferences of terrestrial herbivores centres on the presence and activity of various secondary plant compounds (e.g. Fraenkel, 1959; Feeny, 1975). Indeed, since Feeny (1969) noted that insect host-finding and gustatory behaviour was controlled largely by such substances, the interaction of herbivores and plant allelochemicals has become a rapidly expanding field of scientific inquiry and there is now a substantial accumulation of evidence to implicate many plant secondary substances as antibiotic agents in ecological interactions between plants and their associated biota (Rhoades and Cates, 1976). The products concerned are diverse, ranging from alkaloids to terpenes to phenolics and steroidal, cyanogenic and mustard oil glycosides. Some, such as the phenolics, are ubiquitous in seed plants, while others occur in only a few species (Levin, 1976).

In recent years, comparable compounds have been found in certain marine algae and it has been suggested that these may influence grazing by both vertebrate and invertebrate grazers. Tannins similar to those found in terrestrial plants, have been isolated from many brown algal species (e.g. Ragan and Jensen, 1977, 1978; Geiselman and McConnell, 1981; Glombitza, 1981; Zavodnik and Jensen, 1981), while various halogenated compounds have been reported from members of the Chlorophyta, Phaeophyta and Rhodophyta, but appear most prevalent in the red algae (Fenical, 1975; Geiselman, 1980). Furthermore, Barwell *et al.* (1981) have recently identified pharmacologically-active amines in certain red algae and other workers have shown the aliphatic

amines to be widely distributed (Steiner and Hartmann, 1968). The latter are thought primarily to restrict the growth of epiphytes (Hartmann and Aufermann, 1973) but it is possible that the amines may also influence grazing.

Perhaps the most widely accepted functional division of the so-called "plant defensive chemicals", is that of Feeny (1975). Feeny proposed the term "quantitative" defences to describe those allelochemicals, such as the tannins, which act in a dosage-dependent fashion so that the degree of protection from herbivory is directly related to the tissue concentration of the substance. "Qualitative" defences, on the other hand, include such plant secondary substances as glucosinolates, alkaloids and cyanogenic compounds (Rhoades, 1979), all of which are characteristically present in low tissue concentrations (commonly <2% dry weight) and which, though less costly to plant metabolism than the aforementioned quantitative defences, can confer adequate protection against nonadapted herbivores. However, even at high concentrations, such compounds typically provide little protection against specialised herbivores which may evolve detoxification or tolerance mechanisms (Rhoades, 1979). Quantitative defences are characteristic of apparent plants and plant tissues readily located by the grazer, whereas qualitative defences normally characterise unapparent ephemeral plants and tissues inherently less vulnerable to discovery.

As Rhoades and Cates (1976) recognise, most qualitative defences of "ephemeral" (= unapparent) plants are designed to interfere with the internal metabolism of the herbivore, while the quantitative defences of the "predictable and available" (= apparent) plants (e.g. tannins) act within the gut, inhibiting the digestion of plant tissue and consequently reducing the availability of plant nutrients. As the digestibility-reducers are not required to cross cellular membranes, such substances may be composed of large or small molecules of either hydrophilic or lipophilic nature. The only requirement is active disruption of the digestive process.

As Levin (1976) points out, the utilisation of chemical defensive

strategies against molluscan herbivores is poorly understood. Cates and Orians (1975) have shown that slugs of the genera *Ariolimax* and *Arion* find early successional species more palatable than late successional and climax species. However, the nature of the unpalatable substances was not ascertained. Likewise, Grime *et al.* (1970), working with the land snail *Cepaea nemoralis*, found that the palatability of different plants varied markedly, but failed to identify the chemical(s) responsible.

There is further evidence of selective feeding by molluscs on cyanogenic forms of the same species. Jones (1962) demonstrated that consumption of *Lotus corniculatus* by *Ariolimax* and *Arion*, and by the snails *Ariana* and *Helix*, was inversely correlated to the content of cyanogenic glucosides. Similar feeding behaviour by *Ariolimax* and *Arion* on cyanogenic variants of *Trifolium repens* has also been recorded (see Levin, 1976).

Although the accumulation of metabolically-redundant substances in the cells of marine algae has often been implicated in selective grazing by molluscan herbivores, there is in fact, very little unequivocal evidence of chemically-mediated food selection. There is undoubtedly a great need for inter-disciplinary research linking the ecological and biochemical aspects of selective grazing. What evidence there is, however, does suggest that allelochemicals fulfil an important role. Geiselman and McConnell (1981) demonstrated experimentally, the inhibition of common winkles feeding by polyphenols extracted from the brown algae *Fucus vesiculosus* and *Ascophyllum nodosum*. Other substances have been shown to act as strong phagostimulants to invertebrate grazers (e.g. Carefoot, 1980), while compounds toxic to generalist grazers may prove to have attractive qualities for the specialist. Stallard and Faulkner (1974) recorded preferential grazing of *Laurencia* and *Plocamium* by the sea hare *Aplysia*, which was observed to concentrate halogenated algal metabolites in its digestive gland and may itself utilise these substances as a defence against predation.

6.4.1. Palatability of algal extracts for *Littorina littorea*

Selective grazing at the germling level may represent a response to the nutritional/caloric value or the palatability (taste) of the algae. The two are not mutually exclusive, since differences in food value may reflect the activity of digestibility-reducing and hence distasteful, allelochemicals.

If plant secondary compounds are, in fact, important deterrents to invertebrate grazing in the intertidal, then the well-defined grazing preferences which have been demonstrated must be based, at least in part, on efficient perception of noxious (and possibly also attractive) allelochemicals. Active sampling by ingestion constitutes the most obvious means of testing for the presence of substances stored intracellularly. Evolution will progress such that "harmful" chemicals acquire a "bad taste" (Chapman and Blaney, 1979) readily detected by gustatory receptors. Here, the gustatory response to extracts from the test algae is examined with the common winkle, *Littorina littorea*.

a) Methods

The palatability of aqueous extracts derived from the test algae was assessed using a technique adapted from Grime *et al.* (1970). Three grammes (wet weight) of fresh algal material was homogenised in 10ml. of distilled water and filtered through Whatman no.1 filter paper. Small squares of filter paper (Whatman no.1) were weighed, soaked in the filtered extract and subsequently dried in an oven at 30-40°C. Consumption of treated filter paper was gauged against consumption of untreated filter paper squares of comparable dry weight.

Treated and untreated samples were placed in separate compartments of a partitioned petri-dish set in the base of a shallow Pyrex crystallising dish (9cm. diameter). The filter paper squares and the base of the empty compartment, were each moistened with several drops of filtered sea water

(added by Pasteur pipette). Four common winkles were introduced to the empty compartment and the dish was placed in a growth cabinet (16L : 8D photoregime; $15 \pm 1^\circ\text{C}$). Drops of filtered sea water were added periodically, to ensure that the filter paper remained moist throughout the experiment.

After one to four days (depending on the voracity of the snails), the uneaten filter paper was removed from each compartment and washed in distilled water to remove salt and extract. The samples were then oven-dried and re-weighed. Preliminary trials (without snails) indicated that samples of both test and reference material treated in this way, could be re-weighed consistently to within 0.2 mg. of the original weight. The basic experiment was replicated ten times with each alga.

b) Results

The results are presented in Table 40. Palatability indices represent the ratio of test to reference material consumed (dry weight). Extracts from the green alga *Cladophora*, the highly favoured foliose species *Enteromorpha* and *Porphyra*, and the germling stages of *Enteromorpha* and *Ulva*, significantly increased consumption of the filter paper substrate. Conversely, consumption was inhibited by treatment with extracts from *Laurencia* and both mature and juvenile *Ascophyllum*.

Table 40. Palatability of aqueous extracts for *Littorina littorea*.

Asterisks denote significance of difference between consumption of test and reference material (Wilcoxon signed-rank test).

Alga	Mean P.I. \pm S.E.
<i>Porphyra umbilicalis</i>	4.85 \pm 1.236*
<i>Ulva lactuca</i>	4.39 \pm 2.094 ^{n.s.}
<i>Cladophora rupestris</i>	3.42 \pm 1.241*
<i>Enteromorpha</i> germlings	3.35 \pm 1.099*
<i>Enteromorpha intestinalis</i>	3.12 \pm 1.047*
<i>Ulva lactuca</i> germlings	3.01 \pm 1.073*
<i>Polysiphonia lanosa</i>	1.89 \pm 0.724 ^{n.s.}
<i>Fucus serratus</i> germlings	1.68 \pm 0.728 ^{n.s.}
<i>Gigartina stellata</i>	1.53 \pm 0.650 ^{n.s.}
<i>Fucus serratus</i>	1.40 \pm 0.419 ^{n.s.}
<i>Corallina officinalis</i>	1.28 \pm 0.388 ^{n.s.}
<i>Ascophyllum nodosum</i> germlings	0.71 \pm 0.362*
<i>Laurencia pinnatifida</i>	0.52 \pm 0.255**
<i>Ascophyllum nodosum</i>	0.49 \pm 0.300*

n.s. non significant

* 0.01 < P < 0.05

** 0.001 < P < 0.01

6.4.2. Effect of algal exudates on consumption by *Littorina littorea*

Many brown algae release dissolved organic matter from the intact thallus into the surrounding sea water. Despite claims that some or all of this release is an artefact arising from shock, stress or injury (e.g. Kroes, 1970), it has been demonstrated beyond doubt (Ragan and Jensen, 1979; Zavodnik and Jensen, 1981) that the release of polyphenols from a number of brown algae is, at least primarily, genuine exudation.

At the same time, work at the University of California has attributed differential settlement of the planktonic *Haliotis* larvae to the presence of nondialyzable macromolecules associated with the surface of the crustose red species. Similarly, work on the gregarious settlement of cypids also suggests a response to the molecular nature of the surface (Crisp, 1976).

The prosobranch mollusc is well supplied with sense organs. The entire surface of the body is naturally sensitive to chemical stimulation, although the tentacles and lips of the head, together with the sides of the foot and the mantle edge, appear to be the prime sensory areas (Fretter and Graham, 1962; Bovbjerg, 1968). In addition, the mantle cavity contains a linear ridge parallel to the ctenidial axis (the osphradium) which consists of an epithelium of sensory cells overlying a ganglion (Fretter and Graham, 1962). The osphradium is usually regarded as the seat of a chemical sense, whose function is either olfactory or gustatory.

It is possible that the nature of exudates and/or surface-associated molecules, may be decisive in determining the response of invertebrate herbivores on contact with the algal thallus. In the two-way choice experiments described in Chapter 5, a number of algae were rejected without any visible signs of grazing appearing on the lamina. Exudates or surface coatings could provide a mechanism permitting close-range recognition of unpalatable macroalgae without requiring that the epidermal layer be breached physically. In this section, the influence of exudates on feeding by *Littorina littorea*, is tested experimentally.

a) Methods

The palatability of exudates from *Ascophyllum* and *Enteromorpha* was tested using the technique employed above in section 6.4.1. Approximately three grammes of the test alga was washed in filtered sea water to remove gross contaminants and incidental exudates from damaged parts of the thallus. The algal sample was then placed in a vial containing 10ml. filtered sea water and permitted to stand for 48-120 hours (16L : 8D photoregime; $15 \pm 1^\circ\text{C}$). Subsequently the alga was removed from the vial and the sea water was filtered through a Whatman no.1 filter paper. Test and reference material was prepared as described in section 6.4.1a above and grazing by *Littorina littorea* was assessed over a period of one to four days.

b) Results

Palatability indices (Table 41) record the ratio of test to reference material consumed (dry weight). Consumption of test material exceeded consumption of reference material for *Enteromorpha* (120 hours soaking).

6.4.3. Determination of total phenols in aqueous extracts

Polyphenols isolated from the brown algae are often assumed to be functionally similar to the terrestrial plant tannins, conferring some degree of protection against grazing by both vertebrate and invertebrate herbivores. However, the only positive evidence to support this theory appears to be that of Hunger (1902) and Geiselman and McConnell (1981). Hunger found that the sea hare *Aplysia*, would consume *Dictyota dichotoma* only after the alga was subjected to a fucosan-extracting procedure, while Geiselman and McConnell demonstrated that polyphenols extracted from *Fucus vesiculosus* and *Ascophyllum nodosum*, inhibited feeding by *Littorina littorea*.

The occurrence of phenolic compounds in the brown algae has been established for more than 80 years. Within the algal cell, the polyphenols are stored in specialised vesicles termed physodes, which are reported to

Table 41. Palatability of algal exudates for *Littorina littorea*.

Asterisks denote significance of difference between consumption of test and reference material (Wilcoxon signed-rank test).

Alga	Soaking period (hours)	Mean P.I. \pm S.E.
<i>Ascophyllum nodosum</i>	48	1.24 \pm 0.41 ^{n.s.}
<i>Ascophyllum nodosum</i>	120	1.36 \pm 0.32 ^{n.s.}
<i>Enteromorpha intestinalis</i>	48	1.70 \pm 0.62 ^{n.s.}
<i>Enteromorpha intestinalis</i>	120	1.93 \pm 0.66*

n.s. non significant

* 0.01 < P < 0.05

undergo diurnal and longer-term variations in number (e.g. Hunger, 1902).

The quantity of stored phenolics is much greater than required for metabolic purposes alone and accumulation within the cell is not necessary, since polyphenols may be readily degraded and recycled or respired (Levin, 1971). Extractable phenols may make up as much as 0.5-9.4% dry matter in the brown alga *Ascophyllum* and 0.7-8.5% for *Fucus serratus* (Larsen and Haug, 1959).

A variety of possible physiological and ecological roles have been proposed, including involvement in cellular respiration, metabolic transport and outer cell wall biogenesis, mucilage formation, storage of metabolic reserves and photoprotection (see Ragan and Jensen, 1978). Nevertheless, a considerable body of circumstantial evidence favours the hypothesis originally proposed by Hunger (1902), that polyphenols provide primarily, a chemical defence against predators and/or epiphytes (e.g. McLachlan and Craigie, 1964; Sieburgh, 1968).

Polyphenols are known to reduce digestibility, causing the precipitation

of proteins and polysaccharides. Higher plant tannins reduce the availability of substrate peptide groups to digestive enzymes and may also complex with the enzymes themselves (Rhoades and Cates, 1976). The digestibility of starch and cellulose is reduced by both enzyme and substrate complex formation.

In this study, aqueous extracts from both mature and juvenile *Ascophyllum* were shown to inhibit grazing by the common wrinkle. Here, the polyphenol content of the fucoid extracts is assessed and considered in the light of the observed littorinid grazing preferences.

a) Methods

Colorimetric determination of the relative polyphenol contents of brown algal extracts has been attempted using the Folin-Denis reagent (e.g. Ragan and Craigie, 1976), Brentamine Fast Red 2G salt (e.g. Zavodnik and Jensen, 1981), vanillin-sulphuric acid (e.g. Ragan and Jensen, 1977), diazosulphanilic acid (Haug and Larsen, 1958), sodium carbonate (Haug and Larsen, 1958) and the Folin-Lowry procedure outlined by Lowry *et al.* (1951) and Bruening *et al.* (1970). The Folin-Lowry method has been used recently by R.W. Loveless (pers. comm.) with some success, and was consequently adopted in this study. The assay is not specific for phenols as aromatic amino acids are also measured. However, this is unlikely to interfere significantly with estimated polyphenol levels as these substances are present in negligible quantities.

Aqueous extracts from adult *Fucus serratus* and mature and juvenile *Ascophyllum*, were prepared as described in section 6.4.1a above and analysed according to the experimental procedure outlined in Appendix D. For each species three separate samples were tested. The polyphenol levels of test solutions were established by reference to a colorimeter calibration curve relating the phenol concentrations of standard phloroglucinol solutions to the corresponding absorbance values. Phloroglucinol is one of the major seaweed polyphenol precursors (Ragan, 1981) and is frequently used as a standard in total phenol determinations (e.g. Ragan and Jensen, 1977, 1978).

b) Results

The results are summarised in Table 42. The phenol content is generally small, suggesting that the efficiency of water extraction is low. However, the highest polyphenol content was recorded in the *Ascophyllum* homogenate. Polyphenol levels in extracts from juvenile *Ascophyllum* and adult *Fucus serratus* were considerably lower.

Table 42. Phenolic content of aqueous extracts.

Species	Mean conc ⁿ . in extract, range in parentheses ($\mu\text{g}.\text{ml}^{-1}$)	Mean conc ⁿ . in food substrate (% dry weight)
<i>Ascophyllum nodosum</i>	82 (80-84)	0.009
<i>Ascophyllum</i> germlings	55 (51-59)	0.006
<i>Fucus serratus</i>	52 (45-56)	0.006

6.4.4. Components of the phenolic fraction

In some brown algae, phenolic compounds account for as much as 2-10% by dry weight, of the biomass (Haug and Larsen, 1958). Yet until recently, the precise chemical structure of the algal polyphenols remained largely unknown. However, a number of recent biochemical investigations have isolated and identified a variety of relatively unreactive high molecular weight polyphloroglucinols (e.g. Ragan and Jensen, 1977; Ragan and Jamieson, 1982). Glombitza (1977,1981), whose term "phlorotannin" is now widely used to describe these compounds, was one of the first to succeed in this field. Glombitza identified phloroglucinol unambiguously in 17 brown algae, including *Fucus serratus* and *F. vesiculosus*, but failed to detect the phlorotannin precursor in *Ascophyllum* or *Pelvetia* (Glombitza, 1977,1981).

In a later study, Geiselman and McConnell (1981) characterised the

ecologically active compounds in methanol extracts from *Fucus vesiculosus* and *Ascophyllum nodosum*, as phloroglucinol polymers with a wide molecular weight range (molecular weight $< 30,000$ to $> 300,000$). Nuclear magnetic resonance absorption values were comparable to the observed values for the phloroglucinol monomers, dimers, trimers, tetramers etc. identified by Glombitza (1977, 1981). The occurrence of both carbon-carbon and ether linkages was also confirmed.

In the preceding section, aqueous extracts from *Ascophyllum* and *Fucus serratus*, were shown to contain polyphenols. Here, the extracts are analysed for the presence of phloroglucinol polymers, using thin layer chromatographic techniques.

a) Methods

Aqueous extracts were hydrolysed (see Appendix E) and the hydrolysates (dissolved in 1-2 ml. ether) were separated by thin layer chromatography (cf. Glombitza, 1981), using the solvent systems described by Harborne (1973). Initial trials with phenolic standards showed poor separation with the acetic acid : chloroform, 1:9 solvent system and use of this system was discontinued.

The acid and alkaline hydrolysates were chromatographed alongside a standard phloroglucinol solution (0.01g.ml^{-1}). To detect free phenols, the silica gel plates were sprayed with Folin and Ciocalteu reagent and with ammonia. As a control, the stability of the phloroglucinol standard to hydrolysis was assessed and the efficiency of the ether extraction procedure was also established.

b) Results

Mean R_F values are recorded in Tables 43 and 44. Despite a certain amount of tailing, probably resulting from phenol dissociation (see Rönnerstrand, 1967), R_F values for both *Ascophyllum* and *Fucus* hydrolysates

Table 43. Thin layer chromatography : *Ascophyllum* extract.

Solvent system	Mean R_F values		
	Phloroglucinol	Acid hydrolysate	Alkaline hydrolysate
Ethyl acetate:toluene	0.27	0.02	0.02
9 : 11		0.15	0.10
			0.14
Acetic acid:Ethylacetate:toluene	0.29	0.02	0.02
1 : 8 : 11		0.32	0.11
			0.13

Table 44. Thin layer chromatography : *Fucus serratus* extract.

Solvent system	Mean R_F values		
	Phloroglucinol	Acid hydrolysate	Alkaline hydrolysate
Ethyl acetate:toluene	0.32	0.03	0.02
9 : 11		0.15	0.03
			0.16
Ethyl acetate:benzene	0.34	0.02	0.02
9 : 11		0.03	0.04
		0.23	0.25

were quite distinct from the R_F value of the standard phloroglucinol spot. The spot patterns for the two species were broadly similar. Co-chromatography of phloroglucinol with extracts, also failed to reveal phenolics with an R_F value similar to that of the phloroglucinol standard.

The control experiment demonstrated the high stability of phloroglucinol to hydrolysis. The calculated extraction efficiency was 99.98% for acid hydrolysis and 98.95% for alkaline hydrolysis.

6.5. Chemoreception

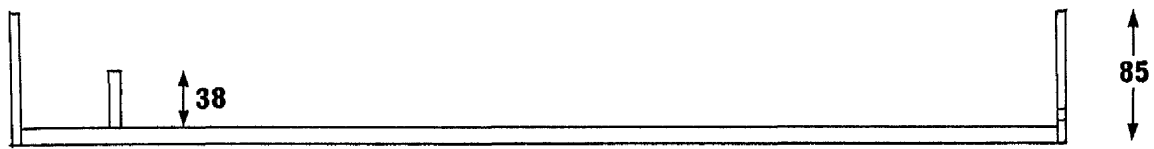
Various gastropods are known to possess an acutely developed olfactory sense. The carrion-eating opisthobranch, *Bullia laevissima*, for example, reacts positively to the chemical products of decomposition, trimethylamine and tetra-methyl ammonium (Brown, 1961). Certain herbivorous species are also thought to utilise chemical stimuli in the location of food (see Bakker, 1959) and, consequently, several studies of grazer feeding preferences have been based on the herbivore's ability to detect chemical "odours" by chemosensory means.

It has been postulated that the exudation of phenolic substances by the brown algae may act (perhaps inadvertently) as an ecological signpost to foraging herbivores. Polyphenol exudation has been confirmed for *Ascophyllum* and for other fucoids, by McLachlan and Craigie (1964), Ragan and Jensen (1979) and Zavodnik and Jensen (1981). This section investigates the chemoreceptive response of the littorinids to *Ascophyllum* exudate.

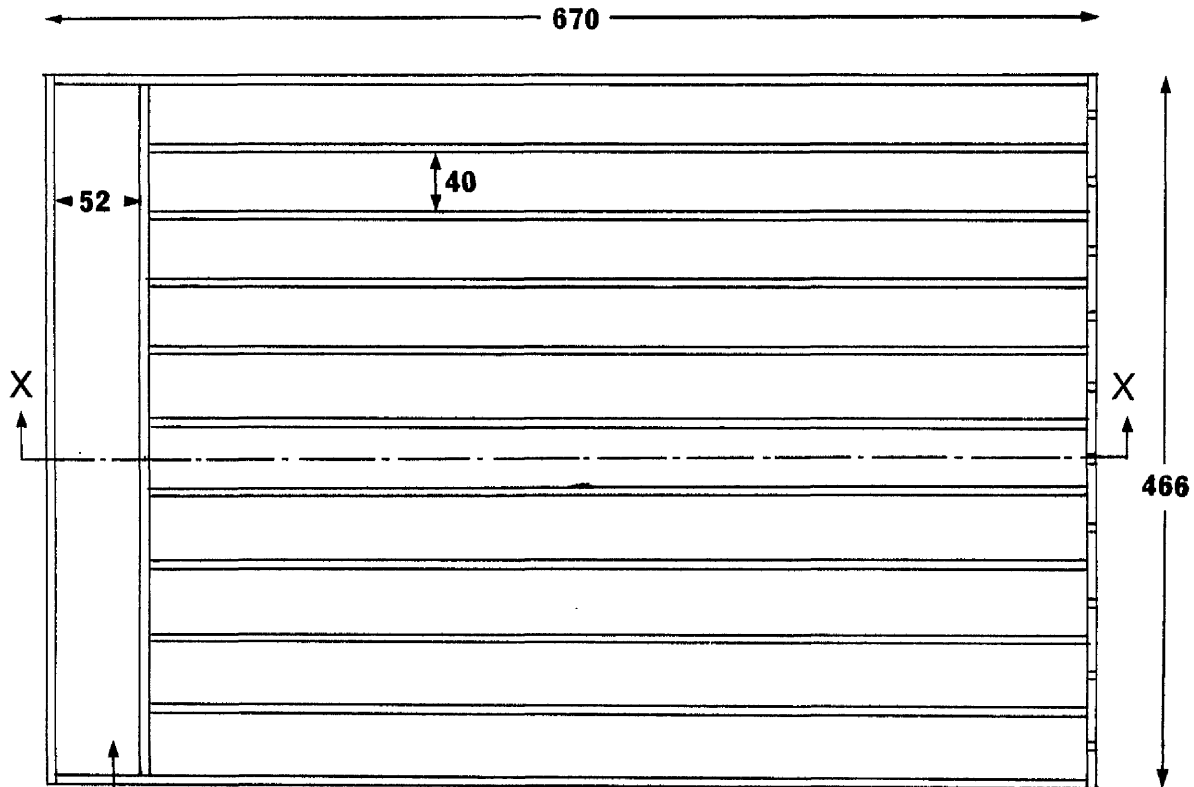
a) Methods

A system of perspex runways was constructed according to the design illustrated in Fig. 48. Two five gallon carboys containing filtered sea water, were placed on a rack above the runway system and connected to the terminal reservoir compartment with rubber tubing. Taps controlled the flow

Fig. 48. System of perspex runways used to test chemosensory response to *Ascophyllum* exudate. (All dimensions in millimetres.)

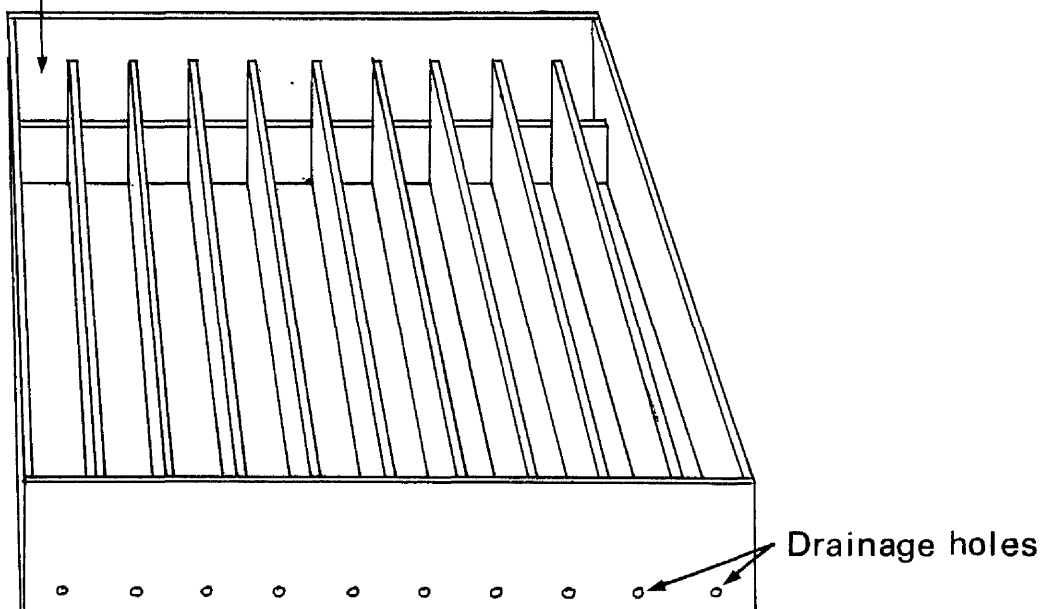


Section X—X , Scale 1:5



Plan , Scale 1:5

Reservoir



Perspective View , not to scale

from each carboy into the reservoir.

From the reservoir compartment, water overflowed into each runway, eventually draining at the opposite end. In a similar experiment Van Dongen (1956) found that a water level of 1cm. or less discouraged wall-climbing. By adjusting the taps it was possible to achieve consistently a depth of 0.4-0.6 cm. in the runways. The velocity of water flow was approximately 5 cm.s.⁻¹.

60 minutes before the commencement of experiments, a net bag containing approximately 15 cm³ of *Ascophyllum* was placed in one of the sea water carboys. The experiments were carried out in darkness (to eliminate light directed crawling) and at a temperature of 10-15°C.

Sieburth (1969) has shown that the exudation of polyphenols by the Fucaceae ceases completely in the dark, while Ragan and Jensen (1979), working with *Ascophyllum*, found that release during active photosynthesis far exceeded dark release. As this work suggests strongly that exudation is a light-mediated process (see also Zavodnik and Jensen, 1981) the carboys were illuminated at approximately 5.0 W.m⁻² during the 60 minutes immediately preceding experiments, to promote release into the sea water medium.

Ten snails (one per runway) were used in each trial. Between trials, the runways were cleaned thoroughly to prevent mucus trail-following, a troublesome behavioural phenomenon observed by D.P. Cheney (pers. comm.) and by the author. At the onset of the trials each snail was placed at a point halfway up the runway. Crawling was permitted to proceed for three minutes (common winkles) or four minutes (flat winkles) and the position of the snails relative to the starting point was then recorded, net crawling into the water flow constituting a positive movement and with the flow, a negative movement. Each batch of snails was tested alternately against filtered sea water from the carboys with and without the test alga. All specimens climbing the sides of the runways were discarded.

With each littorinid, eight batches of ten snails were tested. Four batches were exposed first to filtered sea water (control) and then to sea

water plus algal exudate (trial). The order of testing was reversed for the remaining four batches.

Table 45. Chemoreceptive response of littorinids to *Ascophyllum* exudate.

Asterisks denote significance of difference between movement in trial and control runs (Wilcoxon signed-rank test).

Species	Number of snails	Response to <i>Ascophyllum</i>
<i>Littorina littorea</i>	60	Repelled*
<i>Littorina mariae</i>	61	Nil ^{n.s.}
<i>Littorina obtusata</i>	56	Attracted**

n.s. non significant

* $0.01 < P < 0.05$

** $0.001 < P < 0.01$

b) Results

The results (Table 45) demonstrate repulsion of *Littorina littorea* and attraction of *L. obtusata* by the *Ascophyllum* exudate. The distance moved by *Littorina mariae* did not differ significantly between trial and control runs.

6.6. Discussion

6.6.1. Structural defences

The manner in which the shape and structure of the algal thallus can interact with the body plan and feeding apparatus of the herbivore to influence the efficiency of feeding has already been demonstrated. The importance of finding a food item which can be manipulated easily and with the minimum energy expenditure, is further confirmed by the common winkle's apparent preference for *Ulva* over *Enteromorpha*. In most respects, *Enteromorpha* appears to be the more attractive food. The durometer readings for mature *Enteromorpha* are significantly lower (*Enteromorpha* : 2.0 ± 0.18 ; *Ulva* : 15.6 ± 0.63) while the aqueous extract enhanced significantly the palatability of the standard substrate ($0.01 < P < 0.05$, Wilcoxon signed-rank test). However, observation of feeding individuals quickly reveals the problems encountered by *L. littorea* in its attempts to consume *Enteromorpha*. While the broad, ulvoid thallus is pinned down by the littorine foot to form a relatively stable food substrate, a single filament of *Enteromorpha* is too narrow to carry the entire foot and has a strong, inherent buoyancy which frustrates efforts to hold it in place. When a feeding snail loses its grip - even momentarily - the filament floats rapidly upward and out of reach.

Slocum's (1980) work on the heteromorphic generations of *Gigartina papillata* further emphasised the considerable defensive potential of an "awkward" thallus form. The fleshy, crustose morph appeared to be maintained largely by grazing. Grazer activity reduced overgrowth by other species without adversely affecting the crust itself. The alternative blade phase, however, became abundant only where grazing pressure was substantially lower. In this way, heteromorphic generations can provide an alternative means of solving the inevitable conflict between defence and productivity. Instead of a direct "trade-off" between competitive ability and the efficacy of anti-herbivore defences, fitness is optimised by two distinct morphological phases, each providing an adaptation to different sources of mortality.

Experiments with the durometer and the abrasive wheel show that an objective assessment of algal toughness is not straightforward. The resistance of the lamina to penetration depends as much on the nature of the force applied as the structural properties of the lamina. This is borne out by the marked difference between toughness rankings based on the criteria of puncture strength and resistance to abrasion.

A divergent response to laminar toughness by the common winkle and the flat winkle might be anticipated in the light of the characteristic differences in radular form. The common winkle employs sharp cusps to tear the thallus, a mode of action which relies heavily on the initial penetration of the lamina. This is reflected in a strong correlation between edibility and puncture strength.

The flat winkle adopts a somewhat different approach, rasping the thallus with its much blunter cusps. Surprisingly, there is no clear-cut correlation between algal edibility and susceptibility to abrasion for this species. However, the foliose alga *Ulva* and the non-preferred fucoid *Ascophyllum*, both registered lower tissue losses in abrasion trials than the preferred *Fucus* spp. The buccal musculature of the smaller sibling, *Littorina mariae*, may not be able to exert as much pressure on the thallus and this may explain the particularly low ranking of the abrasion-resistant *Ascophyllum* in edibility trials with this species.

Durometer readings for *Ascophyllum* and *Pelvetia* revealed a substantial gradient in puncture strength between soft apices and tough basal tissue. This trend was reversed in experiments with the abrasive wheel. I would suggest that the puncture strength of a tissue is a function of the strength of the outer wall or cuticle. While this may be weak in young tissue, in older tissue the wall and/or cuticle is fully formed and therefore more difficult to penetrate. However, abrasion resistance is more likely to reflect the "average" toughness of the material abraded. In young tips the tissue consists predominantly of small cells with a high ratio of wall: cytoplasm. In older regions most of the cells have extended and the medullary

tissue is primarily mucilaginous with only scattered cells. Consequently there is a higher preponderance of soft, readily abraded tissue.

The relationship between herbivory and the structural and morphological characteristics of different algal parts has remained largely unexplored. However, Saitô and Nakamura (1961) suggested that intertidal herbivores may attack tougher parts of the thallus only after consuming the more tender portions. My own observations suggest that *Littorina littorea* grazes the *Pelvetia* thallus selectively, preferring the frond tips to the rough, channelled basal tissue.

Laboratory and field observations also indicate selective grazing of plant parts by the flat winkle. Both *Littorina obtusata* and *L. mariae* seldom graze the abrasion-resistant *Ascophyllum* apices. However, this may be related to the body plan of the winkle and not primarily the toughness of the alga, as the apical portion of the frond is often too narrow to accommodate the entire foot - a factor of some importance to the grazing flat winkle.

As well as acting directly as a grazing deterrent, a tough epithelial layer may be detrimental to the browsing snail by accelerating radular wear. The tough cuticle of *Gigartina stellata* undoubtedly confers a high degree of resistance to both penetration and abrasion. *Gigartina* also ranks low in common winkle feeding preferences.

It is a basic premise of optimal foraging theory, that an animal experiences a strong selective pressure to eat those foods yielding the maximum "value" per unit time (Emlen, 1973). The value of a given food item will be determined ultimately by the herbivore's ability to digest, absorb and assimilate the available nutrients and not directly by the quantity and quality of the nutrients potentially available. Certain algae are defended against herbivory by the incorporation of structural calcium carbonate in the cell walls. While this may be a metabolically expensive mode of defence, its efficacy cannot be denied. Of the algae studied, only the calcareous *Corallina* is virtually immune to grazing. Without reinforcement of this nature, however, the cell wall would appear to be

highly vulnerable to breakdown in the common winkle's digestive system and this may explain the remarkably high assimilation efficiency computed by Grahame (1973) for *L. littorea* fed on *Ulva*. However, Dahl (1964) frequently found undigested cells and tissue fragments in the stomachs of various North American littorinids and it seems likely that digestion of less preferred algae is correspondingly less efficient.

Although many invertebrates show only a weak ability to digest cellulose, the level of digestion achieved by the common winkle is high. Digestion by the flat winkle was less efficient but this may reflect the nature of the food substrate (*Fucus*) and not inherent differences in the digestive capabilities of the two species. Despite such strong evidence of effective cellulose digestion, the digestive process itself is probably costly in energy terms. It is perhaps significant that the red alga *Porphyra*, which substitutes the cellulosic component of the cell wall with xylan, ranks very highly in common winkle preferences, while *Cladophora*, a green alga with a high cellulose content, is not readily consumed.

Throughout the digestion experiments, a persistent "background" polysaccharide level was evident in faecal material collected from starved, control specimens. Without further investigation, the source of this carbohydrate must remain a mystery.

Since the cellulase activity of the faeces and the *Ulva* does not differ greatly, it is possible that the faecal cellulase was derived entirely from the food. If so, this would imply that the activity of the ulvoid cellulase remained unimpaired during its passage through the gut. Alternatively, the similarity between the two values may be coincidental, the ulvoid cellulase being degraded in the gut and replaced by cellulase produced by the winkle or by the micro-flora/fauna of the gut.

That *Ulva* contains significant quantities of cellulase is not, in itself, unusual. Most plants contain cellulase and its activity in the intact plant must be strictly controlled to prevent wholesale degradation of structural cellulose. However, such control is lost when the alga is

ingested by the winkle and this could initiate extensive cellulose degradation.

Whatever the origin of the cellulase, it is clear that the gut contains significant amounts of the enzyme and this accounts for the observed cellulose degradation in digestion experiments. Further study of the algal and faecal cellulases would be necessary to determine whether the animal produces its own cellulase or simply makes use of the enzyme present in the alga.

It has been suggested (e.g. Huang and Giese, 1958) that marine herbivores may utilise only those algal constituents, such as protein and starch, which are readily available. However, the littorinids investigated in this study are clearly capable of deriving considerable benefit from a much wider range of algal constituents.

6.6.2. Chemical defences

The widespread occurrence and potentially valuable defensive role of plant secondary chemicals is beyond doubt. Indeed, grazer food selection may be influenced more strongly by the taste of a plant than by the nutritional or caloric value (Prosser, 1973).

Before drawing any conclusions from extract experiments, the procedure adopted must first be placed in strict context. Secondary plant chemicals may be volatile or stable only for brief periods after release (Grime *et al.*, 1968; Glombitza, 1981) and, as a result, noxious or attractive effects may not always be apparent in aqueous extracts. Furthermore, the dilution of allelochemicals during extraction may itself suppress attractive or repulsive properties. Indeed, Feeny (1975, 1976) notes that quantitative plant defences act in a dosage-dependent fashion such that the degree of protection from herbivory is directly related to the concentration of secondary chemicals in the plant tissue. It is also possible that allelochemicals may be produced in the field - particularly by unapparent ephemeral species - only in direct

response to grazing damage and consequently, may not be detected in laboratory extract experiments.

Differential consumption of filter paper treated with algal extracts may represent either caloric/nutritive differences amongst the algae or the presence of defence chemicals or phagostimulants in the homogenates. Where the consumption of untreated filter paper exceeds the consumption of treated samples, however, the presence of a repellent is established without ambiguity.

In the light of Carefoot's (1980) experiments, the phagostimulant properties of the green algal extracts are particularly interesting. Carefoot found that extracts from *Ulva fasciata* acted as strong phagostimulants for the sea hare, *Aplysia dactylomela* and used these extracts to derive an artificial diet.

Despite the low ranking of the species in common wrinkle preferences, homogenates of the branched, filamentous alga *Cladophora*, had a similar effect on grazing to extracts from the foliose greens. This infers that adult *Cladophora* is rejected in gustatory trials for structural and not chemical reasons.

Palatability experiments with algal exudates provide further confirmation of the phagostimulant properties of *Enteromorpha* and indicate that the chemical(s) involved may be leached into the surrounding sea water in sufficient quantities to permit detection.

Although the red alga, *Gigartina*, produces relatively high concentrations of the pharmacologically-active amine hordenine (Barwell and Blunden, 1981), the aqueous extract did not deter grazing significantly. However, grazing was inhibited strongly by a homogenate of the halogenating rhodophyte, *Laurencia*. The red seaweeds synthesise a wide range of halogenated organic compounds, from low molecular weight ketones and aromatic compounds to sophisticated terpenes and products of fatty acid origin. These compounds are not involved in primary metabolic processes but are thought, instead, to constitute the messengers of an exocrine system which has evolved to provide a selective environmental advantage (Fenical, 1975). *Laurencia* spp. have been studied

particularly closely and are known to synthesise halogenated sesquiterpenes, each composed of three isoprene units, as well as halogenated diterpenes and acetylenes. Bromophenols have been identified in *Polysiphonia* and *Corallina*, although aqueous extracts from these species did not appear to inhibit grazing. No significant formation of halogenated metabolites has been recorded in either *Porphyra* or *Gigartina* (Fenical, 1975).

Fucoid polyphenols were most abundant in the unpalatable extract of *Ascophyllum nodosum*. This might be interpreted as an indication of a dosage-dependent response to the inhibitory chemicals. However, the phenolic content of the unpalatable *Ascophyllum* germling extract is much lower, comparable in fact, to that of the palatable *Fucus* extract. Clearly this suggests that significant qualitative differences exist in the phenolic contents of the two homogenates. The ecologically active compound(s) either do not occur or occur at a lower concentration in the *Fucus* extract. There is certainly no attempt to compensate for a lack of structural defences at the germling stage by increasing the total phenol level.

A high proportion of brown algal polyphenols are structurally based on the relatively stable phloroglucinol precursor (Glombitza, 1981; Ragan, 1981). Geiselman and McConnell (1981) characterised the compounds active in the inhibition of littorinid grazing as phloroglucinol polymers with a wide molecular weight range. Consequently, it was most surprising to find no positive indication of phloroglucinol in either *Ascophyllum* or *Fucus* extracts. In this context it may be significant that Glombitza (1981) was himself unable to detect phloroglucinol in *Ascophyllum*. While it is possible that phloroglucinol derivatives were extracted, but not in sufficient quantities to permit chromatographic identification, it is more probable that other water-soluble phenols are also active as grazing repellants, so that chemical protection is afforded by a heterogeneous mixture of polymeric phenols and not by a single compound or type of compound. Despite the apparent preoccupation with phloroglucinol, Ragan (1976) was quick to acknowledge that all physodes do not necessarily contain phloroglucinol, nor do physodes

contain phloroglucinol alone. It is perhaps prudent to remark, strictly in the context of this study, that aqueous extracts are likely to contain free phenols, diphenols, phenol glycosides and lower molecular weight polyphenols as well as the high molecular weight polyphloroglucinols characterised by Geiselman and McConnell (1981). Furthermore, water extraction may not remove all the high molecular weight polyphenols from the plant tissue.

There was no positive evidence of a close-range olfactory or gustatory response to *Ascophyllum* exudate. This does not rule out the possibility that a "tactile chemical sense" (Crisp, 1976) may be used to recognise the molecular nature of the surface before grazing proceeds. Such a means of food selection has already been demonstrated by Bernays and Chapman (1970), with the terrestrial arthropod *Chorthippus parallelus*.

The application of "long-distance" chemosensory perception was clearly demonstrated in experiments with both *Littorina littorea* and *L. obtusata*. *L. littorea* responded to *Ascophyllum* by crawling away from the source of exudation, while *L. obtusata* responded in the opposite manner by crawling towards the source. This illustrates the divergent evolutionary response to an ecological signpost by two species with contrasting behaviour patterns. *Littorina obtusata* responds positively to the presence of a potential food source and a suitable microhabitat. The negative reaction of *L. littorea*, on the other hand, reflects a strong gustatory dislike of the brown alga.

Ascophyllum ranks very low in the food preferences of the sibling flat winkle, *Littorina mariae*, and the two species are seldom found in association on the shore. Consequently, the complete absence of a response to *Ascophyllum* exudate may be based primarily on the winkle's indifference to *Ascophyllum* as a food substrate. Alternatively, the lack of response may constitute a mechanism designed to maintain the low-shore distribution of the species.

The seasonal fluctuation of both exudation and intracellular accumulation of polyphenols is well known. Ragan and Jensen (1978) found a significant temporal correlation between polyphenol content and reproductive state for *Ascophyllum* and *Fucus vesiculosus*, phenol levels peaking during the period

of sterility, which coincides with a period of low grazer activity. Consequently, intracellular polyphenol concentrations will be minimal when grazing is most intense and no special protection will be afforded to the vulnerable fruiting bodies. Any *in situ* defensive role which may be attributed to the physode contents, is therefore probably incidental. However, it should be borne in mind that Zavodnik and Jensen (1981) did record a slight early summer increase in phenol tissue concentrations.

Coincident with declining intracellular phenolics, an increase in exudation has been reported during the spring and summer (Sieburth and Jensen, 1968; Sieburth and Tootle, 1981). Hence the polyphenols may be intended primarily as an olfactory and not a gustatory deterrent. The positive response of *Littorina obtusata* to *Ascophyllum* exudate may typify the "evolutionary arms race" which continually pressurises the specialist herbivore to adapt, and indeed to utilise, the "host" plant's defences. At the same time, *Ascophyllum*'s allelochemicals provide an eloquent example of the potential efficacy of non-specialist quantitative defences in combatting a generalist herbivore, such as the common wrinkle.

CHAPTER 7 - GENERAL DISCUSSION

The recent wealth of ecological studies relating herbivory in the littoral zone to algal community structure emphasises the important ecological role of selective grazing and, at the same time, highlights the critical lack of experimental data concerning feeding preferences. This relatively new field of scientific inquiry has flourished particularly in eastern North America, where the common winkle, *Littorina littorea*, is the dominant invertebrate herbivore of the rocky intertidal.

The common winkle was introduced to America in the early nineteenth century by European settlers and, like many recent introductions, has exerted a profound effect on its new habitat (Cheney, pers. comm.; Lubchenco, 1978, 1980; Sze, 1980; Bertness *et al.*, 1983). Despite the abundance of the common winkle on its native British shores, however, little is known of the ecological role or grazing preferences of the species in this country.

In the North Atlantic, emergent substrata on sheltered-moderately exposed shores are dominated by the canopy-forming furoid algae. At the vulnerable germling stage these species avoid destruction by grazers either by virtue of distasteful chemicals or by growing rapidly to a size at which grazing damage is discouraged by thallus toughness and no longer threatens plant fitness. The net result is that once established, these species are not affected significantly by herbivory. Even the potentially damaging preferential grazing of furoid reproductive tissue by dense flat winkle populations will not influence fitness greatly, as the reproductive period of the "host" algae coincides - at least partially - with the period of grazer inactivity. Consequently, if grazing is to influence the growth of a perennial species, it must do so primarily at the juvenile phase - a phase at which chemically-mediated selectivity has been shown to operate. The longevity of the fucoids is such that only a low level of successful recruitment is required to maintain a healthy stand and dense canopy. Should experimental removal (e.g.

Schonbeck and Norton, 1980) or ecological catastrophe denude an area of shore, however, recolonisation may fail to occur and this is undoubtedly due largely to the combined gustatory attentions of the common winkle and the patellid limpets.

Grazing of adult macrophytes does, nevertheless, occur. Extensive feeding on the highly palatable ephemeral species may indeed be of some considerable ecological significance. Lubchenco (1978) found that grazing of adult ephemeral algae following the initial heavy settlement and growth, rapidly returned high-tidal pools to a state of ecological equilibrium in which the unpalatable red alga *Chondrus* out-survived the faster-growing ephemeral species. On European shores, Lein (1980) has observed the systematic destruction of adult *Enteromorpha* by "fronts" of grazing winkles. In this way, common winkle grazing probably maintains the mid-low shore dominance of *Gigartina* and *Chondrus* underneath the furoid canopy on British shores.

It has been suggested that algal growth form may restrict a herbivore to feeding within a particular vegetative stratum (Maiorana, 1978). This is confirmed by the contrasting capacity of common and flat winkle to graze the adult furoid species forming the canopy and the adult foliose algae of the understorey. Consequently the herbivore will not experience intensive selective pressures to overcome the defences of plants outside its own preferred stratum.

Present theories concerning benthic algal adaptations to survive herbivory, focus mainly on the contrasting policies of defence and escape. It is generally accepted that r-selected species escape temporally by means of short life histories, or spatially as a result of patchy distribution, while the K-strategists normally develop "sedentary" structural or chemical defences (Vadas, 1977). Such defences are inevitably costly in terms of fitness. When grazers are absent, the poorly defended r-strategists are capable of achieving considerably higher levels of fitness (Rhoades, 1979). On the evidence of my study, the prey responses of an alga can be further

sub-divided into five categories similar to those defined by Paine and Vadas (1969b):

- 1) Distasteful - such plants gain some immunity by repelling the herbivore after discovery.
- 2) Daunting structure - morphological adaptations repel or more often inhibit, efficient consumption.
- 3) Low food value - a physiological rather than behavioural mechanism reducing the alga's appeal to the grazer.
- 4) Patchy distribution - ecological defence where the alga adopts an irregular distribution making location difficult.
- 5) Ephemeral life style - grazing avoided by temporal escape.

These contrasting strategies are not mutually exclusive and a single species may adopt any combination of the five.

The foliose species preferred by the common wrinkle are typical r-strategists and counteract an inherent susceptibility to grazing with a rapid growth rate and high reproductive potential which may swamp the grazers temporarily during the early part of the year (e.g. Lubchenco, 1978; Lein, 1980). These species remain highly vulnerable to herbivory throughout their life cycle, relying not only on rapid replacement, but also on a patchy distribution, to provide a spatial escape from grazing. Consequently, the ephemeral algae are by nature, unpredictable species of relatively low "apparency". Plant apparency, as defined by Feeny (1976), includes all characteristics influencing the susceptibility of a plant to discovery by herbivores, including persistence, growth form, population density, and the host-finding adaptations of all relevant herbivores. My findings provide at least partial support for the plant apparency and antiherbivore chemistry theory (Feeny, 1976; Rhoades and Cates, 1976) derived from terrestrial ecosystems. The theory states that dominant perennial plants are defended primarily by chemicals which reduce digestibility. However, the value and widespread use of structural defences is also emphasised by my study.

The high ranking of ephemeral algae in the food preferences of the common

winkle, indicates that the species is basically an opportunist. Total reliance on algae which are ephemeral both in time and space, would constitute a dangerous policy - especially for a grazer with restricted mobility. The well-defined food preferences which dictate the composition of the diet when food is plentiful, are not binding, and the common winkle can revert to a policy of opportunism where population densities are high or preferred food items scarce. Feeding on the shore is therefore a compromise between preferences (as defined in laboratory choice experiments) and availability. This balance is essential to the operation of selective grazing in the field. In terms of optimal foraging theory, the predator should eat only the highest ranking type of prey if the encounter rate with that prey is sufficiently high that the inclusion of lower ranking items in the diet would reduce the average net rate of energy intake (Hughes, 1980). As the highest ranking prey becomes scarce, the predator should expand its diet to include the next prey lower in rank and so on. This rather simplistic, theoretical concept of selectivity may be modified according to the nutritional state of the herbivore. If the foraging animal is "hungry" the search for preferred foods will be truncated and less preferred species will not be passed over so frequently, even if the net energy gain from such items is small.

Several attempts have been made to correlate herbivore preferences with the life-history characteristics of the food plants. Connell and Slatyer (1977) suggest that herbivory may act as a driving force behind succession by enhancing species replacements. If early colonists (r-strategists) retard or prevent the establishment or growth of later successional plants, then removal of the former by herbivores will speed up the rate at which later species become established or grow (Lubchenco and Gaines, 1981). Vadas (1977) found that subtidal sea urchins in the Pacific Northwest preferred the early successional kelp *Nereocystis luetkeana* to the later successional species *Agarum cribrosum*. When urchins are present, *Agarum* occurs; if urchins are naturally rare or artificially removed, *Nereocystis* becomes established and effectively inhibits *Agarum* growth.

My results revealed that the early successional species *Ulva*, *Enteromorpha* and *Porphyra* were strongly preferred by *Littorina littorea*. On Norwegian shores, Lein (1980) has recorded the destruction of *Enteromorpha* by marauding common winkles, leaving behind a vegetation consisting primarily of *Fucus* and various encrusting algae. Similarly, Lubchenco and Gaines (1981) found that preferential grazing of *Ulva* in New England permitted the rapid establishment of the less preferred *Fucus vesiculosus*. Where dense grazer populations occur on British shores, it is possible that a similar mechanism may operate.

A policy of ecological escape will, as Rhoades (1979) suggests, prove least effective against the generalist herbivore. While ephemeral green algae may temporarily swamp the grazer populations during the spring and early summer, the essentially defenceless adult plants constitute a ready food source for the opportunistic common winkle and patches of ephemeral algae are rapidly destroyed by concerted grazing effort (pers. obs.; R.L. Vadas, pers. comm.; Lubchenco, 1978; Lein, 1980). Escape in space and time is more effective against specialist herbivores which have fewer alternative food sources. Thus ephemerality and predictability select for generalisation and specialisation respectively, in the associated herbivores.

The dominant perennial algae *Ascophyllum* and *Fucus serratus*, adopt a strategy based on defence rather than escape and, as a result, are less appealing to the generalist herbivore at both the adult and juvenile stages. As Hughes (1980) predicted, herbivorous browsers feeding on the intractable macroalgae are by nature, specialists. The inevitable price of specialisation, however, is dependence, and as a result, the flat winkles (particularly *Littorina mariae*) suffer a restricted distribution. In the Firth of Clyde neither species is found on mud flats or exposed shores where fucoid algae are scarce.

The characteristic immobility of the benthic herbivore dictates the extension of dietary specialisation to cover, in addition, choice of habitat. The spatial separation of habitat and food requirements would not constitute

an efficient behavioural strategy in terms of energy expenditure for it has been estimated that *Littorina littorea* consumes oxygen at 15 times the basal rate when actively crawling (Newell, 1970).

Underwood (1979) suggested that behavioural adaptations in response to the presence of a favoured microhabitat or preferred food may confine intertidal invertebrates to particular levels on the shore. However, food preferences should place no direct restrictions on opportunist species. *Littorina littorea*, for example, achieves a wide vertical distribution on all sheltered shores. The flat winkle, *Littorina obtusata*, will feed enthusiastically on most canopy species, and happily occupies a wide variety of microhabitats wherever macroalgal cover exists. *Littorina mariae*, on the other hand, forms a remarkably close association with the low-shore furoid *Fucus serratus*. It is common only in the *Fucus serratus* zone and is, indeed, found almost exclusively on the fronds of the dominant species and to a lesser extent on *Fucus vesiculosus*. In laboratory choice experiments, *L. mariae* is strongly attracted to *F. serratus*. Furthermore, the low-shore flat winkle does not demonstrate a positive chemosensory response to *Ascophyllum*. As *Ascophyllum* dominates the algal zone immediately above *Fucus*, this constitutes another factor in favour of a very restricted distribution.

Littorina obtusata is typically less abundant low down the shore and, as a result, excessive competition between the two potentially sympatric species is reduced. Competition is further minimised by the contrasting positive attraction of *L. obtusata* towards the mid-high shore furoid, *Ascophyllum*. But if evolution places such store by the avoidance of competition, what then is the potentially limiting factor driving the two species apart? The vast biomass of canopy-forming algae on sheltered shores is theoretically sufficient to provide adequate space and a surfeit of macroalgal food. I would, however, suggest that the hypothesised reliance of juvenile flat winkles on micro-epiphytic foods may be causal in the partial separation of the two species.

The vertical zonation and microdistribution of *Littorina littorea* and *Patella vulgata* is such that these two morphologically divergent species also

constitute potential competitors. While the common winkle is an opportunist able to utilise a wide variety of food sources it is, by preference, a grazer of the microflora of the rock substratum. The abundance of *Patella vulgata* on open rock at both exposed and sheltered sites, may explain the corresponding absence or low abundance of *Littorina littorea*. By way of contrast, R.L. Vadas and S.L. Miller (pers. comm.) have observed dense populations of *L. littorea* grazing on open rock on the coast of Maine, U.S.A., where there is a general paucity of herbivores. This gives further credence to the theory of competitive exclusion from limpet-dominated sites with poor macroalgal cover.

The relationship between the gastropod grazers and the co-existing algal flora is clearly complex and reflects both the foraging behaviour of the grazers and the corresponding defensive strategies of the algae. By answering MacArthur's (1972) query "Where should an animal feed to get the most food and what items of food should it pursue?", conclusions can be drawn regarding aspects of life in the intertidal of far greater importance than mere dietary balances and caloric intakes.

Appendix AComposition of enriched seawater medium (Boney's medium)

The following nutrient solutions were prepared:

Solution A

- 50 ml. of 0.4% Na_2NO_3
- 2 ml. of $1.47 \text{ g.l}^{-1} \text{ MnSO}_4 \cdot 4\text{H}_2\text{O}$
- 2 ml. of $0.0023 \text{ g.l}^{-1} \text{ CuSO}_4 \cdot 5\text{H}_2\text{O}$
- 2 ml. of $0.064 \text{ g.l}^{-1} \text{ CoCl}_2 \cdot 6\text{H}_2\text{O}$
- 2 ml. of $0.23 \text{ g.l}^{-1} \text{ NaMoO}_4 \cdot 2\text{H}_2\text{O}$

Solution B

- 2 ml. of $4.98 \text{ g.l}^{-1} \text{ ZnSO}_4 \cdot 7\text{H}_2\text{O}$

Solution C

- 15 ml. of 2.6 g.l^{-1} tetrasodium salt of EDTA (ethylene diamine tetraacetic acid) + $0.12 \text{ g.l}^{-1} \text{ FeSO}_4 \cdot 7\text{H}_2\text{O}$

Solution D

- 1.5 ml. of $15 \text{ g.l}^{-1} \text{ Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$

Solutions A, B, C and D were autoclaved at 15 lbs. in^{-2} for 15 minutes. Approximately 100 ml. distilled water was added to 900 ml. filtered seawater and the mixture was heated to c. 65°C and then allowed to cool. This procedure was subsequently repeated. Nutrient solutions A, B, C and D were then added to the pasteurised seawater. The medium was stored at c. 5°C .

Appendix B

Carbohydrate assay (after Dubois *et al.*, 1956)

Sample :

10-70 µg. carbohydrate in 1 ml. water

Experimental procedure :

- a) Add 1 ml. 5% phenol and mix.
- b) Add 5 ml. concentrated sulphuric acid to surface of liquid.
- c) Stand at room temperature for 10 minutes.
- d) Incubate at 25-30°C for 10-20 minutes.
- e) Read optimal density at 490 nm. on spectrophotometer.

Appendix CExtraction of non-cellulosic carbohydrates (after Updegraff, 1969)

Updegraff reagent -

Acetic acid : nitric acid : water

8 1 2 (volume/volume)

Experimental procedure -

- a) Extract for 60 minutes at 100°C with 3ml. Updegraff reagent.
- b) Centrifuge and remove supernatant.
- c) Wash with distilled water, centrifuge and remove supernatant.

Appendix DTotal phenol estimation (after Lowry *et al.*, 1951)

- Reagents :
- 1) Alkaline tartrate reagent.
3.3% sodium potassium tartrate.
 - 2) Copper sulphate solution.
1.25% hydrated copper sulphate.
 - 3) Sodium carbonate solution.
2.5% sodium carbonate.
 - 4) Working alkaline copper reagent.
1 ml. alkaline tartrate reagent and 1 ml. copper sulphate solution were freshly mixed immediately before use and diluted to 100 ml. with sodium carbonate solution.
 - 5) Folin and Ciocalteu phenol reagent (B.D.H. Ltd., Poole, U.K.)
Reagent diluted 1:2 with distilled water when required.

A 1 ml. volume of standard or test solution was added to 4 ml. of working alkaline copper reagent. 1 ml. distilled water was treated in a similar manner to serve as a reagent blank. The solutions were mixed thoroughly and left at room temperature for ten minutes. 1 ml. diluted Folin and Ciocalteu's reagent was then added to each solution and mixed. The tubes were left to stand for 30 minutes at room temperature and the absorbances were subsequently measured against the reagent blank at 660 nm.

Appendix E

Hydrolysis of aqueous extracts

- Acid hydrolysis :
- 1) 4.95 ml. 2M hydrochloric acid added to 27.5 ml. aqueous extract under nitrogen.
 - 2) Solution incubated in water bath at 60°C for four hours.
 - 3) Solution cooled and extracted five times into diethyl ether (1:1).
 - 4) Ether extract rotary evaporated to dryness.

Alkaline hydrolysis :

- 1) 2.2 g. sodium hydroxide added to 27.5 ml. aqueous extract under nitrogen.
- 2) Solution incubated at room temperature for four hours.
- 3) Solution acidified with 2M hydrochloric acid and extracted five times with diethyl ether (1:1).
- 4) Ether extract rotary evaporated to dryness.

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