THE FOOD OF THE TERRESTRIAL SNAIL

Helicella virgata (da Costa)

IN SOUTH AUSTRALIA

by

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SUMMARY

The terrestrial snail Helicella virgata (da Costa) was introduced from Europe into South Australia where it is now widespread. It feeds mainly on decaying plant material, although gut-contents indicate that green tissues of vascular plants do form a part of its diet.

Experiments were conducted to determine whether there is a specific dietary requirement which can only be satisfied by eating 'litter'. It is concluded that this is unlikely.

Certain observations suggested that H. virgata may feed indiscriminately unless inhibited by physical or chemical properties of the food - that is, that it does not respond to positive chemical stimuli to feeding. This hypothesis is rejected; so too is the hypothesis that it responds to one specific stimulus. No attempt was made to test a comprehensive list of chemicals, but substances shown to stimulate feeding include glutathione, glucose and a vegetable oil.

Evidence was obtained that H. virgata is inhibited from feeding on certain plants not only by the toughness of their outer layers but also by chemical components of the plants. A pyrrolizidine alkaloid was shown to be inhibitory. However, toughness may also be an important barrier to feeding. Evidence is presented that this is so with at least two species of grasses. The possible role of gastropods in the evolution of such protective devices in plants is briefly discussed.

Using y-tubes in the laboratory it was confirmed that H. virgata is attracted by the odour of a crushed member of its species. Having found a crushed snail, the live ones feed on it. There is no evidence of the presence of a pheromone and it is tentatively accepted that the snails are attracted by an odour associated with food. In agreement with this, there is evidence of attraction to several other foods.

The stimuli which arouse snails from inactivity are briefly discussed. Evidence is presented which confirms the earlier finding that dormancy is not obligatory in summer and that snails briefly awakened from summer dormancy are able to feed.

Two field experiments were conducted to investigate the likelihood of snails experiencing a shortage of food. In both experiments and in one by a previous worker, various measures of growth, reproduction, activity or survival were found to decrease with increasing density of snails. It is suggested that these results appear inconsistent with the hypothesis of an absolute shortage of completely-accessible food. The results could be explained by a modification of that hypothesis or by the hypothesis that snails at high density interfere with each other by means other than competition for food. The second experiment gave results suggesting that, on the area studied, the food naturally available is not of high quality; the snails may suffer a relative shortage of food, of the kind in which food is abundant but much of it is low in nutrients.

DECLARATION

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university and, to the best of my knowledge and belief, contains no material previously published or written by another person, except when due reference is made in the text of the thesis.

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1. INTRODUCTION

The helicid snail Helicella virgata (da Costa) was introduced into South Australia from Europe early in this century and is now widespread throughout agricultural areas of the state. Pomeroy and Laws (1967) considered that the activities of man have played a great part in the spread of the snail and that its spread is probably still continuing.

Although H. virgata is rare over much of its range it can reach densities of over 200 per square metre in some places (Pomeroy, 1966). It is especially numerous in agricultural areas, along roadsides and the borders of paddocks; relatively few are found amongst native vegetation.

During the hot, dry South Australian summer the snails spend long periods dormant, many of them attached high on plants, fence-posts and other upright objects. Most of them are young animals that hatched from eggs during the preceding winter, but some are aestivating for their second or, rarely, third summer. Because of this behaviour H. virgata sometimes becomes a pest in the croplands; large numbers of snails dormant on the cereal-stalks may be included in the grain when it is harvested.

Most of South Australia's rain can be expected to fall between March and October or November. Temperatures during the winter are mild and at this time H. virgata will be active on most nights and many of the days. Adult snails which have oversummered will feed, grow relatively little and shortly begin to lay eggs. Reproduction can take place over a long period, some young hatching as late as

early October, but most in April-May. The young grow quickly and most of them weigh 250-400 mg by October-November (Pomeroy, 1969).

Pomeroy (1966, 1967, 1968, 1969) undertook a study of the ecology of H. virgata in South Australia. He examined a number of aspects of the general biology of the animal and concentrated particularly on dormancy.

Hodson (1969) continued this work, studying the behavioural, structural and physiological adaptations which enable the snail to survive a prolonged period of dormancy in a hot, dry place.

Results obtained by both workers indicate that dormant H. virgata are remarkably impermeable to water and that death in dormancy is due neither to desiccation nor to the accumulation of metabolic products in the tissues. It appears to be due to exhaustion of their energy reserves.

It seems obvious, then, that the availability of food in winter will be important for the survival of snails in summer; they can feed very little in summer and so their chances to survive will be reduced if they have not accumulated sufficient reserves in winter.

In addition, a priori, one would expect that the availability of food early in winter will influence the time at which adults lay eggs, perhaps the number of eggs laid, the number of young which survive and the rate at which the young grow. Thus, unless food is everywhere abundant, its availability and quality must be important in determining the distribution and abundance of the snail.

And evidence obtained by Pomeroy indicates that food is indeed important. He found the abundance of H. virgata in South Australia

correlated with the amounts of organic matter and calcium in the soil (Pomeroy, 1967). Others of his findings regarding food are discussed in Sections 2.1 and 7.1 of this thesis.

This thesis concerns the food H. virgata in South Australia. In Chapter 2 I comment briefly on the diet of the animal in the field. At first sight, H. virgata seems not to choose the richest food available to it, and Chapters 3 and 4 concern the question of why the snails eat what they do. Chapter 5 reports brief investigations of the ways in which snails find their food, and Chapter 6 discusses the conditions under which they can feed.

In Chapter 7, I consider the question of whether snails are likely to experience a shortage of food and what might be the nature of the shortage.

The work was carried out partly in the laboratory and partly on two study-areas, which are described in Appendix 3.

Helicella virgata is polymorphic for banding of the shell and, one would expect, for many other characters as well. (This polymorphism has been investigated by Baverstock, 1968 and Roberts, 1970). As has been frequently pointed out of late, such variations among animals in a population may be very important in influencing the size of the population (Birch, 1960; Pimentel, 1961; Chitty, 1967; Reddingius and den Boer, 1970; Shorrocks, 1970; Wolda, 1970). For example, Wolda (1970) and others have found that snails, Cepaea nemoralis, of different morphs and from different areas differ in a number of characters which influence their dispersal, survival and fecundity. These variations seem likely to be important in influencing

fluctuations in the sizes of populations.

I have been unable to investigate such differences in this project. Instead, I have worked only on one morph. The part of Buckland Park where I worked is rather unusual in that nearly all the snails present are unbanded. (They have plain white shells - other morphs have various patterns of brown bands on the white background.) Excepting the surveys discussed in Chapter 2 all the experiments described in this thesis were conducted on unbanded snails collected at Buckland Park.

It seems likely that there is a good deal of genetic variation within the unbanded morph - it would be necessary to examine this as well as to compare the different morphs before we could construct an adequate model for the influence of food on the ecology of H. virgata. This project is merely a small step towards such a model.

2. DIET OF HELICELLA VIRGATA IN SOUTH AUSTRALIA

2.1 INTRODUCTION

Previous observations on the feeding-habits of H.virgata in South Australia are virtually restricted to those of Pomeroy, summarised in the following extract from his 1969 paper (p.503):

> The exact food requirements of H. virgata are unknown, but observation and examination of the gut and faeces suggest that decaying leaves, fungi, algae, and other micro-organisms of the litter are the most important. Some parts of the grassland area at Buckland Park have no soil overlying the shell grit and little or no litter; there were few snails in these places. The green parts of the higher plants are rarely eaten by the majority of gastropods, and have never been recorded as being eaten by H. virgata. However, snails were sometimes seen to feed on dead wood, and occasionally on branches of shrubs which had a well-developed bark. Crushed snails and orange peel were also eaten on occasions; in the laboratory they "feed" freely on moist filter paper. These observations suggest that texture is more important as a stimulus to feeding behaviour than chemical composition.

In contrast to Pomeroy's opinion regarding feeding on living higher plants, Mienis (1969) records that in the Netherlands Cernuella virgata * feeds on Plantago lanceolata, P. major, P. coronopus, Achillea millefolium, Taraxacum officinale, thistles and grasses and notes that snails are frequently found on the faeces of rabbits, sheep and horses. It is not clear, however, whether the snails eat healthy, green tissues of the plants.

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* Mienis follows Kennard and Woodward (1926) in assigning this name to the species which Thiele (1929-35), in a major revision of the Mollusca, placed in the genus Helicella.

As Pomeroy (above) points out, gastropods closely related to Helicella seem most often to be litter-feeders. Boycott (1934) lists many examples of the food of British land mollusca, noting that well-grown wild green plants are seldom eaten though seedlings are perhaps taken more freely. He, too, records feeding on sheep and rabbit dung, and a fondness for paper. Graham (1955) gives an estensive list of the diets of different mollusca; whilst many pulmonates are litter-feeders a number are recorded as eating higher plants.

Thus H. virgata might be expected to eat mainly decaying matter but perhaps some green plant tissues. It was of interest that Pomeroy recorded it eating none of the latter.

Early in my project I dissected 40 snails from Buckland Park, Northfield and from vacant allotments near Northfield, and examined their gut contents.

All contained macroscopic plant material; only 6 contained, in addition to plant material, a small amount of grit. In 3 snails all the plant material was green. In 16 no chlorophyll was visible. In the remaining 21 there was both green and brown plant material, generally more brown than green.

It seemed, then, that H. virgata feeds largely on macroscopic plant tissue, and that whilst it eats mostly dead, or senescent, tissue it does eat some green tissue. I also observed, as others had done, that the snails feed on paper (old cardboard cartons, for example) and on rabbit faeces.

To obtain more detailed information about what the snails will or will not eat, I could have gone on to take extensive surveys of gut-contents, identifying the plant fragments and comparing them with the plants available to the snails in the field. (Such identifications

could be made; for example, techniques for identifying plant fragments in stomachs and faeces have been described by Griffiths and Barker (1966) and Williams (1969), and such techniques have been used on gastropods by Grime and Blythe (1969) and Pallant (1969).)

However, I decided that it would be more informative to investigate experimentally the reasons why H. virgata feeds primarily on decaying material. Therefore I only conducted one further small survey of gut-contents, whose purpose was simply to check the above impression about the animal's feeding-habits. That survey is described in Section 2.2.

2.2 FIELD SURVEY OF GUT-CONTENTS AT NORTHFIELD AND BUCKLAND PARK

PROCEDURE

The survey was carried out in September, 1969. The study-areas, which will be mentioned repeatedly throughout the thesis, are described in Appendix 3.

At Northfield, 5 quadrats of area 0.25 m^2 were randomly placed on the study area using the fences as coordinate axes. At Buckland Park, one quadrat was thrown onto the grassland and two under the Olearia shrubs.

All snails were collected from each quadrat, giving totals of 98 from Northfield and 58 from Buckland Park. Each snail was placed in a separate specimen tube.

In the laboratory a piece of No. 1 filter paper wetted with distilled water was placed into each tube. A similar technique was used by Grime and Blythe (1969) to collect faeces from Cepaea and Arianta. Like those species, Helicella eats filter paper readily. Within 5 or 6 hours it produces white faeces indicating that it has voided all the material previously in its gut. Much of the material is easily recognized.

The faeces were stored in neutral formalin.

The shell-diameter of each snail was measured on a millimetre scale and I recorded whether the snail was ≤ 5 mm or > 5 mm in diameter.

For examination of the faeces, temporary slides were prepared as follows:

(a) If the amount of faeces was small enough, the whole string

of faeces was placed on the slide in a drop of formalin, teased gently with glass needles and covered.

(b) If the amount was larger and the faeces appeared homogeneous, several small parts were taken and mounted.

(c) If the faeces were not homogeneous, I firstly recorded an estimate of the proportion of the string made up by each part.(The 'parts' were usually 'green' and 'brown' material.) Then I mounted several small samples from each part.

The slides were examined under 100x magnification; the whole of the sample on each slide was scanned. For each field of vision I recorded a rating of 0, 1, 2, or 3 for the proportion of the plant material in the field which was green and a rating of 0, 1, 2, or 3 for the amount of grit visible in the field.

The ratings were defined as follows:

(a) For green material, 0 = none; 1 = specks of green material, a few percent of the area of plant material in the field; 2 = up to 50% of the area of plant material; 3 = 50% to 100%.

(b) For grit; 0 = none; 1 = a few grains, occupying a few percent of the area of the field; 2 = up to 50%; 3 = 50% to 100%. Fields rated '3' for grit contained very little plant material but nearly all sand or shellgrit.

Now, the proportions (by volume) of green material and grit in the whole faeces of each snail were calculated as follows:

Each rating was taken as representing the mid-point of its class-interval, so that the ratings took on the following meanings:

0 = 0%; 1 = 2%; 2 = 27%; 3 = 75%.

Suppose the faeces could be divided into 2 parts, 'green' and 'brown', making respectively, say, 20% and 80% of the string. Suppose the samples from these parts covered 10 and 12 fields respectively, when examined under the microscope. Then

(a) The 10 ratings for green material in the 'green' part were expressed as percentages as above, and then averaged. This average was multiplied by 0.2.

(b) Similarly the average figure for green material for the 12 fields of the 'brown' part was calculated and multiplied by 0.8.

(c) The results of (a) and (b) were summed to give an estimate of the proportion of green material in the whole faeces of the snail.

(d) The proportion of grit in the faeces was estimated in the same way.

(e) The proportions obtained in (c) and (d) were converted back to ratings 0, 1, 2 or 3 as above.

It is clear that the procedure will be open to wide errors, but it serves as a means of converting the ratings assigned to individual fields into a rating for the whole faeces of each animal. The rating thus obtained is accurate enough for the purpose of this survey.

RESULTS

The numbers of snails given each rating, classified according to size and location of collection, are shown in Tables 2.1 and 2.2.

TABLE 2.1

Number of snails given each rating for the estimated proportion, by volume, of the faeces that consisted of green plant tissue. (Snails collected at two places in September, 1969).

Location / Rating / Size	Northfield			Buckland Park			Totals
	≤ 5 mm	> 5 mm	Totals	≤ 5 mm	> 5 mm	Totals	
0	10	41	51	15	9	24	75
1	4	23	27	4	14	18	45
2	1	19	20	4	12	16	36
3	0	0	0	0	0	0	0
Totals	15	83	98	23	35	58	156

TABLE 2.2.

Number of snails given each rating for the estimated proportion, by volume, of the faeces that consisted of grit. (Snails collected at two places in September 1969).

Rating \ Location / Size	Northfield ≤5mm	Northfield >5mm	Northfield Totals	Buckland Park ≤5mm	Buckland Park >5 mm	Buckland Park Totals	Totals
0	4	3	7	8	3	11	18
1	5	22	27	7	15	22	49
2	6	58	64	8	17	25	89
3	0	0	0	0	0	0	0
Totals	15	83	98	23	35	58	156

INTERPRETATION OF RESULTS

I conducted 3-way tests of independence using the G-statistic (Sokal and Rohlf, 1969, Section 16.5) to determine whether the proportion of snails assigned each rating differs between locations or between size-classes.

The test shows that the numbers of snails gaining the different ratings for green material can be considered independent of both size and location. Thus, it is valid to pool across both size and location to obtain the totals in the righthand column of Table 2.1, which are shown converted to proportions in Table 2.3.

TABLE 2.3

Proportions of snails gaining different ratings for the estimated proportion of green plant tissue in their faeces. Data from Table 2.1

Rating	0	1	2	3	Total
Number	75	45	36	0	156
Proportion	0.48	0.29	0.23	0.0	1.0

The test for independence performed on the data in Table 2.2 - the ratings for 'grit' - shows a significant association between 'rating' and 'location' ($0.025 < P < 0.05$; a higher proportion of Northfield snails gained high ratings) and also between 'rating' and 'size' ($P < 0.001$; proportionately more large snails gained high ratings). Thus, it is not proper to pool across Table 2.2 and merely examine the totals. Inspection of the table as it stands is sufficient for my present purpose.

Table 2.3 confirms the impression mentioned in Section 2.1, that although most of the food of H. virgata at Northfield and Buckland Park consists of higher plant material which is dead or senescent, a substantial minority of snails will be found with up to half their gut-contents looking like fresh green plant material. Contrary to Pomeroy's observations, the snails do not strictly avoid green material, but the general picture remains that a large part of their diet is decaying material.

Regarding the amount of grit present, Table 2.2 suggests that H. virgata does not feed largely by scraping the soil indiscriminately, for we find no snail rated '3' for grit. Indeed, some snails were rated '0', indicating that they selectively ate plant material and, if crawling over soil, did not ingest it. But the largest proportions of snails were rated '1' and '2' - a substantial proportion of their gut contents was grit. Clearly, then, the ingestion of particles of soil may be important in the feeding of H. virgata either because the snails digest microorganisms on the particles or because the particles help to break up plant tissues in the gut, or both. (It has been suggested that the latter is important in the nutrition of Lymnaea - Owen, 1966b.)

I have not pursued further the question of the importance of ingesting soil, but describe in chapters 3 and 4, experiments designed to investigate why so large a part of the plant material eaten by H. virgata is non-green.

3. DIETARY REQUIREMENTS

3.1 INTRODUCTION

The observation that Helicella eats mostly decaying plant material is consistent with two broad hypotheses. The first is that some nutritional requirement is not adequately met by living vascular plants, whereas it is met by decaying material. The second is that, although the animals' nutritional requirements would be met, perhaps best met, by at least some living vascular plants, these plants present physical or chemical barriers to feeding by snails. The hypotheses are not mutually exclusive. In this chapter I tentatively assume the second hypothesis to be wrong in order to explore the first.

The leaves of plants generally contain decreasing proportions of nitrogen and increasing proportions of cell-wall constituents as they advance in maturity (Weston and Hogan, 1971). Even if the digestive tract of a snail contains enzymes capable of degrading cell-wall constituents the decline in nitrogen will reduce the quality of the food - it does so for sheep.

And a dead leaf is likely to be particularly low in nitrogen (Burges, 1967). Its nitrogen-content will increase as it is decomposed to humus, but snails more usually eat senescent leaves on the plant or recently-fallen ones with their structure still very largely intact.

Thus, if young green plants are available as food for snails, it cannot be a need for sources of energy or of protein that makes the animals prefer litter. It must be some specific dietary requirement - a need for a vitamin or a particular amino acid, for example.

Since a decaying leaf contains many more micro-organisms than a live one, it would seem likely that this requirement is associated with a micro-organism. I therefore set out first to test a particular version of the first hypothesis, namely that there is some micro-organism which is essential in the diet of H. virgata.

For example, Newell (1965) studied the role of organic debris in the nutrition of the prosobranch Hydrobia ulvae and the bivalve Macoma balthica. He found evidence that the carbon compounds in the debris were mainly indigestible but that the molluscs digested the bacteria attacking it. Reid and Reid (1969) reported that the stomach contents of various species of Macoma depended on the nature of the siphons and mantle cavity. Each species evidently gained a large proportion of its food from micro-organisms of various sorts - for example, M. secta must live almost entirely on the bacteria which grow on the large sand-grains it eats.

But the only examples that I know of result from the modes of feeding of the animals. They are not cases of animals choosing to eat one thing because other available things fail to meet their needs.

Most of the work on specific dietary requirements of pulmonate snails concerns aquatic members of the order and is still in an early stage. Berrie (1970), quoting a number of studies of snails which are intermediate hosts of African schistosomes, says that algae are the commonest component of diet, green algae and diatoms particularly. In Bulinus globosus he describes a situation a little like the one I am postulating for Helicella;

> 'It has been suggested that in the ecological succession in a pond, B. globosus prefer, and in the very young stage require, to feed on the intial growth of micro-organisms which develop on submerged surfaces and that the heavy growths of algae and higher plants which develop later are less favourable (Pringle and Raybould, 1965). Tissues of higher plants may also be eaten if they are in some stage of decomposition, but there is evidence that snails feeding exclusively on leaves of higher plants grow much more slowly than those feeding on algae'.

But, again, this may not result from a need for a specific nutrient. It could be explained by the fact that mature plants are tough or relatively poor in proteins, when compared with young ones.

There is evidence of another kind of dietary requirement in pulmonates which looks more like a need for a specific nutrient but which does not involve micro-organisms: Bovbjerg (1968) found that four species of Lymnaeids, Lymnaea stagnalis, Stagnicola elodes, S. exilis and S. reflexa, are mainly herbivorous but eat animal food when available. In the last two months of the growth of L. stagnalis a mixed diet of animal and plant material supported greater growth than either animal or plant material alone, and it was the only diet which produced snails exhibiting reproductive behaviour. The giant African snail, Achatina fulica, changes its diet and feeding habits as it grows older. The young are typically herbivorous, but as they become sexually mature they become omnivorous, usually feeding on decaying matter and even small dead animals. This change in diet is accompanied by corresponding changes in enzymes in the digestive diverticula, and it appears that this change cannot be much influenced by forcing the animals to feed only on starch or only on protein (Several papers by A. C. Smith, P.B. van Weel and C. L. Prosser cited by Owen, 1966b).

One wonders if A. fulica, too, needs some animal material in its diet before it can reproduce, a need which is reflected in the ontogeny of its digestive system.

I know of no case where a dependence on micro-organisms such as I postulate for Helicella has been suggested in a land-snail.

Regarding Helicella virgata itself, some support for the hypothesis comes from the observation that the snails are unlikely to be found in great numbers where there is a dense cover of pasture, or under trees; amongst many hypotheses that might account for this, one is that the snail depends on a micro-organism which needs plenty of light, such as a microscopic alga.

I should make it clear that I am discussing micro-organisms as food for snails. I have not investigated at all the possibility that micro-organisms are important as symbionts which assist in the digestion of cellulose and other complex molecules (Owen, 1966b; Koopmans, 1970; Soedigdo et al., 1970). The question under consideration is why the snails choose to eat litter, and the hypothesis is that micro-organisms provide some nutrient not occurring elsewhere. In view of the results in the literature, this hypothesis seems unlikely but I have tested it before proceeding to investigate the second hypothesis which seems more likely.

If some micro-organism in litter is essential as food for Helicella, then sterilization of the litter might be expected to reduce its food value, whereas allowing the micro-organisms to multiply by 'culturing' the litter in a solution of plant-nutrients might be expected to increase its food-value. In Section 3.2 I describe two experiments of this kind.

In Section 3.3. I mention two experiments in which snails were fed on a variety of materials. These substances were crudely-defined, but were chosen because the snails' response to them was likely to give clues regarding the relative importance of the two hypotheses outlined above.

The experiments in Sections 3.2. and 3.3. threw the first hypothesis into great doubt. It seemed that, whilst micro-organisms can serve as food, they are not essential in the sense of providing a specific nutrient not available elsewhere.

If so, it should be possible to keep snails on macroscopic plant material provided it is rich enough in proteins and available carbohydrates. This proved to be so with lettuce. And the rate of growth of the snails should correlate with the amount of protein and carbohydrate in the plant material. I attempted to check this latter prediction using samples of two species of pasture plants kindly provided by Dr. J. P. Hogan of C.S.I.R.O., Division of Animal Physiology, Prospect, N.S.W. These plants had been harvested at various stages of maturity, dried, and their chemical composition had been analysed.

The experiments are described in Section 3.4. I did not find the correlations predicted above, but instead found evidence which contradicts the first hypothesis at the beginning of this section, and strongly supports the second - that there is some reason why snails avoid eating living vascular plants other than a lack of nutritional value.

3.2. THE EFFECT OF 'CULTURING' THE MICRO-ORGANISMS IN LITTER ON THE FOOD-VALUE OF THE LITTER.

3.2.1 FIRST 'CULTURING' EXPERIMENT.

PROCEDURE

In the first experiment three kinds of 'litter' were used. On vacant lots and roadsides near Adelaide, snails are to be found feeding on the first two; they were the dead leaves of the wild artichoke, Cynara cardunculus, and dead stems of the turnip-weed, Rapistrum rugosum. The third was oaten straw. Snails may eat the dead leaves of wild grasses such as wild oats, Avena fatua, although in cereal lands they are mostly to be found in roadside weeds amongst such species as C. cardunculus and R. rugosum and much less in the wheaten or barley stubble. The oaten straw, then, might be expected to prove poor food for snails.

Each kind of litter was treated in two ways which I shall call 'sterilized' and 'cultured'. Sterilized samples were autoclaved at 270°F for 10 minutes, dried in the sterilizer at 270°F for 30 minutes and stored in plastic bags in the refrigerator until fed to the snails no more than 7 days later. Cultured samples were placed in 3" Petri dishes containing c.1 cm. of a solution of plant nutrients, and stood for 3 days at 20 - 25°C under fluorescent lights, before being fed to the snails. The nutrient solution was a modified Hoagland's solution, whose composition is shown in Table 3.1. Fungi were visibly growing on the cultured artichoke and straw by the time they were fed to the snails.

TABLE 3.1

Composition of Nutrient Solution. (After Johnson et al., 1957)

Macronutrients			Micronutrients		
Compound	Stock conc'n. (M)	ml stock per l final sol'n.	Compound	stock conc'n m.mole/l	ml stock per l final sol'n
KNO_3	1.00	6.0	KCl	50	1.0
$Ca(NO_3)_2 .4 H_2O$	1.00	4.0	H_3BO_3	25	1.0
$NH_4 H_2 PO_4$	1.00	2.0	$Mn SO_4 . H_2O$	5.0	1.0
$Mg SO_4 .7 H_2O$	1.00	1.0	$Zn SO_4 .7 H_2O$	2.0	1.0
			$Cu SO_4 .5 H_2O$	0.5	1.0
			$H_2 Mn O_4$	0.1	1.0
			$Fe SO_4 .7H_2O$ (In H_2SO_4, pH 3.5)	2.0	2.0

In order to make the dry sterile litter similar in texture to the cultured litter I wetted it with distilled water when feeding it to the snails. When inspected later, it appeared to be just as sodden as the cultured material.

The snails used were all unbanded, large adults. (Most had shell-diameters, measured as described by Hodson (1969), of between 14 and 16 mm and weighed 500 - 900 mg). They were collected at Buckland Park on 1/5/69 and kept without food and water at about 20°C until the experiment was commenced on 12/5/69. (Under such conditions snails will become dormant at any time of the year and will survive for a long time. For example, in one of Hodson's (1969) experiments snails dormant over silica gel at 20°C lived an average of about 170 days.)

During the experiment snails were kept under the spraying-system described in Appendix 4 with the sprays operating three times every 24 hours. There were two cages for each of the six foods, and 25 snails randomly allotted to each cage. The foods were replaced daily and the cages washed with running tap water every two days.

I discuss in Appendix 1 the choice of dependent variables in experiments such as this, and the methods I used for measuring them. In this experiment I measured the polysaccharide contents of random samples of snails, and the dry weights of separate random samples.

A random sample of 25 snails was frozen on the day of collection for the estimation of the initial values of these variables in all treatments. The timing of later samples was to be chosen on the basis of the following pilot experiment, set up just before the main experiment:

100 snails were fed on each of (i) no food, (ii) filter paper and (iii) filter paper sprinkled with fish food and calcium carbonate. The 'fishfood' to which I refer here will be mentioned repeatedly throughout the thesis. It is marketed as 'Supreme fish pellets,' and also in a granulated form, by Aqua-lab. Products, Caulfield, Victoria. The makers state on the labels of 25-lb. bags that its ingredients include meat-meal, soya bean meal, dried yeast and dried skim milk and they give the following analysis:

"Crude protein	30%	
Crude fat	5%	
Crude fibre	4%	
Minimum vitamin A	6000 I.U./lbs.	(13 I.U./g)
" " D3	1000 I.U./lbs.	(2.2. I.U./g)
" " E	60 I.U./lbs.	(0.13 I.U./g)
Thiamin (B1)	4 Mgs/lb.	(0.0088 mg/g)
Riboflavin (B2)	20 Mgs/lb	(0.044 mg/g)
Pyridoxine (B6)	6 Mgs/lb.	(0.013 mg/g)
Choline	1200 Mgs/lb.	(2.6 mg/g)
Niacin	100 Mgs/lb.	(0.22 mg/g)
B12	35 Mcgs/lb.	(0.077 µg/g)

Also includes Vitamin C (Ascorbic acid), Vitamin K, and the essential amino acids, particularly Methionine and Lysine.

Calcium	5%
Phosphorous	3%
Iodine	30 P.P.M. "

I calculated the figures in parentheses on the assumption that

a) 1 lb. = 453.592 g
b) Mgs. stands for "milligrams"
c) Mcgs. " " "micrograms".

Snails in treatment (i) were expected to lose polysaccharide. There was no knowledge of whether snails could digest cellulose and therefore no knowledge of the likely changes in (ii). Snails had routinely been fed fishfood sprinkled on filter paper when being kept

in the laboratory by previous students and therefore snails in (iii) were expected to maintain or gain polysaccharide reserves. Samples of 10 were to be assayed fortnightly until differences appeared. The results of the readings at 14 days are presented in Table 3.2.

TABLE 3.2.

Polysaccharide contents in mg of "glycogen" per g wet weight, dry body weights and total dry weights* of snails given (i) no food, (ii) filter paper and (iii) filter paper and fishfood and $CaCO_3$ for 14 days.

Treatment	(i)	(ii)	(iii)
No. of snails for P.S.	5	5	5
Mean P.S. content	1.68	3.58	31.51
S.E. of P.S. content	0.38	0.65	1.41
No. of snails for D.W.	5	5	5
Mean D.B.W.	52.93	61.06	63.25
S.E. of D.B.W.	6.44	5.48	20.85
Mean T.D.W.	248.68	228.63	257.91
S.E. of T.D.W.	31.03	21.41	10.67

* Total dry weight includes the weight of the shell.

Since the expected differences in polysaccharide content had already become significant, the pilot experiment was discontinued and the main experiment was sampled at 14 days. (As Table 3.2. shows, differences in dry weight had not become significant, though dry body weight appeared to be moving in the same directions as polysaccharide.)

I present the results of this pilot experiment because they suggest that snails have some small capacity for gaining polysaccharide on a diet of cellulose.

The samples from the main experiment were taken by pooling the 50 snails in each treatment and then randomly choosing 10 from amongst them. These snails were sprayed in empty cages overnight so that they would defaecate and were then frozen by placing them in the direct blast of the cooling-unit of a cold-room which is maintained at $-5^{\circ}C$. Subsequent samples were taken in a similar way after 30 and 40 days.

Only the initial and 14-day samples were assayed for polysaccharide and dry weight. The 5 snails from each treatment to be assayed for polysaccharide were removed from the deep-freeze, weighed frozen to give the weight relative to which polysaccharide contents were expressed, removed from their shells and homogenized under trichloro acetic acid within 15 minutes. Their 'glycogen' content was estimated using the anthrone method described in Appendix 1.

The dry weights of the other 5 snails were estimated as described in Appendix 1.

The 30- and 40-day samples were not assayed because I decided that errors in the technique for polysaccharide were too great to be tolerated. They were kept frozen, awaiting an improved assay for polysaccharides, but a failure in the cooling-plant allowed them to thaw and decompose, and they were discarded.

RESULTS

(1) POLYSACCHARIDES

Table 3.3. shows the estimated 'glycogen' - contents of the 14-

day samples. For comparison, the 10 snails frozen immediately after collection gave a mean value of 4.17 mg/g wet weight frozen with a standard error of 0.818 mg/g.

TABLE 3.3.

Mean 'Glycogen' contents in mg per g wet weight frozen for snails fed on 3 kinds of litter, sterilized and cultured.

Litter		Dead Cynara leaves	Dead Rapistrum stems	Avena straw
Sterile	No. of snails	5	5	5
	Mean mg/g	9.64	5.90	2.90
	S.E. of mean	1.516	1.699	0.452
Cultured	No. of snails	5	5	5
	Mean mg/g	16.25	5.35	2.33
	S.E. of mean	1.478	1.414	0.749

F max (6, 4) = 11.23; $P > 0.05$.

The results of the analysis of variance are shown in Table 3.4.

TABLE 3.4

Two-way AOV of 'glycogen' contents of snails fed on 3 kinds of dead plants, sterilized and cultured.

Source of variation	d.f.	S.S.	M.S.	F_s	P
Treatments	5	675.48	135.10		
Plants	2	564.74	282.37	33.45***	< 0.001
'Sterility'	1	25.089	25.089	2.97	$0.05 < P < 0.1$
Plants x Sterility	2	85.647	42.823	5.07*	$0.01 < P < 0.025$
Within Treatments	24	202.58	8.441		
Total	29	878.06			

Inspection of the figures in Table 3.3 indicates that the significant 'plants' mean square is due largely to snails on artichoke containing more polysaccharide than those on the other plants. Of more interest is the effect of culturing. The 'sterility' mean square is not significant but the interaction is significant. Taking each plant separately and comparing 'sterile' with 'cultured' using a t-test we find:

Cynara	$t_8 = 3.12^*$	$0.01 < P < 0.02$
Rapistrum	$t_8 = 0.25$	$P > 0.10$
Avena	$t_8 = 0.66$	$P > 0.10$

Cultured artichoke produced significantly higher values than sterile artichoke whilst 'sterility' made little difference to the other plants.

(2) DRY WEIGHT

As in the pilot experiment, dry weights do not show the same clear trends as polysaccharides. Table 3.5 gives descriptive statistics for dry body weights and total dry weights. For comparison, 5 snails from the initial sample gave the following figures:
Mean DBW 73.94 mg (S.E. 3.52 mg); Mean TDW 249.78 mg (SE 17.94 mg).

TABLE 3.5.

Mean values of dry body weight (mg) and total dry weight (mg) for snails fed on three kinds of litter, sterilized and cultured.

Litter		Dead *Cynara* leaves	Dead *Rapistrum* stems	*Avena* straw
Sterile	No. of snails	5	5	5
	Mean DBW	59.89	53.27	57.18
	SE DBW	9.31	3.07	3.38
	Mean TDW	203.64	203.25	205.31
	SE TDW	17.67	25.62	16.96
Cultured	No. of snails	5	4	5
	Mean DBW	72.18	53.75	46.71
	SE DBW	2.24	3.07	4.47
	Mean TDW	220.06	248.18	195.52
	SE TDW	13.90	26.69	18.65

For DBW: $F_{max}(6, 4) = 17.32$; $P > 0.05$

An analysis of variance was carried out on the data on dry body weights with the results shown in Table 3.6.

TABLE 3.6.

Two-way AOV of dry body weights of snails fed on 3 kinds of dead plants, sterilized and cultured.

Source of Variation	d.f.	S.S.	M.S.	F_s	P
Treatments	5	1833.03	366.61		
Plants	2	1180.85	590.43	5.74^{**}	$0.005 < P < 0.01$
Sterility	1	7.800	7.800	0.076	$P > 0.75$
Plants x Sterility	2	644.37	322.19	3.13	$0.05 < P < 0.10$
Within treatments	23	2364.58	102.81		
Total	28	4197.61			

Again, inspection of Table 3.5 shows that the significant 'plants' M.S. arises because snails fed on artichoke weighed more than those fed on turnip-weed or straw. Although snails on cultured artichoke weighed more than those on sterile artichoke this is not enough to make 'sterility' or 'interaction' mean squares significant though the 'interaction' mean square is not far from it. It seems likely that, had the later samples not been lost, dry body weights would have shown the same differences found for 'glycogen'.

INTERPRETATION OF RESULTS

It appears that neither turnipweed nor oaten straw is of much value either for the accumulation of polysaccharide or for growth as measured by weight. Nor is their value much affected by sterilization or culturing.

But the mean polysaccharide contents of snails on artichoke are significantly higher than the mean of the initial sample. The material seems to support the increase of snails' polysaccharide reserves, and more so when it has been 'cultured'. However, the artichoke leaves, when wet, become much softer than the other two kinds of litter. Thus the results might be explained by the snails' having to expend less energy in order to eat it. I endeavoured to prevent such a difference between sterile and cultured litter but it is true that initially the snails feeding on cultured artichoke had a very soft, wet food whilst those on sterile artichoke had a crisp, recently-dry one.

If the snails are responding merely to a mechanical difference between cultured and sterile artichoke the difference might be due to the action of micro-organisms in breaking down the structure of the leaf. In that case, micro-organisms are important in providing food for snails but not because they themselves contain or produce some dietary essential. A laboratory mill ought to be able to take their place.

Finally, as discussed in Appendix 1, the measurement of 'glycogen' is open to errors.

Thus, the experiment gives little support for the hypothesis as outlined in Section 3.1. It suggests micro-organisms may be important in providing food for snails but gives no clear indication that they do so by actually providing a 'growth factor' not available elsewhere. It contradicts one very limited form of the hypothesis, namely that snails cannot add polysaccharides without a heat-labile growth-factor provided by micro-organisms.

3.2.2 SECOND 'CULTURING' EXPERIMENT

PROCEDURE

This experiment was designed to clarify the effect of 'sterilization' and 'culturing' on dead leaves of Cynara cardunculus as food for snails. There were four treatments, in which I fed snails on dead artichoke leaves treated as laid out in Table 3.7.

TABLE 3.7

Procedures for preparing Cynara leaves for the four treatments in the second 'culturing' experiment.

	Treatment	Procedure
1.	'Sterilized'	Autoclave at 270°F for 10 minutes in 3" x 1" polypropylene tubes with a few ml of distilled water. (Enough leaves to feed one cage ad lib. in each tube). Store in refrigerator in the closed tubes of water until used.
2.	'Natural'	Place leaves in tubes with a few ml of distilled water and store in refrigerator until used.
3.	'Cultured: Light'	Allow to stand in Petri dishes containing c. 0.5 cm of nutrient solution under fluorescent lights for 7 days and then feed to snails. Make nutrient solution as follows: half-fill plastic jars with a good loam which has been steam-sterilized, fill them with distilled water and for two days shake them frequently to keep the soil suspended in the water. Decant water and filter. Mix this 'soil extract' with Hoagland's solution (Table 3.1) in ratio 1 part of Hoagland's solution : 2 parts of soil extract. Keep in darkness until used.

(continued overleaf)

	Treatment	Procedure
4.	'Cultured: Dark'	As for 3, but enclose the dishes in black cloth bags to exclude light. I had previously shown using panchromatic film that these bags are light-proof if properly closed. The temperature inside the bags was c. 22°C; that on the bench beside the 'light' dishes was c. 21°C.

The artichoke leaves were collected on the study area at Northfield on 15/7/70. Leaves for treatments 1 and 2 were prepared immediately. Those for treatments 3 and 4 were stored in plastic bags in the refrigerator, and samples removed every second day to be placed in culturing dishes 7 days before they would be needed.

Snails were taken from Buckland Park on 22/7/70. I collected small snails, most of them about 5 mm in diameter (50 - 60 mg in weight), because small snails might be expected to grow more quickly than large ones. Pomeroy's (1966, 1969) 'generalized growth curve' indicates that snails grow most rapidly when they are between about 200 and 500 mg in weight. But he found that in the second and, should it enter one, the third winter of a snail's life, growth at any size is much slower than in the first. By taking small snails, I hoped to get mostly young of the year.

The measure of growth chosen was the repeated weighing of marked snails (Appendix 1).

80 snails were used. The 20 snails in each treatment were kept in one cage under the sprays which operated twice a night, at 2030 and 0230. I permitted natural light to come through the windows during this experiment to aid the growth of algae in treatment 3, so the photoperiod was the natural one of about 12 hours and increasing.

I weighed the snails and measured the growth of their shells at 0, 7, 14, 21 and 28 days from the start of the experiment.

RESULTS

Only the changes in weight and increases in shell over 28 days are presented here (Appendix 1). Changes over 7, 14, and 21 days show differences between treatments which are smaller, but in the same directions. Table 3.8 gives descriptive statistics of these two measures of growth.

TABLE 3.8.

Mean changes in weight (mg) and mean shell-growths (10ths of a whorl) in snails fed on dead Cynara leaves treated in four ways

Treatment	Sterile	Natural	Cultured : Light	Cultured : Dark
Number of snails*	19	16	19	18
Mean weight-change	22.47	34.58	36.49	25.97
S.E. of weight-change	2.55	2.72	4.01	2.59
Mean shell-growth	2.00	3.25	3.32	2.44
S.E. of shell-growth	0.22	0.25	0.30	0.26

For weight-change, Fmax (4, 15) = 2.57 ; $P > 0.05$

For shell-growth, Fmax (4, 18) = 1.88 ; $P > 0.05$

*The numbers are <20 due to deaths.

An analysis of variance on the changes in weight gave the results in Table 3.9.

TABLE 3.9.

AOV of weight-changes in snails fed on dead Cynara leaves treated in four ways

Source of Variation	df.	S.S.	M.S.	F_s	P
Between treatments	3	2500.92	833.64	4.91**	<0.005
Within treatments	68	11540.02	169.71		
Total	71	14040.94			

The Student-Newman-Keuls test was employed to find which means differ significantly. The result is depicted in the diagram below, in which the treatments are arranged in ascending order of means and a line under two or more treatments indicates that their means are not significantly heterogeneous at the 5% level. There is significant heterogeneity among any group of means not joined by underlining.

Sterile Cultured : Dark Natural Cultured : Light

In fact, the probability of obtaining a difference at least as great as that between 'Cultured : Dark' and 'Natural' is just greater than 0.05, so the means almost fall into two distinct groups.

The two groups are distinct when compared on shell-growth. The analysis of variance is shown in Table 3.10.

TABLE 3.10

AOV of shell-growths in snails fed on dead Cynara leaves treated in four ways.

Source of Variation	df	S.S.	M.S.	F_s	P
Between treatments	3	22.44	7.48	6.24^{***}	<0.001
Within treatments	68	81.55	1.20		
Total	71	103.99			

The Student-Newman-Keuls test gives the following pattern of significance:

Sterile Cultured : Dark Natural Cultured : Light

'Dark' and 'Natural' differ significantly at the 5% level.

INTERPRETATION OF RESULTS

Although it is less clear what they mean in terms of an animal's chance to survive and multiply, change in weight and growth of shell are measured in trustworthy ways compared with the estimation of 'glycogen' in the first culturing experiment. Further, the foods in all treatments were equally wet when first presented to the snails in this experiment.

It would appear, therefore, that the difference between 'sterile' and 'cultured' artichoke in the first experiment may not have been an artefact due to the wetness of the 'cultured' material.

Instead, it appears that sterilization does reduce the quality of the litter as a food to support the growth of snails. Culturing

in the light does not seem to improve it greatly, though the slight (non-significant) improvement makes one wonder if it would have done, given much longer time. Culturing in darkness significantly reduces the value of the leaves for supporting growth.

3.2.3. DISCUSSION OF THE 'CULTURING' EXPERIMENTS

These experiments suggest that micro-organisms have a measurable effect upon the quality of litter as food for snails. It appears that this is not achieved merely by physically breaking down the texture of the litter, for culturing in darkness reduced the value of the litter in the second experiment. Non-photosynthetic organisms do not seem beneficial to the snails.

The experiments could not disprove the hypothesis that photosynthetic micro-organisms produce a micro-nutrient, such as a vitamin, essential to the snails; but they do not lend it much support. There was some growth on sterile litter in both experiments - so the postulated micronutrient must be heat-stable, or the snails must be able to do without it for some time. It seems a preferable explanation that photosynthetic micro-organisms are providing major nutrients, rather than any unique micro-nutrient. Using the dead leaf as a source of carbon compounds and upgrading the inorganic nitrogen in the plant-nutrient solution, they may make the leaf a richer food for snails, particularly in proteins. On the other hand, non-photosynthetic micro-organisms may reduce the quality of the material by consuming the rather scarce organic nitrogen compounds present in it. Or they might reduce the palatability of the litter.

This hypothesis would predict some reduction in quality by autoclaving, for the heat would denature some proteins.

It further predicts that snails ought to grow on some macroscopic plants or artificial foods; and also that there must be some other reason why they rarely eat green plants, which are better sources of major nutrients than litter (Section 3.1).

3.3 THE GROWTH OF SNAILS ON A VARIETY OF POTENTIAL FOODS

I conducted two experiments in which I fed snails on a number of foods some of which were not even potentially part of their natural diet. Each was likely to give information regarding some aspect of their dietary requirements. The first experiment was to depend on the measurement of polysaacharides, but frozen samples from it were lost along with those from the first 'culturing' experiment (Section 3.2.1). The only data obtained from this experiment were the numbers of snails dying on different foods; the snails began with low energy reserves and the substantial numbers of deaths on some foods were probably due to starvation. In the second experiment I measured changes in dry weight, and in the live-weights of marked snails, as well as recording deaths and the numbers of strings of faeces produced.

I mention here only a few foods which gave results bearing on the main hypotheses of this chapter.

(1) In the first experiment, death-rates were almost zero on powdered brewers' yeast, fishfood, and lettuce. In the second, yeast was not included but fishfood and lettuce supported faster growth than any other foods. These results confirm the prediction at the end of Section 3.2.3 that snails should grow on some macroscopic plants or artificial foods.

(2) Filter paper and finely-ground oaten straw were readily eaten; in the second experiment they gained the highest ratings of all for the amounts of faeces produced by the snails feeding on them. But although death-rates were fairly low, snails lost weight on these foods.

Firstly, this result suggests that Helicella does not possess very effective cellulases. At least, there is no evidence that snails, or their symbionts, can continue to produce such enzymes given the unbalanced diets of filter paper and straw.

Secondly, the result suggests that snails feed indiscriminately unless either satiated or inhibited from feeding, because they appear to have eaten very readily two materials of little value to them. This hypothesis is examined in Chapter 4.

(3) In South Australia, Helicella sometimes occurs under gum trees, Eucalyptus spp, but I have never found it in those stands of native vegetation, dominated by eucalypts, which have little but sclerophyllous litter on the ground. Nor are snails found in stands of pines (Pinus spp., Callitris sp.) or of Casuarina spp, where the ground is littered with the needles of these species.

In the first experiment, 71% (averaged over 4 cages) of the snails fed on recently-dead leaves of the pink gum, Eucalyptus fasciculosa, died within 21 days. Deaths averaged 35% on leaves which had been decaying longer, and 16% on much decayed litter of E. fasciculosa. This finding seems to agree with the 'culturing' experiments, indicating that the action of micro-organisms improves litter as food for snails. But the death-rate is still high on much-decayed gum litter and this is consistent with the field observation above.

In the second experiment both the decaying leaves of E. fasciculosa and the decaying needles of Pinus radiata were finely-ground in a laboratory mill and then fed to snails. The snails ate

very little of either material, and lost weight. The grinding should have removed any effect of the hardness of the leaves, and so this result suggests that they are distasteful. Had they merely been low in nutrients, one would have expected them to be eaten as readily as filter paper or ground straw. The hypothesis that snails can be chemically inhibited from feeding is considered in Chapter 4.

(4) One food in the first experiment was decaying grass-cuttings. This grass, mainly couch-grass, Cynodon dactylon, had been cut regularly from a lawn tennis court and placed on a pile. The growing grass was probably rich in protein when cut. I took material from 10 - 20 cm below the surface of the pile; there, it was laced with fungal hyphae, and I thought that the action of the fungi would have degraded the hard cuticles of the leaves. So this grass was expected to be both soft and nutritious, and therefore good food for snails.

But they ate very little of it, and in the four cages an average of 34.7% of them had died within 21 days. This seems consistent with the hypothesis, to which I shall return in Chapter 4, that there may be inhibitory or toxic chemicals associated with some materials which prevent snails from eating them even though they would otherwise be nutritious.

3.4 THE GROWTH OF SNAILS FED ON DRIED PASTURE PLANTS HARVESTED IN DIFFERENT STAGES OF MATURITY.

3.4.1 PILOT EXPERIMENT ON DRIED LETTUCE

The forage plants supplied to me by Dr Hogan had been dried immediately on harvesting. In case drying should do some unexpected damage to a green plant as food for Helicella, I tested its effect upon a plant known to be good food, lettuce.

Marked snails were fed on (1) filter paper, (2) lettuce dried in an oven at 47°C for 2 - 3 days, (3) lettuce dried in the vacuum unit at room temperature for 2 - 3 days and (4) fresh lettuce, and weighed weekly. Those on fresh lettuce and vacuum-dried lettuce grew about equally quickly. Those on oven-dried lettuce gained weight significantly more slowly; significantly fewer of them grew any shell at all. Those on filter paper lost weight and none grew shell.

I mention this trial because it confirms the suggestion of Section 3.2.3 that autoclaving reduced the food-value of dead Cynara leaves not by killing some essential micro-organisms but, in a more general way, by denaturing some compounds - perhaps proteins - of importance in the snails' nutrition. Here, a lower temperature seems to have had a similar effect on a macroscopic plant.

3.4.2 FIRST DRIED-FORAGE EXPERIMENT

PROCEDURE

Of a number of forage samples made available by Dr. Hogan, I chose to feed snails on 6. They were chosen because, taken together,

they should have given some indication of the importance to the snails of variations in carbohydrate and nitrogen. They were:

(1) Four harvestings of forage oats (Avena sativa cv. Cooba), used by Hogan and Weston (1969).

(2) Two harvestings of subterranean clover (Trifolium subterraneum cv. Clare), used by Weston and Hogan (1971).

The chemical compositions of the plants, taken from the papers cited above, are shown in Table 3.11.

As a seventh treatment, snails were given fishfood as a 'control' known to be good food.

The foods were given chopped, in pieces c. 3 cm long, rather than ground. I had no evidence yet that these plants would be too tough for snails to rasp. Had I ground them, I would have needed to supply them on filter paper, or the particles would have fallen through the mesh floor of the cage, and I wanted to avoid filter paper because snails seem to prefer it to other things (Section 3.3). The foods were given ad lib, placed on the bare floors of the cages, and lightly sprinkled with $CaCO_3$.

The first attempts at this experiment failed because most of the snails died very quickly. Their bodies hung limply out of the shells at death - normally starved or desiccated snails die strongly withdrawn into their shells - and I suspected some disease. I am unable to define the conditions under which this disease develops. It is unlikely to be due to a dietary deficiency, because it strikes rather more heavily in snails fed on fishfood than on other foods.

I have not seen symptoms of it in the field.

I scrubbed the apparatus with 'Ajax' cleanser repeatedly and rinsed it well. I did not want to use antibiotics because of the possible dependence of snails upon symbiotic micro-organisms. After a time, the 'disease' largely disappeared and in later experiments only a few snails died. Given more time, it would be interesting to investigate this possible disease, but I merely kept the apparatus as clean during experiments as could be done without using chemicals, and so kept it at a low level.

TABLE 3.11.

Chemical compositions of forage oats and subterranean clover harvested at different stages of maturity. (HN: grown with addition of nitrogenous fertilizer. LN: unfertilized) (% organic material)

Forage Sample	Oats*				Sub. clover‡	
	Harvest 1 LN	Harvest 2 HN	Harvest 3 HN	Harvest 3 LN	Early Maturity Harvest 2	Late Maturity
Cell-wall constituents	50.7	55.0	60.0	62.0	43.3	56.8
Cellulose	27.0	30.9	34.1	32.9	30.3	39.4
Lignin	3.1	3.2	4.1	4.2	4.3	9.0
Soluble carbohydrate	16.0	5.3	8.4	18.0	12.8	12.8
Crude Protein (N x 6.25)	25.6	29.9	21.7	11.5	25.1	16.5
Alcohol-soluble nitrogen	0.543	0.813	0.729	0.223	0.570	0.460
Ammonia nitrogen	0.046	0.072	0.083	0.024	0.062	0.062
Nitrate nitrogen	0.063	0.335	0.382	0.028	0.044	0.031

*from Hogan and Weston, 1969

‡from Weston and Hogan, 1971.

For the satisfactory run of this experiment, the unbanded adult snails came from Buckland Park (13/12/70). They were dormant at room temperature (c.25 - 30°C) until 23/12 and then at 21°C until the experiment began on 28/12/70. There were 20 marked snails per food in two cages of 10. The laboratory was air-conditioned at 21°C (temperature under the sprays dropped to c.16°C or less when the sprays operated) and the photoperiod was 11 hours (lights on 0500— 1600). The sprays operated twice a 'night', at 1630 and 2230. Although this experiment was run during natural summer, there is evidence that Helicella is able to be active, feed and grow at this time given appropriate conditions (chapter 6), and the conditions in the laboratory were as near as I could make them to winter conditions.

Snails were fed every second day; they were weighed and their shell-growths measured after one night and then on six subsequent occasions up to 27 days.

RESULTS

(1) Changes in weight

I present here the analysis of changes in weight over 14 days (Appendix 1) because the two later experiments related to this one were terminated after 14 days. The use of longer intervals makes no difference to the conclusions reached. Table 3.12 shows descriptive statistics of weight-changes over 14 days in the two cages of each treatment.

TABLE 3.12

Mean weight-changes over 14 days ($\bar{y}$) in 2 cages on each of 7 foods, with sample sizes (n) and standard errors ($s/\sqrt{n}$).

Food	Early Sub. Clover	Late Sub. clover	Oats H_1, LN	Oats H_2, HN	Oats H_3, HN	Oats H_3, LN	Fishfood
n	10	10	8	9	9	10	8
$\bar{y}$	31.16	77.71	- 17.43	- 7.21	- 19.79	2.15	109.94
$s/\sqrt{n}$	7.54	15.66	7.80	19.67	4.86	7.41	20.09
n	10	10	8	6	9	9	10
$\bar{y}$	37.62	60.14	- 7.30	- 29.67	- 11.91	- 16.04	130.92
$s/\sqrt{n}$	11.40	17.85	9.28	6.69	8.12	5.22	30.28

F max (14, 8) = 43.19** ; $P < 0.01$

The sample variances can evidently not be assumed homogeneous. I discuss this problem in Appendix 1, and suggest that it may be difficult to interpret transformed data. In this case, if the cage means are taken as raw data for a single-factor AOV with sample sizes of 2, differences between treatments are found not significant. The results of AOV on the full data are shown in Table 3.13. Here, the differences are found highly significant.

TABLE 3.13.

Nested AOV on changes in live weight over 14 days in snails fed on different dried forage plants.

Source of variation	df	SS	MS	Fs	P
Treatments	6	304414.52	50735.75	45.64***	< 0.001
Cages within treatments	7	7781.90	1111.70	0.557	> 0.75
Within cages	112	223466.28	1995.23		
Total	125	535662.70			

In view of the non-significant 'cages' MS, it seems safe to pool cages and use the 'within cages' MS as an estimate of a weighted average variance in order to carry out a Student-Newman-Keuls test for differences among groups of means. When this is done, the following pattern of significance emerges.

Oats H2, HN	Oats H3, HN	Oats H1, LN	Oats H3, LN	Early Sub. clover	Late Sub. clover	Fishfood

The treatments are arranged in ascending order of means. There are significant differences between, but not within, underlined groups of means. Probability levels for significantly different adjacent means are:

Oats H3 LN - early sub. clover	$P < 0.01$
early clover - late clover	$0.01 < P < 0.05$
late clover - fishfood	$P < 0.01$

It must be remembered that these estimates may be biased by pooling heterogeneous variances. But simple inspection of the data in Table 3.12 would lead to the same conclusion.

(2) Growth of Shell

Table 3.14 shows statistics of the increases in shell circumference in 10ths of a whorl which had occurred by 14 days. The sample variances are again heterogeneous; in Table 3.15 is presented a single-factor AOV treating the cage-means in Table 3.14 as raw data. (This conservative procedure is discussed in Appendix 1.)

TABLE 3.14

Mean increases in shell-circumference ($\bar{y}$) in 2 cages on each of 7 foods, with sample sizes (n), and standard errors ($s/\sqrt{n}$). (Units : 10ths of a whorl).

Food	Early Sub. clover	Late Sub. clover	Oats H1, LN	Oats H2, HN	Oats H3, HN	Oats H3, LN	Fishfood
n	10	10	8	9	9	10	8
$\bar{y}$	1.30	2.80	0.13	0.00	0.11	0.50	2.25
$s/\sqrt{n}$	0.15	0.36	0.08	0.00	0.07	0.13	0.25
n	10	10	8	6	9	9	10
$\bar{y}$	1.40	2.40	0.06	0.00	0.19	0.39	4.00
$s/\sqrt{n}$	0.33	0.49	0.06	0.00	0.08	0.22	0.54

F_{max} (12, 7) = 92.30^{**} ; $P < 0.01$ (Excluding Oats, H2, HN, with variance of zero)

TABLE 3.15

Single-factor AOV on means in Table 3.14

Source of variation	df.	S.S.	M.S.	Fs	P
Foods	6	19.924	3.321	14.28^{**}	$0.001 < P < 0.005$
Within foods	7	1.628	0.233		
Total	13	21.552			

The Student-Newman-Keuls test gives the following pattern of significance:

Oats H2 HN	Oats H1 LN	Oats H3 HN	Oats H3 LN	Early Clover	Late Clover	Fishfood

The difference between late clover and early clover is significant at the 5% level.

INTERPRETATION OF RESULTS

The statistical analysis must be treated cautiously but I think the following points can be made:

None of the dried fodders seems as rich food as fishfood, but this is not surprising. The fishfood was merely to confirm that snails were in fit condition to grow.

The hypothesis that snails need a specific micro-organism in their diet is further contradicted by the growth of snails on subterranean clover.

The subterranean clovers fall into the 'wrong' order, if we base our expectations on their nitrogen-contents (Table 3.11).

The oats, which in two cases contain more nitrogen and in another two cases more soluble carbohydrate than the clovers, prove to be poor foods. Many snails lost weight on them and they supported little growth of shell. The non-significant differences between the oats are opposite from what would be predicted on the basis of nitrogen-content and inconsistent with predictions on the basis of soluble carbohydrate.

Two hypotheses seem consistent with the above results: one is that the proportion of cell-wall constituents is the most important single property of the plants (see Table 3.11 - greater growth was in fact recorded on fodders with greater CWC - contents.) Although snails do not grow on filter paper or straw, it may be that cell-wall constituents provide a source of energy once some minimum requirement for nitrogen has been met. The other is that the less mature plants, though potentially better diets, are unavailable because of some physical or chemical barrier. And indeed, although I did not keep quantitative records, I observed that the amounts eaten by snails placed the foods into the same order as did growth; i.e., they did eat some oats H3 LN, a little oats H3 HN but very little of the earlier oats, and they ate more late clover than early clover.

3.4.3. SECOND DRIED-FORAGE EXPERIMENT

PROCEDURE

In an attempt to disprove the hypothesis that young plants in the last trial were unavailable to snails because their cuticles were too tough to rasp, I repeated the trial, but ground the foods

twice in a laboratory mill using a 1.25 mm sieve. By producing particles small enough to ingest, this should at least have improved the food value of the plants a good deal, if the only barrier was toughness.

The list of foods was changed only by the exclusion of early-harvested subterranean clover, which was temporarily out of stock.

Foods were supplied sprinkled on 3" squares of silk bolting-cloth (64 meshes/inch) which snails had been shown not to eat - this avoided the disadvantage of using filter paper which the snails may prefer to the plants - with a light sprinkling of $CaCO_3$.

The snails, unbanded adults, were collected dormant at Buckland Park on 24/2/71, sprayed the next night to determine which were alive and then left dormant at 21°C until set up on 1/3/71. There were two cages per food, 15 marked snails per cage. Conditions of temperature, photoperiod and spraying were as in Section 3.4.2. Snails were fed, on new silk squares, every 2 days and weighed every 3 or 4 days, a total of 5 times in 14 days although there had been many deaths by 6 days.

RESULTS

(1) Deaths

A large proportion of the snails fed on oats were dead by the 6th day. Table 3.16 shows the number (out of 15 snails) dead in each cage by then.

TABLE 3.16

Number of snails out of 15 in each cage dead 6 days from the start of an experiment in which they were fed different dried, ground forage plants.

Food	Oats H1, LN	Oats H2, HN	Oats H3, HN	Oats H3, LN	Late Sub. clover	Fishfood
Cage A	11	9	5	8	0	0
Cage B	13	8	11	14	0	2

These figures were converted to proportions by dividing by 15, transformed to angles (Snedecor and Cochran, 1967), and an analysis of variance performed with the results in Table 3.17.

TABLE 3.17

AOV on arcsine-transformed proportions of snails dead in 6 days when fed on different dried, ground forage plants.

Source of variation	df.	S.S.	M.S.	Fs	P
Foods	5	5716.06	1143.21	8.29*	$0.01 < P < 0.025$
Within foods	6	827.34	137.89		
Total	11	6543.40			

There are 3 comparisons that might have been made a priori even without a significant analysis of variance. They are listed below with the results of making them by the procedure outlined by

Sokal and Rohlf (1969), Section 9.6.

(a) Oats - vs - others (1 d.f.) $P < 0.001$

(b) Between the four oats (3 d.f.) $0.25 < P < 0.5$

(c) Late clover - vs - fishfood (1 d.f.) $0.5 < P < 0.75$

Thus, we can accept that the foods fall into two groups; oats, with a high death rate, and fishfood and clover, with a low death rate.

Further, I think that the causes of death were different in these two groups. In one replicate on fishfood (B) the first deaths were recorded at 6 days and all but 2 were dead by 14 days. In the other replicate there were no deaths. On clover, 6 and 2 snails respectively were dead in the two cages by 14 days. Deaths on fishfood and clover were associated with a limp body hanging from the shell - "the disease", Section 3.4.2. This was rarely so in the snails on oats, which generally died withdrawn.

The live snails on oats were mostly withdrawn too, often having epiphragms; very few faeces were produced on oats.

(2) Growth

Increases in shell-circumference and in weight seem not worth presenting, firstly because deaths and the lack of feeding on oats appear to have disproved the hypothesis that toughness is the only barrier, and secondly because deaths have made the sample-sizes very small. And the 'disease' appears to have struck the cages on good food so that the actual values of their weight increases are of little value. I merely mention that snails lost weight on oats and gained it on clover and fishfood, at least up to 6 days. There was

no shell-growth on oats. Snails grew shell on clover and significantly more on fishfood ($0.02 < P < 0.05$) over 6 days.

INTERPRETATION OF RESULTS

The results are inconsistent with the hypothesis that the reason why oats are poor food is purely that they are too tough. The snails appeared to avoid eating them, even when ground. I would therefore favour the hypothesis that there is some chemical barrier to eating oats, and that the substance responsible is present at a higher concentration in the younger plants.

3.4.4 THIRD DRIED-FORAGE EXPERIMENT

PROCEDURE

When the early-harvested subterranean clover again became available, I set up a new experiment to check that it supports less growth than late clover (because this conflicts with the hypothesis that nitrogen content is important, or requires a qualifying hypothesis) and to check the effect of grinding. This time, foods were ground through an 0.5 mm sieve to make them even finer. Also, I attempted to estimate the amound of feeding early in the experiment to determine whether oats is immediately unpalatable or whether snails stop eating it after some days, indicating perhaps a toxic effect or a metabolic shutdown.

Oats, harvest 2, HN, was omitted from the list of foods which was otherwise as in Section 3.4.2. All foods were given both chopped, as in Section 3.4.2, and ground.

Snails were unbanded, most about 12 mm in diameter. (Young of

the year were not yet large enough.) They were collected at Buckland Park on 20/5/71, left dormant overnight and set up next day. I used 480 marked snails, 20 in each of the 6 (foods) x 2 (ground or chopped) x 2 (replicates) cages.

Cages and racks were all scrubbed to combat the 'disease'. Conditions of temperature, photoperiod and spraying were as in Section 3.4.2. Snails were fed, using fresh silk squares, generally every 3 days; they were weighed after one night and again 6 and 12 days later.

At each weighing date, I assigned ratings for feeding by counting the strings of faeces in each cage and giving the rating 1 if there were ≤ 25, 2 if there were ≤ 50, 3 if there were ≤ 100 and 4 if there were >100 strings of faeces.

RESULTS

(1) Ratings for feeding

In Table 3.18 are presented the ratings for each cage, averaged over the 3 occasions because no change could be observed with time. Note that the ratings have not been corrected for the different numbers of snails present in the cages by the end of the experiment; this does not very greatly affect the comparison of 'ground' and 'unground' within a particular food - the most interesting comparison.

(2) Growth of Shell

There was no shell-growth in any treatment. Pomeroy (1966) found little growth in adults at this time of year. I found the same in my field experiments (Chapter 7), so this result is not surprising.

TABLE 3.18

Feeding-ratings averaged over the experiment in which snails were fed 6 foods, ground and unground; 2 cages per treatment.

Food	Oats H1, LN	Oats H3, HN	Oats H3, LN	Early sub. clover	Late sub. clover	Fishfood
Unground	1	1	2	3	3	4
	1	1	1	3	4	4
ground	1	3	2	4	4	3
	1	2	3	4	4	3

(3) Changes in Weight

Table 3.19 shows descriptive statistics of the changes in weight over 14 days in the 24 cages.

The analysis of variance on the data on which Table 3.19 is based shows a significant variance between cages within treatments ($P < 0.001$). Between treatments, it shows a significant effect of foods ($P < 0.05$), but none of grinding, or of interaction between foods and grinding. This analysis is based on the unwarranted assumption that the sample variances are homogeneous. As mentioned in Appendix 1, since I am studying the effect of foods and grinding and not asking why cages differ (I suspect it is due to a disease but that is not the question here), it seems better to do the conservative analysis of variance taking cage-means as raw data. The results of such an analysis of the means in Table 3.19 is presented in Table 3.20. Again, it shows a significant effect only of foods.

TABLE 3.19

Mean changes in weight over 14 days ($\bar{y}$), with sample sizes (n) and standard errors ($s/\sqrt{n}$), for snails given 6 foods, ground and not ground.

Food		Oats H1, LN	Oats H3, HN	Oats H3, LN	Early sub. clover	Late sub. clover	Fish-food
Unground	n	18	17	20	18	20	20
Cage A	$\bar{y}$	- 28.44	- 21.35	- 8.82	- 5.01	21.78	51.70
	$s/\sqrt{n}$	6.60	9.19	2.94	3.76	6.25	7.85
Unground	n	20	17	17	20	20	20
Cage B	$\bar{y}$	- 16.07	- 16.79	- 29.37	3.97	9.52	4.89
	$s/\sqrt{n}$	9.53	6.76	7.10	2.96	4.21	3.31
Ground	n	11	15	15	17	20	13
Cage A	$\bar{y}$	- 22.17	- 5.30	- 32.57	26.10	- 4.50	- 6.64
	$s/\sqrt{n}$	8.49	4.42	8.52	9.98	3.84	5.95
Ground	n	5	9	15	20	20	14
Cage B	$\bar{y}$	- 54.35	- 42.74	- 8.93	- 3.62	- 5.79	- 17.82
	$s/\sqrt{n}$	13.92	16.83	7.26	4.72	3.38	6.03

Fmax (24, 8) = 14.73* . By extrapolating from Table T. of Rohlf and Sokal (1969), I estimate $P \approx 0.05$. Bartlett's test gives $X^2_{23} = 142.41$**, $P < 0.001$. Bartlett's test is very sensitive to departures from normality and the high X^2 could be due to the latter (Sokal and Rohlf, 1969, Sect. 13.3).

TABLE 3.20

Two-way AOV using the means in Table 3.19 as raw data.

Source of Variation	d.f.	S.S.	M.S.	Fs	P
Treatments	11	8235.24	748.66		
foods	5	5754.79	1150.96	3.92*	$0.01 < P < 0.025$
grinding	1	855.90	855.90	2.91	$0.10 < P < 0.25$
foods x grinding	5	1624.55	324.91	1.11	$0.25 < P < 0.50$
Within treatments	12	3525.48	293.79		
Total	23	11760.71			

INTERPRETATION OF RESULTS

The ratings in Table 3.18 show rather more feeding on ground than unground plants, but this tends to apply to the ones which were eaten more anyway, in earlier experiments. The ratings show clearer differences between oats and subterranean clover.

The ground foods did not support more growth than unground ones; early clover is a possible exception but the difference between replicates is large there (Table 3.19). Table 3.19 gives some indication that, as in the first dried forage experiment, late clover supports more growth than early clover. This, and the fairly clear difference between oats and subterranean clover, are hard to explain in terms of the chemical compositions of the plants (Table 3.11). They are, however, paralleled by the differences in feeding-ratings.

I think the hypothesis that oats is unsuitable as food purely because of toughness must be rejected. There is no clear indication that the nutritional value of the plants explains the differences either, and it seems the best hypothesis that some toxic or distasteful chemical is present in oats, and that there is a higher concentration of it in immature oats. Some chemical with similar influence may also be present in immature subterranean clover.

This hypothesis is considered further in Chapter 4.

3.5 DISCUSSION OF DIETARY REQUIREMENTS

I have not attempted to discover the detailed dietary requirements of Helicella virgata, an investigation of the nutritional physiology of the animal being quite beyond the scope of this project. My aim was to find whether there is some rather unusual requirement which can only be satisfied by eating litter. Had such a requirement been found, it might have had over-riding importance in determining the distribution and abundance of the snail.

The micro-organisms in litter do appear to be important in providing food for snails, but I concluded in Section 3.2 that the reason for this is unlikely to be that the micro-organisms provide some unique nutrient. Nor does it seem to be that they break down the hard structure of the litter. Rather, they simply seem to improve the litter by increasing its content of major nutrients, and the obvious one to postulate is protein.

This suggestion implies that materials other than litter ought to be good foods unless they are unpalatable, and this prediction is fulfilled by the results of feeding snails on fishfood and lettuce (Section 3.3) and on dried subterranean clover (Section 3.4).

A number of materials, dead leaves of Eucalyptus and Pinus and grass cuttings (Section 3.3), dried forage oats and perhaps young subterranean clover (Section 3.4), did appear to be unpalatable. With all but grass cuttings, the hypothesis that this unpalatability is only due to toughness was eliminated; the evidence indicated that

there must also be toxic or distasteful chemicals present.

Finally, I suggested in Section 3.3, because of the behaviour of snails given filter paper or ground straw, that in the absence of such a chemical deterrent they may feed indiscriminately.

In view of these results, I would discard the hypothesis that Helicella has an unusual dietary requirement which might help in explaining its distribution and abundance.

For an ecology of Helicella, it might be sufficient to accept that the snail eats litter and set out to study the importance to the animal of changes in the abundance or quality of litter ~~so~~ we might decide that it does not matter why it rarely eats green plants.

But I decided to digress only a little and enquire further into the reasons why green plants form only a small part of the snail's diet. These investigations form the subject of Chapter 4.

4. FACTORS DETERMINING WHETHER SNAILS WILL EAT CERTAIN THINGS

4.1 INTRODUCTION

I suggested in the last chapter (Section 3.3.) that *Helicella virgata* may feed indiscriminately unless inhibited from doing so. The observations leading to that suggestion are similar to ones that have been made on the closely related *Cepaea nemoralis*. Grime *et al* (1968), discussing the usefulness of *C. nemoralis* for the study of palatability, noted that it 'is a fairly indiscriminate feeder ...' and that it 'consumes pure cellulose filter paper avidly, and in preference to many natural materials, living or dead.' Grime *et al* (1970) tested the palatability to *C. nemoralis* of the aqueous extracts of 10 flowering plants and found only four which increased the palatability of filter paper. Only one (the extract of the nettle *Urtica dioica*) increased it significantly. An earlier study (Grime *et al*, 1968) had indicated that of 52 species of flowering plants, the extracts of 41 (including *U. dioica*) had no significant effect whilst the rest depressed the palatability of filter paper to varying extents.

One might therefore speculate that positive chemical stimuli to feeding are of little importance for land snails. But the hypothesis seems an unlikely one; the simplest animals are found to react positively to certain chemical stimuli associated with food (Carthy, 1958) and this is true of numerous mollusca (Carthy, 1958; Kohn, 1961; Owen, 1966a; Jager, 1971). *H. virgata* aggregates on fishfood in preference to bare filter paper; there is, if nothing more, an orthokinetic mechanism.

Nevertheless, there remained the possibility that this orthokinesis may be based only on the recognition of texture. (Pomeroy, 1969 - see the quotation in Section 2.1 of this thesis). Snails eating crumbed fishfood merely ingest whole particles, whereas snails eating filter paper have to tear it with their radulae, which presumably requires more energy. It seemed worthwhile testing the hypothesis that, in the absence of chemical inhibitors, it is only this kind of difference ('texture') between potential foods which makes one preferable to another.

I conducted three experiments to test it. In each I attempted to vary one of 'nutritiousness' or 'texture' whilst keeping the other constant. The experiments are described in Section 4.2. They led to the rejection of the hypothesis, and so it was appropriate to ask what chemicals stimulate feeding.

In Section 4.3 I describe experiments which are only sufficient to indicate that H. virgata is not one of those animals requiring a specific stimulus to feed, and to indicate most profitable directions for further work on the stimulation of feeding. The first of these (Sections 4.3.1, 4.3.2) suggested that the stimulus to feeding is present in fishfood and crushed H. virgata, but not in several simpler mixtures and single compounds.

I therefore postulated that glutatione might be stimulatory. Glutathione stimulates feeding in some coelenterates (Carthy, 1958; Reimer, 1971). I do not know of its having been tested in a mollusc, but if Helicella should require some animal material in its diet (Section 3.1, Chapter 7), a response to glutathione might help

it to recognize a dead animal. The experiment described in Section 4.3.3 indicates that gluthathione does stimulate feeding.

This means that if there is a specific stimulus to feeding, it is associated with glutathione.

However, in attempting to determine what is distasteful in forage oats (Chapter 3) I tested the palatability of aqueous and ethereal extracts of the plants and of fishfood. The experiments, which are described in Section 4.3.4, led me to reject the hypothesis that only glutathione is stimulatory.

Finally, therefore, I tested again a number of substances representative of the major classes of foods, (proteins, fats and carbohydrates). The experiment is described in Section 4.3.5. At least two substances, glucose and a vegetable oil, appeared stimulatory. I left the work on stimulation at this point. It was apparent that H. virgata does not respond only to some unique or specific stimulus; further work on the stimulation of feeding, though interesting, was less likely to provide keys to the understanding of the animal's ecology than the topic of Section 4.4. - What inhibits feeding?

The evolution in plants of devices protecting them from herbivores - and the coevolution of the herbivores - is a topic attracting a good deal of attention. (Jones, 1962; Grime et al , 1968; Culvenor, 1970; Grime et al ,1970). It seems likely that terrestrial gastropods have taken an important part in this process. (Grime et al , 1968; Grime et al , 1970).

H. virgata, like C. nemoralis, seems to eat very little green

material and this seems to be the result of physical or chemical barriers in the plants. In forage oats, the barrier appears to be chemical (Chapter 3), and not contained to a significant extent in the water- and ether-extracts (Section 4.3.4). In Section 4.4.1 I describe one further experiment on forage oats. I tested an acid-extract, which had no effect on the palatability of filter paper.

Extraction in acid was of interest because it is the technique used to extract alkaloids (Culvenor et al, 1954) and at this point I decided not to pursue further the reason for the unpalatability of oats, but to examine the effect of alkaloids.

Alkaloids are of wide occurrence (Culvenor, 1970). They are, in various degrees, toxic and distasteful to mammals, but certain alkaloids do have pharmacological effects on molluscs (Tauc, 1966; Walker et al , 1968) and I would suggest that they might provide better protection from small, slower-feeding herbivores such as gastropods than from mammals. A narcotic effect on a snail or slug might act before the animal had eaten very much. A large mammal might eat a great deal before suffering any ill-effect, so the toxin will give the plant little protection from the mammal.

At least one of the plants eaten by H. virgata when dead but very little if at all when alive, namely 'Salvation Jane', Echium plantagineum (Family Boraginaceae), contains alkaloids when young but virtually none by the time the leaves are senescent (J. L. Frahn, pers. comm.). I could not obtain alkaloids from Echium, but Dr. Frahn of CSIRO, Division of Nutritional Biochemistry, Adelaide, kindly supplied me with a sample of lasiocarpine, a pyrrolizidine alkaloid closely similar to those found in Echium. It was obtained

from the 'potato weed', Heliotropium europaeum, an introduced member of the Boraginaceae which grows wild in South Australia. Experiments described in Section 4.4.2 indicate that lasiocarpine does inhibit feeding by H. virgata.

Finally, the finding in Chapter 3 that toughness could not account for unpalatability in oats, or in other substances tested, is surprising. Grime and co-workers found evidence that grasses may often be protected from the attacks of Cepaea nemoralis by toughness and not by chemicals. Toughness is considered to be a barrier to feeding by certain water-snails on macroscopic plants (Calow, 1970; Berrie, 1970).

As Grime et al observed with Cepaea, I have noted that Helicella feeds very little on grasses (it is a pest in the croplands because it climbs, not because it eats the cereal plants), and I decided to try to demonstrate that at least in some cases the cuticle of the grass provides an important barrier to feeding by Helicella. I chose three grasses found in places where snails occur but apparently very little eaten by them and compared them with forage oats. The experiments are described in Section 4.4.3. They suggest that toughness is the only barrier to feeding on these three grasses and that at least two of them would otherwise support growth of snails.

4.2. THE HYPOTHESIS THAT SNAILS FEED INDISCRIMINATELY UNLESS INHIBITED

4.2.1 THE EFFECT OF VARIATIONS IN THE TEXTURE OF FOOD

PROCEDURE

The hypothesis to be tested in this experiment is that the only positive stimulus leading snails to prefer one food over another is 'ease of ingestion'.

To test it, I laid out sixty 2.5 cm squares of filter paper on a sheet of steel wire mesh under the laboratory sprays. Twenty of them, randomly chosen, were left bare. On another twenty I sprinkled tiny pieces of shredded filter paper; particles about the size of the particles of granulated fishfood. Thus, the difference in texture between these two treatments was much like that between filter paper and filter paper with fishfood sprinkled on it, but there was no nutritional difference. The third twenty squares had fishfood sprinkled on them. Thus, there was as little difference in texture as possible between the second and third treatments, but a large nutritional difference for I knew that snails would grow on fishfood but not on filter paper.

Sixty snails collected at Buckland Park about ten days before were wakened by brief immersion in water, and randomly placed on the 60 squares. As soon as a snail moved the lip of its shell to the edge of its square, I removed it. The grid was photographed every two minutes and the photographs therefore provide a record of the number of snails in each "treatment" having reached the edges of their squares by the end of each 2-minute interval.

RESULTS

Table 4.1 shows mean times taken to 'leave' in the three treatments. The mean for treatment (iii) is in fact slightly biased downwards - three snails had not left by the last photograph at 72 minutes and showed no signs of moving. They were assigned a time of 74 minutes. Samples are smaller than 20 because some snails did not emerge from their shells at all.

TABLE 4.1

Descriptive statistics of times to 'leave' squares of filter paper with (i) nothing, (ii) shredded filter paper, (iii) crumbed fishfood added.

Treatment	(i)	(ii)	(iii)
No. of snails	18	16	19
Mean time to leave (mins)	9.89	9.63	35.26
Standard error	1.37	1.14	5.31
95% confidence limits	(7.01, 12.77)	(7.19, 12.07)	(24.10, 46.43)

INTERPRETATION OF RESULTS

No further statistical test is needed. The hypothesis predicts that treatments (ii) and (iii) will differ from treatment (i) but not from each other; that prediction is clearly disproved.

4.2.2 THE EFFECT OF VARYING NUTRITIOUSNESS WITHOUT VARYING TEXTURE

PROCEDURES AND RESULTS

(1) One observation leading to the hypothesis examined in Section 4.2 is that snails will eat at least small quantities of sand - washed beach-sand, for example. This behaviour was used to produce 'foods' of different nutritiousness but very similar textures. Six upturned plastic bucket-lids (with holes punched in them for drainage) were filled with sand which was levelled but not tamped down. Onto this I sprinkled from a salt-shaker c. 3.5 g of dry sand into which I had mixed (i) no fishfood, (ii) 1.5 g of finely-ground fishfood, (iii) 3 g of finely-ground fishfood. Each treatment was applied to two lids. The sand was then tamped down firmly. The surfaces all looked the same except for the slightly reddish colour of the fishfood. Fifteen snails were randomly placed on each of the lids, the sprays set going over them, and photographs taken every two minutes. I had planned to determine from the photographs the speeds at which snails were moving on the different foods, but a simpler analysis proved sufficient.

From the films, the number of snails having crawled off each lid in each 2-minute interval could be counted as a simple measure of the speed at which the animals were moving.

After 44 minutes, 14 snails had left from each of the lids of treatment (i), one snail from one of the lids of treatment (iii) and no snail from treatment (ii).

(2) Another experiment of this kind was designed because of the drop in production of faeces observed in the crowding experiment of

Section 7.2. Briefly, I argued that: if this drop were due to depletion of the good food in the pens, and if the hypothesis were true that snails can recognize nothing but 'texture', then since there appeared to be ample material of the texture of litter still present, the drop in production of faeces would have to result from an internal process. I postulated that when snails had been feeding for a time on poor-quality food their activity might decrease. (The stimulus might be something like a decrease in blood-sugar levels - but that does not matter here.)

To test for the operation of such a mechanism I caged snails in the laboratory on sand impregnated as above with four levels of fishfood ranging from zero to 6 g on a bucket-lid. As a check that the difference in food-value had some physiological effect on the snails I weighed them on two occasions a week apart and found that those fed on sand with fishfood had gained weight while those fed merely sand lost weight. (There was no significant difference between the three treatments given fishfood.)

I collected the faeces produced by the 15 snails in each cage over 5 days and dried and weighed them, obtaining the results in Table 4.2.

TABLE 4.2

Weights of faeces produced in 5 days by two cages of 15 snails fed on sand with each of 4 levels of fishfood.

Level of Fishfood		0	1.5 g on c.450 cm^2	3.0 g on c.450 cm^2	6.0 g on c.450 cm^2
mg. of faeces	Cage A	11.05	282.25	182.60	297.25
	Cage B	29.70	211.25	262.80	239.55

An analysis of variance on the data in Table 4.2 gives $F_s(3,4) = 13.80^*$; $0.01 < P < 0.025$. It seems clear that snails given no fishfood produced significantly less faeces than those given fishfood, all of which produced similar amounts.

It is noteworthy that the snails fed on sand with fishfood did produce faeces containing a great deal of sand, indicating that they were not able to select soft particles of fishfood from amongst the sand. This lends support to the assumption that they were not able to detect a great difference in the texture of the different foods in this or the above experiment.

I mention this experiment only because of the above result, which helps further to discredit the hypothesis that snails can recognize food by nothing but its 'texture'. There were technical problems with my attempts to test for a persistent drop in activity in the snails fed sand without fishfood. But that hypothesis is no longer important since the hypothesis of this section has been disproved. Suffice it to mention that I could find no evidence of such a drop. Snails which had been fed only sand for some weeks, and which were producing small amounts of faeces and losing weight, immediately produced larger amounts of faeces when given sand with fishfood.

INTERPRETATION OF RESULTS

Although there remains the possibility of snails having recognized a subtle difference in texture, I think the results of these two experiments are sufficient to throw into grave doubt the hypothesis that snails cannot taste something in fishfood which

stimulates them to feed.

4.2.3 DISCUSSION

It seems safe to conclude that H. virgata can taste something in fishfood which stimulates them to stay on it, and to feed on it. The results disprove the hypothesis that the only positive stimulus influencing their feeding is associated with texture. We must favour the general picture that, whilst no doubt influenced by texture, the snails can be both discouraged from feeding and encouraged to feed by chemical stimuli.

4.3. WHAT CHEMICALS STIMULATE FEEDING?

4.3.1 EXPERIMENTS USING THE 'SPEED-OF-LEAVING' TECHNIQUE

Experiments described in this section used techniques which appeared to be either unreliable or insensitive. However, there is a possible explanation for their variable results (Section 4.3.6) and so I have decided to include them. I tested some substances of known chemical composition, and some materials which snails do eat and which ought, therefore, to contain the specific stimulus to feeding if there were one.

In the first experiment, I used the technique of experiment 4.2.1. The aim was to find whether snails appear likely to be stimulated only by compounds in one of the groups a) carbohydrates, b) nitrogen-compounds, c) vitamins.

I laid out 100 squares of paper. A randomly-chosen 20 were treated in each of the following five ways:

(i) left bare
(ii) sprinkled with fishfood
} controls of 'bad food' and 'good food'

(iii) sprinkled with AnalaR D (+) Glucose

(iv) soaked in a mixture of nitrogen-compounds of the following composition:

glycyl-glycine	7.35 mg
L-valine	1.05 "
L-proline	1.30 "
L-arginine	1.65 "
L-cysteine	1.00 "
Monosodium glutamate	2.75 "

(continued overleaf)

Hippuric acid	1.10 mg
Riboflavin	1.30 "
Casein	1.25 "
Heparin	1.05 "
Double-distilled water	100 ml

(v) Soaked in a drop of 'ABDEC', an aqueous solution of vitamins marketed by Parke-Davis & Co., Sydney, who give the following composition: 'Each 1 ml represents:

Vit. A	3450 units
" D	1200 "
" B_1	1.7 mg
" B_2	1.5 "
" B_6	1.7 "
Pantothenic acid (as the sodium salt)	3.3 "
Nicotinamide	8.3 "
Vit. C	100 "

One hundred snails, wakened by brief immersion in distilled water, were randomly allocated to the 100 squares, photographed at 2-minute intervals and removed as they 'reached the edge' of their squares. (Defined as moving the leading edge of their aperture to the edge of the square.)

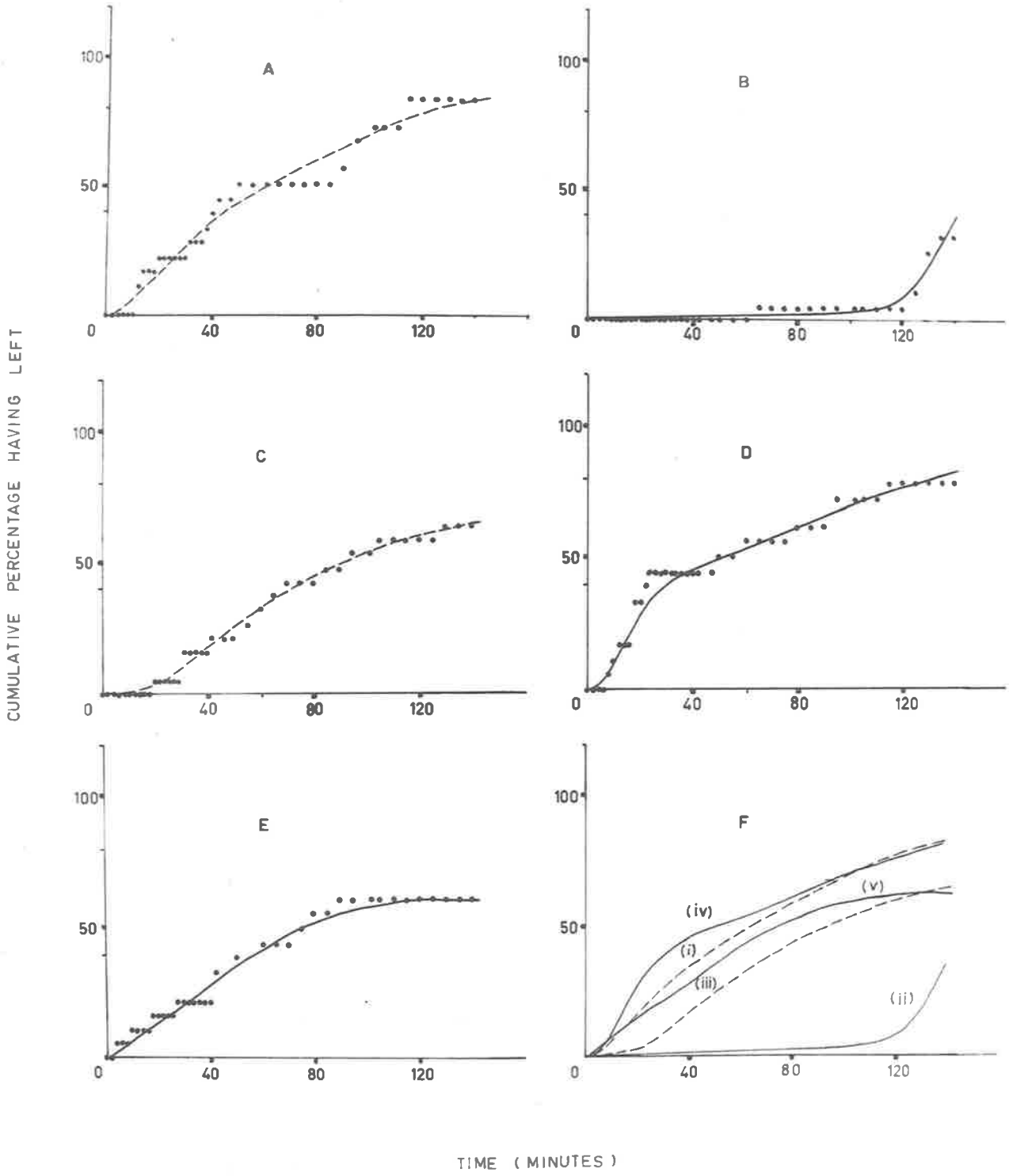
Figure 4.1 shows the (cumulative) percentage of snails removed, plotted against time for each of the 5 treatments. Only on treatment (ii) does the curve appear very different from that on filter paper.

FIGURE 4.1

Percentage of snails having left filter-paper squares treated in 5 ways, plotted against time.

(Curves drawn by eye)

A	(i)	filter paper
B	(ii)	fishfood
C	(iii)	glucose
D	(iv)	N - compounds
E	(v)	vitamins
F	All curves together; points omitted.	


CUMULATIVE PERCENTAGE HAVING LEFT
A
B
C
D
E
F
100
50
0
40
80
120
(i)
(ii)
(iii)
(iv)
(v)
TIME (MINUTES)

When the times taken to 'leave' are compared using the Kruskal-Wallis test for differences of location, a probability of $P < 0.005$ is obtained. The simultaneous test procedure outlined by Sokal and Rohlf (1969), Section 13.10, and based on the Wilcoxon-Mann-Whitney U-statistic gives the following pattern, where there are no significant differences at the 5% level within groups underlined. (The treatments are arranged in increasing order of time spent on them.)

(iv)	(i)	(v)	(iii)	(ii)
N-compounds	Filter Paper	Vitamins	Glucose	Fishfood

(It is of interest that the Us-values for comparison of treatment (ii) with treatments (iii) and (v) are close to the 5% point.) Note that comparisons of treatments (ii) - (v) with each other are of little value, owing to the different concentrations used: comparison of each of them with treatment (i) is, however, valid.

The results are consistent with the hypothesis that snails are stimulated to feed by some substance present in fishfood but not in any of the more clearly-defined substances chosen. But they are not strongly contradictory to the hypothesis that glucose and vitamins might serve to stimulate feeding - and see Section 4.3.5.

I repeated the experiment on different, smaller snails; the design was almost unchanged, except for a considerable (≈50-fold) increase in the concentration of the mixture of nitrogenous compounds and the omission of casein and heparin from it. It was now made up as:

Glycyl-glycine	1.9 mg
L-valine	1.2 "
L-proline	1.0 "
L-arginine	1.3 "
L-cysteine	1.3 "
Monosodium glutamate	1.0 "
Hippuric acid	1.25 "
Riboflavin	1.20 "
Double-distilled water	2 ml

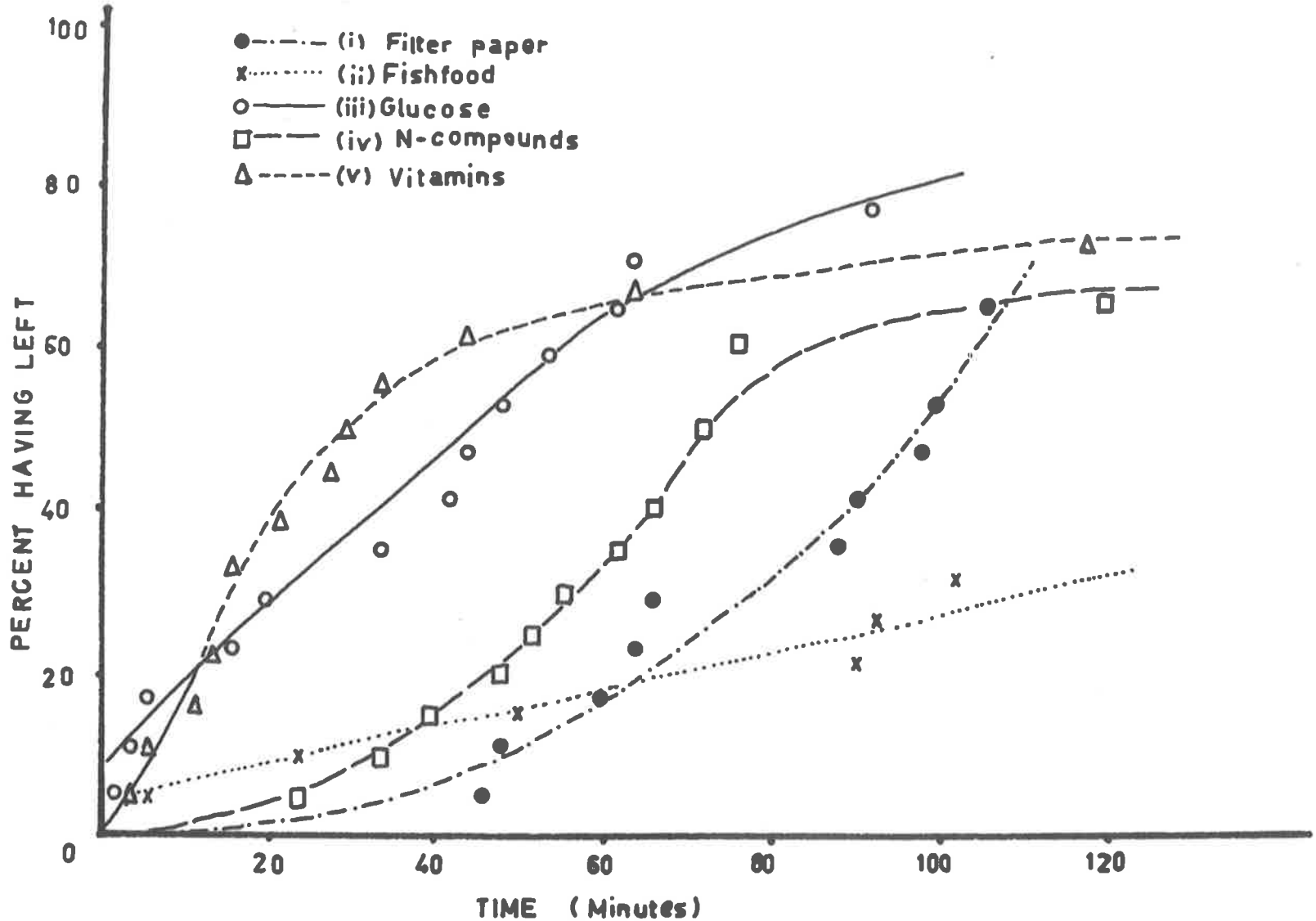
The proportions of snails having 'left' are plotted against time in Figure 4.2.

At 120 mins, fewer snails had 'left' from fishfood than from the other treatments. For comparing the times taken to 'leave' the different foods, all those snails which had not 'left' by 120 minutes were grouped together and considered as having 'left' by one later time. This test is conservative, because the snails on fishfood were observed to be feeding and might have been expected to stay longer than those remaining on the other foods. The Kruskal-Wallis test applied in this way gives $0.001 < P < 0.005$. The simultaneous test procedure applied above indicates that treatment (ii) (fishfood) differs from treatments (iii) (glucose) and (v) (vitamins) at the 5% level, whilst the Us-values for the comparisons (filter paper-glucose) and (filter paper-vitamins) come close to the 5% point (Again, see Section 4.3.5).

The only significant differences here are <u>not</u> among those found in the first run of the experiment. Further, as mentioned above,

FIGURE 4.2

Percentages of snails having left squares of filter-paper treated in 5 ways, plotted againt time.


(i) Filter paper
(ii) Fishfood
(iii) Glucose
(iv) N-compounds
(v) Vitamins
PERCENT HAVING LEFT
100
80
60
40
20
0
20
40
60
80
100
120
TIME (Minutes)

the comparison of treatment (i) with the rest is of most interest. Here, we find no significant differences, and the only differences which look very large are in the opposite direction from the corresponding differences in the first run.

The technique does not seem to give very reliable results. Such as they are, these results do not contradict the hypothesis that snails are stimulated to feed by some chemical present in fish-food but not present in the other substances tested.

The technique seems to suffer from the fact that snails have to move only a small distance to score as 'leaving'. They may often 'leave' by chance, perhaps in the course of 'hunting' for a gradient in the concentration of a stimulatory substance, so that the results have a wide variability. I therefore devised a technique which, in a very crude sense, placed the snails in a concentration-gradient and allowed them to turn up or down it.

4.3.2 AN EXPERIMENT USING THE "TWO-STRIPS" TECHNIQUE

PROCEDURE

In this experiment, two strips of chromatography paper (Whatman No. 1) about 3.5 cm wide were placed about 2 mm apart. Snails were placed with their apertures over the gap between the two strips, so that when the snail's head emerged it was very likely to touch both strips. If it put its foot down squarely, any chemosensory areas on the lateral parts of the foot (Carthy, 1958) would receive information from the two strips. If the snail were attracted by some substance present on one of the strips it could be expected to turn onto that one. Helicella virgata generally moves its head

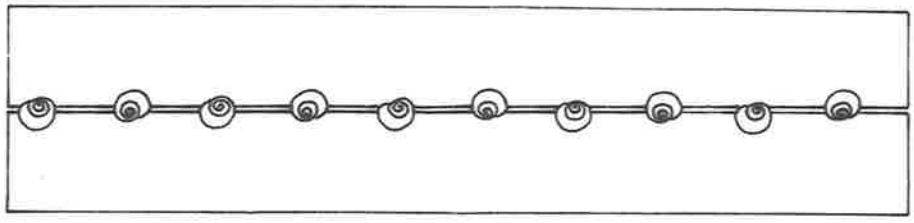
FIGURE 4.3.

Layout for the 'two-strips' experiment.

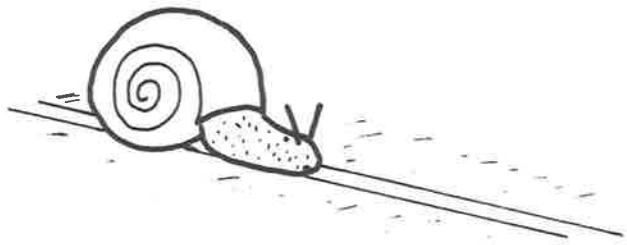
A. Ten snails laid out over the gap between two strips at the start of a 'run'. One strip is soaked in a solution of 'test' material, the other in water.

B. Close view of a snail emerging, and touching both strips.

A



B



about in different directions on emerging from the shell (It may well have important chemoreceptors in the oral region as in Helix (Carthy, 1958; Morton, 1967)) and so a snail which did not initially place its foot on both strips at once would be very likely to touch both before turning and crawling in one direction or the other.

In the absence of stimulation, snails may be more likely to turn one way than the other. To cancel out any such tendency, alternate snails were placed over the gap with their apertures facing in opposite directions. Thus, a pair of strips initially laid out for a test by this technique would appear as in Figure 4.3. If the direction in which the snails turned were determined by nothing other than chance and their anatomy, we might expect the proportion turning a particular way to be not significantly different from 0.5.

I tried the technique initially with an aqueous extract of fishfood and an aqueous extract of crushed Helicella virgata. These snails will eat crushed members of their own species - that they are attracted to them by smell is discussed in Chapter 5. When they encounter the fluid from a crushed snail they turn onto it and commence feeding in nearly every case.

These preliminary trials were encouraging. Snails preferred each of the extracts to water. The probability (by X^2) of getting the result by chance was $0.001 < P < 0.01$ with fishfood extract and $P < 0.001$ with snail extract. I took this to indicate firstly that the technique is a useful one and secondly that if there is a specific stimulant to feeding it may occur in crushed H. virgata as well as in fishfood. But it must be noted that snails in this kind of experiment are scored on their turning-tendency and not on their

feeding. This is true in the experiments described in Section 4.3.1.

An experiment was now set up to check the work described in Section 4.3.1 on several clearly-defined substances, to check the above findings on aqueous extracts of fishfood and of crushed H. virgata, and to investigate another complex material possibly attractive to snails, namely, ground wheat. This last was chosen because a poisoned bait for snails recently placed on the market in South Australia is based on ground wheat and because Michelson (1960) found wheat germ attractive to Australorbis glabratus.

Solutions were prepared as outlined in Table 4.3.

For each run, six pairs of 25.5 x 2.5 cm strips were laid out. I conducted six runs, including each of the six foods (tested against distilled water) in each run. The galvanised wire grid on which I laid the papers was thoroughly washed with fast-flowing tap water between runs.

Each run, I laid out the strips and snails in the same systematic order and then waited five minuted after laying out the last one, before scoring the numbers with their heads on each paper, and the numbers withdrawn (or, in rare cases, touching both papers equally). Thus, it was desirable that by the end of the six runs, each food should have been tested in each of the six positions on the grid. This was achieved randomly by picking a 6 x 6 Latin Square from a table. The other point where a systematic error could have entered was in the choice of which of the two strips in a pair should be the one with the extract on it. Some effect of light or temperature, of my presence, even of more subtle factors (Brown et al, 1960;

TABLE 4.3

Preparation of solutions for the 'two-strips' experiment (see Text).

Material extracted (or dissolved)	PROCEDURE	
(i) Fishfood	Place about 2 g in each of two 3" x 1" (30 ml) specimen tubes with 20 ml distilled water, shake them frequently for two hours and then filter the water and refrigerate it.	
(II) Crushed snail	Crush ten snails (of the collection used in the experiment) and drop 5 of them whole into each of two 3" x 1" tubes with 20 ml of water, shake and filter as above.	
(iii) Wheat	Grind, almost to flour, in the laboratory mill with 1.25 mm sieve. Place about 2 g in each of two tubes, and treat them as above.	
(iv) Casein	Place about 2 g in each of two tubes and treat as above.	
(v) Amino Acid mixture	This contained the same ingredients as the mixture used in the second trial under 4.3.1, but at c.3.5 times the concentration.	
The recipe was:	glycyl glycine	77.6 mg
	L-valine	72.9 "
	L - proline	73.3. "
	L - arginine	32.6 "
	L - cysteine	16.3 "
	Monosodium glutamate	74.9 "
	Hippuric acid	68.7 "
	Riboflavin	74.3 "
	Distilled water	40 ml.
(vi) Glucose	Dissolve 7.2 g AnalaR D (+) glucose in 40 ml of water (a c. $_1$M solution).	

Brown and Webb, 1968) might have influenced the snails' direction of turning. It was too difficult to randomize against this in one set of runs, partly because it was necessary to lay out the papers, treat them with solutions and lay out the snails quickly and without error. I chose simply to run the whole experiment twice. The second Latin Square was chosen quite independently of the first. In the whole of the first square, the solution was on the strip nearer me, water on the distant strip. In the whole of the second square, the solution was placed on the strip further from me.

This procedure did not guard systematically against the odours of any of the substances having an influence on the behaviour of snails on neighbouring pairs of strips. As will be discussed in Chapter 5, snails are attracted to the odours of fishfood and crushed snail. But, inspection of the raw results shows no sign that this has any effect here.

I used 720 snails collected on 2/10/70. They were completely randomly allotted to substances and runs, and each snail was used only once.

RESULTS

The data of interest for each 'unit' of the experiment – where a 'unit' is a pair of strips of paper with 10 snails placed over the gap – are the numbers of snails going towards and away from the extract. It is possible to ask questions about variations in the ratio of these numbers between runs, between positions on the grid and between the Latin Squares, but the most interesting questions are those answered by the G-tests (Appendix 2) in Table 4.4. Only the totals for the foods, not for individual 'units', are presented there.

Each of the G_H-values (with 11 d.f.) in the body of the 5th column tests whether the 12 units on a particular food are heterogeneous with respect to the proportion of snails turning towards the food. Only one is significant - and see the discussion in Appendix 2. Below them, the G_H with 5 d.f. indicates significant heterogeneity among the six foods. The G_p-values in the body of the 6th column ask whether the figures shown in columns 2 & 3 deviate significantly from a 1:1 ratio. In two cases they do so. The G_p at the foot of the 6th column indicates that the overall totals differ significantly from a 1:1 ratio. The 7th column shows the corresponding values of $G_T = G_H + G_p$.

INTERPRETATION OF RESULTS

From Table 4.4 one can conclude that the 72 'units' are significantly heterogeneous and also differ significantly from a 1:1 ratio.

Further, there is significant heterogeneity between the 12 replicates on food (v) (Amino acids) but not on any of the others.

The pooled data deviate significantly from a 1:1 ratio in only two cases, (i) fishfood extract and (ii) extract of crushed snail; but in one of these, fishfood, the total G is non-significant.

This result suggests that, of the substances tested, the only ones containing anything attractive to snails are fishfood and crushed Helicella virgata. The experiment does not, of course, give direct information about the stimulation of feeding.

TABLE 4.4

Total number turning towards and away from the food, summed over 12 runs for each of six foods, and G - statistics (see text).

	Food	Towards Food	Away from Food	Total	G_H(11d.f.)	G_P (1d.f.)	G_T (12 d.f.)
(i)	Fishfood Extract	65	40	105	12.17	6.01*	18.18
(ii)	Extract of Crushed Snail	65	29	94	18.02	14.15***	32.17**
(iii)	Extract of Ground Wheat	61	48	109	6.98	1.55	8.53
(iv)	Casein Suspension	49	52	101	13.23	0.09	13.32
(v)	Amino Acid Mixture	50	54	104	23.95*	0.15	24.11*
(vi)	Glucose Solution	51	51	102	9.96	0.00	9.96
	Column Totals	341	274	615	14.64*(5d.f.)	7.32**(1d.f.)	21.95** (6d.f.)

*P < 0.05; **P < 0.01; ***P < 0.001

4.3.3 THE EFFECT OF GLUTATHIONE ON THE PALATABILITY OF FILTER PAPER

This experiment was based on a technique like that of Grime et al (1968) who offered a standard area of plant material or of filter paper soaked in plant extract, together with the same area of a palatable reference material, to individual Cepaea nemoralis and calculated the 'palatability index' of the test material as

P.I. = area test material consumed/area reference material consumed.

PROCEDURE

A 'unit' of this experiment was a small 'Bates' food container (6 cm diam.; 4.5 cm deep) in which were placed one snail and two 1 - cm squares of Whatman No. 1 filter paper. One square of paper had been twice wetted with a 0.02M solution of glutathione and vacuum-dried, and finally wetted with distilled water before being placed in the container. The other had been treated in the same way but with double-distilled water instead of glutathione solution. The two papers in a pot were about 5 mm apart.

There were 200 such units. The snails were briefly sprayed to waken them and then placed in a random order into the pots, alternate snails with their 'glutathione' paper on opposite sides of them to cancel out any asymmetryin the direction in which snails tend to turn on emerging. After about 8.5 hours the papers were removed to small, numbered plastic tubes, dried in the vacuum-chamber and weighed. I prepared 250 of each type of paper; this provided 50 of each to be treated like the 'test' papers but not fed to the snails; they gave an estimate of the weights of uneaten papers.

RESULTS

For a sensitive test, a 'palatability index' like that of Grime et al (1968) could be calculated, and compared with a postulated value of 1, or with values obtained with other test-materials. But in this case I adopted the simple procedure of assigning to each unit a '+' if the 'water' paper weighed more than the 'glutathione' paper, or a '-' if the reverse were true. If glutathione is neither a stimulant nor a deterrent to feeding, one would expect equal numbers

of '+' and '–' within the limits of random error.

After I had discarded units lost due to snails eating away the pencilled labels on the papers (I had tried several dyes without success) there were 163 left. Of these, there were 56 ($\lesssim$ 34%) scored '–'. Table W in Rohlf and Sokal (1969) gives confidence limits for binomial proportions. It shows that 99% confidence limits for the 'true' percentage '–' in the statistical population from which this sample was drawn are (24.86, 44.10); they do not include 50%. Thus, significantly (at the 1% level) more than half the units had been scored '+', i.e. they were units in which more 'glutathione' paper than 'water' paper had been eaten.

INTERPRETATION OF RESULTS

This result gives encouragement about the usefulness of the 'PI' technique. It also indicates that if there is a single chemical which stimulates feeding, it is, or is associated with, glutathione.

4.3.4 EXPERIMENTS ON EXTRACTS OF PASTURE PLANTS

(1) AQUEOUS EXTRACTS

PROCEDURE

The effect of aqueous extracts of late-harvested subterranean clover, oats H_1LN, oats H_3EN (Table 3.11, Section 3.4) and fishfood on the palatability of filter paper was investigated in two experiments using the technique described in Section 4.3.3.

In the first, the foods, ground in the laboratory mill with 1.25 mm sieve, were kept agitated in double-distilled water for

$2\frac{1}{2}$ hours (1.5 g of ground food in 100 ml H_2O) and then the extract was filtered through Whatman No. 1 paper.

In the second, a little of the ground food was placed in a homogenizer with a little water, ground well and transferred to a flask, and so on until 1.5 g of food had again been ground into 100 ml of water. This was shaken well and filtered.

Extracts were stored in the refrigerator overnight before being used.

In the first experiment 25 snails were tested with each of the 4 extracts; in the second, 50 with each extract.

The 1-cm squares of paper were wetted twice with extract (or water), allowed to dry after each treatment, and then wetted a third time with water immediately before the pots were set up. Different snails were used for the two experiments, collected on different occasions in March and April, 1971.

RESULTS

Table 4.5 is similar in format to Table 4.4. It shows the number of snails scored '+' and the number scored '-' as described in Section 4.3.3, for each experiment on each extract. It shows the total numbers '+' and '-' for each extract. And it shows the results of G-tests for goodness-of-fit to a 1:1 ratio (Appendix 2).

The results for fishfood-extract showheterogeneity between experiments significant at the 0.1% level, and so I calculated the G-values for individual experiments. They differ, but both values indicate significantly more '+' than predicted by a 1:1 ratio.

The results for oats H3HN show a significant G_T but not a significant G_p, so it was of interest to calculate G-values for individual experiments here. They are shown in Table 4.5; one is significant at the 1% level, the other not significant at the 5% level. (Note that the G_H value is almost large enough to be significant.)

It did not appear necessary to partition the total G-values for Oats H1LN and clover.

In view of the above results it does not seem excessively risky to use the pooled data for each of the extracts to ask the next question, Which extracts differ from each other in their proportions '+'? This was done by means of the test outlined by Sokal and Rohlf (1969), Section 16.3, a simultaneous test procedure using the G-statistic. The G_H for the extract totals (the bottom one in the G_H column of Table 4.3) has 3 d.f. Therefore, I take the 5% and 1% points of X^2 with 3 degrees of freedom as critical points for the test. They are $X^2.05(3) = 7.815$ and $X^2.01(3) = 11.345$.

The following G_H - values are obtained for groups of 2 and 3 treatments:

Oats H_3HN - Subclover	$G_H = 0.520^{N.S.}$	$P > 0.05$
Oats H_1LN - Fishfood	$G_H = 0.210^{N.S.}$	$P > 0.05$
Oats H_3HN - Subclover - Oats H_1LN	$G_H = 12.87^{**}$	$P < 0.01$
Subclover - Oats H_1LN - Fishfood	$G_H = 11.85^{**}$	$P < 0.01$

Thus the treatments, arranged in order of proportions '+', fall into two groups:

(1) Oats H_3HN and sub clover (proportions '+' of 0.62 and 0.68)

(2) Oats H_1LN and fishfood (proportions '+' of 0.86 and 0.89)

TABLE 4.5

Numbers of snails scored '+' and '−' in a trial on the palatability of aqueous extracts of four foods; and results of G-tests

FOOD		No +	No −	Total No.	G_H +	G_P +	G_T +
Fish-food	Expt 1	16	6	22		4.717^{*} (1)	
	Expt 2	41	1	42		48.773^{***} (1)	
	Total	57	7	64	8.954^{**} (1)	44.535^{***} (1)	53.489^{***} (2)
Late Sub-clover	1	15	8	23			
	2	33	15	48			
	Total	48	23	71	0.090 (1)	8.995^{**} (1)	9.085^{*} (2)
Oats H3 HN	1	17	5	22		6.917^{**} (1)	
	2	25	21	46		0.348 (1)	
	Total	42	26	68	3.464 (1)	3.800 (1)	7.264^{*} (2)
Oats H1 Ln	1	19	6	25			
	2	45	4	49			
	Total	64	10	74	3.350 (1)	43.974^{***} (1)	47.324^{***} (2)
Total		211	66	277	21.488^{***} (3)	79.817^{***} (1)	101.305^{***} (4)

+ Degrees of freedom are given in parentheses.

Significance is indicated by:

$^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

INTERPRETATION OF RESULTS

All the aqueous extracts seem to contain something stimulatory - enough to override any inhibitory substance extracted. The extract of late-harvested oats seems to contain least of such material. This result is consistent with the hypothesis that glutathione alone stimulates feeding. Glutathione is widely-distributed in plant and animal cells (Fruton and Simmonds, 1958). And an examination of Table 3.11 (the chemical compositions of the dried forage plants) and the maker's analysis of the fishfood (Section 3.2.1) does indicate a correlation, though imperfect, between the above proportions '+' and the 'crude protein' contents of the foods. The 'crude protein' fraction would include the nitrogen in glutathione. However, Table 3.11 suggests more strongly that carbohydrates are stimulatory. The figures for soluble carbohydrate place the three plants into the above order. (I have no figure for carbohydrate in fishfood.) It appears that this hypothesis is worth testing further.

(2) ETHER EXTRACTS

PROCEDURE

In a preliminary trial on an ether-extract of ground oats H1LN, I found that snails ate considerably more of papers soaked in the extract and allowed to dry, than of papers soaked in ether and dried.

Therefore I made ether-extracts of the materials used in (1) above by grinding 1.5 g of each of the foods (already previously ground in the mill as for the aqueous extracts above) in a mortar and pestle under ether, using a total of 50 ml ether, filtering the

extracts through a No. 1 paper. A great deal of the ether evaporated, and I made up each extract to a final volume of 20 ml with ether.

The design used for this experiment was expected to yield the same information as the 'P.I.' technique with considerably less labour. I took 50 12.5 cm discs of Whatman No. 1 filter paper. Ten discs were treated with 2 ml of each extract, and 10 were treated with 2 ml of ether as controls. All were allowed to dry in the fume cupboard. They were left overnight to equilibrate to laboratory humidity and then weighed.

Each paper was placed in a small 'Bates' food-container with 10 snails and 3 ml of double-distilled water, and capped with a plastic lid. (The 500 snails involved were randomly distributed among the 50 pots, which had been washed in tapwater and themselves randomly assigned to the 5 treatments and laid out in a random order on the bench. The snails were wakened by spraying before put into the pots.)

The papers were removed after 9 hours. They were dried in an oven at 105°C and allowed to equilibrate to room humidity. Faeces were carefully removed from them and they were then weighed, again in random order. I could then calculate the amount of each paper that had been eaten by 10 snails in about 9 hours.

RESULTS

The decreases in weight of the papers in the 5 treatments are summarized in Table 4.6.

TABLE 4.6

Descriptive statistics of the decreases in weight of filter papers treated with four different ether-extracts (and stock ether) and each fed to 10 snails for 9 hours.

Treatment	Ether	Extract of Oats $H_1^L N$	Extract of Oats $H_3 HN$	Extract of late subclover	Extract of Fishfood
No of papers	10	10	10	10	10
Mean decrease in weight (mg)	43.05	47.78	41.80	46.71	55.94
Standard error	1.47	3.24	1.80	1.91	2.36

F max (5, 9) = 4.85; $P > 0.05$

The results of the analysis of variance are shown in Table 4.7.

TABLE 4.7

AOV on weights eaten of papers treated with ether extracts of 4 foods and with stock ether

Source of Variation	d.f.	S.S.	M.S.	Fs	P
Treatments	4	1232.99	308.25	6.127^{***}	< 0.001
Within Treatments	45	2264.00	50.31		
Total	49	3496.99			

The student-Newman-Keuls test places four of the means into a group not significantly heterogeneous: fishfood gives a mean significantly greater than all the rest, at least at the 5% level:

Oats H_3HN	Control	Sub. clover	Oats H_1LN	Fishfood

INTERPRETATION OF RESULTS

There is no evidence that the ether extracts of the plants make filter paper less palatable to snails. It is noteworthy that the order of the amounts eaten is the same as that of the 'PI' values of the aqueous extracts. I have no information about the lipid contents of the plants, but it seems likely that they could be higher in the immature plants.

This experiment suggests that something which can be extracted from fishfood by ether stimulates feeding. The Merck Index (7th Ed.) gives no reason to think glutathione would be extracted by ether. (It is "freely soluble in water, in dilute alcohol, liquid ammonia and dimethyl formamide".) If not, then, coupled with the conclusion of Section 4.3.3, this experiment disproves the hypothesis that there is a single, specific stimulant to feeding by H. virgata.

4.3.5. THE EFFECT OF OIL, PEPTIDES AND CARBOHYDRATES

PROCEDURE

I decided to test one representative of each of: sugar, polysaccharide, peptide, protein, lipid, and bare filter paper, and several of these combined in pairs. The list chosen is given in Table 4.8 with the methods of preparing the papers.

The technique used was similar to that described in Section 4.3.4(2). I used 10 snails per paper, 10 papers per food. The 900 unbanded snails, collected on 23/5/71 and kept dormant till 31/5/71,

were completely randomized to the 90 papers. The snails were left to feed for 16 hours in this experiment.

The papers were weighed before treatment with the various materials. After they had been treated, fed to snails and dried at 105°C they were weighed again. Some increased in weight because of the materials added, and there was error due to the papers being stacked in the oven in random order, which allowed oil to seep from one paper to another. However, corrections could be made from the changes in weight of 'control' papers treated at the same time as the experimental papers but not fed to snails.

TABLE 4.8

Procedures for treating papers in an experiment on the effect of oil, peptides and carbohydrates on feeding.

TREATMENT	PROCEDURE
(i) Glucose	Papers wet with 3ml of a 0.1M (or 18 g/l) solution of AnalaR D (+) glucose.
(ii) Starch	Papers wet with 3 ml of a solution of AnalaR 'Soluble Starch' of the concentration by weight needed to make 0.1M glucose above, viz. 18 g/l.
(iii) Glycyl-glycine	3 ml of a solution containing 1.147 g/l glycyl-glycine, on each paper.
(iv) Casein	Each paper wet with 3 ml of a saturated solution of vitamin-free casein (Nutritional Biochemicals Corporation, Cleveland, Ohio).
(v) Oil	A 'polyunsaturated' safflower oil, marketed by Arthur Vale & Co. Pty. Ltd., Port Melbourne, Victoria, was sprayed onto the papers with a perfume-sprayer (a new one which had never

(Continued overleaf)

		contained perfume) using a standard number of squirts for each paper. This procedure introduced considerable variability into the weights of the 'oil' papers. 3 ml of water was added to each paper in the pot. In the absence of water, snails may have become inactive.
(vi)	Glucose and Glycyl-glycine	Each paper wet with 3 ml of a solution containing 1.147 g/l of glycyl-glycine and 18 g/l of glucose.
(vii)	Glucose and Oil	Each paper treated with 3 ml of the solution used in treatment (i), then sprayed with oil as in treatment (v).
(viii)	Glycyl-glycine and Oil	Each paper treated with 3 ml of the solution used in treatment (iii), then sprayed with oil as in treatment (v).
(ix)	Paper	Each paper treated with 3 ml of double-distilled water.

RESULTS

I added to the initial weight of each paper a correction for increases in its weight due to the addition of substances for testing and due to the accidental addition (or loss) of oil. The corrections were calculated from the changes in weight of the appropriate controls. Then I subtracted the final weight of the paper from this corrected initial weight. The means and standard errors of the resulting 'corrected decreases in weight' are shown in Table 4.9.

The variances of these samples are heterogeneous, clearly because of the large variances of the two treatments in which oil was sprayed onto wet papers-(vii)and (viii). Independently of the data, there is reason to expect large variability in the treatments involving oil,

particularly oil sprayed onto water. I think that it is therefore proper to remove treatments (vii) and (viii) from the analysis, and that this is preferable to using a non-parametric 'analysis of variance' which would have rather less power. The variances of the remaining treatments are not significantly heterogeneous (F_{max} (7, 9) = 2.87; $P > 0.05$).

TABLE 4.9

Mean ($\bar{y}$) and standard error ($s/\sqrt{n}$) of corrected decreases in weight of 10 papers treated in each of 9 ways and fed to snails for 16 hours

Treatment	(i) Glucose	(ii) Starch	(iii) Glycyl glycine	(iv) Casein	(v) Oil	(vi) Glucose + Gly. gly	(vii) Glucose + oil	(viii) Gly. gly + oil	(ix) Water
n	10	10	10	10	10	10	10	10	10
$\bar{y}$	107.63	70.12	42.85	54.94	96.49	111.92	142.24	93.46	43.17
$s/\sqrt{n}$	4.0	6.78	5.66	5.65	6.18	5.43	11.73	16.26	6.63

F_{max} (9,9) = 16.54** ; $P < 0.01$

The analysis of variance on the remaining 7 treatments gives the result shown in Table 4.10.

The Student-Newman-Keuls test groups the means as shown in the diagram:

gly. gly. water casein starch oil glucose glucose + gly.gly.

where the underlined groups are not significantly heterogeneous at

the 5% level and, as it happens, all wider groups are significantly heterogeneous at the 1% level.

TABLE 4.10

AOV on decreases in weight of papers treated in 7 ways and fed to snails for 16 hours

Source of Variation	d.f.	S.S.	M.S.	Fs	P
Treatments	6	53617.66	8936.28	26.34^{***}	< 0.001
Within Treatments	63	21372.99	339.25		
Total	69	74990.65			

INTERPRETATION OF RESULTS

It appears that starch significantly increased the palatability of filter paper (for the 'starch' mean is significantly above that of 'water') but we cannot infer that glycyl-glycine or casein had any effect one way or the other. Oil and glucose increased the amount eaten significantly more than starch did, but did not differ from each other. Again, from this upper group of means we find no evidence that glycyl-glycine has a significant effect, because the mean for glucose-and-glycyl-glycine is in the same homogeneous group. The only interesting point lost in the above analysis is that the mean for glucose-and-oil, treatment (vii), is particularly high, as if the effects of glucose and oil are additive.

In summary, the results are inconsistent with the 'specific

stimulant' hypothesis, because they show stimulation by a starch, a sugar and an oil. Note that the concentrations of glycyl-glycine and casein were low, so the results do not throw into strong doubt the possibility that such nitrogen-compounds may also be stimulatory.

4.3.6 DISCUSSION OF THE STIMULI FOR FEEDING

The results in this section seem inconsistent with the hypothesis that there is a single stimulus for feeding by H. virgata.

There is evidence that glutathione and glucose are stimulatory. The stimulation by glucose is consistent with the findings recorded for Nassarius reticulatus (Kohn, 1961) and Lymnaea stagnalis (Jager, 1971). Jager found that sugars which stimulate feeding by L. stagnalis at low concentrations inhibit it at high concentrations. Such an effect on H. virgata could explain my earlier results (Sections 4.3.1, 4.3.2) indicating that glucose had no influence or perhaps even an inhibitory influence on feeding.

The results suggest that starch is stimulatory. It is reported that Nassarius reticulatus responds positively to starch and glycogen in solution (Kohn, 1961). But it must be noted that the manufacturer's analysis of the starch I used includes 'Reducing substances (as glucose) 0.2%.' The response may have been one to simple sugars.

One might speculate, given a response to a polysaccharide or even just to a simple sugar, that cellulose might stimulate feeding. Thus, the observation that snails readily eat filter paper should not have been taken to mean that they 'eat everything in their path.' Rather, if cellulose is of no food value (that is in doubt), I should

have considered that the animals' chemical senses had been 'tricked'.

I do not know what impurities are present in the safflower oil I used, but the results with that oil (Section 4.3.5) strongly suggest that feeding is stimulated by lipids. I do not know of a recorded example of this in a gastropod.

The details of these responses, such as the sites of receptors, the effects of different concentrations and of course the effects of a wider range of substances, remain to be worked out. My experiments are preliminary tests, sufficient to indicate that the snail does not feed indiscriminately unless inhibited, but can 'taste' certain substances associated with food, substances which one might have expected it to taste.

4.4. WHAT INHIBITS FEEDING?

4.4.1 PALATABILITY OF AN ACID-EXTRACT OF EARLY-HARVESTED FORAGE OATS

PROCEDURE

An acid-extract of oats, H1LN (Table 3.11) was prepared as follows: about 30 g of chopped (not ground) plant material was beaten under 750 ml of 0.1N hydrochloric acid for 10 minutes in a Ronson domestic blender. The extract was filtered twice through Whatman No. 1 paper using Buchner apparatus then neutralized with 0.1N sodium bicarbonate.

A solution with which to treat control papers was prepared by neutralizing 750 ml of 0.1N HCl with $NaHCO_3$ in the same way.

The palatability of the extract was tested in the following experiment.

400 snails were randomly divided into 40 lots of 10. Eighty 9-cm filter papers were randomly divided into 4 lots of 20. The first 20 papers were dipped into acid-extract of oats and put into Petri dishes with 10 snails per dish. The second 20 were dipped into acid-extract and put into dishes without snails. Similarly the third and fourth groups were treated with 'control' solution, with and without snails respectively.

The dishes were covered and left 8 hours, then the papers were removed, dried at 105°C for $2\frac{1}{2}$ hours, left to equilibrate to room conditions for 15 hours and weighed.

Table 4.11 shows the mean weights of the papers in the four 'treatments'; Table 4.12 shows the results of the analysis of variance.

TABLE 4.11

Descriptive statistics of the weights of papers in an experiment to test the effect of an acid-extract of early-harvested oats on the palatability of filter paper

Treatment	Acid extract, fed to snails	Extract, not fed to snails	Control Sol'n, fed to snails	Control, not fed to snails
No. of papers	20	20	20	20
Mean Wt.(mg)	486.40	549.38	484.72	555.24
S.E. of mean	6.68	7.90	5.98	6.98

Fmax (4, 19) = 1.75; $P > 0.05$

TABLE 4.12

AOV on the data on which Table 4.11 is based

Source of Variation	d.f.	S.S.	M.S.	Fs	P
Treatments	3	89,483	29,827	31.15^{***}	< 0.001
Within Treatments	76	72,772	957.53		
Total	79	162,255			

Partitioning the 'treatments' sum of squares by the procedure outlined by Sokal and Rohlf (1969), Section 9.6, confirms that the means fall into two homogeneous groups, the difference between which is significant at the 0.1% level. The groups are shown in the diagram:

Control fed to snails	Extract fed to snails	Extract not fed to snails	Control not fed to snails

Evidently the snails did eat the papers soaked in both solutions, and ate them equally readily.

INTERPRETATION OF RESULTS

This experiment was of a satisfactory design, one which did show significant differences in other experiments. Thus, the fact that it did not do so here can be taken as evidence that there is little that is stimulatory or deterrent in the acid-extract of early-harvested oats as I prepared it. I may, of course, have prepared too dilute an extract. But I decided that, rather than look further for the inhibitory substance in a grass which might in any case be too tough for snails, it would be of more interest to examine an alkaloid which might explain why H. virgata does not eat certain soft-leafed plants.

4.4.2 EXPERIMENTS WITH A PLANT ALKALOID

(1) THE EFFECT OF LASIOCARPINE ON THE PALATABILITY OF FILTER PAPER

PROCEDURE

One hundred mg of solid lasiocarpine was treated with 2 ml of approximately 0.1N HCl which neutralized it, according to pH

papers. From a sample of this stock solution I prepared a solution of concentration 0.250 mg/ml by dilution with double-distilled water. This concentration was chosen because the concentration of alkaloids such as lasiocarpine in plants is of the order of 0.05% dry weight (Frahn, pers. comm.); when a 5.5. cm disc of filter paper is wetted with 0.5 ml of this solution, it will contain c. 0.05% of lasiocarpine by weight. The following experiment was conducted to compare papers treated with this solution and papers treated with water.

Twenty papers were placed in Petri dishes. To each of 10 of them, randomly chosen, I added 1 ml of lasiocarpine solution. To each of the other 10 I added 1 ml of water. This was in excess of the amount needed to saturate the paper; the extra, free water would help keep the snails active. As most of it lay on the dish around the paper, this excess probably did not raise the percentage of lasiocarpine on the papers very much. It could not possibly have raised it above about 0.1% dry weight, and the figure of 0.05% dry weight is possibly low for my purposes. For example, suppose that the alkaloid extracted by chemists from the whole leaf and estimated at c. 0.05% dry weight were localized near the surface; then a snail feeding on the plant might encounter a concentration of alkaloid considerably above 0.05%. Thus, I think I used what might be considered a realistic dose for a preliminary experiment.

I divided 200 snails randomly into 20 lots of 10, sprayed them briefly to wake them and put them into the dishes where they remained for 5 hours. I then let the papers drain and dry in room conditions for 13 hours, dry at 45°C for 3 hours and equilibrate 19½ hours before weighing them.

RESULTS AND INTERPRETATION

The results are presented in Table 4.13.

TABLE 4.13

Descriptive statistics of the weights of 10 papers soaked in each of a) water and b) lasiocarpine solution and each fed to 10 snails for 5 hours

Treatment	Water	Lasiocarpine
No. of papers	10	10
Mean wt. (mg)	182.305	202.750
S.E. of mean	2.3423	2.5576
95% confidence limits for mean	(177.01, 187.60)	(196.96, 208.54)

The difference between the means is clearly significant, since the 95% confidence limits do not overlap. The papers treated with lasiocarpine weigh more. The mere 0.1 to 0.2 mg of lasiocarpine added cannot account for this; as one could tell by looking at the papers, those treated with lasiocarpine had been eaten less, though not entirely refused.

This result suggests strongly that lasiocarpine inhibits feeding by H. virgata.

(2) THE EFFECT OF LASIOCARPINE ON THE PALATABILITY OF FILTER PAPER IN THE PRESENCE OF GLUCOSE

This experiment was conducted to test whether lasiocarpine tended to inhibit feeding in the presence of a substance known to stimulate feeding. If it did not do so, it would be hard to see how

the alkaloid could be important in inhibiting snails from eating a young plant.

PROCEDURE

The procedure was that of the experiment in Section (1) above, this time with 4 treatments. The papers in the 4 treatments were soaked with 1 ml of (1) water,

(2) lasiocarpine solution prepared from the same stock described in Section 4.4.2(1) but now at a concentration of 0.5 mg/ml, producing a concentration of lasiocarpine on the paper of less than 0.2% dry weight and probably about 0.1% dry weight.

(3) a solution containing 0.1M glucose and 0.5 mg/ml lasiocarpine,

(4) a solution containing 0.1M glucose.

The experiment was conducted twice on consecutive days, using the same snails but randomizing them anew for the second run.

RESULTS

Descriptive statistics of the weights of the papers in each treatment and in each run of the experiment are shown in Table 4.14.

To analyse the weights as they stand is conservative because there was about 9 mg of glucose added to each of the papers in treatments 3 and 4. (0.5 ml of a solution of 18 mg/ml). Papers in these treatments are expected to show low weights, due to snails more readily eating them, unless lasiocarpine is inhibitory. By not making a correction for the weight of glucose added, I decrease my chance of showing that these treatments have significantly lower

weights than treatments (1) and (2). The weight of lasiocarpine can be neglected, and so the error is unimportant in comparing treatment (1) with treatment (2) or in comparing treatment (3) with treatment (4).

TABLE 4.14

Means ($\bar{y}$) and standard errors ($s/\sqrt{n}$) of the weights in mg of papers from two tests of the influence of lasiocarpine in the presence of glucose

Treatment		Water	Lasiocarpine	Lasiocarpine + glucose	Glucose
Run 1	n	10	10	10	10
	$\bar{y}$	172.91	193.52	175.36	165.79
	$s/\sqrt{n}$	4.71	2.64	5.07	3.20
Run 2	n	10	10	10	10
	$\bar{y}$	166.85	200.22	170.99	149.35
	$s/\sqrt{n}$	3.32	3.07	3.17	2.72

$F_{max}(8,9) = 3.70$; $P > 0.05$

A two-way analysis of variance on the data on which Table 4.14 is based gives the results in Table 4.15.

There are several comparisons which are of interest a priori. They are:

(i) Compare treatment (1) with treatment (2). Does the experiment confirm the results presented in Section 4.4.2(1)?

(ii) Compare treatment (1) with treatment (4). Does the experiment confirm the results of Section 4.3.5?

TABLE 4.15

AOV on the data on which Table 4.14 is based.

Source of Variation	d.f.	S.S.	M.S.	Fs	P
Subgroups	7	18054.65	2579.24		
Treatment	3	16199.76	5399.92	41.97^{***}	< 0.001
Runs	1	509.04	509.04	3.96	Just > 0.05
Interaction	3	1345.84	448.62	3.49^{*}	$0.01 < P < 0.025$
Within Subgroups	72	9263.86	128.66		
Total	79	27318.51			

(iii) The most interesting comparison is between treatments (3) and (4). Does lasiocarpine significantly inhibit feeding in the presence of glucose?

Having found significant interaction between runs and treatments, I think it best to make these comparisons separately for the two runs.

Run 1

A simple analysis of variance on the data for the first run gives the results in Table 4.16.

TABLE 4.16

AOV on results of the first run, statistics from which are shown in Table 4.14.

Source of Variation	d.f.	S.S.	M.S.	Fs	P
Treatments	3	4179.48	1393.16	8.558^{***}	< 0.001
Within treatments	36	5860.41	162.79		
Total	39	10039.89			

When the 'treatments' sum of squares is partitioned (Sokal and Rohlf, 1969, Section 9.6) to make the above three comparisons, the F-statistics obtained are:

(i) (Treat.(1) vs. Treat.(2)) $F_{1,36} = 13.047^{***}$; $P < 0.001$

(ii) (Treat.(1) vs. Treat.(4)) $F_{1,36} = 1.557^{N.S.}$; $0.1 < P < 0.25$

(iii) (Treat.(3) vs. Treat.(4)) $F_{1,36} = 2.813^{N.S.}$; $0.05 < P < 0.1$

Run 2

An analysis of variance on the data for the second run gives the results in Table 4.17.

TABLE 4.17

AOV on the results of the second run, statistics from which are shown in Table 4. 14.

Source of Variation	d.f.	S.S.	M.S.	Fs	P
Treatments	3	13366.13	4455.38	47.119^{***}	< 0.001
Within treatments	36	3404.02	94.56		
Total	39	16770.15			

F-statistics obtained in partitioning the 'treatments' S.S. are:

(i) (Treat.(1) vs. Treat. (2)) $F_{1,36} = 58.88^{***}$

(ii) (Treat.(1) vs. Treat.(4)) $F_{1,36} = 16.18^{***}$

(iii) (Treat.(3) vs. Treat.(4)) $F_{1,36} = 24.76^{***}$

For all three, $P < 0.001$

INTERPRETATION OF RESULTS

The first run does not show a significant depression of feeding by lasiocarpine in the presence of glucose, though it does show a depression without glucose, confirming the results of Section 4.4.2(1). Nor does it show a significant increase in feeding in the

presence of glucose alone, though that comparison is made less powerful by not correcting for the weight of glucose added.

In the second run, all three comparisons are highly significant. It appears, on inspection of Table 4.14, that on the second day both the stimulatory effect of glucose and the inhibitory effect of lasiocarpine acted more strongly.

Overall, the results make it seem likely that this alkaloid does inhibit feeding in the concentrations used, and continues to have its effect (which is not an absolute one) in the presence of a sugar known to enhance feeding.

Alkaloids might, then, serve to discourage H. virgata from eating plants otherwise suitable as food for it.

4.4.3 THE TOUGHNESS OF GRASSES

(1) THE EFFECT OF GRINDING ON THE PALATABILITY OF FOUR GRASSES

PROCEDURE

I collected barley, Hordeum distichon, L., and couch-grass, Cynodon dactylon Pers., from a roadside near Aldinga, S.A. on 6/6/71 and buffalo grass, Stenotaphrum secundatum (Walt.), from a suburban garden near Adelaide. Snails are sympatric with the first two at Aldinga (and elsewhere) and with the last at Moana, S.A.; there is no sign that they eat any of the three grasses.

The barley was c. 60 cm tall, headed but still green. The couch was quiescent; the buffalo green, being regularly watered. Oats, H_1LN (Table 3.9) was included as a fourth grass expected to be distasteful. I picked only green leaves from each species. The

leaves were vacuum-dried, half of each sample was ground in the laboratory mill with 0.5 mm sieve, and all were stored over silica gel.

I collected 400 snails on 17/7/71 and randomized them into 40 lots of 10. 'Units' of the experiment were 40 numbered Petri dishes, 5 for each of the 4 (grasses) x 2 (ground and unground) 'treatments', with a small amount of the appropriate grass and 2 ml of distilled water in each. The snails were wetted to wake them, put into the dishes in a random order (10 to a dish) and left 17½ hours. In no dish was the food completely consumed.

Most of the snails were still extended and so they would not have defaecated. They were transferred to new dry dishes with numbers corresponding to those of the first dishes. There, they would defaecate on withdrawing into their shells. The first dishes were upturned to drain and all were left another 24 hours. Finally, I collected faeces from the first dish, second dish and the snails' shells and apertures. All the faeces from one such 'unit' went into one tared tube to be vacuum-dried, allowed to equilibrate, and weighed.

RESULTS

Descriptive statistics of the weights of faeces obtained from each of the 40 experimental units are shown in Table 4.18. Table 4.19 shows the analysis of variance.

TABLE 4.18

Means ($\bar{y}$) and standard errors ($s/\sqrt{n}$) of the weights of faeces produced by snails fed on four different grasses, ground and unground

Grass	Oats H_1LN	Barley	Buffalo	Couch
n	5	5	5	5
Unground $\bar{y}$	4.99	17.12	12.64	10.35
$s/\sqrt{n}$	1.75	2.49	1.52	1.51
n	5	5	5	5
Ground $\bar{y}$	4.69	33.52	25.60	59.10
$s/\sqrt{n}$	1.27	3.73	1.90	3.15

$F_{max}(8,4) = 8.66$; $P > 0.05$

TABLE 4.19

AOV on the data on which Table 4.18 is based

Source of Variation	d.f.	S.S.	M.S.	Fs
Subgroups	7	11751.12	1678.73	
Grasses	3	4717.18	1572.39	58.66***
Grinding	1	3784.00	3784.00	141.16***
Interaction	3	3249.94	1083.31	40.41***
Within subgroups	32	857.79	26.8058	
Total	39	12608.90		

*** $P < 0.001$

INTERPRETATION OF RESULTS

Further statistical testing seems unnecessary. An examination of Table 4.18 suggests that early-harvested forage oats was eaten less than the rest (hence the significant 'foods' variance) and was not affected by grinding. Grinding led to increased feeding on the other three (hence the significant 'grinding' variance). Couch was more affected by grinding than barley or buffalo (this, and the lack of effect on oats, would account for the significant interaction).

The results support the hypothesis that in all but oats, and especially in couch, toughness is a very important barrier to feeding by H. virgata. The possibility remains, however, that the picture could be different at other times of the year, e.g. when barley is less mature.

(2) NUTRITIONAL VALUE TO SNAILS OF TWO OF THE GRASSES USED IN (1)

Despite the results above, I have not shown that toughness actually eliminates a food which would otherwise be of value to snails. I therefore conducted a feeding experiment similar to those in Section 3.4 to determine whether, with the effect of toughness removed, barley and couch do support the growth of snails.

PROCEDURE

The grasses were collected near Aldinga (28/7/71), vacuum-dried, half of each ground twice through a 0.5 mm sieve and stored in plastic bags. The snails were collected on 2/8/71. They were all large young-of-the-year; the latter could be inferred from their pigmentation and the fact that all showed recent growth of shell. Most were c. 1 cm, or a little less, in diameter.

The experiment involved 5 treatments; barley and couch both ground and unground, and filter paper, unground, as a control of 'no value'. There were 2 cages per treatment, 10 marked snails per cage. Conditions of temperature, photoperiod and spraying were as in Section 3.4.2.

Whatman No. 1 filter paper was given as two 5.5 cm discs per cage. The other foods were given on 5.5 cm discs of bolting-silk, two per cage. A little calcium carbonate was sprinkled on each. The amounts of food were small but enough for snails to feed ad lib. The foods were changed every 2 days, using fresh silk discs. Snails were weighed after 1, 8 and 15 nights.

RESULTS

Table 4.20 shows descriptive statistics of the changes in weight over 14 days (Appendix 1).

An inspection of Table 4.20 suggests the interpretation that grinding does increase the growth supported by the grasses, just as it increased the amounts eaten in (1) above.

The sample variances are significantly heterogeneous, and so I present the analysis of variance taking the cage-means as raw data (Appendix 1). The analysis was done in two parts. The first question is whether the unground grasses are of any value as foods. To answer it, a single-factor AOV was performed on the means in the top half of Table 4.20 with the results in Table 4.21.

TABLE 4.20

Descriptive statistics of the changes in weight (mg) over 14 days in snails fed on filter paper and on two grasses, ground and not ground (n = sample size. $\bar{y}$ = mean, $s/\sqrt{n}$ = standard error.)

Food		Filter paper	Barley	Couch
Unground	n	10	9	10
Cage A	$\bar{y}$	-17.98	17.11	11.47
	$s/\sqrt{n}$	5.06	5.54	6.30
Unground	n	10	10	9
Cage B	$\bar{y}$	- 8.76	36.06	- 1.88
	$s/\sqrt{n}$	3.21	8.30	3.13
Ground	n		10	10
Cage A	$\bar{y}$	-	25.52	59.64
	$s/\sqrt{n}$		11.02	8.55
Ground	n		8	9
Cage B	$\bar{y}$	-	47.04	64.50
	$s/\sqrt{n}$		13.46	10.61

$F_{max}(10,7) = 16.41^{*}$; $P < 0.05$

TABLE 4.21

AOV on changes in weight of snails fed on filter paper, barley and couch-grass (Data from Table 4.20)

Source of Variation	d.f.	S.S.	M.S.	Fs	P
Foods	2	1600.24	800.12	7.716	$0.05 < P < 0.10$
Within Foods	3	311.10	103.70		
Total	5	1911.34			

Even without a significant 'Foods' variance it is valid for a priori reasons to partition the 'Foods' S.S. and make two comparisons (Sokal and Rohlf, 1969, Section 9.6):

(i) Test the null hypothesis that the mean for filter paper is equal to the mean for barley and couch taken together.

$F_{1,3} = 10.85^{*}; \quad 0.025 < P < 0.05$

This null hypothesis is rejected at the 5% level.

(ii) Test the null hypothesis that the mean for barley is equal to the mean for couch.

$F_{1,3} = 4.58^{N.S.} \quad (0.10 < P < 0.25)$

This null hypothesis is accepted. Test (ii) is the one point where analysis of cage-means differs greatly from the analysis of all the raw data, and from the analysis of data transformed to logarithms: both those analyses give F-values for tests (i) and (ii) significant at the 1% level. This conservative analysis leads to the

conclusion that there is no significant difference between barley and couch as foods when not ground. But it indicates that both are better foods than filter paper.

The next question is whether grinding improves significantly the food-value of the grasses. To answer it, I disregarded the filter-paper treatment and could then calculate a two-factor AOV with the results in Table 4.22.

TABLE 4.22

AOV on changes in weight of snails fed on two grasses, ground and unground. Data from Table 4.20

Source of Variation	d.f.	S.S.	M.S.	Fs	P
Subgroups	3	3382.64	1127.55		
grasses	1	8.00	8.00	0.063	> 0.75
grinding	1	2242.69	2242.69	17.52*	$0.01 < P < 0.025$
interaction	1	1131.94	1131.94	8.84*	$0.025 < P < 0.05$
Within Subgroups	4	512.15	128.04		
Total	7	3894.79			

Once again, there is no evidence that one food is better than the other. Grinding is found to have a significant effect. But because of the significant interaction this analysis does not answer the question adequately. I therefore considered each food separately and carried out a t-test to compare ground with unground, still using the

cage-means as raw data. The results were:

Barley: $t_2 = 0.676^{N.S.}$; $P > 0.10$

Couch : $t_2 = 8.063^{*}$; $0.01 < P < 0.02$

Thus couch, which seems to support rather less growth than barley when not ground, is significantly improved by grinding; barley appears to be somewhat improved butthe difference is not significant on this particular test.

INTERPRETATION OF RESULTS

The results of (1) and (2) above are in general agreement. It appears that couch-grass, barley and buffalo grass are not entirely refused by snails and do support some growth when dried but not ground. But grinding makes them more palatable, particularly in the case of couch-grass, and at least with couch-grass the ground plant supports significantly more growth.

Thus, as would have been expected from the literature, toughness evidently can be an important barrier to feeding by H. virgata.

4.4.4 DISCUSSION OF THE INHIBITION OF FEEDING

H. virgata can be inhibited from feeding by chemical stimuli, as shown for forage oats. There is no evidence about the range of chemicals likely to have such an effect, but one which does so is a pyrrolizidine alkaloid.

Or the barrier can be physical, as shown for one and to a lesser extent for two more species of graminae.

Thus my limited experiments agree with the findings of Grime and co-workers for Cepaea nemoralis (Section 4.1). The implications

for an ecology of H. virgata are much as Grime et al (1970) saw them for C. nemoralis. As Taylor (1896, cited by Calow, 1970) suggested, these land-snails appear not to live 'in the midst of plenty' - many plants are unavailable to the snails while the plants are still alive and the snails usually have to eat mostly decaying material, which may be low in nutrients.

And my findings are consistent with the suggestion of Grime et al (1968) regarding the evolution of protective devices in plants. These authors suggest that certain physical and chemical barriers may be much more effective against land molluscs than against mammals, and that 'The widespread occurrence of features which deter gastropods from eating foliage suggests that these animals, possibly in conjunction with other invertebrates, have exerted considerable selection pressures during the evolution of flowering plants.'

In further studies it would be of interest to examine the behaviour of snails in response to toxins. Is a snail deterred on contact with an alkaloid, as if by taste, or does it suffer a toxic or narcotic effect after feeding for some time? An immediate effect would confer the best protection on the plant. I found a less than absolute effect of lasiocarpine, but is it more effective at higher concentrations and would a snail feeding on a plant encounter a high concentration? A study of the micro-distribution of toxins in plants may help in deciding the importance of different herbivores in their evolution. For example, if gastropods have been at least as important as mammals in the evolution of pyrrolizidine alkaloids in Boraginaceae, then one might expect the alkaloids to be localized

near the surfaces of leaves and stems. This would make the most effective use of the alkaloids against a mollusc - it would be of no account against a mammal.

5. MOVEMENT OF SNAILS WITH RESPECT TO FOOD

5.1 INTRODUCTION

Although individuals have been recorded moving considerable distances at the beginning and end of summer (Hodson, 1969), Helicella virgata seems to move little during winter. Pomeroy (1966) found evidence that at Buckland Park they move about with random changes in direction, so that the net distance moved by an individual is not great. In a capture - recapture survey at Buckland Park early in my project I found that between July and November 1968, 63 snails recaptured after 14-day intervals had moved a mean distance of only 1.98 m (S.E. 0.59 m) in 14 days, which seems to confirm Pomeroy's results. (For, released on a laboratory bench, snails will crawl that distance in 20 minutes or less.) Thus, it is a plausible hypothesis that H. virgata moves randomly, stopping to feed whenever it recognizes food by contact chemoreception, and that it does not perceive food at a distance.

Despite the apparent lack of directed movement towards food over any great distance, local movements might be influenced by more than an orthokinesis. It seems unlikely that the eyes of H. virgata form suitably precise images for the recognition of foods. The snails undoubtedly possess shadow receptors, but my observations confirm what one would expect from studies of other snails and slugs (Land, 1968; Newell and Newell, 1968); that there is no reason to think they have sharp vision. But distant chemoreception may direct the movement of snails.

Hodson (pers. comm.) observed that under certain weather

conditions if a specimen of H. virgata is crushed others of its species will come and feed upon it, apparently attracted to it from a distance of up to 25 cm. I was able to repeat this observation in the field, although I could never see signs of attraction over any more than about 15 cm.

I decided to test the hypothesis that H. virgata is attracted by the smell of a crushed snail and, if so, to explore the reasons for this. In Section 5.2.1 I describe the laboratory technique developed for this purpose. The hypothesis was supported by results obtained using this technique.

Two classes of hypotheses might account for the attractiveness of crushed snails.

The first is that there is a pheromone in snails. It may in the normal life of the animals be important in finding mates, or in finding other snails which have discovered good food, or in homing (Kohn, 1961; Owen, 1966a; Charles, 1966). It may be released into the air or laid down in the mucous trails left by snails. We might postulate that there is a reservoir of this material in a snail's body and that it is released in abundance when the animal is crushed.

The second is that a crushed snail releases odours which others associate with food. The body of a snail may well be a source of nutrients normally in rather short supply. This hypothesis says that even though a crushed snail may be a very rich source of some odorous material, it is not the only source. The odour is not snail-specific, but belongs to certain foods.

In Section 5.2.2 I describe several experiments concerned

with the first hypothesis. They do not give any support for the idea that Helicella produces a pheromone.

The second hypothesis is considered in Section 5.2.3. Although there a number of examples of the attraction of carnivorous and scavenging gastropods to food at a distance (Kohn, 1961; Owen, 1966a) there are fewer for herbivores. Michelson (1960) found that the planorbid Australorbis glabratus gave no reaction to lettuce, water-cress, chlorophyll extract or human faeces and urine, but a positive reaction to wheat germ. It is attracted to an alginate-based snail food containing wheat germ. The herbivorous Littorina obtusata is found to be attracted to various species of Fucaceae from 100 cm downstream in a flow-chamber. Different species appear to differ in attractiveness (Van Dongen, cited by Kohn, 1961).

Grime et al (1970) found some evidence that the leaves of one species of plant (Urtica dioica) attracted Cepaea nemoralis by odour, whilst another (Hedera helix) had a somewhat inhibitory odour.

Bovbjerg (1965) found that the lymnaeid Stagnicola reflexa aggregates on algae by orthokinesis alone, with no directed movement towards the algae from a distance, and Storey (1971) has reported similar behaviour in Lymnaea peregra. However, Bovbjerg (1968) found that four species of Lymnaeids showed contact chemoreception of plant and animal food (pondweed and crayfish respectively) - more strongly of animal food - and distant chemoreception only of the animal material. Bovbjerg suggested that it is adaptive for these snails, which seem to require some animal food but are largely herbivorous (Section 3.1), to show only contact chemoreception to the omnipresent plant food but distant chemoreception and oriented

movement towards the occasional animal carrion.

It would not be surprising, therefore, to find that H. virgata is attracted by the odours of some foods. If it is like Lymnaea stagnalis, and possibly Achatina fulica (Section 3.1), in needing a certain amount of animal material in its diet, then it may well be attracted to animal material. Experiments on the attractiveness to H. virgata of various substances on which it feeds are described in Section 5.2.3.

5.2. ATTRACTION AT A DISTANCE

5.2.1 TECHNIQUE FOR STUDYING THE BEHAVIOUR OF HELICELLA IN Y-TUBES

PROCEDURES

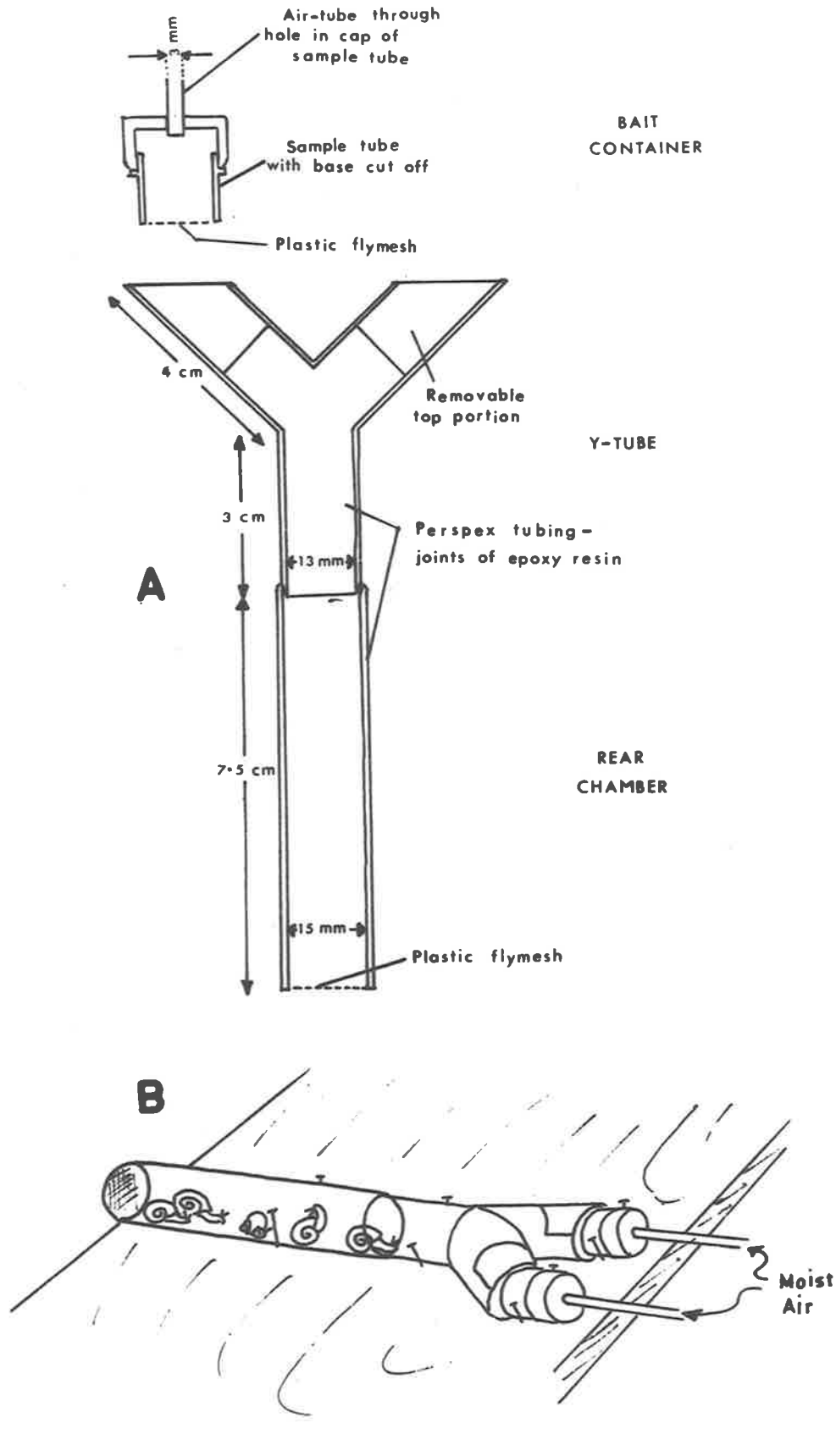
The basic procedure in my experiments on distant chemoreception is to place a snail near the junction of a Y-shaped tube with the test-material at the end of one arm, about 2 cm beyond the junction, and record which arm the snail crawls down. Figure 5.1 A shows the type of Y-tube I found most suitable. Conditions remained fairly moist in the rear chamber where the snails were placed initially, so that most of them remained active. The removable covers on the arms of the Y allowed me to remove a snail when it had made its 'choice' and so make way for others, without disturbing the baits.

The procedure which I finally adopted was to allocate randomly 10 snails to each of the 20 Y-tubes, and put them into the rear chamber. Then I set up the front, 'Y'-parts of the tubes which were held in place by nails driven into a long wooden plank (see Figure 5.1 B), put the baits into their containers which were also held in place by small nails, and attached an air-tube to each bait-container. The tube carried a slow current of air, saturated with water-vapour by passing it through a flask of distilled water. Of the 20 Y-tubes, 4 had no baits, 4 were baited with fishfood which was known to be attractive, and 12 were baited with the material under test. The Y-tubes were numbered 1-20, permanently. It was always randomly determined which treatment (no bait, fishfood or unknown) would be assigned to each tube and which of the 20 positions on the

FIGURE 5.1

A. Y-tube for the study of distant chemoreception in *H. virgata*.

B. A Y-tube set up for an experiment.


mm
Air-tube through hole in cap of sample tube
Sample tube with base cut off
Plastic flymesh
BAIT CONTAINER
4 cm
Removable top portion
Y-TUBE
3 cm
Perspex tubing – joints of epoxy resin
13 mm
A
7·5 cm
REAR CHAMBER
15 mm
Plastic flymesh
B
Moist Air

board would be occupied by each tube, Of the n tubes of each treatment, n/2 had the bait in the left-hand arm, and n/2 in the right, and it was randomly decided which.

Finally the chambers containing the snails were briefly immersed in cool distilled water (14-18°C) to awaken the snails, and attached to the Y-tubes. Snails could then not crawl out of the tubes except *via* one arm of the Y. Some, of course, did not crawl out at all during the 60 minutes chosen as an optimum time for observation. I made no attempt to force them to do so. I recorded a snail as having 'entered' an arm of the Y when its head reached the mid-line of that arm (the point where the removable cover began). When the lip of the shell reached that point I removed the snail with forceps. If it turned back before then, this was recorded, and if it later entered the other arm, this was recorded in a separate column. However, the data I present in the sections below are the number of snails making their first 'entry' in each arm.

Snails for each experiment were sprayed without food on the preceding night. The experiments were conducted under a safelight (a 20.3 x 25.4 cm Ilford S902 (light brown) filter with a 25 W globe behind it) just after 'lights out' in a room in which the photoperiod was 11 hours light - 13 hours dark. The temperature on the bench beside the Y-tubes was recorded at the beginning and end of each experiment. It was in the region of 17-20°C.

After a trial, the tubes were washed well with tap water and a clean test-tube brush. Bait-containers were discarded.

The above procedures were adopted as standard although preliminary trials had shown that, providing they were not too well-fed, neither

the treatment of snails prior to a test nor the time of day at which the test was conducted made much difference to their behaviour.

THE ATTRACTIVENESS OF CRUSHED SNAILS

In the course of these preliminary experiments, it was found that a crushed snail is a highly attractive bait. In the test on the effect of prior treatment, snails treated in seven different ways and then tested with crushed snails as baits gave the pooled results and G-values (Appendix 2) shown in Table 5.1. In the test on time-of-day, snails tested at four different times with crushed snails as baits gave the results in Table 5.2.

TABLE 5.1

Results (from a test on the effect of different prior treatments on responsiveness in Y-tubes) to illustrate the attractiveness of a crushed snail as bait

Treatment	No. entering bait side	No. entering other side
1. Dormant 3 days, then test	51	15
2. Test immediately on collection	42	20
3. Test snails from 2. again after 3 days dormant	46	21
4. Feed on fishfood overnight. Test	39	7
5. Test snails from 4. again after 3 days dormant	43	16
6. Dormant 1 night, Spray overnight with no food. Test.	47	22
7. Test snails from 6. again after 3 days dormant	50	14
Total	318	115

$G_H = 7.39^{N.S.}$ (6 d.f.; $0.1 < P < 0.5$)

$G_P = 49.53^{***}$ (1 d.f.; $P<0.001$)

$G_T = 56.92^{***}$ (7 d.f.; $P<0.001$)

TABLE 5.2.

Results (from a test on the effect of time-of-day on responsiveness in Y-tubes) to illustrate the attractiveness of a crushed snail as bait

Time	No. entering bait side	No. entering other side
0900	50	1
1200	48	6
1600	52	3
2100	32	4
Total	182	14

G_H = 4.95 N.S. (3 d.f.; $0.1 < P < 0.5$)

G_P = 170.87*** (1 d.f.; $P < 0.001$)

G_T = 175.82*** (4 d.f.; $P < 0.001$)

THE PROBLEM OF HETEROGENEITY

In many of the Y-tube experiments I conducted, the G-tests showed heterogeneity between tubes. That is, regardless of whether or not they conformed to my hypothesis of a 1:1 ratio of numbers entering the 'bait' and 'other' arms, the results for different tubes could not be regarded as coming from statistical populations with the same 'true' proportion of snails choosing the 'bait' arm.

This could be due to G_H being badly approximated by X^2 when samples are small (Appendix 2). But I tested other possibilities. I conducted tests which indicated that the heterogeneity could not be

due to inadequate washing of the tubes or bait-containers. (In any case I adopted as a precaution the practice of using the bait-containers only once and then discarding them.) Other tests indicated it could not be due to snails following each other's mucous trails or to unevenness of the air-flow.

The heterogeneity remains unexplained by anything but that the G-test makes 'type I' errors too often with small samples. It occurs less often with attractive baits, like fishfood, than with baits than seem unattractive, or with no baits.

5.2.2 ATTRACTION TO CRUSHED SNAILS - A PHEROMONE?

(1) ATTRACTION TO LIVE SNAILS

A simple form of the hypothesis that H. virgata possesses a pheromone is that snails are always attracted by the smell of other snails. Such a response would make them gregarious, as it has been suggested they may be (Pomeroy, 1966), and then they would gain certain advantages in finding food and mates. If such a response is to be of any use at all to snails, it seems reasonable to expect it to act over a distance of 2 cm, and so one would expect the response to be shown in my Y-tubes. I tested this twice, before the air-flow system was in use.

Each test involved 10 tubes containing 10 snails. In the bait-container in one arm of each tube was a live, adult snail randomly chosen from amongst the snails used for the test (which were collected on 7/12/70 and run on 11/12 and 17/12). The bait-snail was wetted like the rest, and the opposite bait-container was wetted in separate clean water. Random samples of snails from the same

collection tested before and after these experiments, though not concurrently with them, showed significant attraction to fishfood, no significant trend without baits, and no significant heterogeneity. This was as near as I was prepared to go to running true controls before the construction of the airflow-apparatus, because odours diffusing from one tube might have interfered with the test of a different material in a neighbouring tube. But I think these 'controls' are sufficient to show the present results trustworthy. (This series is mentioned again in Section 5.2.3(1).)

The results are summarized in Table 5.3.

Only one G-value is significant, that for the pooled data of the second experiment. But the total G for that experiment is not significant and the two experiments, combined, give no evidence of a pheromone, detected at a distance, which might lead snails to aggregate in December. This is not, of course, a test for the presence of a sex-pheromone, for snails are not mating in December. The response to crushed snails <u>was</u> demonstrated at that time of year but there remains the possibility that a sex-pheromone may be stored but not normally released then.

(2) ATTRACTION TO LIVE SNAILS WHEN FEEDING

A second form of the hypothesis of a pheromone is that snails when feeding emit an odour detected by other snails.

This was tested before the use of the air-flow using lettuce as a source of food. A piece of lettuce about 1 cm square was placed in each bait-container, and a snail in one. Ten snails were tested in each Y-tube. (They were collected 7/1/71 and tested on 10/1.)

TABLE 5.3

Results of two Y-tube experiments on the attractiveness of live adult snails collected in early December

	No. entering Bait side	No. entering Other side	G-values	d.f.	P
First experiment	27	44	G_H = 8.08	9	$0.5 < P < 0.9$
			G_P = 3.13	1	$0.05 < P < 0.1$
			G_T = 11.20	10	$0.1 < P < 0.5$
Second Experiment	44	29	G_H = 8.93	9	$0.1 < P < 0.5$
			G_P = 4.13*	1	$0.025 < P < 0.05$
			G_T = 13.07	10	$0.1 < P < 0.5$
Both Experiments Pooled	71	73	G_H = 24.20	19	$0.1 < P < 0.5$
			G_P = 0.072	1	$0.5 < P < 0.9$
			G_T = 24.27	20	$0.1 < P < 0.5$

I did not have clear evidence as to whether lettuce is attractive or not, but at that time it was the least attractive substance which I knew snails would eat. Later, having also found that dead leaves of Cynara cardunculus, which snails eat, are only mildly attractive if at all, I repeated the experiment using dead Cynara leaf instead of lettuce, and this time using the air-flow. (Snails collected 1/5/71, tested 2/5.) This second trial involved 4 tubes without baits, 4 with fishfood as baits and 12 with feeding snails as baits.

The results of the two experiments are shown in Tables 5.4. and 5.5.

TABLE 5.4.

Results of a test of the attractiveness of snails feeding on lettuce

No. entering bait side	No. entering other side	G-values	d.f.	P
29	35	G_H = 6.59	9	0.5 < P < 0.9
		G_P = 0.582	1	0.1 < P < 0.5
		G_T = 7.18	10	0.5 < P < 0.9

There is no indication of any attraction to feeding snails. Unfortunately, the four control-tubes with fishfood did not show an attraction strong enough to be significant. (The numbers are small but in other experiments four tubes were enough for a significant result.) This casts some doubt on the responsiveness of snails in the second experiment; but the trend towards fishfood was stronger than any trend in the 'test' tubes, where the only significant G

TABLE 5.5

Results of a test of the attractiveness of snails feeding on dead Cynara leaves

Treatment		Bait side	Other side	G-values	d.f.	P
No baits		Left 16	Right 20	G_H = 5.56	3	0.1 < P < 0.5
				G_P = 0.456	1	≤ 0.5
				G_T = 6.01	4	0.1 < P < 0.5
Fishfood		24	13	G_H = 2.74	3	0.1 < P < 0.5
				G_P = 3.33	1	0.05 < P < 0.1
				G_T = 6.07	4	0.1 < P < 0.5
Feeding Snails	Tube 1	5	2			
	" 3	7	3			
	" 5	1	9			
	" 8	6	2			
	" 10	1	8			
	" 12	6	4			
	" 14	5	4			
	" 15	8	2			
	" 16	3	5			
	" 17	4	5			
	" 18	6	4	G_H = 24.58*	11	0.01 < P < 0.025
	" 20	6	3	G_P = 0.483	1	0.1 < P < 0.5
	Total	58	51	G_T = 25.07*	12	0.01 < P < 0.025

was due to heterogeneity between tubes. Thus, there is no support here for the hypothesis that these snails are attracted to feeding members of their species by odour.

(3) TENDENCY TO FOLLOW SLIME-TRAILS

If snails possess a pheromone it may well be laid down in their mucous trails.

As with the postulated airborne pheromone of sections (1) and (2), such a pheromone may be released only at certain times. Suppose it is a sex-pheromone; then even in the breeding season, since this period seems to be several months long, only some snails might be releasing the odour at any one time.

So a negative result means little, but a positive result might provide convincing evidence that snails follow slime-trails.

A small amount of information concerning this hypothesis might be obtained from Y-tube experiments in which there were no baits. Assume Y-tubes were initially clean. Suppose the first snail in each tube were equally likely to enter either arm, and suppose subsequent snails were extremely likely to follow the slime-trail of the first. One would expect that in about half the tubes there would be a significant trend to the left; in the other half a significant trend to the right. The G-tests would show no significant trend in the pooled data, but a significant total G and significant heterogeneity.

Suppose, however, that the tendency for a snail to follow the trail of another were only weak. By chance, then, a considerable proportion of the second snails would not follow the first; and

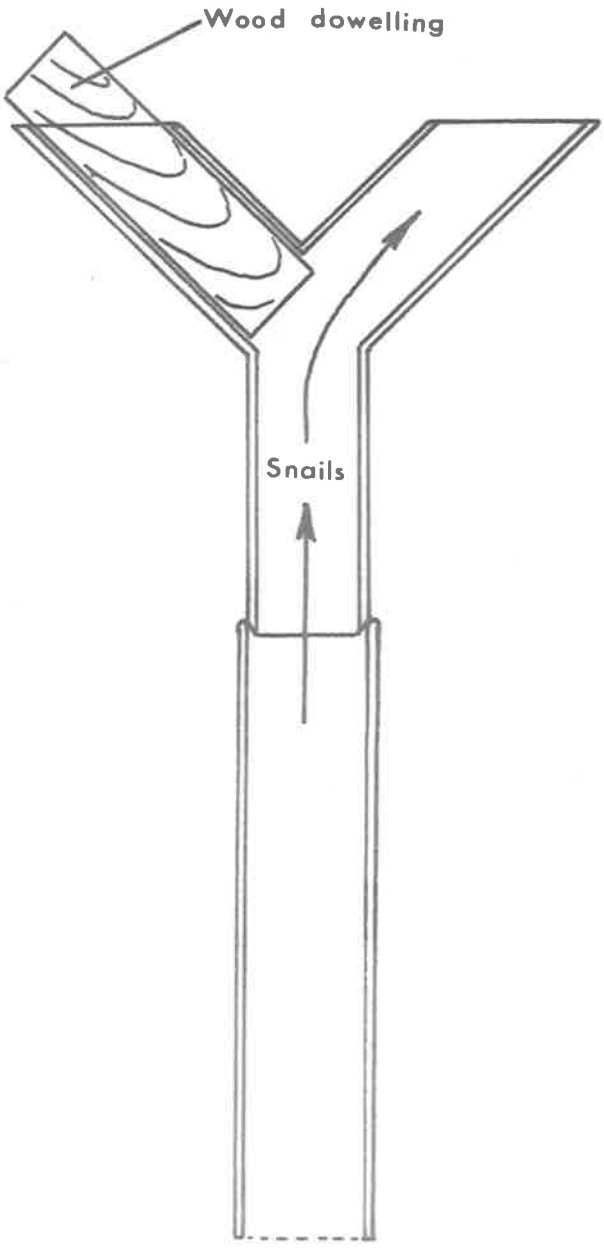
once the second snail had entered the opposite arm from the first, that Y-tube would provide no further test of any tendency to follow slime-trails.

I have conducted 14 independent tests with no baits, most of them involving 4 Y-tubes, as controls in experiments on other substances. Of these, 8 gave no significant G at all; 3 gave significant trends to the right and no significant heterogeneity. One gave heterogeneity significant at the 5% level but non-significant G_P and G_T values. And 2 gave the results predicted above – highly significant heterogeneity, highly signigicant G_T, and non-significant G_P. (These two tests were run in February and May.) This result gives little support for the idea that snails tend strongly to follow each other's slime-trails, though it suggests there might be a weak tendency to do so.

I conducted twice a similar experiment, specifically to seek a tendency to follow mucous trails. For the first run, 20 new Y-tubes were divided into two lots of 10. They had never had snails in them, nor had they been washed in anything but distilled water. I used random numbers to choose the left or right arm of each tube to be blocked with a piece of dowelling as in Figure 5.2, and permitted up to 10 snails to crawl freely through the open arm from the chamber to the outside. Then I removed the dowelling and conducted a standard test with 10 snails in each Y-tube and no baits. The snails used in the first 10 tubes had been kept in the laboratory, dormant except for one Y-tube test, for over a month; those used in the second 10 tubes had been collected the day before and sprayed over-

FIGURE 5.2.

Y-tube with one arm blocked prior to a test on the following of mucous trails.


Wood dowelling
Snails

night. This test was conducted in January. Thus it would not be expected to detect a sex pheromone and perhaps not a 'feeding' pheromone. A positive result would be exciting but a negative one means little.

The experiment was repeated in April and again in May. These tests were made not with new tubes but with tubes washed thoroughly in dilute HCl followed by distilled water, a procedure which should have removed all traces of mucus. Snails for these tests had been collected the day before and sprayed overnight.

The results of these four experiments are shown in Table 5.6.

These tests give no evidence that snails follow slime-trails.

Now, as noted above, a highly significant result would only have been expected in the event of a strong tendency to follow trails.

A more powerful test of a weak tendency could be made by running only one snail in each Y-tube before washing the tube and again letting snails walk though one arm to prepare it for the next test. In fact, in the above experiments I did record which arm the first snail entered. The experiments, combined, thus give respectively 10, 10, 18, and 19 observations of the behaviour of one snail in a Y-tube with one 'soiled' arm, and I show these data with the appropriate G-values in Table 5.7.

Again, there is no evidence that snails tend to follow slime-trails. It must be concluded that I have found no evidence of the existence of pheromones in H. virgata.

TABLE 5.6

Results of four experiments on the tendency of snails to follow mucous trails in Y-tubes

1st experiment 18/1/71. Snails collected 12/12/70.

'Slime side'	Clean side	G-values	d.f.	P
36	41	G_H = 13.46	9	$0.1 < P < 0.5$
		G_P = 0.348	1	$0.5 < P < 0.9$
		G_T = 13.81	10	$0.1 < P < 0.5$

2nd experiment 19/1/71. Snails collected 18/1/71.

'Slime side'	Clean side	G-values	d.f.	P
29	36	G_H = 6.75	9	$0.5 < P < 0.9$
		G_P = 0.777	1	$0.1 < P < 0.5$
		G_T = 7.53	10	$0.1 < P < 0.5$

3rd experiment 22/4/71. Snails collected 21/4/71.

Tube	SS	CS	Tube	SS	CS	G-values	d.f.	P
1	4	2	11	1	1			
2	2	8	12	2	8			
3	2	7	13	9	1			
4	8	2	14	0	9			
5	5	5	15	3	1			
6	3	1	16	3	4			
7	2	3	17	3	4			
8	8	2	18	2	5			
9	5	4	19	4	6	$G_H = 44.88^{***}$	18	< 0.005
10	7	3	20	–	–	$G_P = 0.107$	1	$0.5 < P < 0.9$
			Total	73	76	$G_T = 44.99^{***}$	19	< 0.005

4th experiment 24/5/71. Snails collected 23/5/71.

Tube	SS	CS	Tube	SS	CS	G-values	d.f.	P
1	2	7	11	0	6			
2	1	5	12	5	5			
3	2	7	13	3	7			
4	3	1	14	4	4			
5	3	7	15	–	–			
6	2	8	16	3	7			
7	4	5	17	7	3			
8	4	5	18	5	5	$G_H = 29.98^{**}$	18	$0.025 < P < 0.05$
9	1	2	19	6	3	$G_P = 5.60^{*}$	1	$0.01 < P < 0.025$
10	8	2	20	3	7			
			Total	66	96	$G_T = 35.58^{*}$	19	$0.01 < P < 0.025$

TABLE 5.7.

The arm chosen by the first snail to 'enter' in each tube in each of the four experiments of Table 5.6.

Experiment	No. of first entries on soiled side	No. of first entries on clean side	G-values	d.f.	P
1	5	5			
2	3	7			
3	9	9	G_H = 4.43	3	$0.1 < P < 0.5$
4	4	15	G_P = 4.01*	1	$0.025 < P < 0.05$
Total	21	36	G_T = 8.44	4	$0.05 < P < 0.1$

5.2.3 ATTRACTION TO FOODS

Experiments on the attractiveness of substances known to serve as food for snails are described in Section (1) and a limited number of experiments designed to give clues regarding the reasons, in Section (2).

(1) ATTRACTIVENESS OF SUBSTANCES ON WHICH SNAILS FEED

I tested five materials on which snails feed: all but one, rabbit faeces, had been shown in the laboratory to support growth to varying degrees. They were: fishfood, rabbit faeces, lettuce, dead leaves of Cynara cardunculus and late-harvested subterranean clover.

FISHFOOD was tested a number of times because I used it as a control to check that snails were responsive to a known attractant when unknown substances were under test. Suffice it to mention here that of 10 such control tests, all showed a preference for the

'fishfood' arm. This was significant in 8 of the tests (6 at $P < 0.005$, 2 at $P < 0.05$). Three of the tests showed significant heterogeneity (These particular 3 also gave highly significant G_P-values).

Fishfood was first tested along with RABBIT FAECES in a series of experiments before the airflow apparatus was working; for this series, a large sample of snails was collected and random samples from it were tested with fishfood, live snails, rabbit faeces and no baits in that order on consecutive days. Further random samples were tested on the same substances in reverse order on the following four days. This meant that, although I did not run controls on a given day to ensure that the snails were responsive, I had experiments on snails from the same collection with which to compare the results; and repeating the series in reverse order enabled me to check for any effect due to the time snails spent dormant in the laboratory before being tested. I found no such effect of time. (This series has already been mentioned in Section 5.2.3(1) where I reported the results of the tests with live snails as baits.)

The results of the tests on fishfood and rabbit faeces are presented in Tables 5.8 and 5.9 respectively.

Rabbit faeces might vary a great deal. Sometimes I have observed snails clustered on piles of faeces - generally old ones - and sometimes I have seen faeces with no snails on them although there were snails close by. I have tried, on days when I could demonstrate the attractiveness of crushed snails in the field, to do so with rabbit faeces. I had no success, even though I used faeces on which snails had been feeding.

TABLE 5.8

Results of two experiments on the attractiveness of fishfood in Y-tubes, December 1970

Experiment	No. entering Bait side	No. entering Other side	G-values	d.f.	P
1	69	17	G_H = 8.09	9	0.5 < P < 0.7
			G_P = 33.74***	1	< 0.001
			G_T = 41.83***	10	< 0.001
2	59	15	G_H = 11.81	9	0.1 < P < 0.5
			G_P = 27.99***	1	< 0.001
			G_T = 39.81***	10	< 0.001

TABLE 5.9

Results of two experiments on the attractiveness of rabbit faeces in Y-tubes, December 1970

Experiment	No. entering Bait side	No. entering Other side	G-values	d.f.	P
1	46	33	G_H = 10.75	9	0.1 < P < 0.5
			G_P = 2.18	1	"
			G_T = 12.92	10	"
2	23	24	G_H = 4.13	9	0.9 < P < 0.975
			G_P = 0.034	1	0.5 < P < 0.9
			G_T = 4.17	10	0.9 < P < 0.975

Finally, in connection with these two tests, it remains to mention that the tests with no baits in the same series showed no significant trends to left or right and no significant heterogeneity.

I tested LETTUCE four times because it gave variable results. The first two, conducted in December 1970 before the use of air-flow, were not concurrent with control-tests on fishfood to confirm that snails were responding normally. In fact, the snails had been dormant in the laboratory for 18 and 20 days and rather few of them 'entered' the tubes. The two experiments gave different results which are shown in Table 5.10.

The third test was part of an experiment in which several substances were run at one time using air-flow. There were only 5 Y-tubes for each substance. I ran the whole experiment 3 times, to give a total of 15 tubes for each substance. Fishfood was included in this series of tests and snails were significantly attracted to it, indicating that they were in a fit condition to respond in the normal way. The results for the 15 tubes with lettuce as bait are shown in Table 5.11.

Finally, another test on lettuce was run in the standard way adopted because the above type of experiment, with a number of foods at once, proved difficult to set up without some of the baits deteriorating. Results of this test are shown in Table 5.12.

TABLE 5.10

Results of two experiments on the attractiveness of lettuce in Y-tubes, December 1970

	Tube	No. entering Bait side	No. entering Other side	G-values	d.f.	P
Expt. 1	1	3	1			
	2	7	1			
	3	1	2			
	4	6	2			
	5	0	6			
	6	6	1			
	7	1	0			
	8	5	1			
	9	0	1			
	10	4	0	G_H = 25.13**	9	0.001 < P < 0.005
				G_P = 6.93**	1	0.005 < P < 0.01
	Total	33	15	G_T = 32.06***	10	< 0.001
Expt. 2	1	2	5			
	2	5	2			
	3	4	3			
	4	2	5			
	5	1	5			
	6	3	3			
	7	1	5			
	8	4	2			
	9	2	2	G_H = 13.75	9	0.1 < P < 0.5
	10	0	4	G_P = 2.43	1	"
	Total	24	36	G_T = 16.19	10	0.05 < P < 0.1

TABLE 5.11

Results of an experiment in which 5 Y-tubes were used with lettuce as bait on each of 3 consecutive days

	Tube	No. entering Bait side	No. entering Other side	G-values	d.f.	P
Day 1	4	2	3			
	11	0	6			
	17	0	5			
	18	2	8	G_H = 11.61*	4	0.01 < P < 0.025
	19	4	2	G_P = 8.38**	1	0.001< P < 0.005
	Total	8	24	G_T = 19.99**	5	0.001< P < 0.005
Day 2	1	1	5			
	3	0	6			
	10	4	4			
	16	1	6	G_H = 9.05	4	0.05 < P < 0.1
	20	0	6	G_P = 14.47***	1	< 0.001
	Total	6	27	G_T = 23.52***	5	< 0.001
Day 3	9	0	9			
	13	1	4			
	14	6	1			
	18	6	1	G_H = 31.49***	4	< 0.001
	19	0	9	G_P = 3.33	1	0.05 < P < 0.1
	Total	13	24	G_T = 34.82***	5	< 0.001
3 days pooled	Total	27	75	G_H = 54.79***	14	< 0.001
				G_P = 23.54***	1	< 0.001
				G_T = 78.33***	15	< 0.001

TABLE 5.12

Results of an experiment on the attractiveness of lettuce in Y-tubes

	Tube	No. entering Bait side	No. entering Other side	G-values	d.f.	P
No. Baits	2	Left 4	Right 4			
	6	" 1	" 5			
	10	" 4	" 0	G_H = 8.56*	3	0.025 < P < 0.05
	14	" 3	" 4	G_P = 0.047	1	0.5 < P < 0.9
	Total	12	13	G_T = 8.61	4	0.05 < P < 0.1
Fish-food	Total	19	2	G_H = 5.57***	3	0.1 < P < 0.5
				G_P = 15.91***	1	< 0.001
				G_T = 21.48***	4	< 0.001
Lettuce	1	1	3			
	3	1	8			
	4	3	0			
	7	5	3			
	8	7	0			
	9	5	1			
	11	0	7			
	13	6	2			
	15	3	2			
	18	1	1			
	19	5	3	G_H = 36.45***	11	< 0.001
	20	5	3	G_P = 1.11	1	0.1 < P < 0.5
	Total	42	33	G_T = 37.55***	12	< 0.001

Thus we have what can be considered as 6 different tests on lettuce. One showed what appeared to be significant attraction, but also significant heterogeneity. One showed neither attraction nor heterogeneity. Two showed significant repulsion although one of these also showed heterogeneity. And two showed neither attraction nor repulsion, but highly significant heterogeneity.

At this stage I think that there is little evidence of an attraction to lettuce. Lettuce may vary in condition and also in attractiveness; snails may also vary.

I decided not to pursue this problem any further. Lettuce is not a natural food of H. virgata; it was chosen because it serves as a food when offered to snails in the laboratory, and because it might therefore have given a clue to the kinds of substances attractive to snails. The variable results are little help in that regard. All these results did was to strengthen my feeling that snails may only be attracted to substances present in animals (like the Lymnaeidae studied by Bovbjerg - Section 5.1). But this idea was to be disproved by the following two experiments.

DEAD LEAVES of the wild artichoke, Cynara cardunculus (the species used in the 'culturing' experiments of Chapter 3) were tested in a single trial of the standard type, with the results in Table 5.13.

TABLE 5.13

Results of an experiment on the attractiveness of dead leaves *Cynara cardunculus* in Y-tubes

	Tube	No. entering Bait side	No. entering Other side	G-values	d.f.	P
No baits	Total	left 15	right 19	G_H = 0.332 G_P = 0.482 G_T = 0.814	3 1 4	0.9<P<0.975 0.1<P<0.5 0.9<P<0.975
Fish-food	Total	33	2	G_H = 2.55 G_P = 33.20*** G_T = 35.75***	3 1 4	0.1<P<0.5 < 0.001 < 0.001
Dead *Cynara* leaves	1	9	1			
	2	8	2			
	3	6	4			
	5	8	2			
	7	1	8			
	10	8	1			
	11	2	8			
	12	8	2			
	13	7	3			
	15	8	3			
	19	3	7	G_H = 35.05***	11	< 0.001
	20	5	5	G_P = 6.22*	1	0.01<P<0.025
	Total	73	46	G_T = 41.27***	12	< 0.001

There seems to be a weak attraction to dead leaves of *Cynara*, but the heterogeneity is great.

LATE-HARVESTED SUBTERRANEAN CLOVER, ground, as used for the second dried forage experiment (Section 3.4.3), was tested in a single experiment of the standard type. The results are shown in Table 5.14.

TABLE 5.14

Results of an experiment on the attractiveness of late-harvested, ground subterranean clover in Y-tubes

	Tube	No. entering Bait side	No. entering Other side	G-values	d.f.	P
No baits	Total	left 10	right 24	G_H = 4.55 G_P = 5.95* G_T = 10.50*	3 1 4	0.1<P<0.5 0.01<P<0.025 0.025<P<0.05
Fish-food	Total	33	7	G_H = 2.43 G_P = 18.37*** G_T = 20.80***	3 1 4	0.1 < P < 0.5 < 0.001 < 0.001
Clover	1	9	1			
	2	11	0			
	3	9	1			
	5	6	4			
	6	8	2			
	7	4	5			
	10	1	6			
	11	10	0			
	14	3	7			
	18	9	1	G_H = 41.57***	10	< 0.001
	19	7	2	G_P = 22.58***	1	< 0.001
	Total	77	29	G_T = 64.15***	11	< 0.001

Again, there is unexplained heterogeneity. (There is a trend to the right in the tubes without baits but this cannot influence any trend to or from baits in the other treatments.) Nevertheless it appears that we should reject the null hypothesis that there is no attraction to clover.

(2) TESTS ON SUBSTANCES WHICH MIGHT GIVE CLUES REGARDING THE CHEMICALS ATTRACTIVE TO SNAILS

Seeking further support for the hypothesis that snails are

attracted to some materials released by animal but not by plant tissues, I included crushed mealworms (larvae of Tenebrio molitor) in the trial mentioned under section (1) in which lettuce was tested on 3 consecutive days. The mealworms, in the bait-containers, were crushed immediately after the wetted chambers of snails had been attached to the Y-tubes, so that they would be as fresh as possible. The results of the third test must be discarded because of an accident. The first two provide two sets of 5 tubes. The results are shown in Table 5.15.

TABLE 5.15

Results of an experiment in which 5 Y-tubes were used with crushed larvae of Tenebrio as baits on each of 2 consecutive days.

	Tube	No. entering Bait side	No. entering Other side	G-values	d.f.	P
Day 1	3	5	1			
	5	3	4			
	7	7	2			
	13	1	6	G_H = 13.49 **	4	0.005 < P < 0.01
	14	8	1	G_P = 2.68	1	0.1 < P < 0.5
	Total	24	14	G_T = 16.17 **	5	0.005 < P < 0.01
Day 2	5	6	1			
	8	3	2			
	9	3	6			
	14	4	5	G_H = 10.06 *	4	0.025 < P < 0.05
	17	7	1	G_P = 2.54	1	0.1 < P < 0.5
	Total	25	15	G_T = 12.60 *	5	0.025 < P < 0.05
2 Days Pooled	Total	49	29	G_H = 23.56 **	9	0.005 < P < 0.01
				G_P = 5.21 *	1	0.01 < P < 0.025
				G_T = 28.77 **	10	0.001 < P < 0.005

There was a tendency to turn towards the crushed mealworms which, when the 10 tubes are considered as a set, is significant at the 2.5% level. But there was significant heterogeneity. More work would be needed to confirm this result but again I pursued this question no further because other experiments disproved the hypothesis that snails are attracted only to animal materials.

I have not conducted a useful number of tests on the attractiveness of well-defined substances. It will be of interest to continue such tests. I have found non-significant trends towards yeast, glutathione and glycogen, all these tests showing significant heterogeneity.

5.3 DISCUSSION

These experiments indicate that the Y-tube technique is a useful one although it can give variable results, for example with lettuce.

Using the technique, it has been confirmed that H. virgata is attracted by the odour of a crushed member of its species. There is no evidence of the presence of a pheromone; it is appropriate with present knowledge to accept the hypothesis that the attraction is an attraction to food. In agreement with this, there is also evidence of attraction to other foods - fishfood, dried subterranean clover and perhaps dead Cynara leaves and crushed Tenebrio larvae.

It is necessary now, as in the work on contact chemoreception, to examine a wider range of substances for attractiveness, and to examine more clearly-defined materials.

And it is necessary to examine the ecological relevance of this 'sense of smell.' We have only one field observation - that of an attraction to crushed snails. I conducted these experiments in the hope that the animal's sense of smell might give clues regarding dietary requirements which are in short supply. It may still do so- nothing very clear-cut has emerged yet. But apart from the search for information about the animal's requirements is the question of what importance this sense has in influencing dispersal and determining a snail's chance of finding food. This question has not been investigated.

An inportant final point regarding ecological relevance is that the variability in my results could well reflect variability in the

snails. Their responsiveness may vary with short-term changes in their condition; this would be worth studying. But perhaps of more interest is the likelihood that snails vary genetically in their chemical senses - they are noted for variability with respect to other characters and this is likely to be very important in gaining an understanding of their ecology (Chapter 1).

I think that my procedure of using strictly random samples was appropriate for the preliminary experiments I was conducting, but it would be of interest to examine variability in chemical senses, which possibly reflect dietary preferences, both within and between populations of H. virgata.

6. CONDITIONS UNDER WHICH SNAILS CAN FEED

6.1 THE STIMULI WHICH AROUSE SNAILS FROM INACTIVITY

In sections 6.1 and 6.2 I mention briefly experiments which, although conducted largely for technical reasons, bear on the condititions under which H. virgata can feed in the field.

I conducted a few experiments on the stimuli which arouse snails from 'inactivity' and possibly also from 'dormancy.' (I use the two terms as Hodson (1969) did. 'Inactivity' refers to the condition of being withdrawn into the shell during the season in which snails are normally feeding and reproducing - winter, for H. virgata. 'Dormancy' refers to the condition in the unfavourable season - summer, for H. virgata - when snails withdraw into their shells for long periods, generally producing calcareous epiphragms and showing certain metabolic changes.) In order to be able to keep snails in the laboratory, and in the hope of developing an assay for food based on the behaviour of the snails, I decided to investigate briefly the stimuli which wake them; mainly the effect of temperature.

Wells (1944) found that Helix pomatia undergoes fluctuations in weight due to changes in water-content which are largely due to internal processes; it enters 'aestivation' ('inactivity' in Hodson's terms) from the troughs of these fluctuations and in the absence of water will not arouse. Howes and Wells had previously observed an exception: snails in an experiment had to be moved to another building and 'the snails, stimulated presumably by the shaking, crept around a little and nibbled at their food.' Wells (1944) suggested that when rain aroused snails from 'aestivation' the immediate

mechanism was such a mechanical stimulation, rather than hydration of the tissues; hydration would follow after, and 'perhaps make a secondary contribution to its great rise in metabolic rate."

Dainton (1954 a, b) studied the activity of slugs and found that atmospheric moisture had no direct effect in inducing activity; nor did darkness, though switching lights off or on would induce short-lived activity. Air-currents would induce activity, the slugs tending to move away from them, but such an effect seems unlikely to be important in arousing an inactive snail which is withdrawn into its shell. Dainton's results indicated that the most important stimulus was changing temperature: between 4° and 20°C activity in Agriolimax reticulatus was induced by falling temperatures and suppressed by rising temperatures, whilst between 20° and 30°C activity was induced by rising temperatures and suppressed by falling temperatures. Dainton discussed the appropriateness of these responses in the conditions under which the slugs live. At constant temperature, she found a steadily deteriorating diurnal rhythm of activity and rest.

Blinn (1963) suggested that activity might be induced in a similar way in the land-snails Mesodon thyroidus and Allogona profunda.

However, records of activity, temperature and light obtained by Newell (1968) suggested that in the slug Agriolimax reticulatus in the field changes in temperature were unlikely to provide the cue for changes in behaviour: light could do so, reinforcing the endogenous daily rhythm of the animals.

Lewis (1969) found with the slug Arion ater in the laboratory that temperature changes had no effect on the timing of activity; it

appeared that cycles of light and darkness might be the most important factor in the control of activity.

And Cameron (1970), studying the proportion of time spent active by three species of Helicid snails at various constant temperatures, at 100% relative humidity and with 16 hours light: 8 hours dark, found different effects of temperature and different degrees of nocturnality, which appeared appropriate in terms of the distributions of the snails. These patterns of activity could not have been induced by changes in temperature.

I conducted some experiments very similar to those described by Dainton (1954a), pp. 168 ff., on the effect of temperature at high humidity. I used a chamber like that of Dainton and some modifications of it; snails were enclosed in an upturned glass Petri dish with its lid lined with filter paper and the whole chamber immersed in a water-bath which gave convenient control of temperature, and made the paper wet so that humidity in the chamber must have been near 100%.

I could not show any clear effect of rising or falling temperature. Snails would begin to emerge in about 15 minutes regardless of the value, direction of change or rate of change of the temperature. The only consistent effect of temperature was that at 25–30°C it became inhibitory. Snails on wet paper at about 28– 30°C would often remain partly extended, but very few would be fully extended or moving. (Hodson, 1969, in describing his experiment to test whether dormancy is obligatory – see Section 6.2 – noted that snails wakened by the sprays at midday on a hot day would move very slowly over the hot, wet ground.)

In some later experiments, snails were placed on a dry substrate

of cork although they were over water and must have been at a humidity near 100%. And I found that unless the cork was wet on top, no snails would emerge no matter how long I waited and no matter in what way the temperature changed in the region 14^{o} - 30^{o}C. After one such experiment I removed the apparatus from the water-bath, carefully removed the covers of the chambers and placed into the aperture of each snail a drop of water from the bath. The snails began emerging in the usual 10-15 minutes.

I have conducted no experiments on the effect of humidity in waking snails, but the above observations suggest that high humidity *per se* is not sufficient; a much more effective stimulus is the presence of free water in the snail's aperture. On the other hand, I have not conducted experiments at temperatures below 14^{o}C, and Pomeroy (1969) comments that '... in the laboratory, *H. virgata* remains dormant at 10^{o}C when the humidity is kept low, but soon wakes if the humidity becomes high.'

I have also repeatedly made observations like those of Howes and Wells, that knocking or shaking a container of snails will arouse some of them, although they will move little if not also wet.

Clearly, further experiments are needed to elucidate the stimuli which arouse snails. It seems likely that the relationships will prove complex. With our present very limited knowledge I would suggest that field measurements of free water on leaves or soil could be expected to correlate with activity of *H. virgata* more closely than any other single measurement. Even this may be complicated, however, by the tendency of *Helicella* to climb away from excess water.

6.2. IS DORMANCY OBLIGATORY DURING SUMMER?

It has been suggested that land-snails in the condition which Hodson calls 'dormancy' - a condition often called 'hibernation' in colder climates - when they are withdrawn for long periods and secrete heavy calcareous epiphragms, may be in diapause and unable to awaken until they have been dormant for a sufficient length of time (Blinn, 1963, and a number of earlier authors cited by Hodson, 1969).

Hodson conducted an experiment on H. virgata in which he penned snails in the field in summer and sprayed them with water, at midnight in one pen, at midnight and midday in another. The snails in an unwatered pen formed thick calcareous epiphragms and became dormant in the way typical of H. virgata in summer, but those in the sprayed pens were awakened whenever the sprays operated and did not form calcareous epiphragms. There was no evidence that dormancy is obligatory.

However, in one of my early experiments in the laboratory the amount eaten by the snails had fallen to a very low level within seven days. This experiment had been conducted in November and so I decided to test again the possibility that dormancy was obligatory even though it seemed unlikely. H. virgata will move about in response to rain in summer. If it is unable to feed at such times this may be of some importance. As I have mentioned in Chapter 1, there is evidence that, at least in the laboratory, death in dormancy is due to the exhaustion of food reserves and not to desiccation. Thus it would be advantageous for a snail awakened in summer to be able to feed, supposing food were available. If snails enter an obligatory dormancy, there is no possibility of this; but if they do not, then it is of

interest for an ecologist to consider the likelihood of food being available during brief summer rains.

The experiment described in this section was designed to test whether snails which have been dormant for some time are capable of feeding when awakened by spraying in the laboratory.

PROCEDURE

One hundred and fifty adult snails were collected on 18/11/69, kept dormant until 20/11 and then randomly divided into three lots of 50. These three groups were treated as in Table 6.1.

TABLE 6.1

Treatments in an experiment on Depth of Dormancy

Treatment No.	Procedure
(i)	Kept permanently dormant in a laboratory cage (Appendix 4) in a 'constant temperature' room at 27 - 29°C with a 12-hour photoperiod.
(ii)	Kept dormant in the same room but removed fortnightly to be placed under the laboratory sprays for two days with no food. Sprays operated once a night, at 1800 early in the experiment and later at 2000; thus these snails were awakened twice during each two-day period under the sprays.
(iii)	As for treatment (ii), but a sheet of filter paper sprinkled with fishfood and a little calcium carbonate was placed in the cage before it was sprayed.

On each of seven occasions I took a random sample of five snails from each cage. These samples were taken two days after the snails in treatments (ii) and (iii) had been replaced in the warm room

following a spell under the sprays. (The first sample was taken two days after the experiment was set up.) The snails in these samples were dried individually and their dry body weights and dry shell weights recorded (see Appendix 1).

RESULTS

The snails in all three treatments withdrew into their shells and secreted thickened calcareous epiphragms. These were, of course, broken (probably partly eaten - Hodson, 1969) every 14 days in treatments (ii) and (iii) but the snails secreted new ones on being returned to the warm room. Some snails whose positions I marked did not move during 12 days in the warm room.

The dry body weights of the random samples taken at intervals are shown graphically in Figures 6.1 and 6.2. The animals varied considerably in size. Their dry body weights and dry shell weights thus showed large variances. Although some snails in treatment (iii) were growing thin, new shell by five weeks (i.e., after two periods of spraying) it must have been light relative to the rest of the shell because there was no sign of any trend in shell-weight throughout the experiment in any treatment. This being so, I decided to calculate the ratio dry body weight/dry shell weight for each snail in order to obtain estimates of dry body weight which were to some extent corrected for differences in size of the animals.

Figure 6.1 shows the mean dry body weights of the samples taken throughout the experiment, with their 95% confidence limits.

Figure 6.2 shows corresponding statistics for dry body weight/dry shell weight. It can be seen that the differences between treatments

FIGURE 6.1

Results of a laboratory experiment on depth of dormancy.

Dry Body Weight (means of samples of 5, with 95% confidence limits) plotted against time.

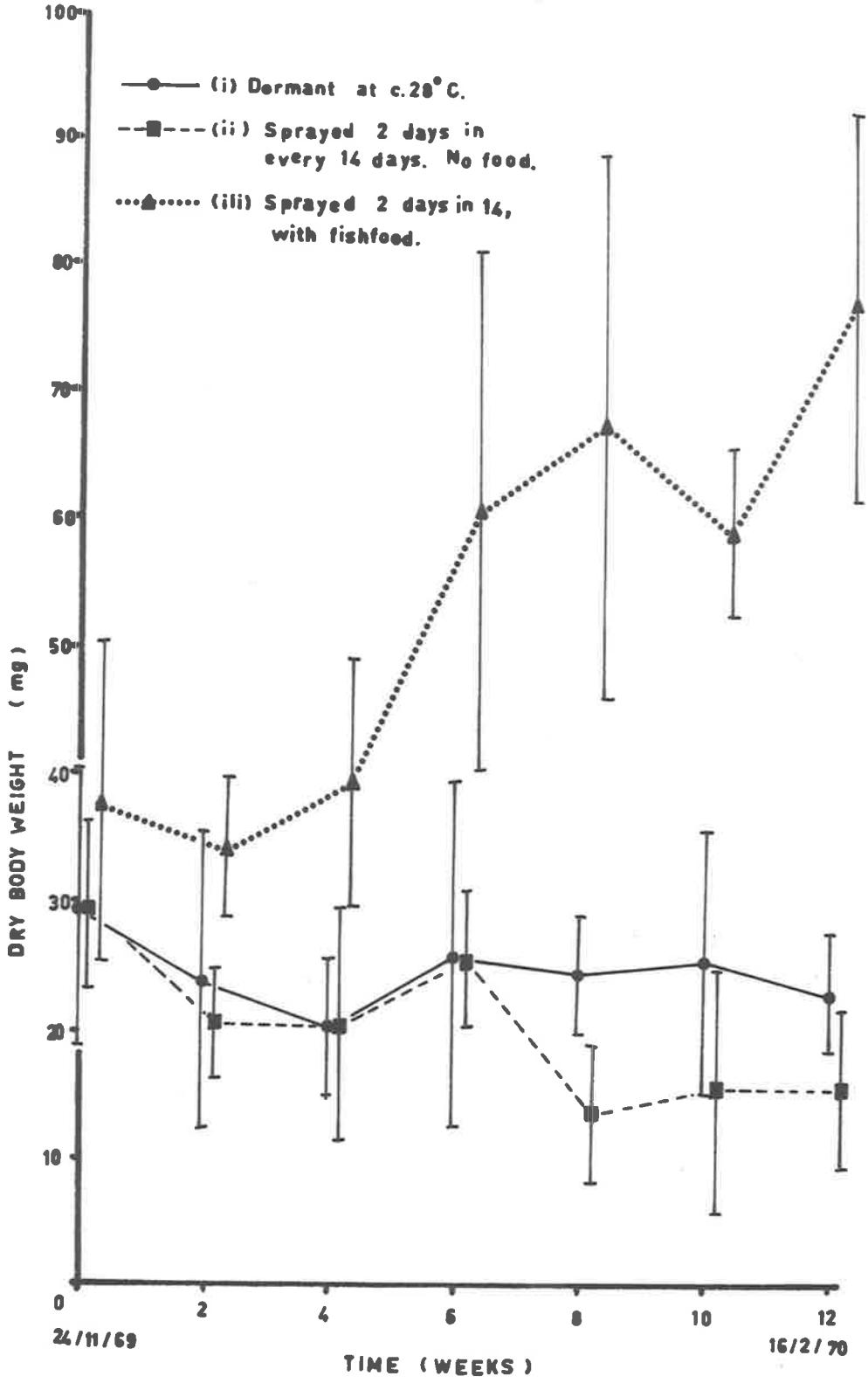
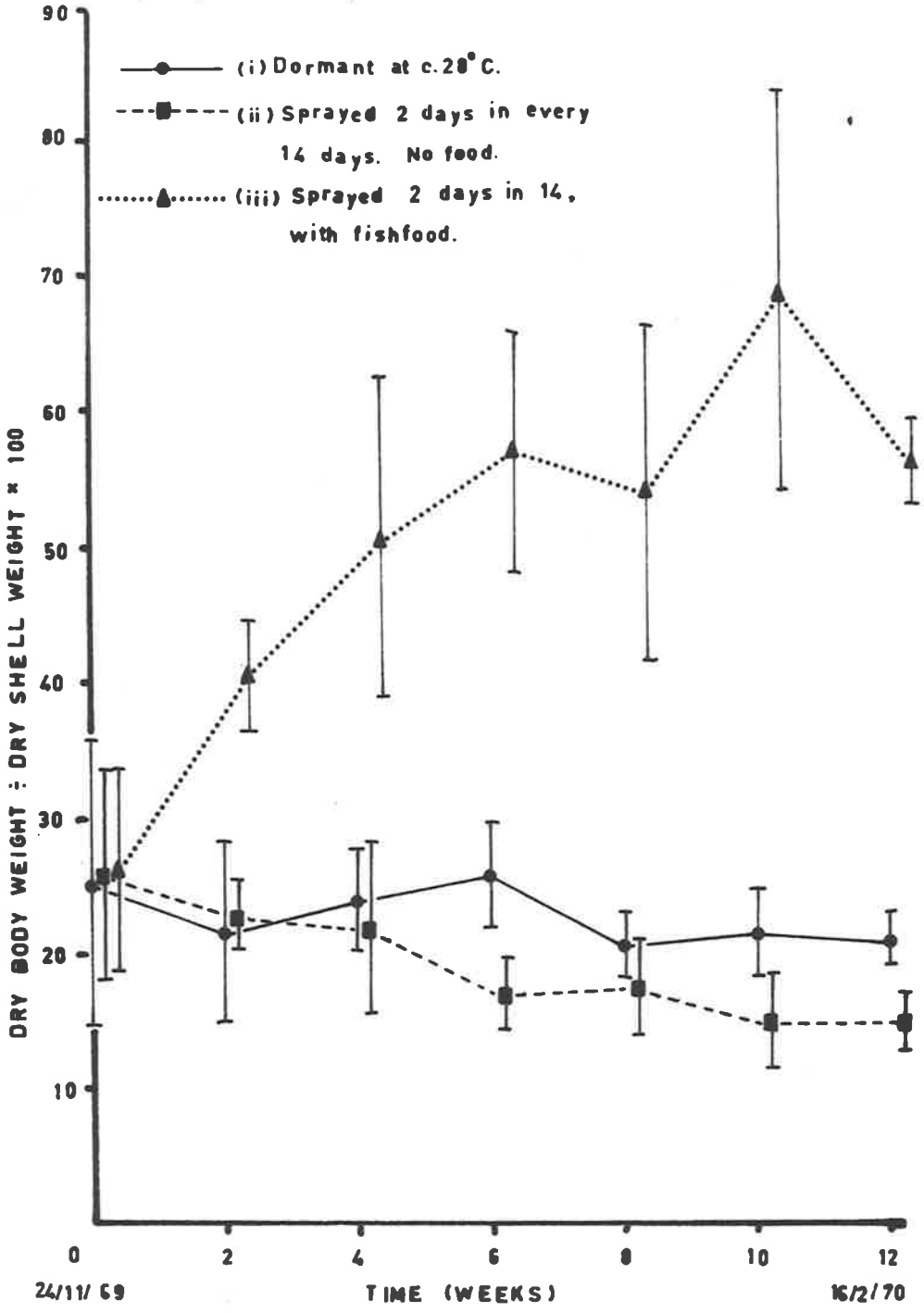

(i) Dormant at c.28°C.
(ii) Sprayed 2 days in every 14 days. No food.
(iii) Sprayed 2 days in 14, with fishfood.
DRY BODY WEIGHT (mg)
100
90
80
70
60
50
40
30
20
10
0
2
4
6
8
10
12
24/11/69
16/2/70
TIME (WEEKS)

FIGURE 6.2.

Results of a laboratory experiment on depth of dormancy.

Dry Body Weight/Dry Shell Weight (means of samples of 5, with 95% confidence limits) plotted against time.


(i) Dormant at c.28°C.
(ii) Sprayed 2 days in every 14 days. No food.
(iii) Sprayed 2 days in 14, with fishfood.
DRY BODY WEIGHT ÷ DRY SHELL WEIGHT × 100
90
80
70
60
50
40
30
20
10
0
2
4
6
8
10
12
24/11/69
TIME (WEEKS)
16/2/70

show up a little more clearly using the measure of dry body weight/ dry shell weight than using uncorrected dry body weights.

I do not think that any further statistical analysis is needed to answer the main question, Can snails feed when they are briefly awakened from dormancy? It is clear from Figure 6.2 that there is no significant difference between treatments at the start but that by the end of the experiment all three treatments differ at probability-levels lower than 2.5%, because their 95% confidence limits do not overlap.

One gains the same impression from Figure 6.1, although at the end of the experiment 95% confidence limits for the means in treatments (i) and (ii) still overlap, and a t-test shows that the difference between the means of dry body weight in treatments (i) and (ii) at 12 weeks is not significant ($t_8 = 0.378$; $0.5 < P < 0.9$).

It seems clear that snails in treatment (i) lost very little weight*, snails in treatment (ii), which were periodically induced to crawl about without food, lost a little more, and snails in treatment (iii), given food, gained weight.

On a number of occasions I have observed snails which were woken by summer rains appearing to feed. On one occasion in December 1970, snails had been dormant for some time, there had been several hot days, and then it rained. On this day I observed that snails at four different places in the Adelaide suburbs appeared to be feeding

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* Pomeroy (1966) and Hodson (1969) found this in dormant snails. I shall not discuss this here. Hodson does so in some detail.

and I collected five snails from each place and put them in tubes with wet filter paper. All of them produced faeces of dead plant material. In the field, as in the laboratory, H. virgata appears able to feed as soon as it is woken from summer dormancy.

Thus, I must conclude as Hodson did that 'It would be wrong ... to conclude that dormancy was obligatory in Helicella virgata during the summer, and the observation that in the laboratory, H. virgata cannot be prevented from entering dormancy under conditions of moisture, constant temperature and in the presence of food is not relevant to the situation in the field during summer.'

I think that the laboratory observation might indeed be partly due to the presence of food. In my experiment in which snails became inactive under the sprays in November (beginning of Section 6.2.) they had been feeding on fishfood for a week when I noted the drop in activity. In my preliminary experiments using Y-tubes I found that snails fed well on fishfood were slow to emerge (Section 5.2.1; Table 5.1). Finally, I conducted an experiment concurrently with the above experiment, in summer, in which three groups of snails were fed respectively with (i) a 15-cm sheet of filter paper, (ii) a filter paper with c. 0.5 g of fishfood sprinkled on it and (iii) a filter paper with c. 5 g of fishfood sprinkled on it. Samples were taken on 6 occasions up to 24 days for drying and weighing.

The snails given fishfood grew. The ones in treatment (iii) grew faster than those in treatment (ii), but their weight increased very little after 10 days whereas those in treatment (ii) continued to increase in weight, at least until 16 days.

I scored activity by assigning subjective ratings (1, 2 or 3) to the number of faeces produced. After 5 days, the score had dropped to rating 1 in treatment (iii) and to rating 2 in treatment (ii). In treatment (i) the snails did not gain weight, and there was no diminution during 24 days in the number of faeces they produced; it stayed at rating 3.

I think we can conclude that this inactivity in the laboratory is an artefact, possibly resulting from satiation with a very rich food. We can conclude that dormancy is not obligatory and that snails woken by rain in summer would be able to feed if food were available to them. It has not been investigated whether there is very often likely to be food which is both palatable and nutritious available to snails in summer.

7. THE ABUNDANCE OF FOOD

7.1 INTRODUCTION

The scope of this project was to study the importance of food in the ecology of Helicella virgata. Food is obviously part of the component of environment which Andrewartha and Browning (1961) called 'resources' and discussed at some length; and the question we generally want to ask about resources is whether or not they are likely to be in short supply. That is the question of this chapter.

One obvious approach to answering the question is to find out what the resource is, to find out how much of it an animal needs in order to perform its various functions, and then to go into the field and measure how much of the resource is present. This approach is implicit in the discussion of food by Boycott (1934), who discussed numerous observations of the feeding habits of terrestrial molluscs, noted that the kinds of things they eat appear to be abundant, and concluded that 'food has no influence either by its quality or quantity on the recurrence of our land Mollusca, excepting Testacella and such meagre habitats as shifting sand-dunes.' (emphasis Boycott's)

Such an approach would be particularly useful if one clearly-defined resource could be identified - and of course such an approach was implicit in the choice of the hypotheses I tested in Chapters 3 and, to some extent, 4 and 5, where I looked for specific dietary requirements or specific stimulants to feeding. But in the absence

of a clearly defined resource this approach could be very difficult, or misleading, because the idea of 'food' could cover various materials differing in their abundance and their importance to the animal; and my findings in the earlier chapters suggest that this is the case with H. virgata.

In these circumstances an approach different from the above is better, at least initially. That is to increase the density of animals in the field and try to assess whether they show any signs of a shortage of food.

Eisenberg (1966, 1970) has studied the pond snail Lymnaea elodes in this way. He confined snails at various densities in pens in a pond in Michigan and found that whilst this only had a small effect on the size to which the adults grew, and none on their mortality, it had a very marked effect on the numbers of eggs and young produced per adult snail. In the experiment reported by Eisenberg (1966), adult densities had been changed to 1/5 and 5 times the natural density in the pond in spring (the first half of June) yet when the snails were aestivating in fall (September - October) the numbers of young in the different pens were found not to differ significantly.

Addition of food, in the form of frozen spinach, seemed to remove the effect of density. The snails in the fed pens grew larger, produced larger egg-masses and produced an enormously greater total number of eggs than those in unfed pens.

Eisenberg interpreted his results to mean that

> this snail is existing at two distinct levels in regard to food. At the first level there is a requirement, which apparently is met quite easily, for what might be called "maintenance nutrition." At this level the snails do not grow particularly well and are barely capable of reproducing, but at the same time they do not die. At the second level there is a more or less open-ended requirement, which apparently is not met easily, for what might be called "positive nutrition" which allows the organism to realize some portion of its potential for growth and reproduction.

He calculated that only a few milligrams (dry weight) of spinach were available per snail per day in his fed pens, and suggested that 'the spinach additions were not supplying the snails their grosser nutritional needs but perhaps something of the nature of an accessory growth factor like a vitamin or a mineral.'

I have already mentioned the possibility that certain snails may require both plant and animal material in their diet and that whilst the former may be abundant the latter may not; the snails seem to have chemical senses adapted to this situation (Bovbjerg, 1965, 1968; and see Sections 3.1, 5.1 of this thesis). Eisenberg's Lymnaea elodes was one of the snails studied by Bovbjerg (1968). It is tempting to surmise that the need for animal food discovered by Bovbjerg might have been met by spinach in Eisenberg's experiment - that is, that Eisenberg's 'maintenance nutrition' corresponds to Bovbjerg's 'plant food' whilst Eisenberg's 'positive nutrition' corresponds to Bovbjerg's 'animal food'.

The different qualities of foods postulated by Eisenberg could well be different types of plants, for Calow (1970) showed that

Lymnaea pereger prefers epiphytic organisms to macrophytic tissue, and green filamentous algae to diatomaceous species. It assimilates green filamentous algae better than diatoms.

Thus, there is reason to think that 'food' for snails might include more than one resource, and that the abundance of the different parts of 'food' might differ.

[I cannot agree with Eisenberg's interpretation of his results as demonstrating a negative feedback mechanism. Whilst they are consistent with such an hypothesis they are also consistent with the hypothesis that there is a constant supply of some resource, all of which is used up to produce a certain number of young regardless of the number of adults producing them. This mechanism, although it would keep the population at a certain size and would produce inverse relationships between adult density and number of offspring per adult, is not negative feedback. The experiment cannot distinguish between the two kinds of mechanism.]

In this chapter I describe two experiments similar to Eisenberg's (and also mention one of Pomeroy's). I do not consider them tests for 'regulation' of the population-density of H. virgata. The abundance of the snails will obviously be influenced by a number of factors. Food may be one, and my concern has been simply to try to determine whether food is a resource likely to be in short supply for H. virgata and, if so, what might be the nature of the shortage.

Pomeroy (1966) conducted an experiment at Buckland Park in which he penned snails at artificially-adjusted densities of about 56, 111, 278 and 556 snails per m^2. The natural density outside the cages

was about 120/m^2. In four of his eight cages he removed the litter and the topsoil to a depth of about half a centimetre. He found that adults (most of which can be expected to die after reproducing in winter) died more quickly the higher the density, and he found that the length of life of adults was shorter in pens with the litter removed. But the snails in the most crowded pens lived for three-quarters of the time of those in the least crowded pens (c.f. Eisenberg's finding that crowding had little effect upon adult mortality).

Pomeroy marked the young snails that were present in early September and subsequently measured their shell-growth. He found less growth at higher density, and less growth with litter removed.

Pomeroy interpreted his results as indicating a 'relative shortage' of food. He considered an absolute shortage unlikely in nature because there must always be some organic matter in the soil; but a relative shortage would occur if its density were so low that the effort needed to obtain the food exceeded the nutritional value gained from it. This suggestion seems reasonable to me, but he goes on to say without evidence, ' ... relative shortages may occur often. They will be the special sort of relative shortages characteristic of grazing animals: the snails influence the chances to survive and multiply of the organisms on which they feed.' If one is to guess about the influence of H. virgata on its food-supply, surely a better guess would be to class the animal as a saprophage and assume that, as such, it will have little influence on the rate of supply of its food, which will depend on the rate at which litter falls from plants and on other factors beyond the influence of snails. (Wiegert

and Owen, 1971).

Pomeroy (1969) now explains the results of his experiment, which I have mentioned above, in terms of an absolute shortage of food, 'because the cages were small in comparison to the area which a snail may search.' I think this a more reasonable explanation than the idea of a relative shortage, not because of the size of the cages but because of the nature of the results.

If snails experience purely a relative shortage of food, of the kind experienced by the mosquito larvae which Andrewartha and Browning(1961) used as an example - that is, a shortage due to the food being abundant but too 'dilute' - then their performance should be no different if there are more of them. If, on the other hand, there is food available which is better in quality, but in absolutely short supply - that is, food which the snails can find and of which there is not enough to go round - then we might expect the results obtained by Pomeroy.

I think that it is reasonable to expect both kinds of shortage to affect H. virgata because I think it is reasonable to consider 'food' as not being homogeneous. Food of poor quality - such as dead grass-leaves - may be abundant. Given this alone, snails would experience a relative shortage of the 'mosquito larva' type. There may also be food materials of high quality - either simply because they are more concentrated or because they contain different nutrients, such as particular vitamins - and these may be in absolute shortage.

This is the kind of situation postulated by Eisenberg to explain his results, and it would be consistent with Pomeroy's results.

Indeed, Pomeroy's findings look very much like those of Eisenberg, although Pomeroy did not present data on the numbers of young produced which could be compared with Eisenberg's.

I describe in Section 7.2 an experiment like that of Pomeroy, which I conducted at Northfield in 1970. I attempted to confirm Pomeroy's results and also to obtain more data on growth and reproduction. There were two broad kinds of outcome possible:

Firstly, I might have found no effect of density. This would have been inconsistent with the hypothesis that there is an absolute shortage of food at the densities used. It would not have told anything about a relative shortage of the 'mosquito larva' kind.

Secondly, I might have found inverse relationships between density and some of the population parameters, such as mortality or egg-production. Such a result would have been consistent with the hypothesis that some part of the animals' food was in absolutely short supply, although not necessarily the whole of it.

It would also have been consistent with the hypothesis that some effect of crowding, quite independent of food, inhibited snails at high density. Such effects are, of course, known in a number of animals including certain snails: Berrie (1970) cites work showing that growth-rate and fecundity of snails that are intermediate hosts for African schistosomes are reduced if the animals are kept at high density. The inhibition appears likely to be due to some chemical substance secreted by the snails.

Some information, though not conclusive, regarding this possibility could have been obtained by supplementing the food in some cages. I did not use supplementary food in the 1970

experiment because the experiment was conducted in large pens containing many snails and I could not cope with a duplicate set of pens. I thought it better to have replicates of the unfed treatments than to use supplementary food and have no replication.

The 1970 experiment had been conducted at Northfield because Buckland Park is prone to the attentions of vandals, but the area which once supported a large population of snails seems to have become less suitable for them as Oxalis has invaded it. Also, I had to use snails imported from Buckland Park, with the obvious risk of their being ill-adapted to the different conditions. Finally, cows broke into the area and trampled some of the cages. For these reasons the experiment was unsatisfactory and I decided to repeat it at Buckland Park in 1971. I used smaller cages and fewer densities and this enabled me to use three different food-supplements as well as one set of cages without the addition of food. This experiment is described in Section 7.3.

In Section 7.4 I discuss the interpretation of the results of these experiments.

7.2 CROWDING EXPERIMENT - NORTHFIELD

PROCEDURE

In his experiment which I mentioned in Section 7.1, Pomeroy used densities of c. 56, 111, 278 and 556/m^2. Natural density on that area at that time was c. 120/m^2. The maximum density recorded by Pomeroy at Buckland Park was about 250/m^2. The maximum density recorded by Hodson (1969) at Northfield was about 120/m^2 although when I set up my experiment it was only about 10/m^2. So I chose the densities of 10, 40, 160 and 640/m^2 which should have been a wide enough range to demonstrate an absolute shortage of food if it had any ecological relevance at all.

I used 8 pens of area c. 4.65m^2, two for each density, and in order to approximate the above densities I enclosed in the pens the numbers of snails shown in Table 7.1.

TABLE 7.1

Densities used in the crowding experiment, Northfield, 1970.

Treatment	Proposed Density snails/m^2	Proposed density converted to snails/pen	Actual number of snails used per pen.
(i)	10	46.5	45
(ii)	40	186	180
(iii)	160	744	720
(iv)	640	2976	2880

The study area at Northfield and the pens are described in Appendix 3. The treatments were completely randomly assigned to pens.

I collected a large number of unbanded snails all between 9mm and 15 mm in diameter at Buckland Park on 3, 4, 5/4/70, because there were not enough snails to be found at Northfield. They may well have been ill-adapted to conditions at Northfield, but this should have influenced them all in a similar way, and so the experiment on the effect of density is still a valid one although it must be remembered that it has the status of a laboratory experiment rather than that of an experiment conducted in the natural habitat of these particular snails.

From the large collection I took haphazardly 630 and assigned them randomly to 14 lots of 45. The snails in 8 of these lots - one for each pen - were individually numbered and marked on the lip of the shell, so that their weights could be measured repeatedly and their shell growth recorded. Those in the other 6 lots were simply marked with a spot of nail-varnish so that they could be recognized as ones introduced for the experiment. These 6 lots were randomly assigned one to each of the pens in treatments (ii) - (iv).

To make the numbers up to those in Table 7.1 it was necessary to add 90 snails to each pen of treatment (ii), 630 to each pen of treatment (iii) and 2790 to each pen of treatment (iv). To distribute them using random numbers would have been too laborious, and the 7020 snails were distributed by a procedure like 'dealing' in cards.

The snails were placed into the pens on 9/4/70.

I used the marked snails to obtain data on growth and survival of adults. On each of four occasions (15/4, 29/4, 12/5 and

26/5/70) I searched the pens in a random order for 30 minutes (on the third occasion, 45 minutes) a pen, collecting all marked snails I found. I took them back to the laboratory, about 7 miles away, in large plastic food-containers with holed covers, weighed them in a random order measuring their shell-growth at the same time, kept them without spraying at about 20°C overnight and returned them to their pens next morning.

Shell-growth was measured in this experiment as millimetres of circumference added, instead of the usual angular measurement. This was done by placing the snail with its painted mark against a datum line on a millimetre rule and then rolling the shell along the rule until its lip touched the rule, giving a measure of the increase in circumference since the shell was painted. This was recorded to the nearest 0.5 mm. This measure of shell-growth cannot easily be compared with the units used elsewhere in the thesis but that is unimportant here; different treatments within this experiment can be compared.

I found, as Pomeroy (1966) did, that adult snails did not grow very much in their second winter, and growth seemed to have ceased by 26/5/70. That is one reason why I discontinued the fortnightly weighings at this time.

Mortality in the different treatments could be compared simply by recording the number of those marked snails collected which were dead. Herein lies another reason for discontinuing weighing - the proportion dead in the crowded cages by 26/5/70 was very high.

The snails in the plastic pots waiting to be weighed generally crawled around a little and then, on withdrawing, defaecated.

I collected all the faeces produced on 29/4, 12/5 and 26/5/70. Microscopic examination showed nothing unusual in them and I could detect no differences in appearance between the faeces from different pens. But the quantities of faeces were markedly different. I kept them in formalin and later dried them in the vacuum unit and weighed them.

The most interesting variable, fecundity, was also the most difficult to measure. H. virgata lays its eggs in the soil at a depth of 1 cm or a little more. Snails spend some time with their heads buried in the soil, laying, so on a winter's day when conditions are suitable for activity it is often possible to find snails laying. When they remove their heads from the soil, the walls of the hole generally collapse but do not always entirely cover the clutch of eggs. Thus it is possible to find some clutches merely by inspecting the surface of the ground.

I proposed to sample for eggs by taking cores of soil and sieving them, but found that the number of quadrats I could handle would be too few. They would detect few eggs and so leave room for wide random variations. Instead, on one morning (6/5/70) a week after I had first found eggs in a pen of treatment (i), I searched the floors of the pens for clutches. I searched each pen in the same systematic manner, allowing 15 minutes per pen. The order of the pens was random and most of the snails were inactive throughout the search, so there is unlikely to be any bias due to snails laying during the search.

I also counted the numbers of snails found laying when I searched for marked snails to weigh them; but these searches took a long

time and some of the days were sunny, so that snails may have been laying when I searched the first pens but inactive by the time I searched the last. Worse, I tended to look only at marked snails on these occasions. These data are therefore biased and I have not included them.

I intended to repeat the search for clutches of eggs later, but terminated the experiment for reasons given below.

If we assume that in each pen I discovered the same proportion of those clutches which were present, then even though that proportion is unknown the numbers of clutches found in different treatments can be validly compared. The major fault in this procedure is that I have no estimate of the number of eggs per clutch. I could have dug out clutches of eggs for counting but decided that it would be better to leave them to hatch, and later to count the number of young in each pen. This proved an unfortunate decision because, before the young were large enough to be counted, some of the cages were damaged by cows and later by, probably, cats. This damaged the vegetation, crushed a number of snails and permitted many escapes. It would have been of little value to count young after this, or to search again for clutches, although I did once more weigh the marked snails which could be found.

RESULTS

(1) Changes in Weight

As discussed in Appendix 1, the best way to analyse my data on live-weights is to calculate the change in weight of each snail from the start to some later date. In this experiment, since changes

in weight over different time-intervals are of interest, I have calculated the changes from 15/4/70 to each of the three later dates - that is, changes over 2, 4 and 6 weeks respectively.

I did not find all the marked snails in a given pen on any one date, and the data for a particular pen over different time-intervals do not refer to the same snails. Descriptive statistics of the changes in weight of marked snails over each of the three time-intervals are shown in Table 7.2.

In the case of the changes over the initial two weeks, the sample variances are significantly heterogeneous. Although this is not so for the changes over 4 and 6 weeks, I have adopted the procedure of taking the cage-means - 2 for each density - as raw data from which to calculate linear regressions of weight-change on log density. The regressions are significant for changes over two weeks ($0.005 < P < 0.01$) and six weeks ($0.025 < P < 0.05$) but not for changes over four weeks ($0.10 < P < 0.25$). The cage-means and the calculated regression lines are plotted in Figures 7.1A, B and C.

There were gains in weight in all pens over the first two weeks - greatest in the least-crowded pens, and the regression was significant. Over the second two weeks there were losses in weight, greatest in the least crowded pens. This can be shown by calculating weight-changes from 29/4 to 12/5/70 but, as pointed out in Appendix 1, this could be misleading. It is better shown by the fact that in the changes over 4 weeks the Y-intercept is lower and the slope of the regression line is not significantly different from zero. Over the

TABLE 7.2

Changes in weight (mg) of numbered snails recaptured alive on 15/4/70 and again 2, 4 or 6 weeks later in an experiment on the effect of crowding at Northfield. (n = sample size, $\bar{y}$ = mean, $s/\sqrt{n}$ = standard error.)

Time-Interval		Density (i) 10/m² A	(i) 10/m² B	(ii) 40/m² A	(ii) 40/m² B	(iii) 160/m² A	(iii) 160/m² B	(iv) 640/m² A	(iv) 640/m² B
2 wks	n	15	19	23	21	14	14	19	16
15/4–29/4	$\bar{y}$	63.26	82.38	58.73	56.39	8.16	49.58	8.68	25.13
	$s/\sqrt{n}$	13.78	7.73	6.25	4.65	5.82	10.23	4.51	9.17
4 wks	n	14	17	16	26	6	8	8	6
15/4–12/5	$\bar{y}$	9.81	46.94	14.98	35.67	−25.83	23.54	−6.73	12.36
	$s/\sqrt{n}$	14.02	8.97	9.19	6.28	21.66	18.20	7.29	8.51
6 wks	n	16	13	14	16	2	7	0	2
15/4–26/5	$\bar{y}$	−10.16	33.62	19.47	12.43	−41.65	−21.94	–	−40.65
	$s/\sqrt{n}$	11.71	9.92	11.00	7.60	9.70	14.69	–	26.20

2 wks: F max (8, 14) = 7.39** ($P \approx 0.01$)

4 wks: F max (8, 5) = 6.61 N.S. ($P > 0.05$)

6 wks: F max (5, 15) = 2.37 N.S. ($P > 0.05$) (Considering only samples of size ≥ 7.)

FIGURE 7.1

Weight-change in snails kept at four densities

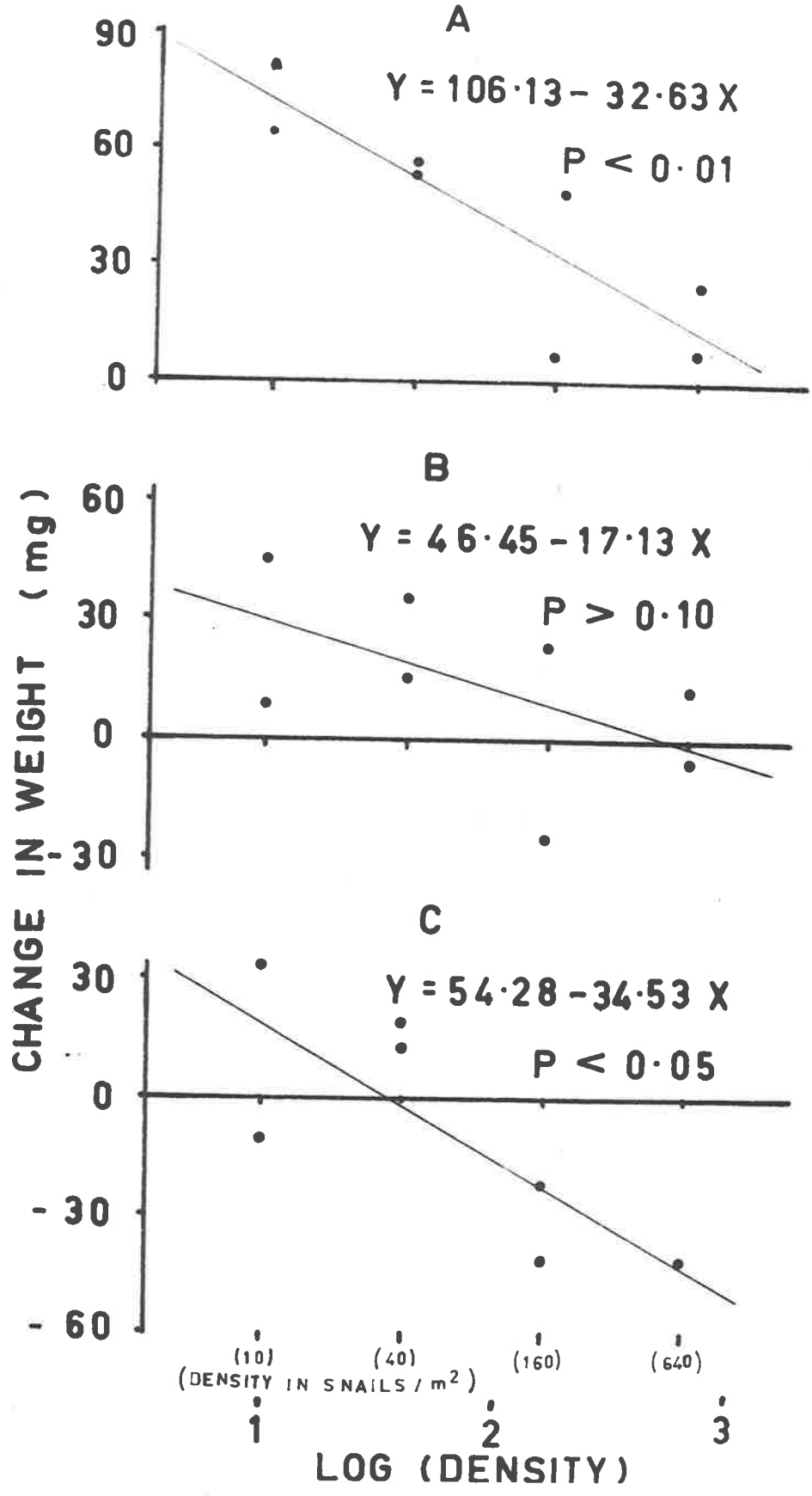
Y = mean change in weight (mg)

X = log (density)

A. changes over 2 weeks

B. changes over 4 weeks

C. changes over 6 weeks


A
Y = 106·13 – 32·63 X
P < 0·01
90
60
30
0
B
Y = 46·45 – 17·13 X
P > 0·10
60
30
0
- 30
C
Y = 54·28 – 34·53 X
P < 0·05
30
0
- 30
- 60
CHANGE IN WEIGHT (mg)
(10)
(40)
(160)
(640)
(DENSITY IN SNAILS / m²)
1
2
3
LOG (DENSITY)

third two weeks there appear again to have been losses of weight in all treatments, but rather greater losses in the most crowded pens, so that the changes over six weeks show small gains only in the least crowded pens and a regression line with a significant negative slope. The possible interpretation of these changes will be discussed below.

(2) Growth of Shell

Table 7.3 shows descriptive statistics of the increases in shell-circumference in marked snails recaptured after almost 3, 5 and 7 weeks.

(The snails had been caged for 6 days when first collected for weighing. Although I have not analysed and presented it here, there was some shell-growth by the first weighing on 15/4/70. Thus shell-growth recorded on 29/4 is growth over not 14 but 20 days, whereas weights taken on 29/4 could only give information about growth over 14 days.)

As for changes in weight, I have used the cage-means as raw data to calculate analyses of variance, taking out linear regressions of shell-growth on log density. As an inspection of Table 7.3 indicates, there was very little shell-growth after the first three weeks, with the result that the analyses of growth over 5 and 7 weeks give results very similar to that of growth over 3 weeks.

The three regression equations, none of which has a slope significantly different from zero, are:

3 weeks: $Y = 5.55 - 1.71 X$

5 weeks: $Y = 4.44 - 1.06 X$

7 weeks: $Y = 4.49 - 1.02 X$ where Y = shell-growth in mm

TABLE 7.3

Increases in circumference of shell (mm) of marked snails recaptured alive after 3, 5 and 7 weeks in an experiment on the effect of crowding at Northfield.

(n = sample size, $\bar{y}$ = mean $s/\sqrt{n}$ = standard error).

Time-interval		Density (i) $10/m^2$ A	(i) $10/m^2$ B	(ii) $40/m^2$ A	(ii) $40/m^2$ B	(iii) $160/m^2$ A	(iii) $160/m^2$ B	(iv) $640/m^2$ A	(iv) $640/m^2$ B
3 wks	n	32	36	36	33	34	33	30	34
(recaptured 29/4)	$\bar{y}$	5.17	3.49	2.83	1.35	0.35	3.26	1.50	0.50
	$s/\sqrt{n}$	0.845	0.497	0.494	0.296	0.098	0.569	0.341	0.123
5 wks	n	28	33	28	36	19	20	15	14
(12/5)	$\bar{y}$	3.89	3.50	2.80	1.26	0.47	4.58	2.30	0.50
	$s/\sqrt{n}$	0.760	0.580	0.637	0.405	0.185	0.846	0.449	0.189
7 wks	n	28	23	23	24	3	18	2	7
(26/5)	$\bar{y}$	4.41	3.59	3.28	0.98	0.50	3.67	3.00	0.93
	$s/\sqrt{n}$	0.860	0.714	0.767	0.321	0.500	0.739	1.50	0.400

2 weeks: F max (8, 31) = 69.29** (P<0.01)

4 weeks: F max (8, 13) = 32.35** (P<0.01)

6 weeks: F max (5, 23) = 8.37** (P<0.01) (considering the samples of size >7)

and X = log density. (Density in units of snails/m^2)

Thus, although there are signs of a trend for snails to grow less shell at higher densities, this is not significant.

(3) Mortality

No marked snails were found dead on 29/4, after almost 3 weeks, but there were some dead on 12/5 and large numbers dead in some pens on 26/5. Table 7.4 shows the total number of marked (numbered or not) snails found in each pen on each of these two dates, and the proportion of these which were dead.

Dead snails were not returned to the cages. Thus, subject to errors in searching, the top half of Table 7.4 shows estimates of the proportions of snails having died between 29/4 and 12/5 whilst the bottom half shows estimates of the proportions, of those still alive on 12/5, which had died by 26/5 in the different pens.

It appears that the proportion found dead increases with density, and this was tested by calculating Kendall's coefficient of rank correlation, τ (Siegel, 1956), separately for each date. The values were: 12/5/70: $\tau = 0.926^{***}$ $P < 0.001$

26/5/70: $\tau = 0.617^{*}$ $0.025 < P < 0.05$

The correlation is significant on both dates.

(4) Production of Faeces

The dry weight of faeces voided by the marked snails (both numbered and not) collected from each pen on 29/4, 12/5 and 26/5, divided by the number of snails producing the faeces, gives the weight of faeces per snail, shown in Table 7.5.

Most of the snails had withdrawn into their shells and produced

TABLE 7.4

Number of marked snails found (n) and the proportion of these that were dead (p) in each pen of an experiment on the effect of crowding at Northfield.

Density		(i) 10/m^2		(ii) 40/m^2		(iii) 160/m^2		(iv) 640/m^2	
Date / Pen		A	B	A	B	A	B	A	B
5 weeks	n	28	33	49	65	40	43	50	34
12/5/70	p	0.00	0.00	0.00	0.015	0.175	0.023	0.400	0.235
7 weeks	n	30	24	55	47	18	31	19	27
26/5/70	p	0.033	0.042	0.073	0.00	0.778	0.065	0.895	0.593

TABLE 7.5

Dry weight (mg) of faeces produced per snail by the n marked snails collected on each of 3 dates from each pen of an experiment on the effect of crowding at Northfield.

Date	Density / Pen	(i) $10/m^2$ A	(i) $10/m^2$ B	(ii) $40/m^2$ A	(ii) $40/m^2$ B	(iii) $160/m^2$ A	(iii) $160/m^2$ B	(iv) $640/m^2$ A	(iv) $640/m^2$ B
29/4	n	32	36	70	68	61	58	60	62
	mg/snail	3.41	7.50	7.37	5.42	1.11	7.08	0.97	1.51
12/5	n	28	33	55	64	31	42	30	25
	mg/snail	6.00	10.23	6.33	5.87	0.26	7.10	0.46	0.19
26/5	n	28	23	51	47	4	29	2	11
	mg/snail	6.86	18.93	5.10	7.41	0.21	3.26	4.13	0.35

faeces by the time I weighed them and collected the faeces from the pots. I always collected faeces from the apertures of the snails. Weights of faeces from pots weighed early in any given weighing-period are likely to be under-estimates because some animals will not have defaecated. But the order of weighing was random so this cannot bias a comparison between treatments - it merely increases random error.

Table 7.5 can be considered as a 4 x 3 table with two replicates per cell. Taking it that the assumptions of analysis of variance are met by these data, I first carried out a two-factor AOV, mainly to determine whether it was justifiable to pool the data for the three dates and so obtain larger samples for a test of the correlation of faeces/snail with density. The analysis showed a significant variance due to densities ($0.01 < P < 0.025$) but none due to dates or to interaction between dates and density (both gave $0.5 < P < 0.75$). Accordingly I treated Table 7.5 as a one-factor table containing 4 samples of 6 replicates. To show the trend more clearly, descriptive statistics of these samples are shown in Table 7.6.

TABLE 7.6

Mean ($\bar{Y}$) and standard error ($s/\sqrt{n}$) of the six values of 'mg of faeces per snail' for each density in Table 7.5.

Density	$10/m^2$	$40/m^2$	$160/m^2$	$640/m^2$
$\bar{Y}$	8.82	6.25	3.17	1.27
$s/\sqrt{n}$	2.21	0.40	1.32	0.61

$F_{max}(4, 5) = 30.87^{**}$; $P < 0.01$

Inspection of Table 7.6 suggests a monotonic association between density and faeces/snail. Parametric regression analysis should not be used with heterogeneous variances: no simple relationship is apparent between variance and mean, such that it could be removed by a transformation. Therefore I have calculated Kendall's coefficient of rank correlation:

$$\tau = -0.565 \; ; \quad P < 0.00007.$$

Thus the trend for the production of less faeces by snails kept at higher density is highly significant.

(5) Production of clutches of eggs

My data on egglaying are scanty and I do not wish to draw firm conclusions on their basis. Nevertheless, the numbers of clutches found in the search on 6/5/70 are of interest. They are shown in Table 7.7, along with values of 'clutches per snail' obtained by dividing the number of clutches found in a pen by the number of snails initially confined there.

TABLE 7.7

Numbers of clutches of eggs found in the eight pens of an experiment on the effect of crowding

Density	(i) $10/m^2$		(ii) $40/m^2$		(iii) $160/m^2$		(iv) $640/m^2$	
pen	A	B	A	B	A	B	A	B
Clutches	10	1	4	4	6	9	6	5
Clutches/ snail	0.2222	0.0222	0.0222	0.0222	0.0083	0.0125	0.0021	0.0017

The numbers of clutches found seem similar at different densities. My searching was, as near as I could make it, equally thorough in different pens. Assuming the same proportion of clutches to be visible in different pens, and if the same proportion of snails had laid in different pens, I should have found more clutches in more crowded pens. The number of clutches per snail appears to be negatively correlated with density, and indeed Kendall's

$\tau = -0.898^{**}$; $0.001 < P < 0.01$.

DISCUSSION

The results point convincingly to an effect of density on the chance of snails in this experiment to survive and multiply. By 29/4/70 - it is not known how much sooner than that - the snails at high density were producing less faeces per head. It seems a reasonable assumption to take it from this that they were eating less by 29/4.

Consistent with this inferred difference in feeding, there was a difference in mortality by 12/5 and later, such that significantly more animals died at high densities.

Two other variables show similar trends but should not be interpreted strongly: Firstly, shell-growth appeared less at high density, but the snails, adults in their second winter, mostly grew shell only early in the experiment and the regression of shell-growth on log density was not significant. Secondly, in one search similar numbers of clutches of eggs were found in different cages, so that the number of clutches per snail was significantly negatively correlated with density.

Finally, the most extensive data are on changes in weight. For changes over 2 and 6 weeks there are significant, negatively-sloped regressions on log density.

It must be noted that in these field measurements water content of the snails may vary more than in laboratory trials, because of differences in the weather at different times. Thus differences between treatments, and changes in one treatment with time, might reflect differences in water-content.

However, the results can also be interpreted as indicating changes in tissue-weight. To confirm this, we have differences in shell-growth, though they were not significant, and differences in mortality. It seems reasonable to tentatively postulate a process like the following (with reference to Figure 7.1):

Snails in the more crowded pens ate less than those at low density. Because of this, over the first two weeks snails at low density could grow more, or build up more reproductive tissue, and as a result we find a greater increase in weight at the lower densities. Over the second two weeks many snails laid eggs. The resultant loss of weight was again greatest in the lowest densities where more snails laid eggs, so that in the changes over four weeks we find rather less difference between treatments. There seem to have been losses of weight in all treatments over the third two weeks, rather greater at high density - perhaps these snails tended to be laying eggs later - and finally we might consider the changes in weight over six weeks as being the resultant of growth, accumulation of reproductive products and subsequent egglaying, the whole process having produced little weight-change at low density

but considerable decrease at higher densities.

Now, there is no information as to why the snails at high density seem to have eaten less. There are several hypotheses that seem likely:

(1) Material recognized as food may have been scarce (though this was not apparent to me on inspection of the faeces and of the abundant litter on the floors of the pens). Evidence presented in Sections 4.2.2 and 6.2 suggests that if good food were available the snails would have been able to eat it even if they had been short of food or dormant for a while. The results of the present experiment can throw a little more light on the hypothesis of a shortage of food. If there were an absolute shortage of some high-quality food, and if this material were essential for growth and reproduction, then we might expect the total amount of growth and reproduction to be the same in all treatments. This rather looks to be the case with production of clutches of eggs, but the data are scanty.

For growth, I have taken the average change in weight per snail in each pen over the first two weeks (Table 7.2) and multiplied it by the number of snails confined in the pen. I have multiplied the corresponding standard error by the number of snails in the pen to obtain a standard error for this estimate of 'total weight-change.'*

---

*It is a theorem of mathematical statistics that if a sample of values Y_1, Y_2 ...Yn has mean $\bar{Y}$ and variance s^2 then the values KY_1, KY_2, ...KYn (where K is a constant) have mean $K\bar{Y}$, variance K^2s^2 and therefore standard error Ks.

I used data for the first two weeks because egglaying had not yet complicated the picture. The results are shown in Table 7.8.

TABLE 7.8

Calculated 'total weight-change' (mg) over 2 weeks in each pen of an experiment on crowding at Northfield. Data derived from Table 7.2 (see text).

Density	$10/m^2$		$40/m^2$		$160/m^2$		$640/m^2$	
Pen	A	B	A	B	A	B	A	B
Total Wt. change	2847	3707	10,571	10,150	5,875	35,698	24,998	72,374
S.E. Total Wt. change	620	348	1,125	837	4,190	7,366	12,989	26,410

Although the standard errors are large, it appears that a greater total amount of weight was added in the crowded pens, and the simple model of an absolute shortage of completely accessible food seems inadequate.

Several variations on the hypothesis can be suggested, such as that there is a continuous gradation in quality of food, the crowded snails having to make do with a proportion of their diet consisting of poorer-quality but not completely useless food.

(2) Another possibility is that a pen contains abundant food of homogeneous (not necessarily very high) quality, but that part of it

is made unpalatable to snails simply by other snails crawling in it. Thus the snails may create an absolute shortage but it could be a shortage such that the amount still palatable in the crowded pens is greater than the amount eaten in the uncrowded pens.

(3) Finally, the crowded snails may have eaten less not because food was scarce but because of some other mechanism which inhibits their activity when they contact many other members of their species.

7.3 CROWDING EXPERIMENT - BUCKLAND PARK

The 1971 experiment was a 'two-factor' experiment in which density was one factor. I chose three densities, 50, 150 and $450/m^2$. On the basis of Pomeroy's results (Section 7.1) and my 1970 results (Section 7.2) I expected to find in cages without supplementary food some negative correlation between density and such variables as growth-rate and production of eggs.

The second factor, chosen to throw some light on the reason for the negative correlation, was 'supplementary food' and I used four 'levels' of this factor;

(i) no food added

(ii) fishfood added *ad lib*.

(iii) a limited amount of fishfood added so that at least in the crowded pens it was all consumed before I added more

(iv) dead leaves of the wild artichoke, *Cynara cardunculus*, added *ad lib*.

The reasons for choosing these supplements can be explained by reference to Figure 7.2, which represents graphically a rather simplified version of the kinds of results that might have been expected given various hypotheses. The results are represented as plots of 'performance' - which might be growth-rate, fecundity or survival - against density.

Firstly, suppose a result had been obtained with supplement (i) like that of Pomeroy and of my 1970 experiment at Northfield. Then one hypothesis to explain it would be that there was an absolute shortage of food - possibly an absolute shortage only of some part of

FIGURE 7.2

Graphical representation of the results expected from the 1971 crowding experiment given various hypotheses (see text).

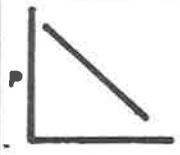
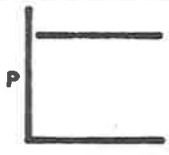
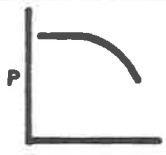
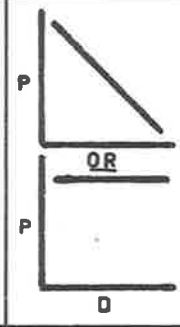
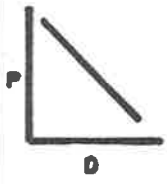
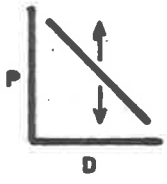
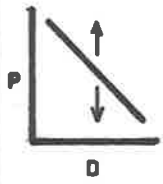
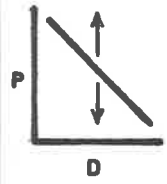
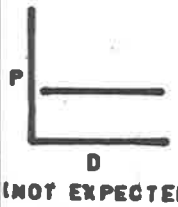
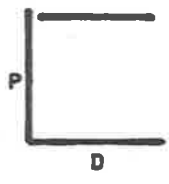
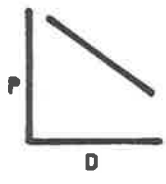
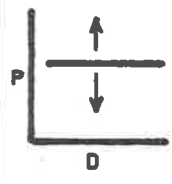
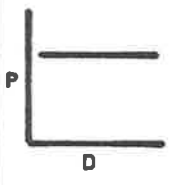
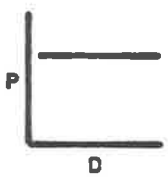
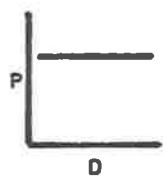
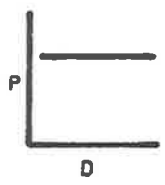
P = "performance"; D = density.

Hypothesis 1. Absolute shortage of some part of food.

Hypothesis 2. Crowding is inhibitory but not through food.

Hypothesis 3. No absolute shortage or inhibitory effect of crowding but a 'mosquito larva' type of relative shortage: food abundant but of poor quality.

Hypothesis 4. None of the above : food is abundant and its quality just as high as that of fishfood or dead Cynara leaves.

FOOD-SUPPLEMENT → / HYPOTHESIS ↓	(i) NO FOOD	(ii) AD LIB FISHFOOD	(iii) LIMITED FISHFOOD	(iv) AD LIB DEAD LEAVES OF CYNARA
1	 (THE 1970 RESULT)			
2	 (THE 1970 RESULT)			
3	 (NOT EXPECTED GIVEN PREVIOUS RESULTS)			
4	 (NOT EXPECTED)			

the snail's food, as postulated by Eisenberg to explain his findings (Section 7.1). The top row of Figure 7.2 indicates the expected results with the different food-supplements given this hypothesis, and assuming that fishfood supplied the requirement which was in absolute shortage. The effect of density should have been removed by supplement (ii), and removed by supplement (iii) except possibly at the highest density where there was an absolute shortage of fishfood. Supplement (iv) was introduced into the experiment because of the possibility, suggested by Bovbjerg's work which I have mentioned in Section 7.1 and elsewhere, that food of animal but not of plant origin might be a 'high quality food in short supply.' If so, the effect of density might persist with ad lib Cynara leaves; but if not - that is if Cynara leaves are of 'high quality' - then supplement (iv) should have the same effect as supplement (ii). Thus I have drawn these two alternatives in column (iv).

A second hypothesis to explain a result for supplement (i) like the 1970 result is that crowding is inhibitory for some reason other than through food. The most likely result on this hypothesis is shown in the second row of Figure 7.2 - whilst the heights of the curves might be moved (for example, upwards with supplement (ii) because fishfood is rich food) the negative slopes should remain.

However, a result like that of 1970 might not have been obtained. The line might have been horizontal instead. This would be inconsistent with the first two hypotheses but consistent with the hypothesis that there is a 'mosquito larva' type of relative shortage (Section 7.1). If so, and assuming fishfood is a 'high quality'

food, a higher, horizontal line should be obtained with supplement (ii); a slope might be obtained with supplement (iii) because there the fishfood was in short supply . . . it would be a slope with a y-intercept higher than that of supplement (i). Supplement (iv) should give a horizontal line, whose height would depend on whether dead Cynara leaves are richer food than that naturally available.

Finally, there may be no absolute shortage, nor a relative shortage of any consequence to the snails; four horizontal lines of about equal height would be expected on this hypothesis, which is represented in the fourth row of Figure 7.2.

PROCEDURE

The pens for this experiment were 60 cm square and had removable covers to facilitate searching for snails and eggs. They are described in Appendix 3. There were 24 pens, 2 for each of the 3 (densities) x 4 (food supplements) treatment-combinations. They were set out, 1 m apart, on a rectangular grid in an area of grassland at Buckland Park, and cleared of snails on 22/3/71. (I found a mean of 12.5 snails/pen ; 95% confidence limits 10.06, 14.95 snails/pen) Treatments were randomly allotted to pens.

The 400 snails removed from the pens, plus over 200 collected from the surrounding grassland, were taken back to the laboratory for marking. They were randomly divided into 24 lots of 20, individually numbered and marked for the measurement of shell-growth and returned to Buckland Park next day to be placed in the pens. The numbers of snails placed in the pens (of area approx. 0.36 m^2) to produce

densities of approximately 50, 150 and 450/m^2 were 20, 60 and 180/pen. There were 20 marked snails in every pen and I made up the numbers with 1600 snails collected from surrounding grassland and randomly divided up so as to provide 40 or 160 snails/pen as required.

The snails were finally placed in the pens late in the afternoon of 23/3/71, when it was cool. (The weather was still warm at this time, daily screen-temperatures rising to about 25°C, although there had been rain earlier in the month and light rain on 22/3/71.)

The grid of pens was surrounded by a fence to exclude cows; in the fortnight before the fence was constructed cows walked through the area, knocking some pens aside but not feeding on the grass in them. A few snails escaped but there was no other damage.

There was some rain on 26/3/71, and the season could be considered to have 'broken' by 14/4/71, on and after which my notebook nearly always records rain or dew, and cool temperatures.

The supplementary food was added on 23/3/71 as follows:

300 g of crumbed fishfood per pen in treatment (ii). This was, at later dates, changed to 100 g of fish pellets.

1.5 g of crumbed fishfood per pen in treatment (iii) - changed, at later dates, to 1.5 g of pellets.

4 dead leaves of C. carduncules per pen in treatment (iv) - which was an ad lib supply.

Food was added again on 3 occasions; 17-18/4, 9/5, 20/6, and in addition treatment (iii) was fed on 24-25/4 and 23/5 because all the fishfood appeared to have been consumed even at low density.

The feeding may have had effects other than directly providing

food for snails - for example the large amount of fishfood in treatment (ii) seems to have provided fertilizer which resulted in the plants, notably Avena sp. and Bromus sp., growing much larger than in the other pens. Not only may this have had important microclimatic effects but also the fishfood might have fertilized microscopic plants on which the snails were feeding. This difference in the vegetation in different cages was so marked by mid-June that it was one reason for discontinuing the experiment.

Weighing the marked animals took two days: I searched the pens in a random order, placing on the randomization the restriction that one replicate of each treatment-combination should be sampled on each day. Thus, for example if pen 4 (density (ii), supplement (i), replicate B) were searched on the first day, pen 3 (the 'A' replicate of the same treatment-combination) would be included in the random order for the second day. The 12 cages to be weighed on a particular day were searched consecutively in the morning and the marked snails placed in plastic pots. They were then weighed to the nearest 0.5 mg (in the order of collection - random order within each pen) on a Sauter torsion balance set up in the back of a van, and returned to their pens when all had been weighed.

They were weighed first on 17-18/4/71 and again on 24-25/4 and 3-4/5/71. As expected, the adult snails were growing very little by the third weighing.

Egglaying was assessed by searching for snails which were in the egglaying position, with their heads buried in the soil. This was done when collecting snails to be weighed on 24-25/4 and 3-4/5. If eggs were found when the snail was pulled from its hole, it was scored

'laying'; if not, it was scored 'head-down '. If the snail was marked its number was recorded.

The time taken for searching was quite long - some $1\frac{1}{2}$ hours - making it possible that a greater proportion of snails were active in the first pens searched than in the later ones, or vice versa if rain began during the search, but this should introduce only random error and not a systematic bias, because of the random order of searching the pens.

I continued searching for laying snails after I stopped weighing the marked ones. On these later occasions I used the same kind of randomization (one replicate of each treatment-combination in the first half and the other replicate in the second half of the random order) but searched all 24 cages on one morning, starting at first light.

Most of the activity of H. virgata is at night (Pomeroy, 1966) but many are still active on the dewy ground at dawn on a winter's day, and a number are to be found laying. What proportion of those snails which lay eggs on a given night are to be found still laying at dawn is unknown, but does not matter for comparison of treatments in my experiment provided we assume the proportion is the same in different pens.

Seven such searches were made, on 9/5, 16/5, 23/5, 30/5, 6/6, 20/6 and 27/6/71, making a total of 9 occasions on which laying snails were counted. By late June few were found laying, and many adults were dead.

The pens were not entirely snail-proof. Adult snails could not escape from them when the pens were carefully placed on their

bases, but wind, or rabbits, often moved them aside just enough to open cracks. Accordingly, the number of snails in each pen was recorded each time the pens were searched. On one accasion (9/5/71) the numbers were replenished with snails from surrounding grassland, even though there was a risk of some error due to introducing snails not suffering from crowding. Young snails, which are little more than 1 mm in diameter, could escape freely and so, although I did collect and count young snails at the beginning of July, these counts are of little value.

RESULTS

(1) Changes in Weight

As for the Northfield experiment (Section 7.2) I have calculated changes in weight from the start to each of the two later dates, i.e. over approximately 7 and 16 days respectively. Descriptive statistics of the changes over 7 days are shown in Table 7.9. Very similar differences between foods and between densities are found in the changes over 16 days; sample-sizes are, however, much smaller in some pens due to deaths and escapes. I therefore present only the analysis of changes over 7 days.

The means from Table 7.9 are plotted against density in Figure 7.3. The lines are drawn by eye. The diagrams are intended merely to illustrate that there is some downward trend with increasing density, but that there are much greater differences between food-supplements.

FIGURE 7.3

Means of weight-changes over c.7 days in the Buckland Park crowding experiment, plotted against Density.

A. Supplement (i) - no food

B " (ii) - ad lib. fishfood

C " (iii) - limited fishfood

D " (iv) - dead leaves of Cynara

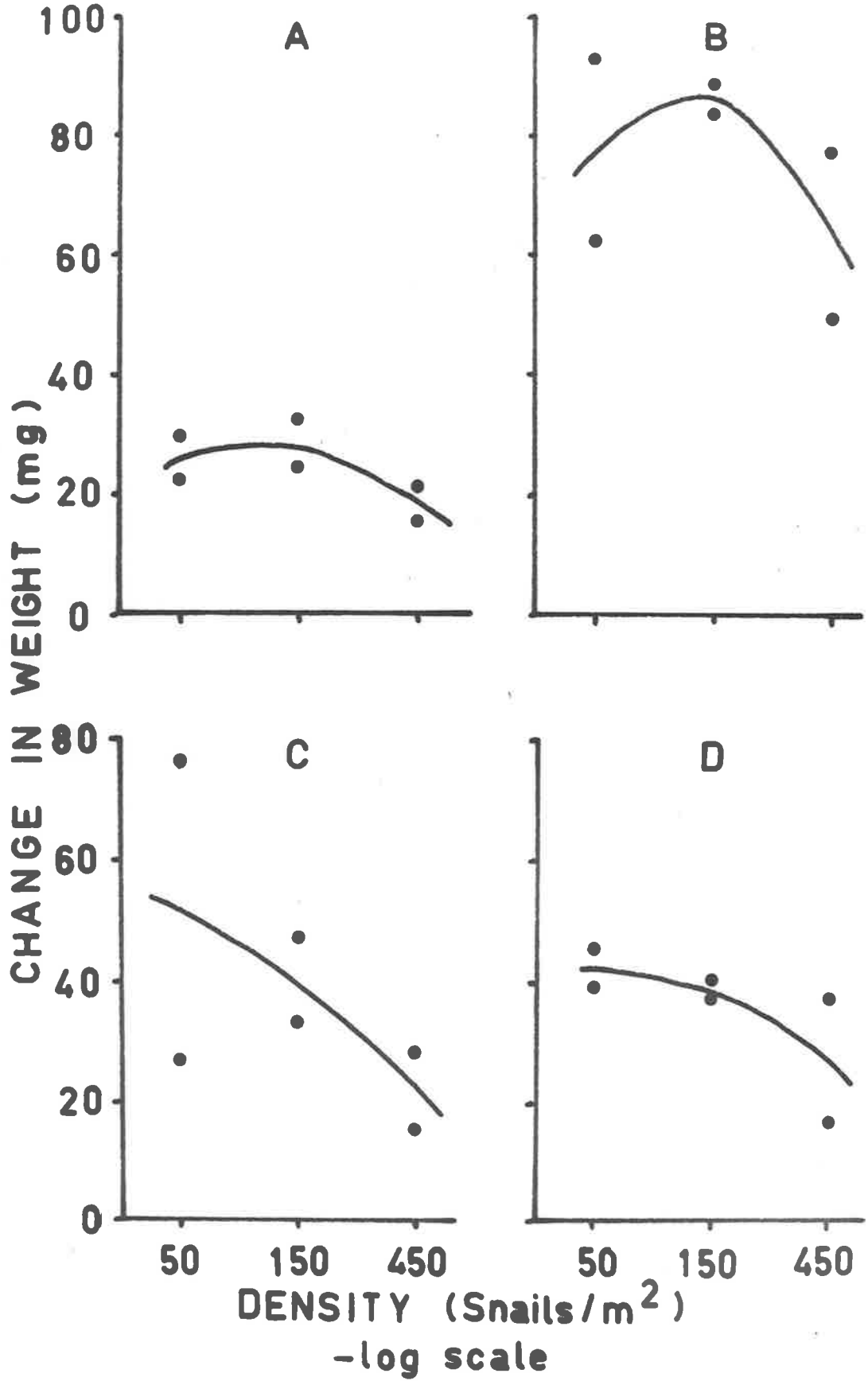

A
B
C
D
100
80
60
40
20
0
80
60
40
20
0
CHANGE IN WEIGHT (mg)
50
150
450
50
150
450
DENSITY (Snails/m²)
-log scale

TABLE 7.9

Mean changes in weight over c. 7 days ($\bar{y}$) with sample-sizes (n) and standard errors ($s/\sqrt{n}$) for snails kept at 3 densities with 4 food-supplements

Density / Food / Pen		$50/m^2$ A	$50/m^2$ B	$150/m^2$ A	$150/m^2$ B	$450/m^2$ A	$450/m^2$ B
(i) None	n	17	17	16	10	12	9
	$\bar{y}$	29.97	22.03	24.03	32.20	21.25	15.44
	$s/\sqrt{n}$	3.70	3.09	3.53	7.81	3.80	4.27
(ii) ad lib. fishfood	n	16	19	17	16	14	18
	$\bar{y}$	62.59	92.08	83.50	88.78	77.18	49.61
	$s/\sqrt{n}$	11.19	12.37	10.97	11.82	15.30	6.34
(iii) limited fishfood	n	19	6	15	13	8	12
	$\bar{y}$	76.00	26.58	33.17	46.69	28.06	15.25
	$s/\sqrt{n}$	9.38	5.57	3.99	3.93	4.10	3.73
(iv) dead Cynara leaves ad lib.	n	20	20	15	9	13	10
	$\bar{y}$	45.45	39.20	40.47	37.33	37.38	16.90
	$s/\sqrt{n}$	4.28	4.09	2.43	5.63	4.21	2.60

F max (24, 9) = 48.5** (Extrapolation of Table T in Rohlf and Sokal (1969) gives the 1% point as ≈ 25)

I have performed the analysis of variance on the cage-means (Appendix 1). Firstly, a two-factor AOV gave the results in Table 7.10.

TABLE 7.10

AOV on the means in Table 7.9

Source of Variation	d.f.	S.S.	M.S.	Fs	P
Subgroups	11	10750.33	977.30		
Densities	2	1389.73	694.87	3.28	$0.05 < P < 0.10$
Foods	3	8970.60	2990.20	14.13^{***}	< 0.001
Interaction	6	389.99	65.00	0.307	> 0.75
Within Subgroups	12	2539.38	211.62		
Total	23	13289.71			

As Figure 7.3 suggests, the analysis shows no difference between densities but a highly significant difference between foods. Nevertheless, I calculated individual regressions of weight-change on density for each food taken alone; all four were non-significant. The slight downward slopes of the curves in Figure 7.3, although interesting, could be attributed to chance.

Now, it is of interest to ask which food-supplements differ significantly, and in view of the non-significant variance due to densities I have pooled the data across densities to obtain samples of 6 means which could be compared by the Student-Newman-Keuls test.

The test gives the following pattern:

(i) No food (iv) Dead Leaves (iii) limited fishfood (ii) *ad lib* fishfood

The mean for supplement (ii) is significantly ($P<0.01$) higher than the rest which are not significantly heterogeneous.

(2) Growth of Shell

There had been some shell-growth by the first weighing at 17-18/4/71, and so we can consider growth over three time-intervals; from the start of the experiment to, respectively, 17-18/4, 24-25/4 and 3-4/5/71. However, as with weight-changes, I present the analysis of growth up to 24-25/4/71 only. The changes up to 17-18/4 show the same differences between pens, but the amount of shell-growth is smaller; changes up to 3-4/5/71 also show similar trends but some of the sample-sizes are much smaller.

Descriptive statistics of the growth of shell up to 24-25/4/71 are shown in Table 7.11 and the means are plotted against density in Figure 7.4.

A two-factor analysis of variance on the cage-means in Table 7.11 gives the results in Table 7.12. Since this indicates a significant difference between densities I calculated an individual single-factor analysis of variance for each of the food-supplements, taking out linear regression of shell-growth on density. These showed no significant difference between densities and no significant regression with one exception - supplement (iv), dead Cynara leaves, which gave the result in Table 7.13.

FIGURE 7.4

Means of shell-growth up to 24-25/4/71 in the Buckland Park crowding experiment, plotted against Density.

A Supplement (i) - no food

B " (ii) - *ad lib.* fishfood

C " (iii) - limited fishfood

D " (iv) - dead leaves of *Cynara*

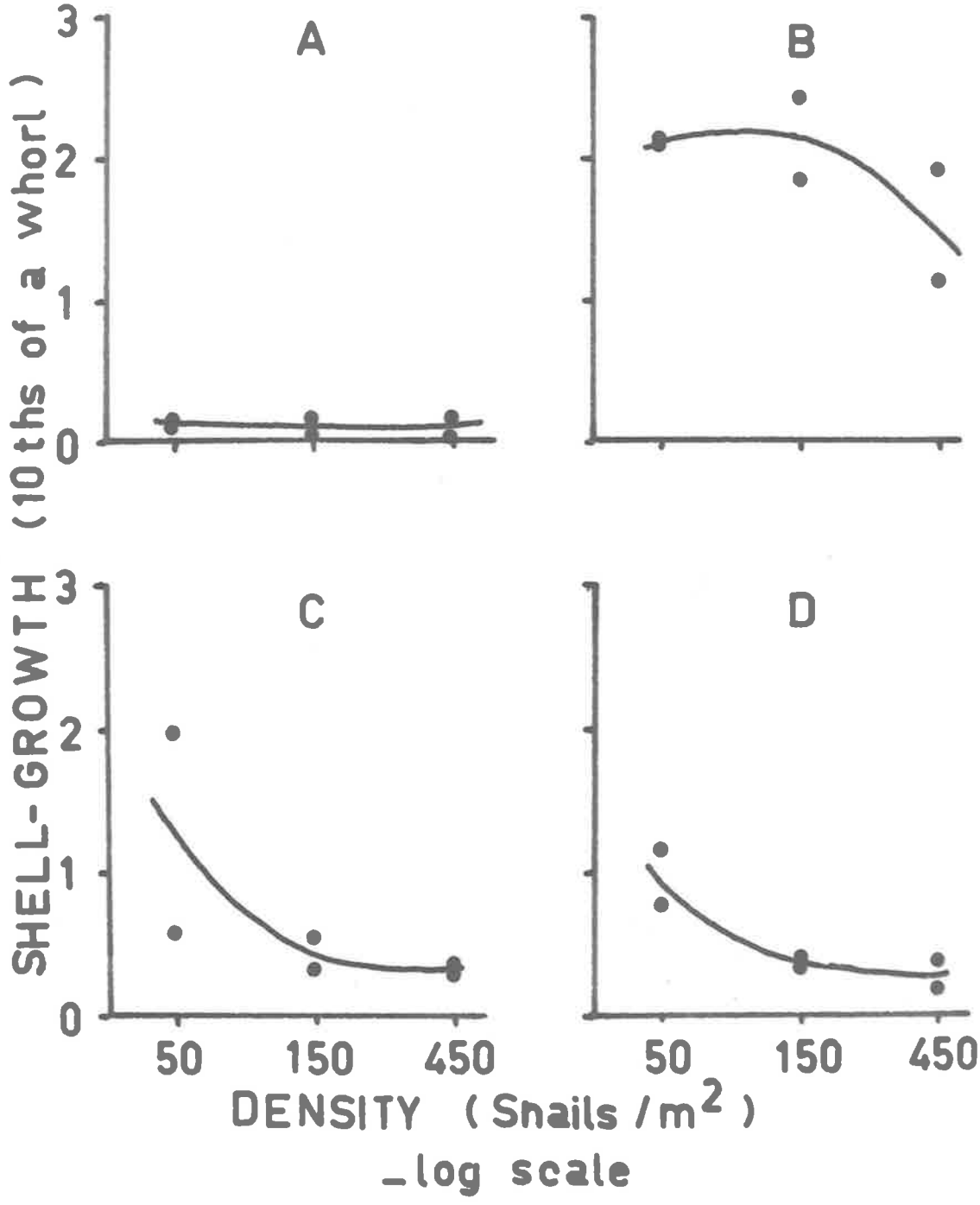

A
B
C
D
SHELL-GROWTH (10ths of a whorl)
0
1
2
3
50
150
450
DENSITY (Snails / m2)
log scale

TABLE 7.11

Means ($\bar{y}$) and standard errors ($s/\sqrt{n}$) of growth of shell (10ths of a whorl) in snails kept at 3 densities with 4 food-supplements. Period of growth was approx. 1 month but this included less than 2 weeks of 'winter' conditions.

Density / Food — Pen		$50/m^2$ A	$50/m^2$ B	$150/m^2$ A	$150/m^2$ B	$450/m^2$ A	$450/m^2$ B
(i) None	n	18	18	20	11	17	13
	$\bar{y}$	0.17	0.15	0.16	0.05	0.04	0.17
	$s/\sqrt{n}$	0.06	0.07	0.06	0.05	0.02	0.12
(ii) ad lib fishfood	n	17	19	18	17	16	19
	$\bar{y}$	2.12	2.11	2.42	1.85	1.92	1.13
	$s/\sqrt{n}$	0.36	0.42	0.39	0.32	0.40	0.22
(iii) limited fishfood	n	19	8	15	15	9	14
	$\bar{y}$	1.96	0.56	0.52	0.30	0.33	0.27
	$s/\sqrt{n}$	0.37	0.17	0.15	0.11	0.24	0.10
(iv) ad lib dead Cynara Leaves	n	20	20	17	9	16	12
	$\bar{y}$	1.13	0.76	0.37	0.36	0.36	0.19
	$s/\sqrt{n}$	0.19	0.12	0.14	0.14	0.14	0.09

F max (24, 16) = 345.3^{**} (Extrapolation of Table T in Rohlf and Sokal (1969) gives the 1% point as < 10)

TABLE 7.12

AOV on the means in Table 7.11.

Source of Variation	d.f.	S.S.	M.S.	Fs	P
Subgroups	11	13.01			
Densities	2	1.32	0.66	5.05*	$0.025 < P < 0.05$
Foods	3	10.89	3.63	27.76***	< 0.001
Interaction	6	0.79	0.13	1.01	$0.25 < P < 0.5$
Within Subgroups	12	1.57	0.13		
Total	23	14.58			

TABLE 7.13

AOV with regression of shell-growth on density, for the means for supplement (iv) in Table 7.11

Source of Variation	d.f.	S.S.	M.S.	Fs	P
Densities	2	0.529	0.264	9.85*	$0.025 < P < 0.05$
Linear Regression	1	0.320	0.320	11.94*	"
Deviations from "	1	0.209	0.209	7.79	$0.05 < P < 0.1$
Within densities	3	0.080	0.027		
Total	5	0.609			

95% confidence limits for regression coefficient $\approx(-0.038, 0.031)$

Regression equation $Y = 0.822 - 0.0034X$ (where Y = shell-growth, X = density.)

Thus the downward slope which appears to some extent in all the lines is significant only in the case of supplement (iv).

The difference between foods is, however, of greater influence than the difference between densities – see the different heights of the lines in Figure 7.4. In order to test which foods differ significantly I have pooled the data across densities to give a single-factor analysis, as done with changes in weight above. Despite the significant difference between densities, this procedure is unlikely to introduce serious error because the influence of density appears similar with the different foods and is just significant in only one case. (The 'interaction' mean square in Table 7.12 is not significant.)

The resultant samples of six means, compared by the Student-Newman-Keuls test, fall into the following pattern:

(i) No food (iv) dead leaves (iii) limited fishfood (ii) ad lib fishfood

Treatment (ii) differs from each of the others significantly at the 1% level. Treatments (i), (iii) and (iv) are not significantly heterogeneous at the 5% level. The same pattern was found for changes in weight in (i) above.

(3) Numbers of Snails Laying Eggs

The data on egglaying consist of counts of the numbers found 'laying' and 'head-down' in each pen on each date. I have also counts of the numbers present on each date and can therefore express the number laying, or the number (laying and head-down) as a percentage of the number present. This serves as a measure of the

amount of 'effort' being devoted to laying in each pen on each date.

An inspection of the table of such figures shows that egglaying activity was greatest about 9-16/5/71 (about one month after the season 'broke'), declined slowly to about 6/6/71 and was virtually zero on 20/6 and 27/6/71. The timing of the peak in egglaying seems no different in different pens. The question of interest here is whether the amount of egglaying over the whole period of the experiment differs in different pens. To examine this question I have calculated for each pen an average figure of egglaying-activity over the whole time, by calculating the total number found laying and dividing it by the average density in the particular pen over the whole time. I have also calculated similarly the total number found laying and head-down and divided this by the average density.

If we make two assumptions this latter figure can be used to compare the fecundity of snails in different pens. (i) The first assumption is that the snails scored 'head-down' were in the act of laying. This assumption, which was also made by Wolda (1963) in scoring the egglaying of Cepaea nemoralis, seems reasonable because their behaviour was just like that of laying snails - they differed only in that when I pulled them out of their holes I saw no eggs at the bottom. (ii) The second assumption is that clutch-sizes were equal in different treatments. This assumption might well be wrong. I have not tested it. If it is wrong, then it seems likely a priori that the difference would be such as to accentuate the differences I have found between treatments. Thus, by not

including information on clutch-size I expect I have made a conservative test of the effects of density and food-supplement on fecundity.

These figures for 'average % laying' and 'average % laying and head-down' are shown in Table 7.14.

TABLE 7.14

'Percentage laying' and 'percentage laying and head-down' - see text- in each pen over the whole period of an experiment on the effect of crowding and supplementary food at Buckland Park.

Density / Food	Pen	$50/m^2$ A	$50/m^2$ B	$150/m^2$ A	$150/m^2$ B	$450/m^2$ A	$450/m^2$ B
(i)	L	0	10.2	4.4	3.9	5.7	0.9
	L+HD	88.9	66.0	41.9	39.5	43.2	29.8
(ii)	L	35.1	22.8	9.5	28.8	18.1	27.3
	L+HD	122.8	114.2	89.4	74.9	61.5	64.1
(iii)	L	6.1	8.6	8.3	6.7	2.1	4.3
	L+HD	42.9	60.3	55.1	42.6	49.1	32.8
(iv)	L	27.5	30.3	15.0	6.5	12.2	11.1
	L+HD	65.9	106.1	96.7	78.1	71.5	54.7

Assumption (i) above need not be made if we analyse only the data on percentages laying in Table 7.14. The result of an analysis of variance on these figures is shown in Table 7.15. The analysis has been done on untransformed data. Although these figures are

'percentages' they can be greater than 100% - a number of marked snails were found in the laying position on two or three different dates, though none was ever scored 'laying' more than once- and therefore I have not used the angular transformation but have assumed that the raw percentages are close enough to normality and homoscedasticity not to seriously bias the analysis.

TABLE 7.15

AOV on the figures for 'percentage laying' in Table 7.14

Source of Variation	d.f.	S.S.	M.S.	Fs	P
Subgroups	11	2076.28			
Densities	2	282.39	141.20	4.08*	0.025 < P < 0.05
Foods	3	1532.21	510.74	14.76***	< 0.001
Interaction	6	261.67	43.61	1.26	0.25 < P < 0.5
Within Sub-groups	12	415.35	34.61		
Total	23	2491.63			

The numbers found laying in the low-density cages are very small (I inspected a pen containing a mere 20 snails for only a few minutes in each week - it is not surprising that I did not find many of them just having laid eggs on these occasions) and so random error can become very important. It therefore seems worthwhile to make assumption (i) and analyse the 'proportions laying and head-down' also.

The analysis of variance is shown in Table 7.16.

TABLE 7.16

AOV on the figures for 'percentage laying and head-down' in Table 7.14.

Source of Variation	d.f.	S.S.	M.S.	Fs	P
Subgroups	11	13325.55			
Densities	2	4267.15	2133.58	12.90**	0.001 < P < 0.005
Foods	3	7229.82	2409.94	14.57***	< 0.001
Interaction	6	1828.58	304.76	1.84	0.1 < P < 0.25
Within sub-groups	12	1984.82	165.40		
Total	23	15310.37			

Because of the significant effect of densities it is of interest to calculate regressions of 'percentage laying' and 'percentage laying and head-down' on density. The question is, which density?

I have calculated Kendall rank correlations of 'percentage laying' with the average density used to calculate it, and found the negative correlation significant (P = 0.028, one-tailed) only in the case of supplement (iii), limited fishfood. I found significant negative correlations of 'percentage laying and head-down' with average density, only in the cases of (i) no food (P = 0.028, one-tailed) and (ii) ad lib. fishfood (P = 0.008, one-tailed).

However, in the 1970 experiment at Northfield I was unable to

take account of changes in density because I had no estimate of them; there, I calculated regressions of the dependent variables on initial density. I did the same in (1) and (2) above because over the period of time concerned there, early in the experiment, there was very little difference between the initial densities and the numbers I counted in the pens. Plotted on graph paper, the data on egglaying look very similar whether the independent variable used is 'initial density' or 'average density.' Therefore it seems consistent to calculate regressions of 'percentage laying' and 'percentage laying and head-down' on initial density, treating the latter as an independent variable measured without error.

The data are plotted in Figures 7.5 and 7.6. It appears from the graphs that a linear relationship is more likely to be found if both variables are transformed to logarithms. Accordingly I made this transformation. The two-factor analyses of variance on the transformed data give the same probability levels as those on untransformed data shown in Tables 7.15 and 7.16. I calculated for each food-supplement a single factor AOV taking out regression of log (percentage laying) or log (percentage laying and head-down) on log (density).

One of them, the analysis of log (percentage laying and head-down) on supplement (ii), ad lib fishfood, showed a significant variation among densities but not a significant regression. All the rest showed no significant variation among densities and no significant regression.

Since the effect of densities seems small and not significantly different with different foods (the interaction is not significant -

FIGURE 7.5

Buckland Park crowding experiment -

'Average Percentage Laying' (see text)

plotted against Initial Density.

A Supplement (i) - no food

B " (ii) - *ad lib.* fishfood

C " (iii) - limited fishfood

D " (iv) - dead leaves of *Cynara*

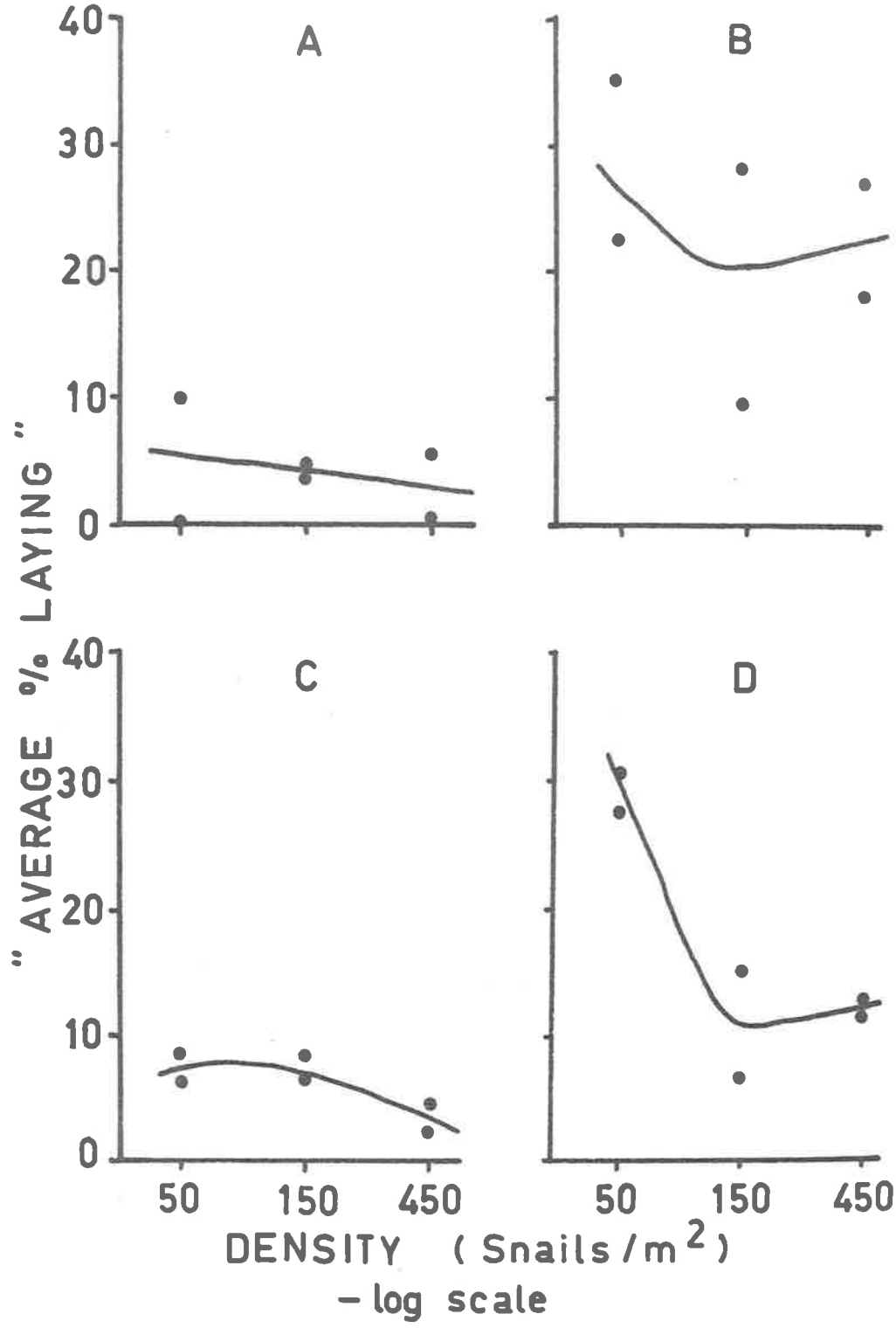

A
B
C
D
40
30
20
10
0
" AVERAGE % LAYING "
50
150
450
DENSITY (Snails / m 2)
– log scale

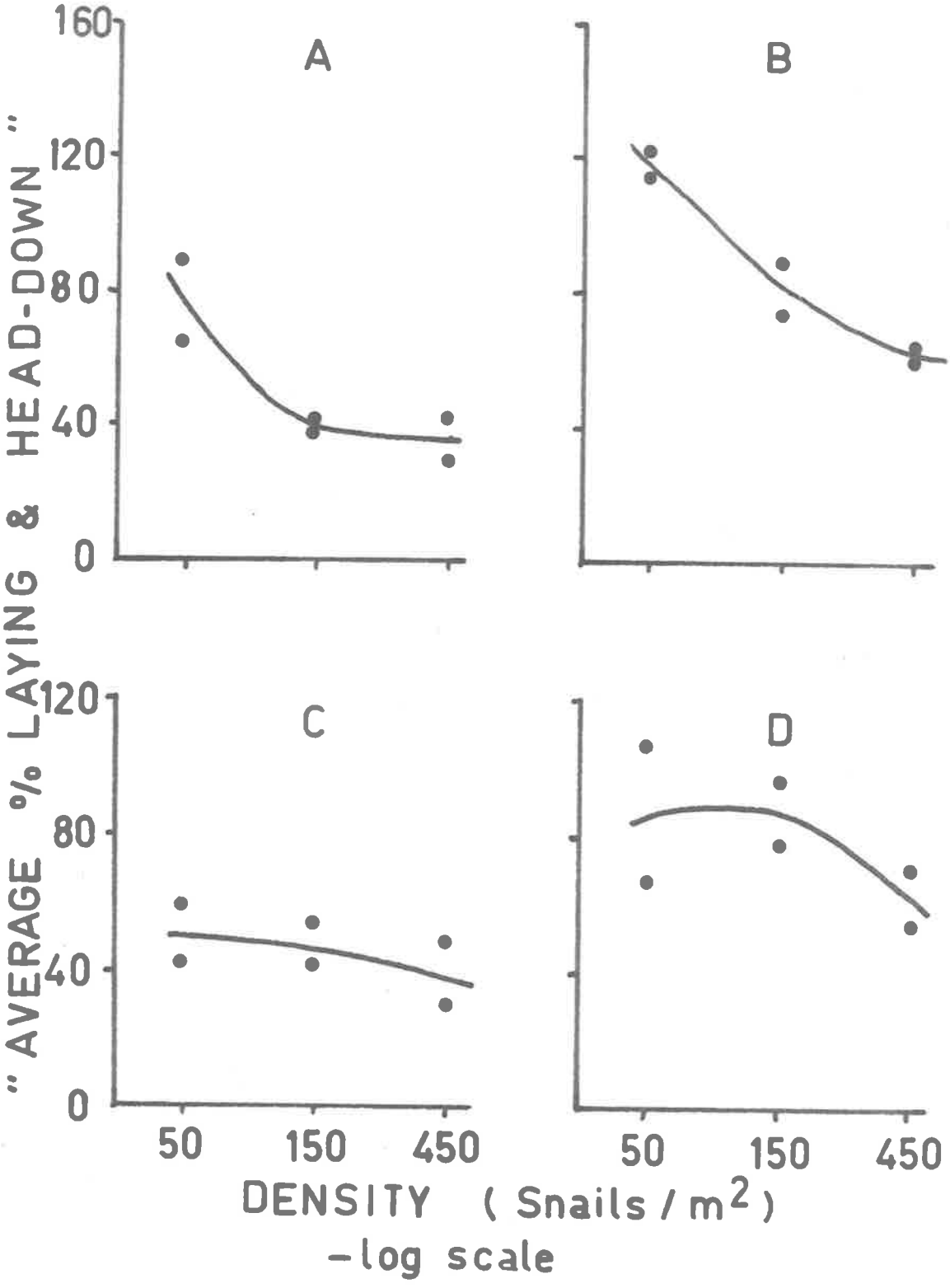
FIGURE 7.6

Buckland Park crowding experiment -

'Average Percentage Laying and Head-down'

(see text) plotted against Initial Density.

A	Supplement	(i)	-	no food
B	"	(ii)	-	*ad lib* fishfood
C	"	(iii)	-	limited fishfood
D	"	(iv)	-	dead leaves of *Cynara*


A
B
C
D
160
120
80
40
0
120
80
40
0
" AVERAGE % LAYING & HEAD-DOWN "
50
150
450
50
150
450
DENSITY (Snails / m2)
-log scale

Tables 7.15, 7.16) it again seems justifiable to pool the data across densities in order to examine the differences between foods, using the Student-Newman-Keuls test. When this is done on the untransformed data in Table 7.14 the following patterns are found. (Treatments are in increasing order of magnitude of their means and the means in underlined groups are not significantly heterogeneous at the 5% level.)

For 'percentage laying':

(i) No food (iii) limited fishfood (iv) dead leaves (ii) ad lib fishfood

For 'percentage laying and head-down':

(iii) limited fishfood (i) no food (iv) dead leaves (ii) ad lib fishfood

INTERPRETATION OF RESULTS

Perhaps not surprisingly the results do not fit exactly into any row of Figure 7.2. There is a general tendency for 'Performance'- growth or egglaying - to be lower at higher density, but this tendency is small. It is significant in only a few cases. Nevertheless on one measure or another there was a significant regression (or rank correlation) with each of the food-supplements. Perhaps more of them would have been significant if the degrees of freedom had been greater. So, with present data, we cannot rule out the hypothesis represented in the second row of Figure 7.2, that crowding is inhibitory in some way other than through food. The hypothesis in the top row is, however, thrown into doubt because amongst the significant correlations were

(a) a significant rank correlation of 'percentage laying and head-down' with density on supplement (ii) and

(b) a significant regression of shell-growth on density on supplement (iv).

The former, at least, is contradictory to the first hypothesis.

I think that the effect of density in this experiment cannot be interpreted strongly. There is little evidence of an absolute shortage of food. There is some sign of an inhibition not due to shortage of food, but we cannot be confident of it. (And the densities used went much higher than natural densities at Buckland Park or elsewhere.)

The difference between foods, however, is large. It contradicts the hypothesis in the fourth row of Figure 7.2, that there is no shortage of food and the food is of high quality. 'Performance' of each kind was higher on fishfood than on other foods. On the measures of egglaying, though not significantly on the measures of growth, dead Cynara leaves also supported higher 'performance' than no food or limited fishfood.

These results are what would be predicted on the hypothesis of a 'mosquito larva' type of relative shortage of food, that is, on the hypothesis that natural food on the area was abundant but of poor quality. (Such a situation may belong in the third row of Figure 7.2, but it may also be superimposed on the hypothesis of the second row, as indicated by the upward and downward arrows in that row.)

Of course, we might not expect natural food ever to be as rich as fishfood, but in some places dead Cynara leaves are eaten by

H. virgata in the field, and these results suggest that in such places the snails have a richer food than they do on the grassland at Buckland Park (where no Cynara occurs).

Thus, it seems that food may be important in determining the numbers of snails at Buckland Park and their size. At the densities naturally occurring there, it appears that each snail has more than it can eat, but the food is not rich. Should it find richer food, this experiment suggests, the snail would grow faster, lay more often and - if its clutch-size stayed constant or increased - produce more eggs.

Should the food-supply remain rich (for example, if the area should be invaded by Cynara cardunculus), and should other factors which influence the proportion of young surviving remain constant, presumably these offspring would grow larger and produce more young in turn.

A limit would be reached when, for other reasons, the large numbers of snails present would reduce each other's chance to survive and multiply - but my results suggest that this limit would be well above the present natural density.

It would be absurd to suggest that food is the only important variable. I am speculating merely that, at Buckland Park, if other factors continued to kill the same proportions of snails at various ages, and if the food-supply were increased not in quantity but in quality, then we might expect to find more and bigger snails there.

7.4 DISCUSSION

The two experiments in this chapter have been discussed fairly extensively at the ends of Sections 7.2 and 7.3, and I merely want to collate them briefly here.

In Pomeroy's experiment and in each of mine, various measures of growth, reproduction or activity were found to decrease with increasing density. This tendency was particularly marked only in my Northfield experiment (Section 7.2) - the one in which densities were greatest of all - but it occurs elsewhere and it occurs in the presence of seemingly abundant food (Section 7.3). I think it best, with present evidence, to attribute this trend to some interaction between snails which is independent of the abundance of food. It may be, for example, the accumulation of toxic wastes or of mucous trails - but I have no information regarding the mechanism and shall discuss it no further.

The experiments were designed to investigate possible shortages of food. The Northfield experiment gave results consistent with an absolute shortage although, according to the calculations shown in Table 7.8, perhaps not a simple one where there is one kind of completely-accessible food in absolutely short supply.

The Buckland Park experiment did not show any sign of an absolute shortage, unless the small effect of densities with all food-supplements can be attributed to an absolute shortage of some nutrient not present in either fishfood or dead Cynara leaves. What that experiment did show was evidence that food at Buckland Park is not of high quality.

I speculated in Section 7.3 that if the quality, though not the quantity, of food at Buckland Park were raised the population of snails might increase. Conversely, one might expect that if the quality of food were lowered snails would become more rare. Buckland Park supports a large population of snails. Its vegetation includes a number of species which produce litter edible to snails. But some parts of Buckland Park support fewer snails. There are fewer under the Acacia ligulata whose dead sclerophyllous leaves may prove poor food because of toughness, and virtually none under a stand of Casuarina sp. which carpet the ground with their dead leaves. If such species became more numerous the snails might find themselves amid an abumdance of very poor food, and searching for the small amount of rather more nutritious litter which was available. Then each of them might grow less, lay fewer eggs, and the population might decrease.

I suspect that snails may be in such a condition where they occur on roadsides on which eucalypts are growing but with some grasses and herbs under them. Snails in such places are usually small and not very numerous.

This may also be the lot of snails in grass or cereal crops. On the other hand, snails on roadsides where there are soft-leafed weeds such as Cynara and Echium grow large and numerous.

In summary, these experiments suggest that food may be an important variable in determining the distribution and abundance of H. virgata in South Australia. They suggest that this is not because there is some specific requirement of which there is sometimes not

enough to go round (though this may happen in some places and Northfield may have been one such place) but because food varies in quality; there is likely to be enough of it but it may be low in nutrients.

The results suggest that any attempt to control snails by means of their food should not be based on removing their food, a difficult problem with serious side-effects, but on reducing its quality - diluting it. The obvious long-term course in South Australia is to replace the introduced weeds on many roadsides and wastelands with sclerophyllous native vegetation.

APPENDIX 1.

THE CHOICE AND MEASUREMENT OF DEPENDENT VARIABLES IN FEEDING-TRIALS AND CROWDING EXPERIMENTS

It is to be expected that a snail's response to food (and crowding) might be reflected in its capacity to grow, to produce eggs and to survive during hard times, especially to survive the period of exposed dormancy during summer. It would be convenient if one measurement (e.g., the rate at which food reserves accumulate) were correlated with all these responses. For this reason I investigated the accumulation of polysaccharides in experimental snails. There is reason from the literature to expect that Helicella might store energy mainly as polysaccharides - glycogen and, in the reproductive tract, galactogen (Goddard and Martin, 1966; Meenakshi and Scheer, 1969; Hodson, 1969).

THE ESTIMATION OF POLYSACCHARIDES

I attempted initially to measure 'glycogen' by the anthrone method of Carroll et al.(1956). The body of a snail was homogenized thoroughly under trichloro acetic acid (T.C.A.) at room temperature. The homogenate was centrifuged, the supernatant filtered and samples of it treated with 5 volumes of 95% ethanol to precipitate the polysaccharide. The polysaccharide was redissolved in water and treated with 5 volumes of an 'anthrone reagent' containing 0.05% anthrone, 1% thiourea and 72% (by volume) H_2SO_4. It was placed at 100°C in a water bath for 15 minutes and then cooled again in a cold water bath. A blank of water and a standard glucose

solution were treated with anthrone reagent at the same time. The blue colour produced by the reaction of anthrone with the hydrolysis products of the polysaccharide, and with the standard glucose, was read at 620 mμ in a Unicam SP500 spectrophotometer. Following Carroll et al, I multiplied the calculated amount of glucose in the sample by 0.9 to convert it to the weight of glycogen hydrolysed.

But a number of tests indicated that, without a rapid technique for separating and estimating both glycogen and galactogen, the estimation of polysaccharides was likely either to be fairly inaccurate, or to be very time-consuming.

Preliminary experiments indicated that the grinding in T.C.A. would not extract more polysaccharide if it were done more thoroughly, and that washing and decanting involved very small losses. A known solution of commercially-available oyster glycogen was estimated at 95% or more of its true concentration.

However, I generally obtained fairly low glycogen levels in Helicella (0.5% to 1% of the live weight, compared with published values of up to 4% for Helix - Goddard and Martin, 1966).

In some tests I had killed snails by brief immersion in boiling water, removed them from their shells and dried the bodies at room temperature in a vacuum-unit before estimation. Drying took about 12 hours, which means that autolysis or bacterial decomposition could have reduced the polysaccaride levels considerably. In other tests, I had frozen snails, and later thawed them in order to remove them from the shells. Here, the dead snail, killed by freezing instead of boiling, spent less than 15 minutes at room temperature

before being homogenized under T.C.A. but, again, autolysis or bacterial action could have taken place. A different cause of the low values could be that T.C.A. does not extract all the glycogen (Roe and Dailey, 1966).

Another source of error stems from the possible presence of galactogen. I made some preliminary tests for the presence of galactogen, and found some evidence that it is present although I did not carry the tests far enough to make them of much interest to a biochemist.

I extracted polysaccharide from snails using hot alkali (Roe and Dailey, 1966, returned to a modification of this classical method in preference to using cold T.C.A.), hydrolysed it with sulphuric acid and ran one-dimensional ascending paper chromatograms of the hydrolysate using isopropanol and water (4 : 1 by volume) as solvent (Smith, 1958). Glucose and galactose were not quite separated in this system: the snail-extract gave a long, oval spot reaching from the bottom of the galatose spot to the top of the glucose spot, which suggests that the snails contained both glycogen and galactogen. I did not attempt to produce better chromatograms or to identify two faint spots, one faster and one much slower than glucose, which appeared in some of the chromatograms.

This suggestive evidence, together with the findings in the literature on other pulmonates, made it seem very likely that galactogen would be present at times, and I went on to consider the effect for my purposes of the presence of both glycogen and galactogen in Helicella.

I measured the 'glycogen' contents of a series of mixtures of glucose and galactose by the anthrone method, and found that a solution of galactose gave only 0.6 of the reading obtained with a glucose solution of the same concentration by weight. A snail might, at certain times, lay on most of its polysaccharide as galactogen; if snails differed in their reproductive condition, or if different foods should differ in quality so that one stimulated snails to enter reproductive condition whilst another did not, then some animals in an experiment might be depositing galactogen and others glycogen. For comparing different foods, the technique could thus have an error of up to 40%, and in a serious direction; a food which stimulated snails to enter reproductive condition might be scored as of rather low value.

This problem could be overcome by estimating the proportions of the two polysaccharides present in a snail, and indeed that would be very interesting. However, I had no automated equipment and the task would have been laborious.

These findings meant that, although Roe and Dailey's (1966) technique might remove the problem of incomplete extraction, the estimation of polysaccharides was still likely either to be fairly inaccurate, or to be very time-consuming. So, for use on large numbers of snails, it had nothing to recommend it over merely weighing the animals, and I decided that it was not worthwhile measuring polysaccharides in my feeding-trials and crowding experiments. This is not to say that, for more specific purposes in the future, it will not be very interesting indeed to measure polysaccharides.

Before I decided to discontinue the measurement of polysaccharides, I had used it in one experiment, which is described in Section 3.2.1. The procedure there was to estimate the polysaccharides as 'glycogen', as described above, after extraction in T.C.A. The results are presented in Section 3.2.1 although, for the various reasons above, they must be interpreted with great caution. The dry weights of random samples of snails were also measured in that experiment.

THE MEASUREMENT OF DRY WEIGHT

In the experiment described in Section 3.2.1, the dry body weights of random samples of snails gave results similar to those for polysaccharides but showing less difference between treatments. Thus, dry body weight seems a less sensitive measure of 'well-being' than polysaccharide. On the other hand it may be less subject to unknown errors. By 'dry body weight' I mean the weight of dry matter in the body of a snail without its shell.

In the experiment of Section 3.2. the samples of snails were frozen. To dry them, I let them thaw, removed the bodies from the shells, placed each body and shell in a separate tared tube and placed the tubes as quickly as possible in a vacuum chamber at room temperature. Drying to constant weight took about 12 hours. The dry bodies and shells were weighed on a Mettler type H16 balance to the nearest 0.05 mg.

THE REPEATED MEASUREMENT OF THE LIVE WEIGHTS OF MARKED SNAILS

The weights of the snails used in my experiments differed, sometimes widely. This meant that a sample taken for dry weight at a given time would show a considerable variance not only because the animals differed in their response to food, due to differences in age, reproductive condition or whatever, but also because they had been of different weights at the start.

Consequently, I tried repeatedly weighing snails alive. It is easy to mark snails individually. I wrote a number on the shell in Indian ink, allowed it to dry and painted a spot of clear nail polish over the number to protect it (Hodson, 1969) as shown in Figure A 1.1 (a).

The live-weights of snails vary greatly depending on their water-content, which itself varies in complex ways (Wells, 1944; Blinn, 1964). Gut contents will also affect their weight. In any laboratory experiment, I always commenced weighing about the same length of time after the snails were last sprayed, and always randomized the order of weighing snails within and between treatments. (The 'initial weight' was always taken after one night's spraying with the foods, so that it would be comparable with later weights.)

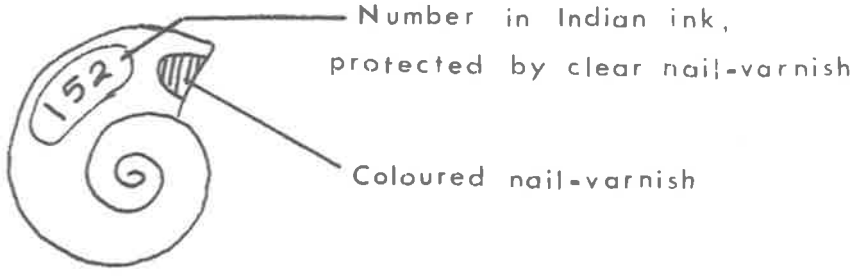
I first measured live-weights of marked snails in one of the experiments mentioned in Section 3.3, in which I also measured dry weights of random samples. Even with small samples, live weights proved nearly as sensitive as dry weights. They took much less effort, required fewer snails to be housed and, in field experiments, did not necessitate changes in density due to snails being removed

FIGURE A 1.1

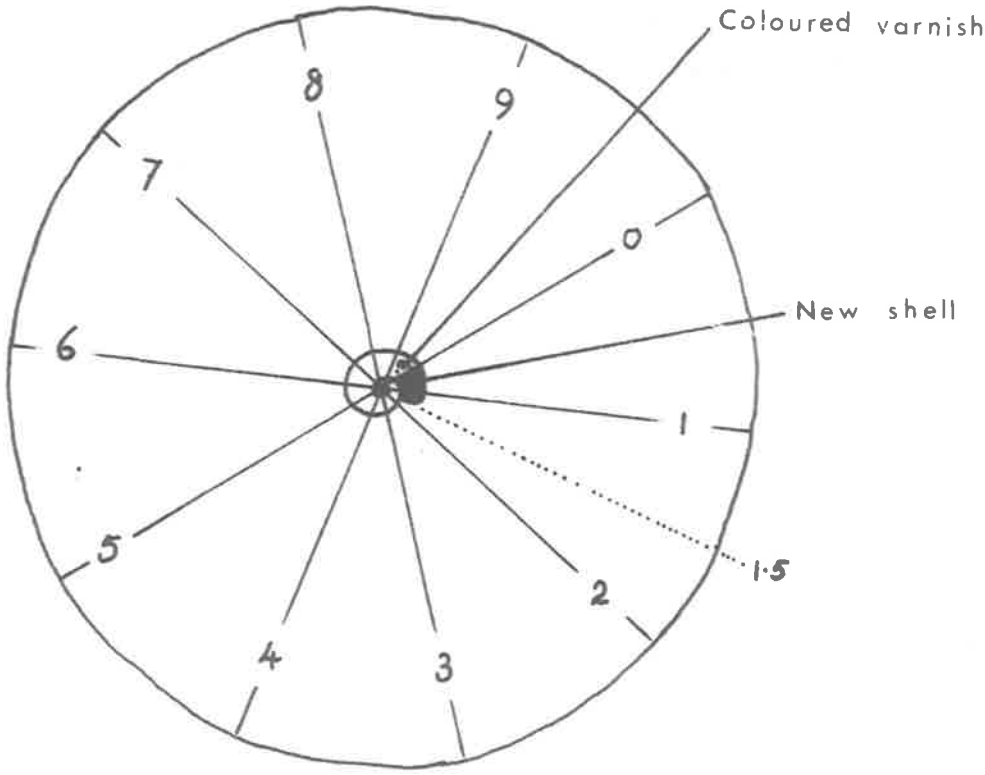
A. Markings on a snail to permit identification and the measurement of shell-growth.

B. Celluloid protractor in place for measuring the shell-growth of a snail which will be scored "1.5".

A


Number in Indian ink,
protected by clear nail-varnish
152
Coloured nail-varnish

B


Coloured varnish
8
9
7
0
New shell
6
1
1·5
5
2
4
3

and killed. I therefore measured live-weights in subsequent experiments.

THE ANALYSIS OF THE RESULTS OF EXPERIMENTS IN WHICH MARKED SNAILS WERE WEIGHED REPEATEDLY.

The question to be asked of live weights of marked snails is, Did snails in one treatment gain significantly more weight than those in another?

I considered asking this question by calculating 'instantaneous relative growth rates' and correcting them for a standard-sized animal (Krebs, 1966; Wheeler, 1970), or by fitting a logistic equation to the growth-curve of each snail (Blumberg, 1968; Raghunandanan and Srinivasan, 1970; Antle et al, 1970). But the adaptation of these methods to my data did not seem warranted.

Figure A 1.2 shows the changes in weight of two randomly-selected snails from each of six treatments in the second experiment in Section 3.3.

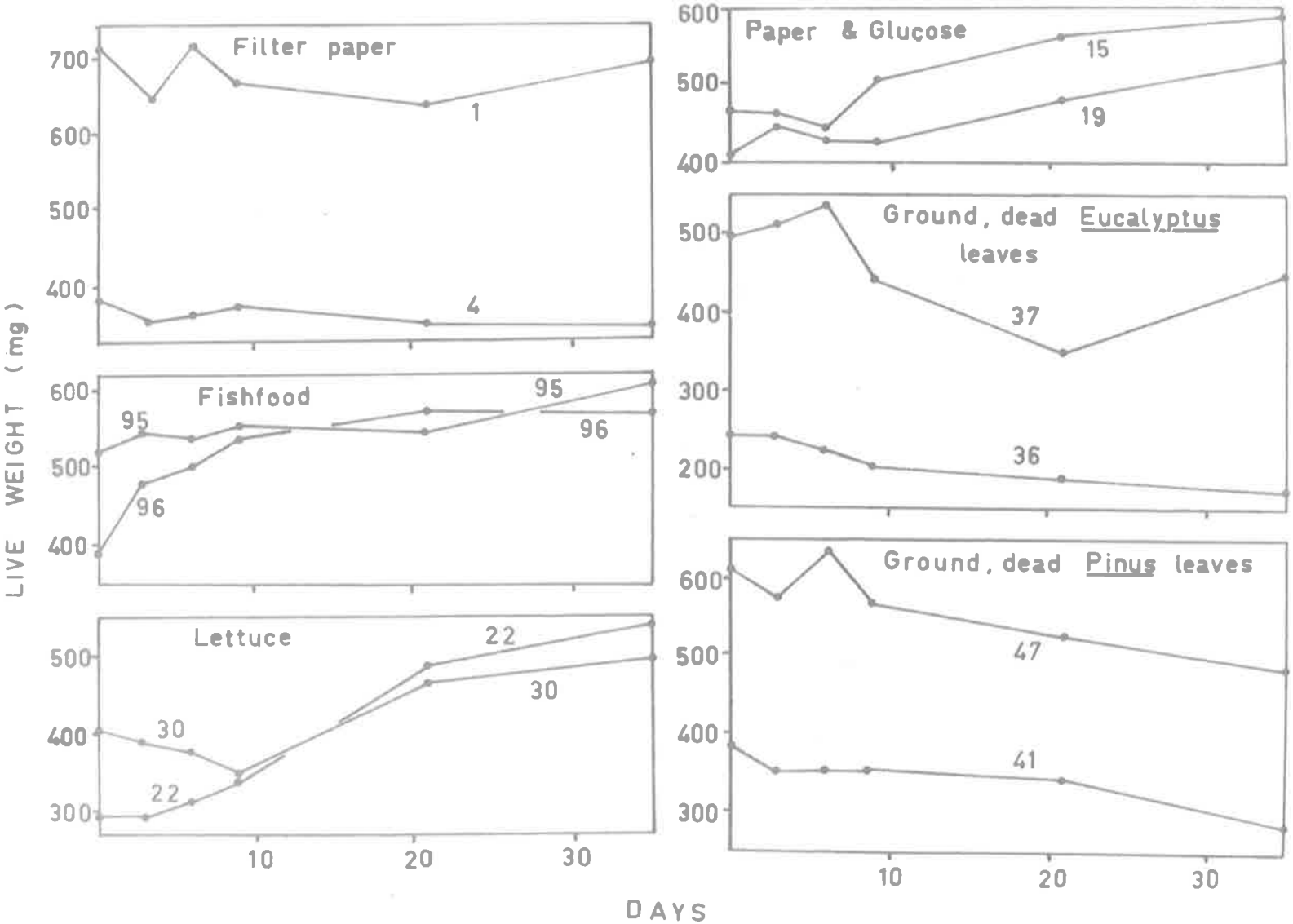
The calculation of 'instantaneous relative growth rates' is based on the assumption that $\frac{dW/dt}{W} = k$ (Brody, 1945) where W is an animal's weight, t is time and k is a constant. Clearly, such an exponentially-increasing curve will not fit many of the curves in Figure A 1.2.

It appears that, over the time-interval used (35 days - in other experiments I used less), most of them would fit a straight line as well as any other curve. There is little reason to fit complex equations.

I therefore considered calculating a linear regression equation

FIGURE A 1.2

Records of live-weights of two randomly-chosen snails from each of six treatments in the second experiment of Section 3.3.


Filter paper
1
4
Paper & Glucose
15
19
Fishfood
95
96
95
96
Ground, dead Eucalyptus leaves
37
36
Lettuce
22
30
30
22
Ground, dead Pinus leaves
47
41
LIVE WEIGHT (mg)
DAYS
700
600
500
400
300
200
10
20
30

of weight on time for each snail, so that the average regression coefficients for different treatments could be compared. However, it seemed just as appropriate and less laborious not to fit any kind of curve to the data, but to calculate some estimate of growth which could be examined independently of the form of the growth-curve.

Two such measures are (W2 - W1) and $\frac{W2}{W1}$ where W1 is the initial weight of a snail and W2 its weight at any later time in the experiment. It is intuitively reasonable that either of these measures will be greater the more favourable a particular 'treatment' is for growth. Both will depend upon the value of W1, upon other initial states of the animal, and upon conditions under which the measurements were made; but since snails were randomly-allocated to treatments, and since the order of weighing was random, these factors should merely increase the random error in a comparison between treatments and should not introduce any bias.

The more straightforward of these measures, for calculation and discussion, is (W2 - W1). There is no reason to prefer $W2/W1$, or its logarithm, (log W2 - log W1), unless (W2 - W1) fails to conform to the statistical model of analysis of variance. (AOV is the statistical technique most useful for comparing treatments in a number of my experiments.)

Of those likely to be wrong in my experiments, the most important assumption of AOV is that all the samples come from populations with the same error variance (Sokal and Rohlf, 1969, Chapter 13). Taking the results of one experiment (that in Section 3.4.4), I calculated (W2/W1), log (W2/W1), (W2 -W1) and log (W2 - W1 + 50) and performed the Fmax test for heterogeneity of the variances of each (Appendix 2).

The values of Fmax decreased in the above order: the variances of W2/W1 and of its logarithm were significantly heterogeneous at the 1% level, those of $(W_2 - W_1)$ just so at the 5% level, those of log (W2 - W1 + 50) not significantly heterogeneous.

This result suggested that the best measure to use was log (W2 - W1 + 50). However, I decided to use (W2 - W1) unless it failed to conform to the assumption of homoscedasticity. If it did so, it could be converted to a logarithm, but, since some snails lost weight, a constant would have to be added first to make all the values non-negative. (The constant chosen above was 50 mg). The choice of the size of this constant is arbitrary, and statisticians I spoke to were uneasy about the procedure. I found, in fact, that in the experiment of Section 3.4.4 the analysis of (W2 - W1) led to the same conclusions as that of log (W2 - W1 + 50); but the former gave more conservative probability levels. For these reasons I have not presented analyses of transformed data.

Where the Fmax test is significant, I have in some cases performed the AOV on the cage means instead of the values for individual snails.

This procedure was suggested to me by Mr. P. I. Leppard. It assumes without test that the cage means are distributed with equal variances, which is quite likely to be untrue. But the procedure has the other recommendation that it is conservative, considerably reducing the number of degrees of freedom for the analysis.

In other cases, I have proceeded with the full analysis of variance, pointing out that this is not strictly valid and interpreting the results cautiously.

THE MEASUREMENT OF SHELL-GROWTH

In most of the experiments in which snails were numbered for repeated weighing, I also marked the lips of their shells with coloured nail-polish as shown in Figure A 1.1(a). Any new shell added during the experiment could then be measured. I measured it in units of 10ths of a whorl by holding above the spire a celluloid disc marked into 10 equal sectors (Figure A 1.1(b)), and I estimated the amount of new shell to the nearest 0.5 unit (that is, the nearest 20th of a whorl) except between 0 and 0.5 unit where I estimated it to the nearest 0.25 unit (40th of a whorl) to help distinguish snails which grew very little from those which did not grow at all.

This measurement of shell-growth is analogous to the absolute change in weight (W2 - W1) chosen as a measure of growth above, because it is the difference between the number of whorls in a snail's shell at the start of the experiment and the number of whorls at some later time, measured to the nearest 0.05 whorl. And I analysed the data in a similar way. Mostly there was no evidence that the assumptions of analysis of variance were not met. When there was such evidence, I proceeded as with changes in weight.

APPENDIX 2.

STATISTICAL METHODS AND CONVENTIONS FOR PRESENTING RESULTS

The statistical tests in this thesis are all straightforward applications of well-documented procedures. I referred mainly to the textbooks by Siegel (1956), Snedecor and Cochran (1967) and Sokal and Rohlf (1969).

When an analysis of variance has been conducted, I give under the table of sample statistics the value of Fmax (k, n - 1), the ratio of the greatest to the least of the k sample variance with n - 1 degrees of freedom. If the degrees of freedom of the maximum and minimum variances are not equal (strictly, the k samples should have equal sizes) then n- 1 is the lesser of the two. Rohlf and Sokal (1969), Table T, gives upper 5% and 1% points of this ratio on the null hypothesis that there is no heterogeneity among the k variances; it affords a quick test for conformity to the most important assumption of those likely to be wrong (Sokal and Rohlf, 1969, Section 13.3). Procedures adopted when the assumption appears to be wrong are discussed in Appendix 1, and in appropriate places in the text.

The 'log likelihood ratio test' (G - test) for goodness-of-fit is used in Chapter 4 and extensively in Chapter 5. This is used in a very similar way to the χ^2 test for goodness-of-fit. Consider an experiment in which animals are placed into two classes and suppose the experiment is replicated k times. I use the G-test to ask whether in each replicate the numbers of animals falling into the two classes have a ratio significantly different from 1 : 1. The test-

statistic, G (sometimes called 2I), is distributed approximately as χ^2 with 1 degree of freedom in this case. G is additive (the statistic X^2 is only approximately so), so a total G for all the k replicates can be obtained. It has k degrees of freedom. This statistic is labelled 'G_T' in the tables in Chapter 5. Then the numbers of animals falling into the two classes are summed over the k replicates and a G (with 1 degree of freedom) for conformity of these 'pooled' data to the 1 : 1 ratio is calculated. It is labelled Gp. Finally, I calculate a G which is distributed approximately as χ^2_{k-1} on the null hypothesis that there is no heterogeneity among the replicates. It is labelled G_H. This procedure is described by Sokal and Rohlf (1969).

Yates' correction for continuity has not been made in calculating the G values in Chapter 5 because they are to be pooled and partitioned, and G is not additive after the application of Yates' correction (Sokal and Rohlf, 1969, Section 16.3). Also, with the application of the correction the test is likely to be excessively conservative (Grizzle, 1967).

The uncorrected G values may be too large with small samples such as the $n \leq 10$ snails in one of my Y-tubes. If my G values for individual tubes are too large for this reason, their total, G_T, will also be too large. The value of Gp will not be greatly inflated by lack of continuity because its sample size is large, and $G_H = G_T - Gp$ will consequently be too large. This may account for the problem of heterogeneity which I discuss in Chapter 5 and which occurs particularly in cases where Gp is small.

To save space, statistics - generally means, standard errors and sample sizes - rather than raw data have been presented in a number of cases. There seem to be various meanings attached to the term 'standard error'. Throughout this thesis the term refers to the standard error of the mean. Thus, if a sample variance is s^2, and the sample size is n, then by 'standard error' I mean $\sqrt{s^2/n}$.

In Chapter 5, only G values are shown for a particular experiment unless G_H is significant, in which case I show the raw data.

APPENDIX 3

STUDY-AREAS

BUCKLAND PARK

Buckland Park is a large property about 40 km north of Adelaide and near to the coast. I worked on the same area as Pomeroy (1966, 1969), who describes it in detail.

The area is just above sea-level; several hundred metres of saltmarsh lie to the west of it. The soil on the study-area is only a few cm deep, overlying shell grit of Recent marine origin. Thus it is well-drained and calcium is abundant. The population of H. virgata is dense; Pomeroy recorded up to 250/m^2 and frequently in the order of 200/m^2. In the particular area where I worked, the snails are virtually all of the unbanded morph.

Two habitats can be conveniently recognized; 'grassland' and 'shrubland'. The grassland is grazed on occasion by sheep and cattle as well as by the fallow-deer and particularly the numerous rabbits which occur throughout. This habitat is dominated by herbs, notably Avena sp., Bromus sp. and Vulpia sp. with scattered bushes of the composite Olearia axillaris and the legume Acacia ligulata. The shrubland is dominated by O. axillaris and A. ligulata.

NORTHFIELD

The study-area at Northfield was on the property of the South Australian Department of Agriculture, adjacent to the Department's Northfield Research Laboratories, about 11 km north-east of Adelaide.

The area, approximately 18 m x 52 m, had been grazing-land when it was fenced to exclude cattle in 1966 (Hodson, 1969). At that time the vegetation consisted of clover and annual grasses.

When I commenced work there, the area had become dominated by Oxalis sp.; Brassica tournefortii and Asphodelus sp. were common. There were several large plants of Cynara cardunculus.

The area supports a lower density of snails than Buckland Park does. Hodson (1969), who discusses changes in numbers and movements of snails in the areas of short and long grass, most often found numbers in the order of $30/m^2$; the numbers were lower when I worked there.

When setting up the experiment described in Section 7.2, I mowed the vegetation in and surrounding the pens (see below) to a height of about 10 cm with a rotary lawn-mower. At this time the Oxalis had hardly begun to regenerate after dying off in summer, and the mowing served mainly to cut off taller plants such as Asphodelus and dead stems of Rapistrum rugosum and Brassica tournefortii so that the pens could be covered.

PENS USED ON THE STUDY-AREAS

Northfield Hodson (1969) describes the construction of 10 large pens at Northfield; eight of these were modified for the experiment in Section 7.2. The pens were rectangular, 3 m x 1.5 m. The walls, about 20 cm high, were made of galvanized wire fly-mesh supported on a trellis-wire framework which was soldered to a strip of galvanized sheet-iron driven about 10 cm into the ground.

The tops of the walls had been turned inwards and fringed with plastic fibres, a device which no longer made the pens snail-proof with the changed vegetation . . . the Oxalis grew to the tops of the walls and the snails could escape by climbing the plants. I therefore covered each pen with a sheet of plastic-coated fibreglass fly-mesh clipped onto the walls with wire hooks. The cover could be removed to permit searching for snails. Thus, each pen became essentially a large fly-mesh box, 3m x 1.5m x 20 cm (Figure A 3.1).

Buckland Park Pomeroy (1966) describes the construction of 8 smaller pens, 60 cm square, of similar design to the above but having sheet-metal bases set in the ground and walls which are clipped onto the bases and can be removed to facilitate searching for snails. For the experiment in Section 7.4 I modified these by again adding roofs of plastic fly-mesh, this time sewing them on with fishing line; and I constructed a number of additional pens of the same design (Figure A 3.2).

FIGURE A 3.1

A pen of the type used at Northfield, 1970 (Section 7.2).

The sheet-metal base, which is set in the ground, is shown shaded. The rest of the walls are covered with galvanized iron fly-mesh. The roof, of plastic-coated fibreglass fly-mesh, is stretched tight and attached with wire hooks.

For clarity, the trellis-wire frame is shown only on the front walls.

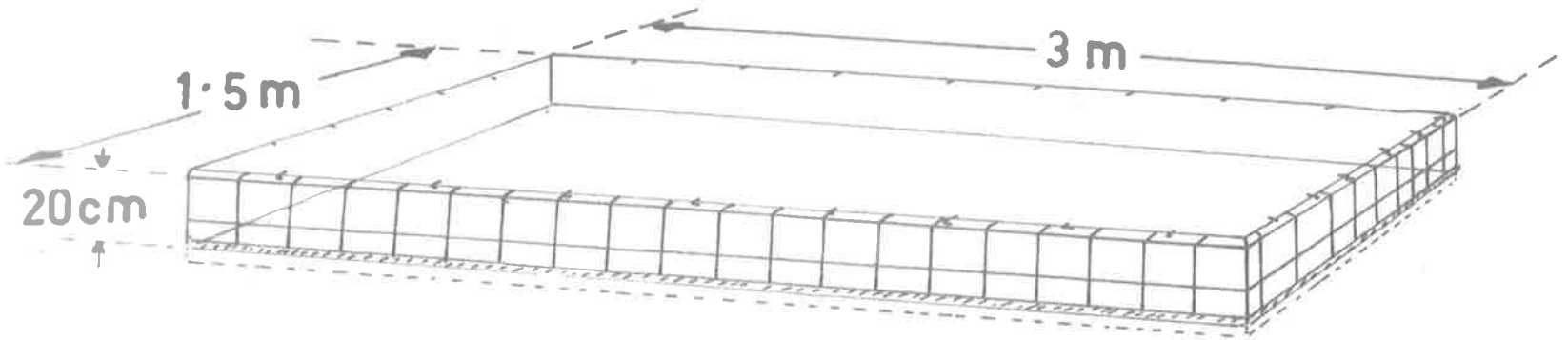
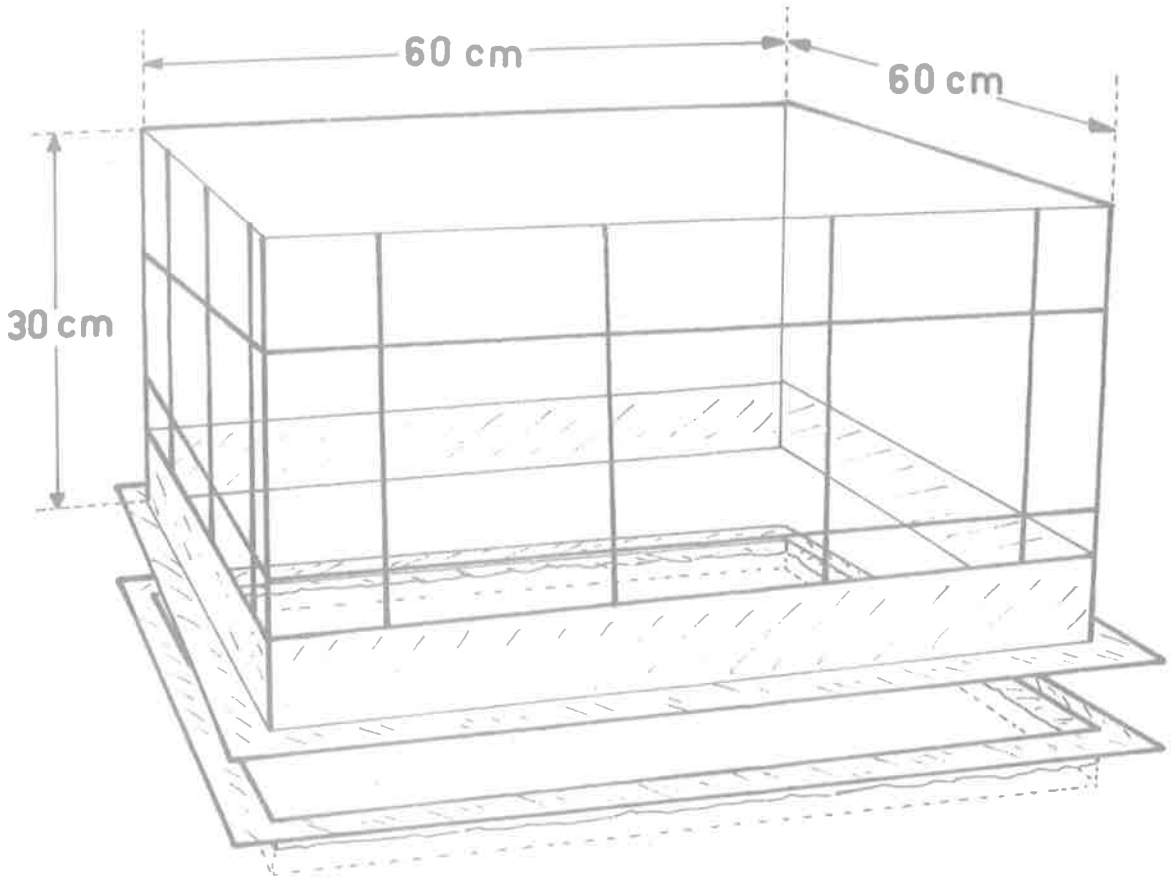

3 m
1·5 m
20 cm

FIGURE A 3.2

A pen of the type used at Buckland Park, 1971 (Section 7.3), shown raised above its base which is set in the ground. Sheet-iron parts are shown shaded. The remainder of the pen is covered with plastic-coated fibreglass fly-mesh. The wire framework is shown only on the front walls. The pen is clipped to the base with clothes pegs.


60 cm
60 cm
30 cm

APPENDIX 4

KEEPING SNAILS IN THE LABORATORY

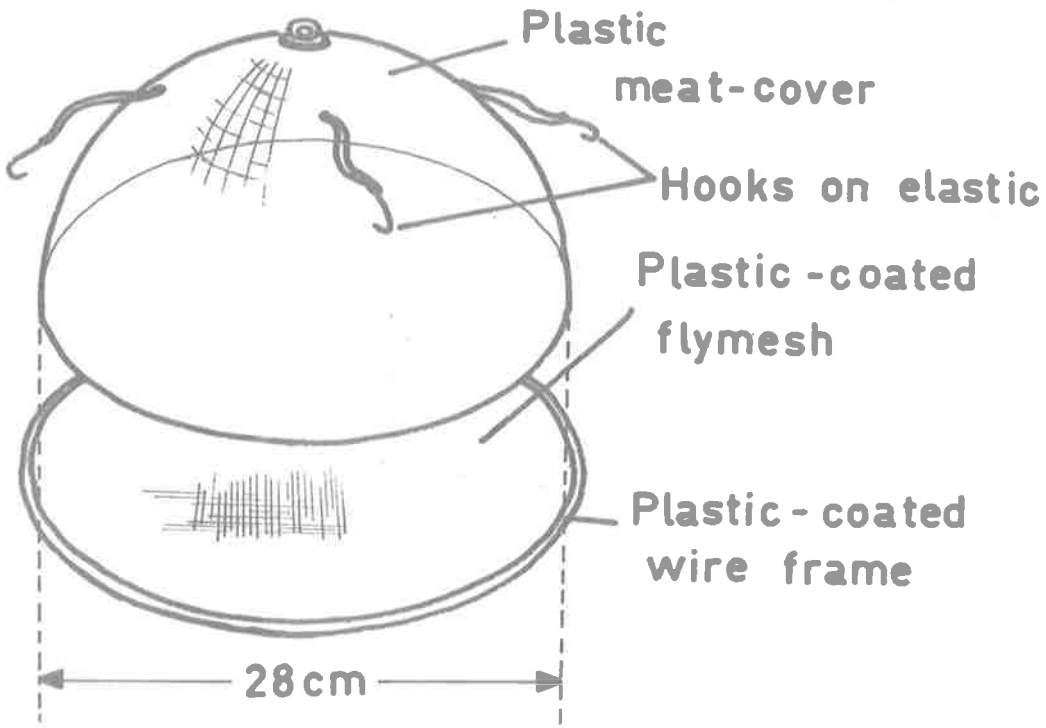
H. virgata proves more difficult to keep active in the laboratory than some other land-snails. If conditions are too dry the snails of course become dormant; but if they are too wet the animals appear to become water-logged, and soon die. (In the field, H. virgata can be seen climbing vegetation and becoming inactive if the ground is very wet.)

In my laboratory experiments snails were housed in small cages made from plastic meat-covers about 28 cm in diameter. Each cage had a base made from a circle of heavy wire coated with plastic tubing, to which was attached (by melting the plastic slightly) a disc of plastic-coated fibreglass fly-mesh. The meat cover was clipped onto its base using wire hooks attached to rubber bands (Figure A 4.1).

The cages were supported on rods of wooden dowelling so that air could circulate freely around them. Above each rack holding 9 cages was a jet (Tee Jet T N-3W - Spraying Systems Co.) which produced a mist-spray of distilled water. There were 5 such racks and 5 jets. The jets were all fed by a small rotary pump (Jabsco Pump Co., Herts. England) powered by a 1/4 horsepower electric motor. An electric time-switch could be set to make the sprays operate as many times as desired during 24 hours; they operated for about 30 minutes each time. The pump took distilled water from a tank which was automatically refilled from the building's supply. Each rack stood in a metal pan from which excess water drained away through plastic

FIGURE A 4.1

A cage of the type used to house snails under sprays in the laboratory.


Plastic
meat-cover
Hooks on elastic
Plastic-coated
flymesh
Plastic-coated
wire frame
28cm

hose, and the racks were surrounded by walls of sheet plastic on two or three sides (Figure A 4.2).

When I commenced work the time-switch was set so that the sprays operated 6 or 8 times in 24 hours. As the sprays were switched off, fans were simultaneously switched on and these served to dry out the cages in the interval between sprays. However, I found it more satisfactory to reduce the number of sprays to, usually, 2 in 24 hours and dispense with the fans. The timing of the sprays is recorded with the 'Procedure' for each experiment.

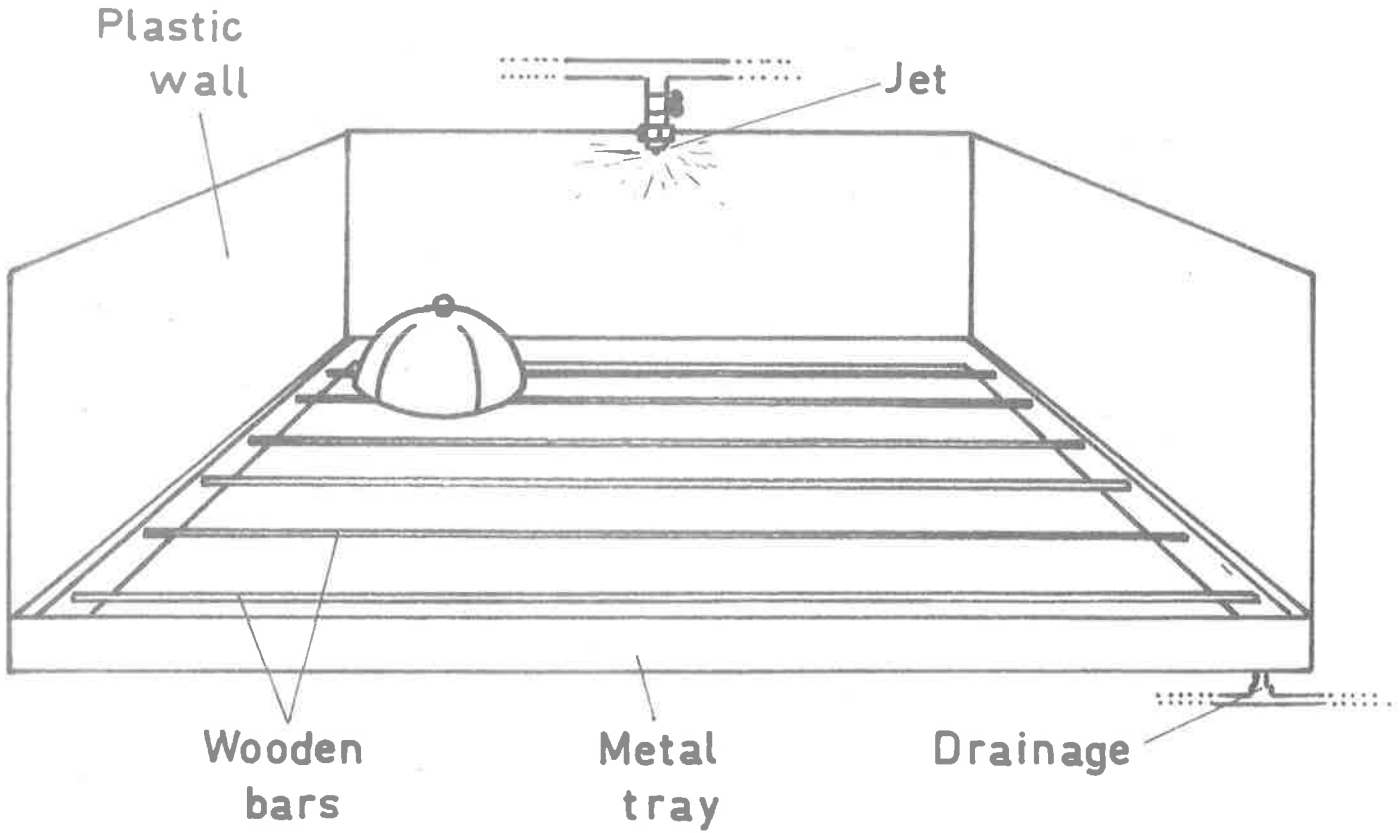
The room was air-conditioned to maintain it at approximately 21°C; under the sprays the temperature fell to about 16°C or less when the sprays operated. These temperatures are higher than those likely to be experienced by snails active in winter; but I had no control over the temperature nor was there evidence that it had any ill-effect.

A bank of fluorescent lights in the room was controlled by a time-switch and was generally set on a 'winter' photoperiod of 11 hours. Again, the regime used is recorded with the 'Procedure' for each experiment.

The sprays did not wet the cages on the racks equally, particularly in the experiment of Section 3.3.1 where there were 48 cages to be housed in the space of 39, and 9 cages were therefore stacked on the tops of others. To avoid any bias due to some snails spending more time active than others, I rotated the cages through the positions on the racks in a systematic order. Generally I moved them all one position each day. In addition, the initial positions occupied by the different treatments in an experiment were randomly-

FIGURE A 4.2

Rack for housing snails in the laboratory. (The rack holds 9 cages of which only one is shown.)


Plastic
wall
Jet
Wooden
bars
Metal
tray
Drainage

chosen.

This apparatus for keeping snails may not have been entirely satisfactory. Some snails lived for a long time in the apparatus (in excess of six months, when I removed them to make space for other experiments) but many died. However, because of the variability in the health of the snails I think it likely that the apparatus itself is satisfactory for housing this species, and that disease (which I discuss in Chapter 3) or dietary deficiency are more likely to be the sources of the problems.

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