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An Evaluation of the Interactions Between Freshwater Pulmonate Snail Hosts of Human Schistosomes and Macrophytes

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AN EVALUATION OF THE INTERACTIONS BETWEEN FRESHWATER PULMONATE SNAIL HOSTS OF HUMAN SCHISTOSOMES AND MACROPHYTES

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An account is given of a laboratory investigation designed to evaluate the extent to which the freshwater pulmonate snail *Biomphalaria glabrata* (Say) can utilize various species of aquatic plants, mainly macrophytes, when presented in the following forms

over different time scales: (i) normal plants; (ii) dried plant material; (iii) homogenized plant material in calcium alginate matrices; (iv) water-soluble filtrates of plant homogenates in the medium.

The following propositions, derived from the theory of phased coevolution of components of the module consisting of the epiphytic bacteria, algae, snails and macrophytes, are evaluated on the basis of the present results and others including those obtained in this laboratory. (i) That as the snails had become specialized to exploit surface communities of epiphytic algae, decaying plant material and dissolved organic matter (DOM) early in their evolutionary history they would continue to exploit these resources when they later become associated with aquatic macrophytes. (ii) That pulmonate snails would tend to be feeding generalists capable of adapting to food of varying chemical composition, given sufficient time, provided it was sufficiently small or flaccid. (iii) That although macrophytes and snails show a strong positive relationship, the living macrophyte tissue would be little used by the snails. (iv) That the hard outer envelope, inherited from their terrestrial ancestors, would remain as the major defence mechanism of aquatic macrophytes against attack by snails and other aquatic invertebrates. (v) That aquatic macrophytes would invest little in the nutrient deficiency strategy to reduce attack by invertebrates such as snails. (vi) That truly aquatic submerged macrophytes would not possess secondary plant compounds (SPC) that would be molluscicidal. (vii) Emergent parts of subaquatic or aquatic plants might be expected to be better sources of SPC with molluscicidal factors than submerged aquatic plants. (viii) Species of epiphytic or planktonic algae might be better sources of SPC with molluscicidal effects than aquatic macrophytes. (ix) That the strategies developed by pulmonate snails for obtaining their energy supplies would not be conducive to rapid speciation. The analysis of the present and other related results supports these propositions.

Predictions based on the theory of mutualism involving the snails, macrophytes and other components of the module also receive some support from an analysis of the present results. The additional empirical work that could be undertaken to test this theory is briefly discussed. Possible reasons are given for the differences between the nature of the interactions involving herbivores and plants in terrestrial and freshwater ecosystems.

1. Introduction

It is generally accepted that freshwater pulmonate snails, such as *Biomphalaria glabrata* (Say) and *Biomphalaria pfeifferi* (Krauss) tend to be positively associated with both subaquatic and aquatic macrophytes or their decaying remains (Ferguson 1977; Pimentel & White 1959). Quantitative evidence in support of this generalization was recently provided by Thomas & Tait (1984*b*) although they also found some negative associations at the microdistributional level.

Two hypotheses, based on evolutionary considerations, can be advanced to explain these negative and positive associations. First, it can be postulated that the negative associations might be due to the release of allelopathic substances by the plants. Such factors might act as repellents, antifeedants or even as toxicants, thus either preventing the snails from approaching or ingesting the plants. A considerable body of information has accumulated on the occurrence of such allelopathic factors in terrestrial plants and some of these, such as nicotine or pyrethrin, or their derivatives, are currently being used to control insect pests (Harborne 1977; Brooks & Johnson 1979; Crawley 1983). If aquatic plants also proved to contain comparable factors, either they or their derivatives might have some potential application as molluscicides.

The second hypothesis explains the positive associations, and postulates that the plants are

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releasing kairomones which act as attractant, arrestants and phagostimulants and that the plants are being utilized as a food source. However, Thomas (1982), Thomas *et al.* (1983*a*, 1985) and Thomas & Tait (1984*b*) have argued that the interrelationships between the pulmonate snails and macrophytes are more complex than this and that there may be strong mutualistic aspects to the associations, resulting from coevolution over the past five hundred million years. This is a departure from current trends as modern theories of community structure tend to ignore mutualism (Wilson 1983) and most community studies omit decomposers or herbivore-decomposers such as *B. glabrata* or else treat them as a kind of consumer species (May 1983).

The possible benefits that both snails and macrophytes may derive from coexistence are illustrated in figure 1. The benefits to the plants are less obvious than those to the snails and include the following. First, the snails may remove excessive growths of epiphytic algae which might harm the macrophytes by competing for light, CO_2 and other nutrient factors. Secondly, the removal of senescent or decaying tissues by the snails reduces the probability of the plant tissue being invaded by pathogenic micro-organisms. Thirdly, the snails facilitate the release and recycling of plant nutrients (for example, Ca^{2+}, Mg^{2+}, K^+, Na^+, NO_3^-, PO_4^{2-}, Cl^-). Fourthly, the snails may assist the plants by removing potentially toxic oxygen. Fifthly, the snails release excretory products such as ammonia, urea and CO_2 which serve as nutrients to the plants. Sixthly, mucus secretions by snails may protect the plants from attack by other

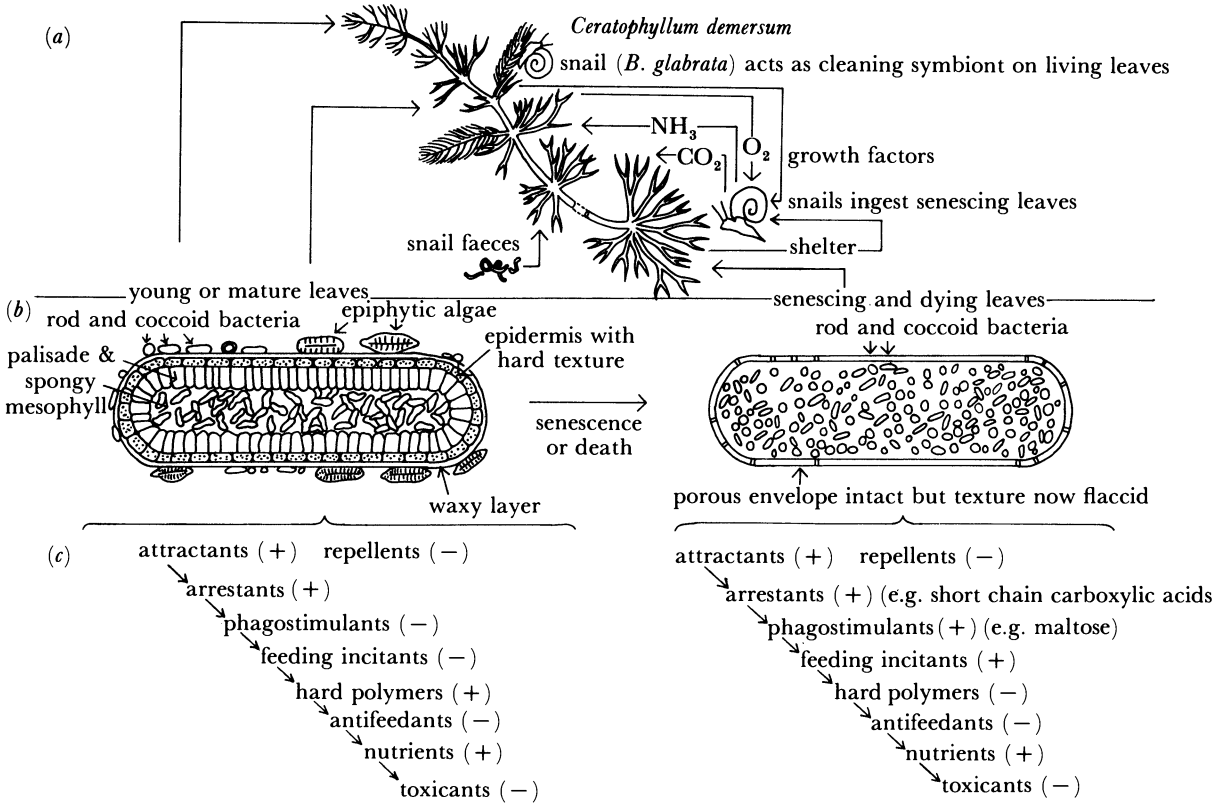

FIGURE 1. Interactions between snails (*B. glabrata*) and aquatic macrophytes *Ceratophyllum demersum*: (*a*) some of the beneficial interactions between snails and plants; (*b*) a diagrammatic representation of mature and senescing or dying leaves; (*c*) the classes of chemical factors which may influence the sequential steps involved in feeding by the snails (− denotes absence). (See table 3 for bioassays that can be used to identify these.)

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herbivores. As a result of recent studies on the feeding behaviour of *B. glabrata* (Thomas *et al.* 1985) this concept of mutualism has been extended further to include the epiphytic algae and the micro-organisms involved in decomposition in addition to the macrophytes and the snails. These components together may be considered as a module with six subsets (Paine 1980). Many of the linkages between the subsets are positive or mutualistic and are suggestive of coevolution.

In addition to the benefits that the macrophytes may derive from association with snails, they may also incur costs. Thus, for example, if the snails occurred at high density, relative to food availability, they might conceivably attack living macrophyte tissue. It is therefore necessary to postulate that as a prerequisite for such mutualistic reactions to occur, it would be essential for the macrophytes to be shielded from attack by snails and other herbivores. However, if on balance the plants benefit from the presence of snails, one might predict that they would release chemical factors which would act as attractants or growth factors to the snails. However, before the theory of mutualism can be properly evaluated, it is necessary to consider it within the context of the theory of phased coevolution, involving the major components of the module: the bacteria, epiphytic algae, snails and macrophytes.

In the present paper, an account is given of laboratory experiments designed to give comparative information on the survival and growth of snails provided with whole macrophytes, their homogenized tissues in uniform matrices and plant homogenates at various concentrations in the medium, as potential sources of food or allelopathic factors. These results will be used to evaluate the propositions and predictions derived from the theories of co-evolution and mutualism and also to explain the differences between the nature of the interactions between aquatic pulmonate snails and macrophytes, on the one hand, and terrestrial plants and herbivores on the other.

2. Materials and methods

The *B. glabrata* used in the experiments was an albino strain originating from Venezuela. Lettuce, *Lactuca sativa* L., which is normally used as a food source for the snails in the laboratory, and 32 species of aquatic plants (figure 1) were evaluated as potential food sources. The families to which they belong and their source of origin are given in table 1*a*. These include plants which have been identified in *B. glabrata* habitats, such as *Nymphaea*, *Lemna*, *Ceratophyllum*, cosmopolitan or tropical plants which are likely to be found in water bodies where *B. glabrata* occurs, as well as some novel species whose ranges are not likely to have overlapped those of *B. glabrata*. Fifteen of these species were also evaluated as possible sources of allelopathic, toxic factors (figure 9). The various methods used are outlined below.

The various categories of *B. glabrata* used in the experiments were carefully selected on the basis of size and health from stock cultures (Thomas *et al.* 1983*b*). All the snails were acclimated for three days before experimentation in standard snail water (ssw2) (Thomas *et al.* 1975). During this time and in subsequent experiments they were kept at a density of one snail per 50 or 100 ml ssw2 in an environmental unit maintained at a temperature of 26 ± 1 °C and a photoperiod of 12:12, L:D. Food was provided in the form of two 1.7 cm diameter lettuce discs (mean mass 38.81 ± 0.78 mg per disc).

The procedures used in the various experiments are summarized in table 1*b*. Three kinds of matrices were evaluated as systems for holding plant homogenates or filtrates in a form that would allow them to be ingested by the snails. These were: (i) 10% gelatine in ssw2 diluted

TABLE 1*a*. THE PERCENTAGE GROWTH RATES, COMPARED WITH THE MEAN GROWTH RATES OF LETTUCE CONTROLS, ACHIEVED BY SNAILS FED ON STANDARD QUANTITIES OF MACROPHYTE LEAVES AND ON THEIR HOMOGENATES IN CALCIUM ALGINATE MATRICES

family	plant species	whole plant	matrices
Potamogetonaceae	‡ *Groenlandia densa* (L.) Fourr	80.2	76.5
	G† *Potamogeton crispus* L.	−2.0	—
	G† *Potamogeton natans* L.	—	45.9
Cruciferae	† *Nasturtium officinale* R.Br. Hayek	22.7	30.5
Cabombaceae	† *Cabomba aquatica* Aubl.	10.7	69.6
Hydrocharitaceae	G† *Vallisneria spiralis* L.	14.9	53.9
	† *Elodea canadensis* Mich X.	12.2	68.6
	§ *Hydrocharis morsus-ranae* L.	33.2	55.1
	G† *Limnobium laevigatum* Humb & Bomplex Willd)Heine	16.5	—
Araceae	S† *Pistia stratiotes* L.	6.5	96.2
Lemnaceae	G† *Lemna minor* F.	24.2	—
	† *Lemna paucicostata* Hegelm ex Engelm.	—	34.2
Ceratophyllaceae	S† *Ceratophyllum demersum* L.	2.8	71.8
Nymphaeaceae	G† *Nymphaea lotus* L.	20.5	−11.8
Ranunculaceae	† *Ranunculus aquatilis* L.	10.2	79.4
Hippuridaceae	§ *Hippuris vulgaris* L.	35.8	—
Alismaceae	G *Sagittaria sagittifolia* L.	15.3	41.8
	† *Alisma plantago-aquatica* L.	52.4	31.1
Polygonaceae	G *Polygonum amphibium* L.	19.7	−8.8
Umbelliferae	† *Apium nodiflorum* (L.) Lag.	17.1	121.5
	‡ *Oenanthe aquatica* (L.) Poir.	11.5	74.8
Gramineae	† *Glyceria fluitans* (L.) R.Br.	4.3	125.4
Boraginaceae	‡ *Myosotis scorpioides* L.	7.3	96.1
Haloragaceae	† *Myriophyllum spicatum* L.	9.8	91.8
Pontederiaceae	† *Eichhornia crassipes* (Mart.) Solms-Laub.	7.1 2.1	— —
Callitrichaceae	† *Callitriche obtusangula* Le Gall.	27.8	80.2
Amaranthaceae	† *Alternanthera sessilis* (L.) R.Br. ex Roth.	14.3	snails died
Musci	‡ *Fontinalis antipyretica* Hedw.	37.2	−23.8
Salviniaceae	† *Azolla africana* Desv.	11.7	—
	S† *Azolla caroliniana* Wild	9.8	48.9
	G† *Salvinia nymphellula* Desv.	15.8	45.7
Characeae	G† *Chara* sp.	22.3	58.2

Cited at generic (G) or specific level (S) as being present in *B. glabrata* habitats in Puerto Rico (Pimental & White 1959).

† Species recorded in South American or with cosmopolitan or almost cosmopolitan distribution according to Cook *et al.* (1974).

‡ Europe, Asia, Africa.

§ North temperate zone.

1:1 with plant extract (50% homogenized in distilled water); (ii) 5% Agar in ssw2 diluted 1:1 with plant extract; (iii) calcium alginate matrix (Standen 1951). The latter was the only one that proved satisfactory. It was prepared as follows: 100 g (wet mass) of a particular plant species was ground in liquid nitrogen with the aid of a pestle and mortar, and 2 g of sodium alginate and 125 ml ssw2 added. After homogenization the mixture was poured into a shallow, standard tray measuring 29 cm × 22 cm × 3 cm to a depth of 1.75 mm. Gelling was achieved by spraying the surface with a 2% $CaCl_2$ solution. The gel was cut into 1.7 cm diameter discs each weighing approximately 420 mg (187 and 9 mg wet and dry mass of plant material,

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TABLE 1*b*. DETAILS OF EXPERIMENTAL PROCEDURES

experiments	plant species used	initial snail mass (mg)	weighing intervals (days)	duration of experiment (days)	volume of medium (ml)	frequency of changing (days)	quantity plant material used per snail	reference in text
normal plant tissue								
short-term experiment	lettuce (control) + 31 species	80	4	4	100	daily	2, 1.7 cm discs or equivalent daily	figure 2
medium-term experiment	lettuce (control) + 15 species	80	3	9	100	3	2, 1.7 cm discs or equivalent daily	figure 3
long-term experiments	lettuce	4–6						
	Nasturtium officinale	20	3–4	17	50	3–4	0.5 g every 3–4 days	figure 4
	Myosotis scorpioides	110						
	Callitriche obtusangula							
	Lemna minor							
very long-term experiments	lettuce	egg masses†	3–4	56–86	100	3–4	1, 1.7 cm discs (snails under 100 mg) 2, 1.7 cm discs (snails over 100 mg)	figures 5 and 6 table 2
	Nasturtium officinale	4–6 20						
dried plant	lettuce (control)	40	3	9	100	3	equivalent to 2, 1.7 cm discs	
	powdered and dried lettuce	300					equivalent to 2, 2.9 cm discs daily	figure 7
plant material in calcium alginate matrix		‡						
short-term experiment	lettuce (control) + 25 species	80	4	4	100	daily	2 calcium alginate discs = 374 wet mass, 18 mg mass of plant	figure 2
long-term experiments	lettuce	4					1, 1.7 cm discs	
	Nasturtium officinale	20	3–4	17	50	3–4		
	homogenates and homogenate filtrates	100					daily	figure 8
water-soluble filtrates	lettuce + 15 plant species	9 80	3	9	100	3	0, 50, 200, 400, 800 mg of homogenates in 100 ml + 2, 1.7 cm lettuce discs daily	figure 9

All treatments replicated 10 times except those marked with † (25 replicates) and ‡ where treatments involving *Chara*, *Pistia*, *Salvinia*, *Polygonum*, *Oenanthe* and *Myriophyllum* which were only replicated five times.

respectively). The matrices were stored at −5 °C. The water-soluble filtrates used in some of the experiments were prepared by filtering the quantity of homogenate normally used through a Whatman number 1 filter.

The snails were weighed as described by Thomas & Benjamin (1974). The relative growth rates of the snails were calculated as follows: $100\ (W_t - W_0)/\{W_0\,(t_n - t_0)\}$ where W_0 and W_t are the wet mass in milligrams on days 0 and t, respectively, for short time intervals of four days or less or as $\{\ln\,(W_t/W_0)\ t\}\ 100$ for longer time intervals (Wilbur & Owen 1964). Over the time interval of four days the two methods gave almost identical results.

3. Results

The results of the first experiment (figure 2A, table 1*a*) show that although the various lettuce cultivars, used as controls for comparison purposes, may differ in nutritional quality they are on average better food sources to the snails than any of the 31 species of aquatic plants presented in their normal form over the time course of this experiment. Thus only five of the 31 aquatic plants tested, namely *Groenlandia densa*, *Hydrocharis morsus-ranae*, *Hippuris vulgaris*, *Alisma plantago-aquatica* and *Fontinalis antipyretica* resulted in the assay snails growing at rates that were more than 30% of the mean value achieved by snails fed on lettuce. However, the assay snails did achieve some slight positive growth in all the other treatments containing aquatic macrophytes except for those with *Potamogeton crispus* and *Lyngbya* species. None of the 31 plant species tested influenced the survival of the snails over the time scale of this experiment.

The results in figure 3, which were based on feeding experiments carried out over a longer period of nine days, confirm that the snails find it difficult to utilize most species of aquatic macrophytes as food sources. Thus, over the initial three-day period the growth rates achieved by the snails fed on lettuce were significantly greater ($p < 0.05$) than those of snails fed on aquatic plants with the exception of *Groenlandia*, *Callitriche*, *Hippuris* and *Alisma*. By the end of the sixth and ninth days the growth rates achieved by the snails fed on lettuce were significantly greater ($p < 0.05$) than those of snails fed on all the species of aquatic plants with the exception of *Groenlandia*. It would appear that the snails find it difficult to adapt to the aquatic plants offered to them, as the growth rates of the snails feeding on them continued to decline during the course of the experiment. In contrast the growth rates of snails fed on lettuce continued to increase. Nevertheless some slight positive growth was achieved by the snails in all the treatments containing aquatic plants, with the exception of that containing *Lyngbya*. There was no evidence that the survival of the snails was influenced by any of the plant species during the course of this experiment.

Figure 4*a–c* shows that the changes in the absolute growth achieved by snails with initial masses of approximately 4–5, 20 and 100 mg over a time scale of up to 17 days when provided with four species of aquatic macrophytes and lettuce as food sources in five separate treatments. It is evident that the absolute growths achieved by the snails in the 20 and 100 mg mass categories are significantly higher in the treatments containing lettuce as a food source than in those with aquatic macrophytes by the end of the 14th and 17th days. The growth achieved by the snails in these two mass categories was particularly poor in the treatments containing *Myosotis* and *Lemna* as food sources. This was also the case with the smaller snails in the 4–5 mg mass category. However, in contrast to the larger snails, the smaller ones had achieved absolute growths by the end of the 14th day in the *Nasturtium* and *Callitriche* treatments which were

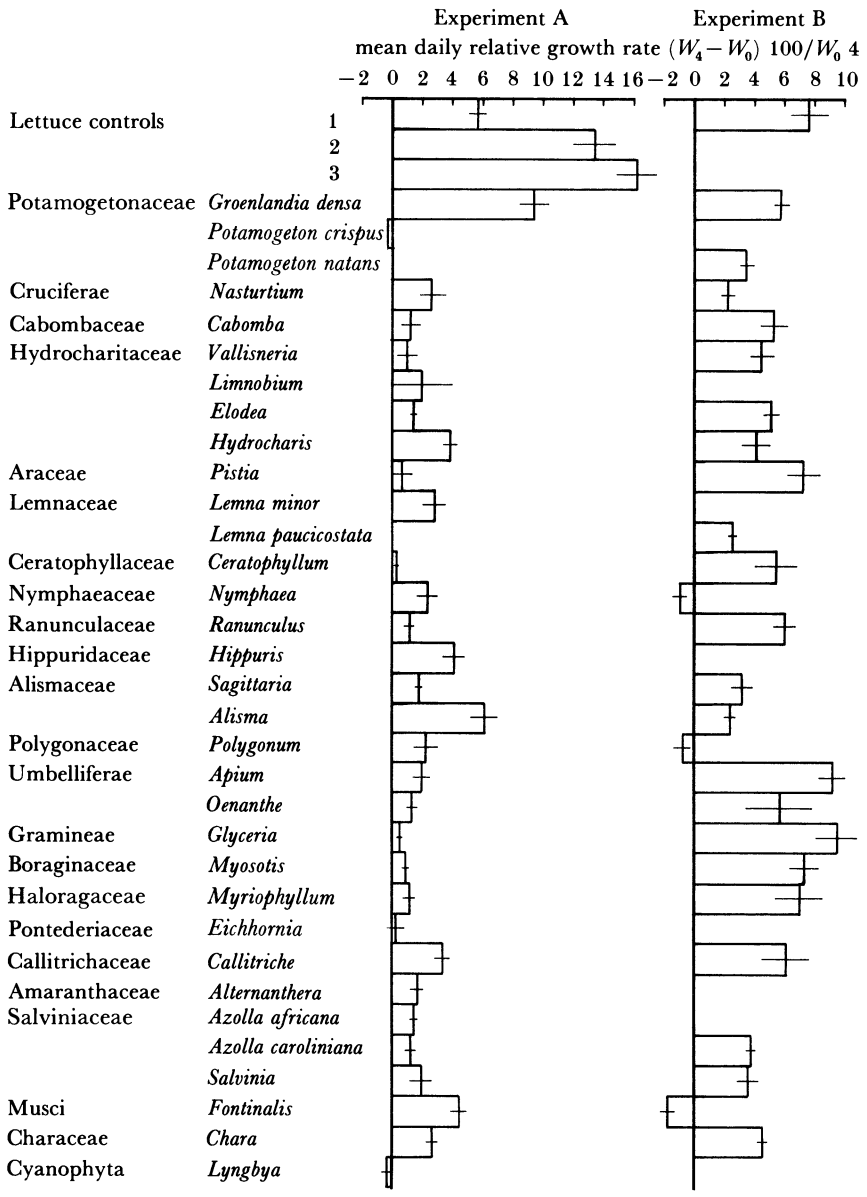

FIGURE 2. The mean daily growth rates $(W_4-W_0)\ 100/W_0 4$ where W_0 is the initial mass in milligrams and W_4 the mass in milligrams after four days achieved by *B. glabrata* provided with two 1.7 cm diameter lettuce discs or the equivalent biomass of aquatic plant as a food source (experiment A) and homogenates of the same plant in calcium alginate (100 g plant, 125 ml water, 2 g alginate). See text for further details. The specific names of the plants are given in table 1.

not significantly different from that of snails provided with lettuce as food. By the 17th day the absolute growth in the *Callitriche* treatment was significantly higher ($p < 0.05$) than in the lettuce controls.

In the experiments involving a comparison of watercress and lettuce as food sources, the results of which are summarized in figures 5 and 6, the observation period was further extended to 56 days in the case of those involving snails with initial masses of 4, 20 and 100 mg and 86 days in the experiment commencing with snails at the egg stage. Figure 5 shows that the absolute growths achieved by the snails in the 4, 20 and 100 mg mass categories are significantly

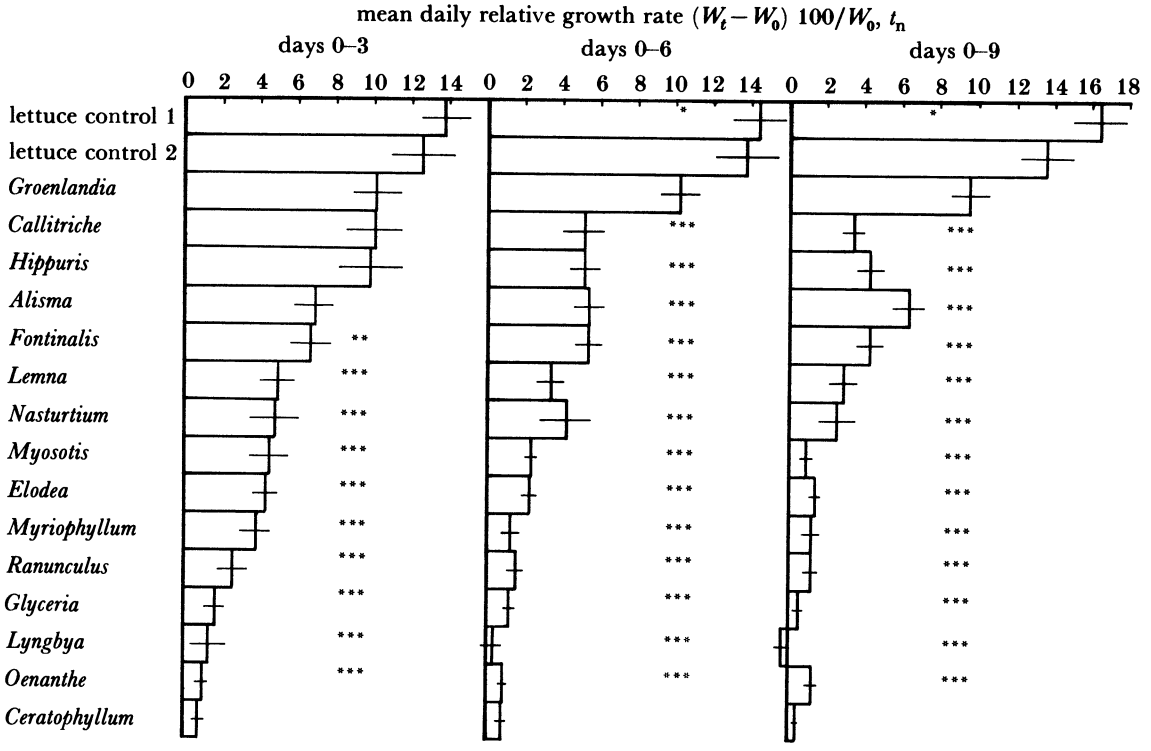

FIGURE 3. The mean daily relative growth rates $(W_n - W_0)\ 100/W_0 n$ at three time intervals of up to nine days (where W_0 is initial mass in milligrams, W_n is mass in milligrams after n days) achieved by *B. glabrata*, provided with two, 1.7 cm diameter lettuce discs or the equivalent biomass of aquatic plants as a food source (See table 1 for specific names).**, ***, Significant differences between experimental plants and lettuce controls at $p < 0.01$, and $p < 0.001$, respectively.

higher in the treatment in which they are provided with lettuce as food than in those in which they were given watercress. Although the absolute growths achieved by the snails in all three mass categories indicate relatively slow growth when they are provided with watercress as a food source there is evidence from the increases in growth rates that they have the ability to adapt to this novel food source. It is of interest that the extent to which the snails can adapt to watercress as a diet appears to be inversely related to their size and age. Thus the time required to reach the phase when they show an acceleration in growth, becomes progressively longer with increase in the initial mass of the snails. As a result, the snails with an initial mass of approximately 4 mg fed with watercress achieve a mean mass at the end of the experiment which is not significantly different from that of snails with an initial mass of 20 mg provided with the same food source. However, none of the snails with initial mass of 4, 20 and 100 mg entered the exponential growth phase (Thomas & Benjamin 1974*b*) during the course of this experiment. In contrast, this was achieved by snails reared on watercress from the egg stage after about 50 days (figure 6). The lag phase preceding the logarithmic phase of growth was, however, appreciably longer for the snails reared from the eggs on watercress than was the case with those reared from the eggs on lettuce (figure 6). The duration of the lag phase was positively correlated with the mortality rate. Thus, for example, the snails reared from the egg on watercress suffered a mortality of 19.2% from day 46 to day 83 compared with only 4.4% in the case of snails reared from the egg on lettuce over the same period.

Table 2 shows the mean egg mass production achieved by snails in the various size categories,

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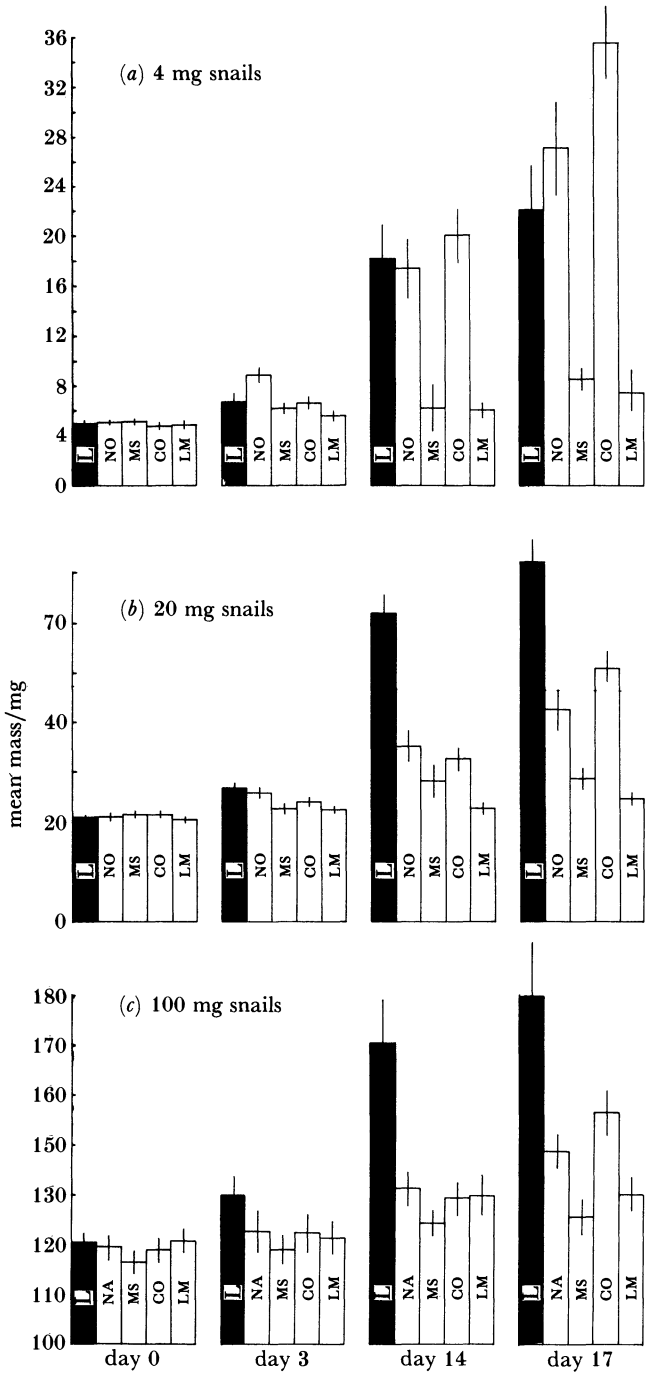

FIGURE 4. Mean absolute growth in milligrams achieved by snails with initial masses of 4, 20 and 100 mg, fed on one, two and four 1.7 cm diameter discs of lettuce and equivalent masses of *Nasturtium officinale* (NO), *Myosotis scorpioides* (MS), *Callitriche obtusangula* (CO) and *Lemna minor* (LM), respectively, at 3, 14 and 17 day intervals. Standard errors are given.

when provided with either lettuce or watercress as food sources. In the case of snails reared from the egg stage those in the lettuce treatments begin ovipositing soon after they enter the exponential growth phase when their mean mass is 63.7 mg. In contrast those in the watercress treatments do not oviposit until their mean mass is 130.6 mg, 17 days later. Egg production

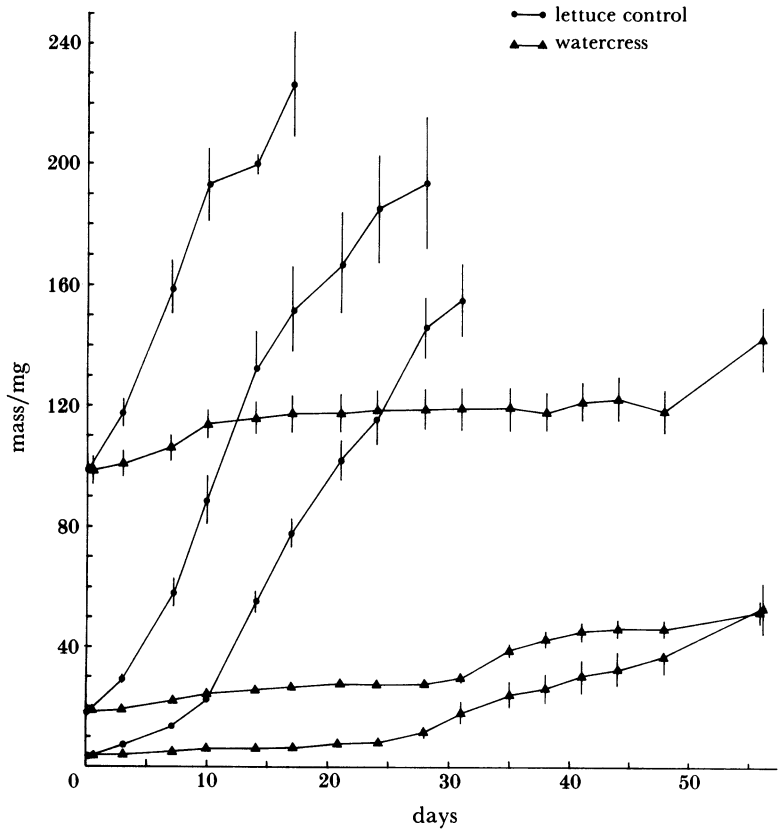

FIGURE 5. Mean absolute growth in milligrams achieved by snails with initial masses of 4, 20 and 100 mg fed on watercress, *Nasturtium officinale*, and lettuce in excess of requirements over a period of 56 days (bars show standard errors).

is also considerably delayed in the case of snails with initial masses of 4 and 20 mg reared on watercress compared with snails in the same size categories provided with lettuce as a food. It is of interest that although the snails in these two mass categories, fed on watercress, are much older when they begin ovipositing than was the case with those fed on lettuce, they are able to do so when relatively quite small, their mean masses ranging from 46 to 53 mg. Although many of the snails in the 100 mg mass range had already reached the oviposition stage at the beginning of the experiment it can be seen that switching to feeding on watercress tended to result in a reduction in the number of egg masses deposited.

The results of experiments described subsequently provide further information on the effects of the 'reatments on the value of various plants as food for the snails. Figure 7 shows that when assay snails in the 300 mg mass category were provided with normal lettuce discs as food, their relative growth and oviposition rates at the end of the nine-day period were significantly greater than those of snails in the same mass category provided with dried discs or dry powdered lettuce in equivalent amounts. This treatment effect is less apparent on days 0–3 and 3–6 so far as the relative growth results are concerned for the following reasons. First, the deprived snails achieved positive growth over these two time periods possibly as a result of shell growth resulting from the accumulation of calcium carbonate from the medium or alternatively as a result of water uptake resulting from malfunction of the osmoregulatory mechanism. Secondly, the relative growth rates were low as a consequence of the large size of the snails. Thirdly, the

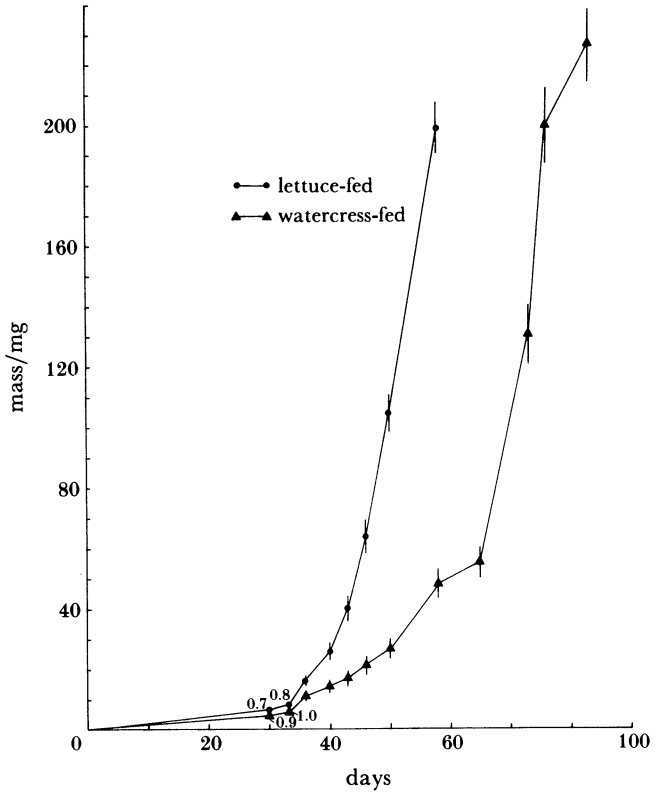

FIGURE 6. Mean absolute growth in milligram achieved by *B. glabrata* reared from eggs on lettuce and watercress, *Nasturtium officinale*, in excess of requirements over a period of 86 days. Bars show standard errors.

variance were relatively high, particularly in the case of the deprived snails. Similar trends were observed in the case of snails in the 40 mg mass categories and by the last period (days 6–9) the relative growth rates achieved by the snails in the treatments receiving fresh lettuce discs were significantly higher than in those provided with lettuce in the form of dry powder or discs.

The results of the short-term experiments with homogenates of various aquatic plants in calcium alginate matrices (figure 2B, table 1*a*) show that assay snails provided with these achieved relatively high growth rates, compared with lettuce controls, in many cases. Thus, for example, the snails given homogenates of *Glyceria* or *Apium* achieved higher mean relative growth rates than those in the lettuce controls, whereas those given homogenates of *Myosotis* and *Myriophyllum* achieved relative growth rates in excess of 90% of the control values. The assay snails also achieved relative growth rates greater than 50% of the control values in several other treatments, namely those containing matrices with homogenates of *Chara*, *Groenlandia*, *Cabomba*, *Vallisneria*, *Elodea*, *Hydrocharis*, *Ceratophyllum*, *Ranunculus*, *Oenanthe* and *Callitriche*. Positive growth was also achieved by the assay snails in all the other treatments with the exception of those given homogenates of *Nymphaea*, *Polygonum*, *Fontinalis* and *Alternanthera*. No snail mortality occurred in treatments containing matrices with *Nymphaea*, *Polygonum* and *Fontinalis* but all snails in the *Alternanthera* treatment died within the first day of the experiment. It is noteworthy that in all the treatments involving plant homogenates, with the exception of those containing the last four species, the assay snails achieved higher relative growth rates

Table 2. The mean number of egg masses per day per snail deposited by snails fed, from various stages of development, on either lettuce (L) or watercress (WC)

initial mass / mg	food type							
		days from start ...	3	7	10	14	17	
100	L	mean masses in milligrams	117.56	158.61	193.36	200.42	226.51	
	L	mean number of egg masses per day per snail	0.6	0.6	2.2	0.2	2.0	
	WC	mean masses in milligrams	100.6	106.0	118.74	116.05	117.94	
	WC	mean number of egg masses per day per snail	0.1	0.6	0.1	0.1	0.1	
		days from start ...	17	21	24	28	31	48	56
20	L	mean masses in milligrams	151.55	167.56	185.62	194.73	—	—	—
	L	mean number of egg masses per day per snail	0.6	1.7	3.5	0.4	—	—	—
	WC	mean masses in milligrams	26.89	27.89	27.73	28.09	30.5	46.35	51.90
	WC	mean number of egg masses per day per snail	—	—	—	—	—	0.5	0.2
		days from start ...	21	24	28	31	35	56	
4	L	mean masses in milligrams	102.07	115.81	145.58	155.20	—	—	
	L	mean number of egg masses per day per snail	0.7	1.8	1.3	2.2	—	—	
	WC	mean masses in milligrams	8.11	8.12	12.1	18.80	24.40	53.17	
	WC	mean number of egg masses per day per snail	—	—	—	—	—	0.1	
		days from start ...	46	50	58	73	76	83	
from egg	L	mean masses in milligrams	63.7	104.71	199.36	—	—	—	
	L	mean number of egg masses per day per snail	0.07	0.11	2.04	—	—	—	
	WC	mean masses in milligrams	21.24	26.24	48.44	130.61	200.80	226.79	
	WC	mean number of egg masses per day per snail	—	—	—	0.83	2.0	0.94	

than when they were provided with whole plant material of the same species in roughly equivalent amounts.

Figure 8 shows the relative growth rates achieved by *B. glabrata* in three mass categories when provided with discs of calcium alginate containing filtered and unfiltered homogenates of watercress or lettuce and also discs of pure calcium alginate as a control over a 14-day period. Not unexpectedly, the snails in all three size categories achieved little or even negative growth in the treatment containing pure calcium alginate during the first four days. However, as the experiment progressed there was some improvement in the growth achieved by the snails in all three size categories in the calcium alginate control treatment. This was particularly marked in the case of the smaller snails in the 4 mg mass category. In this case there was no significant difference between the relative growth rates achieved by snails provided with discs containing lettuce homogenate and the control treatment by the 11th and 14th days. There are, however, two observations that indicate that the calcium alginate matrices are relatively poor, from the nutritional viewpoint, to the snails. First, there is a tendency for the growth rates of the snails in all three size categories to decline as the experiment proceeded. Secondly, the relative growth rates achieved by the snails in this experiment were low compared with those given fresh lettuce discs.

The results in figure 8 also show that the growth rates of snails in the 100 mg mass category

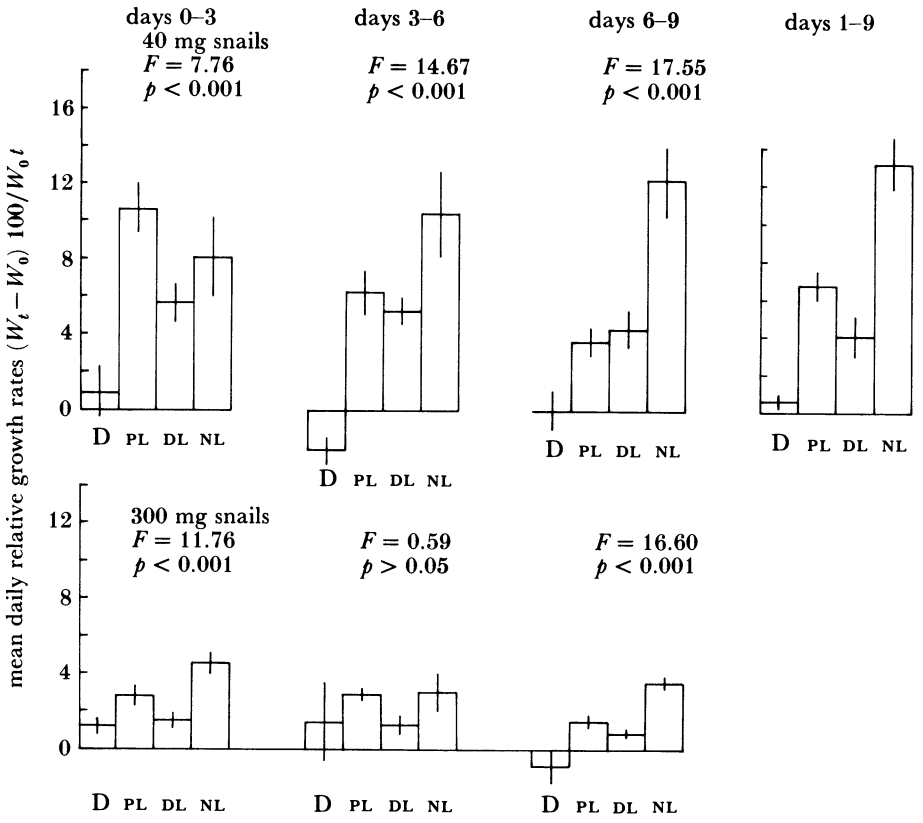

FIGURE 7. The mean daily relative growth rates $(W_t - W_0)\ 100/W_0\, t$ where W_0 and W_t are the initial and final masses in milligrams over a period of 14 days achieved by *B. glabrata* with initial masses of 40 and 300 mg subjected to the following treatments: normal lettuce discs in excess, and similar quantities of dried lettuce discs and powdered lettuce. Control snails were deprived of food over the same period. The mean number of eggs laid per day by the snails in the 300 mg mass category is also given. D, deprived snails; PL, powdered lettuce; DL, dried lettuce; NL, normal lettuce.

were significantly less in some cases when they were fed on discs containing filtrates of either lettuce or watercress than when they were fed on discs containing the unfiltered homogenates of these two plant species. These differences are less marked for snails in the 2 and 4 mg mass categories but there are some instances where the growth rates of snails fed on filtrates of lettuce were significantly less than when they were fed on unfiltered homogenates.

Figure 9 shows that the presence of filtered homogenates of *Myosotis*, *Groenlandia*, *Glyceria*, *Myriophyllum*, *Elodea*, *Hippuris*, *Alisma*, *Ranunculus* and *Oenanthe* at concentrations ranging from 200 to 800 μg l^{-1} resulted in statistically significant growth promotion compared with controls without homogenate. However, with the exception of *Oenanthe* there was no evidence that the response increased proportionately with concentration of the homogenate.

In contrast some of the treatments containing homogenates of *Ceratophyllum*, *Myosotis*, *Callitriche* and *Lemna* resulted in statistically significant growth inhibition compared with controls. There are however, some unexpected aspects to the results. First, the inhibitory effects occurred at the low concentration of 50 mg l^{-1} but promoted growth at a concentration of 200 mg l^{-1}. Secondly, there was a lack of consistency in the results obtained when experiments

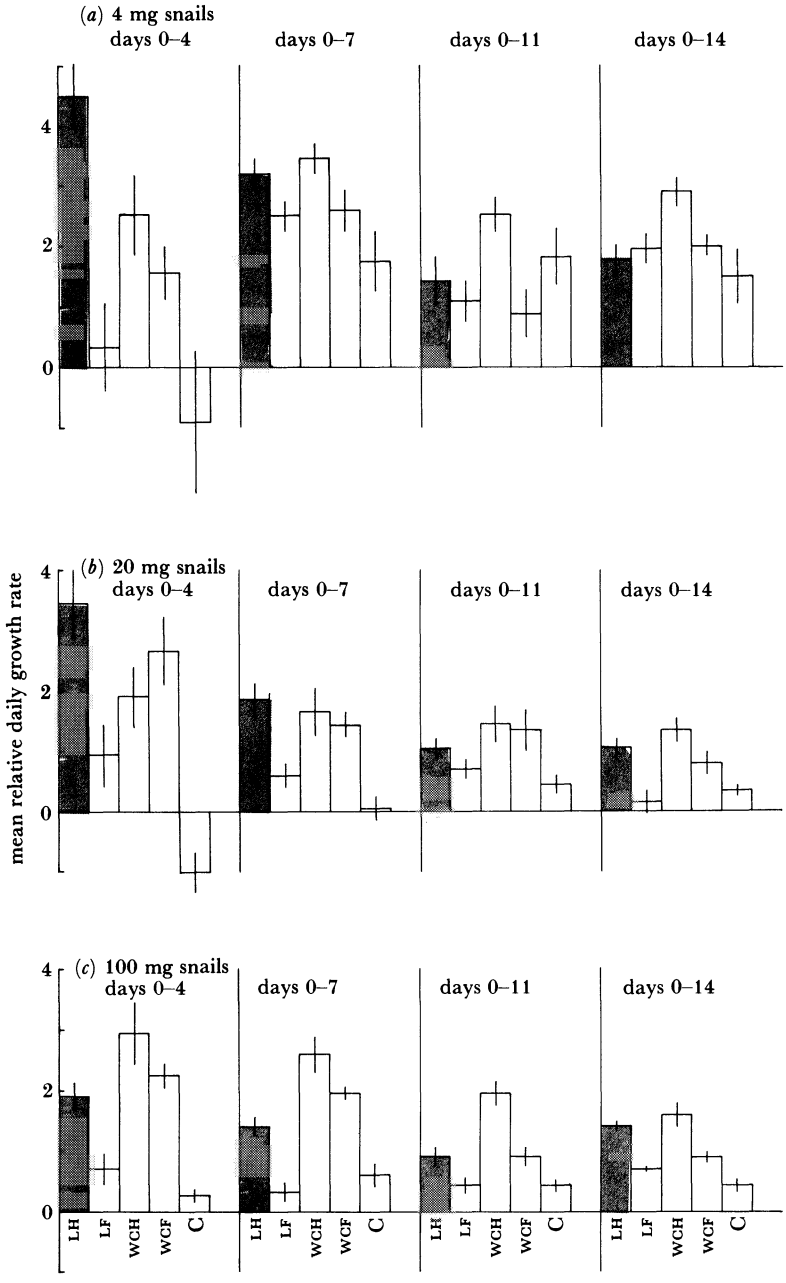

FIGURE 8. The mean relative growth rates, $(W_t - W_0)\ 100/W_0 t$, where W_0 and W_t are the initial and final masses in milligrams over a period of t days achieved by *B. glabrata* with initial masses of approximately 4, 20 and 400 mg fed on calcium alginate matrices (C) (as a control); C plus lettuce homogenate (LH); C plus filtrate of lettuce homogenate (LF); C plus watercress homogenate (WCH); and C plus watercress filtrate (WCF).

involving *Ceratophyllum* and *Myriophyllum* were repeated. None of the homogenates from the plant species featured in figure 9 resulted in snail mortality during the course of this nine-day experiment. In contrast homogenates of *Alternanthera sessilis* caused 100 % snail mortality in less than a day when present at concentrations of 50 mg l^{-1}.

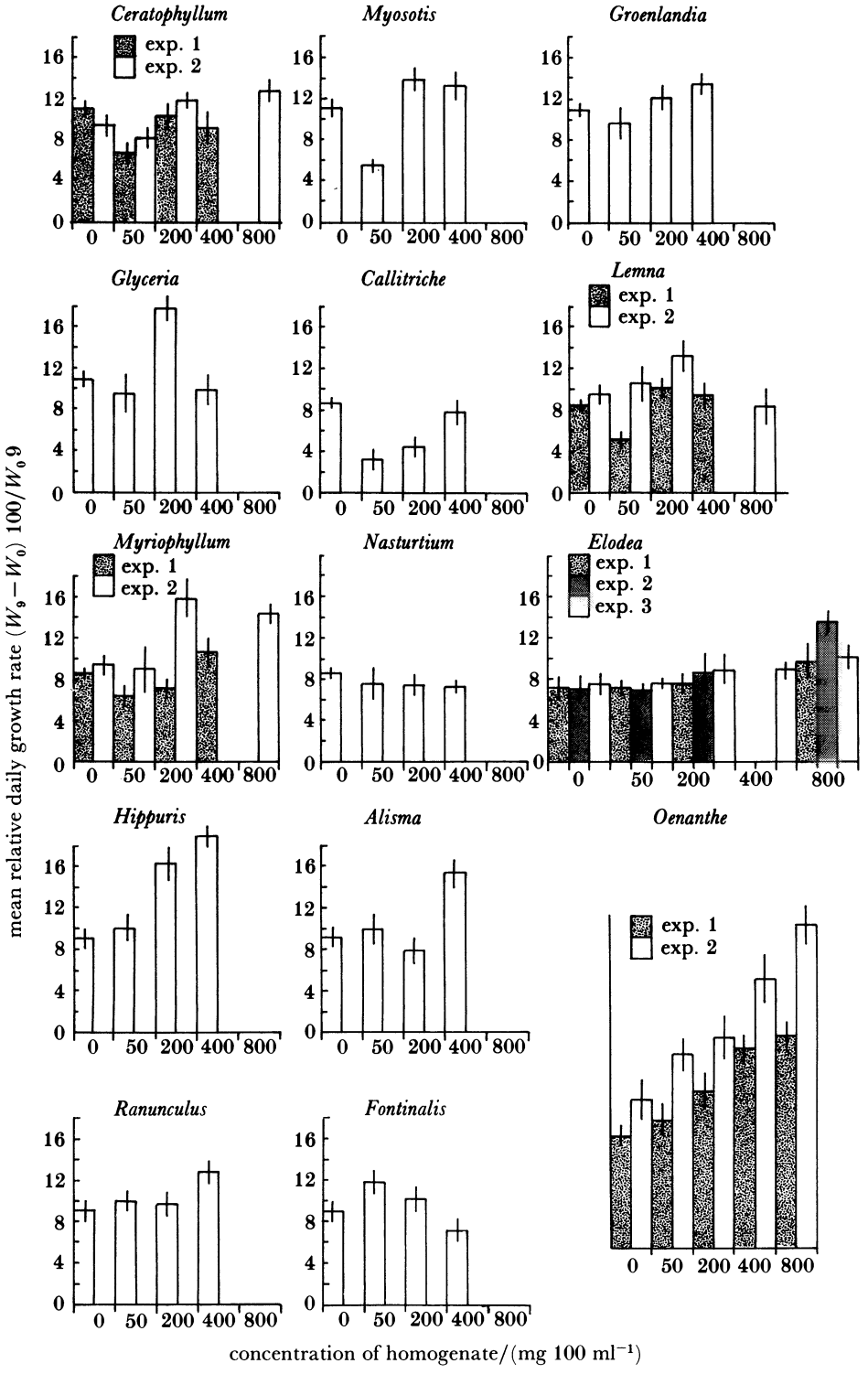

FIGURE 9. The mean daily relative growth rates $(W_9 - W_0)\ 100/W_0\,9$ where W_0 and W_9 are the initial and final masses in milligrams achieved by 80 mg *B. glabrata* placed in filtered homogenates of various species of aquatic plants and fed on standard rations of lettuce.

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4. Discussion

4.1 *Interactions between snails and water plants; the theory of phased coevolution and mutualism*

An attempt will be made here to analyse the present results within the context of the theory that the components of the module, namely the epiphytic bacteria, algae, the macrophytes and the snails have converged to form very close associations during the course of evolution and that the relationships between the various components of the module, including the snails and plants, have become essentially mutualistic. The various components in the module first appeared at widely different times on the evolutionary scale. The main events can be divided into four phases.

(i) Phase 1 (figure 10). The heterotrophic bacteria, which were the first to appear about three billion years ago, were spheroidal prokaryotes, perhaps comparable to modern bacteria of the clostridial type found on the surfaces of macrophytes today. They probably obtained their energy initially by fermenting organic matter of non-biological origin. However, as they were followed initially by photosynthesizing and nitrogen-fixing bacteria, and later, about two billion years ago, by cyanobacteria similar to the blue-green algae of today, vast quantities of organic matter of a biological nature, became available as an energy source for these heterotrophic bacteria (Schopf 1978).

(ii) Phase 2 (figure 10). The organisms in phase 1 were joined by eukaryotic algae, and also possibly by fungi, about 1.5 billion years ago. These probably existed on rock or sandy surfaces in shallow water as dense, mat-like communities, bearing some resemblance to the 'aufwuchs' seen on such surfaces today (Round 1981). The members of this community helped to bind each other to the sand thus forming stromatolites. It is probable that it was in communities such as these that the symbiotic relationships involving bacteria–algae and algae–fungi evolved (Round 1981).

(iii) Phase 3 (figure 11). Gastropods first appeared much later (500–600 million years ago) in the late Cambrian or Ordovician. By this time they had already evolved radulae which were well adapted for raking the highly productive 'aufwuchs' or detritus on sediments in the shallow, eutrophic, nearshore waters into their mouths (Calow 1975; McMahon *et al.* 1974; Russell-Hunter 1978). Alternatively they could also take up flaccid or fine particulate matter by suction through the mouth or by pinocytosis in the floor of the mantle (Cheng & Sullivan 1974). It is also probable that they were able to take up and metabolize dissolved organic matter (DOM) in the form of short-chain carboxylic acids (C_2–C_5) produced during glycolytic fermentation of decaying plant material by heterotrophic bacteria (Patience *et al.* 1983; Sterry *et al.* 1985). This has been shown to be the case with *B. glabrata* today (Thomas *et al.* 1984*a*).

The pulmonates appear to have arisen in the Devonian from a prosobranch, monotocardian, archaeogastropod from which also diverged the Cephalaspidea, the probable stem group of the Opisthobranchia (Morton 1955). The pulmonate line was one whose evolutionary history was characterized by the continual invasion of successively higher and more aerial levels of estuaries or intertidal shores (Ellis 1926; Morton 1955; McMahon 1983). This eventually led to a totally terrestrial, air-breathing and reproductive schesis. As pointed out by McMahon 1983) the freshwater pulmonates that originated from terrestrial ancestors are represented by a continuum commencing with primitive, nearly terrestrial, occasionally amphibious species living on the shore of lakes, pools and stream, such as *Lymnaea truncatula*, through intermediately adapted forms, such as the Physidae, some of the Lymnaeidae and Planorbidae, which are

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TABLE 3. A CLASSIFICATION OF THE SEQUENTIAL INTERACTIONS INVOLVING SNAILS AND AQUATIC PLANTS

Possible assay methods are also given.

spatio-temporal relationship	stimuli exogenous plant substances	responses	assay methods
precontact	(*a*) attractants	+snail turns towards source	turning assay[1]
	(*b*) repellents	−snail moves away from source	diffusion[2] and flow[3] olfactometers
	(*c*) toxicants	−snail mortality	
immediately after contact	(*a*) arrestant or tactile stimuli	+inhibition of movement	disc olfactometer[2]
	(*b*) repellent	−movement away	
prefeeding	(*a*) physical barrier	−inability to penetrate	matrix olfactometer[4]
	(*b*) epiphytic micro-organisms as decoys	consume decoys	
	(*c*) exogenous antifeedant	−inability to feed	
	(*d*) exogenous phagostimulant	+radula movement initiated	buccal mass olfactometer[4]
stage after penetration	(*a*) endogenous factors act as feeding incitants	+radula movement continues	
	(*b*) endogenous factors which induce continuous feeding	+radula movement continues intermittently, ingestion	matrix olfactometer[4]
	(*c*) nutrient rich	+somatic and reproductive growth	growth assay[5]
	(*d*) endogenous antifeedant	−stoppage of buccal mass activity, no ingestion	matrix olfactometer[4]
	(*e*) toxicant = secondary plant compounds	−mortality or loss of mass	
	(*f*) nutrient poor	−loss of mass	growth assay[5]

[1] Townsend (1973); [2] Thomas *et al.* (1983*b*); [3] Bousfield (1978); [4] Thomas *et al.* (1985), [5] Thomas & Benjamin (1974*a*).

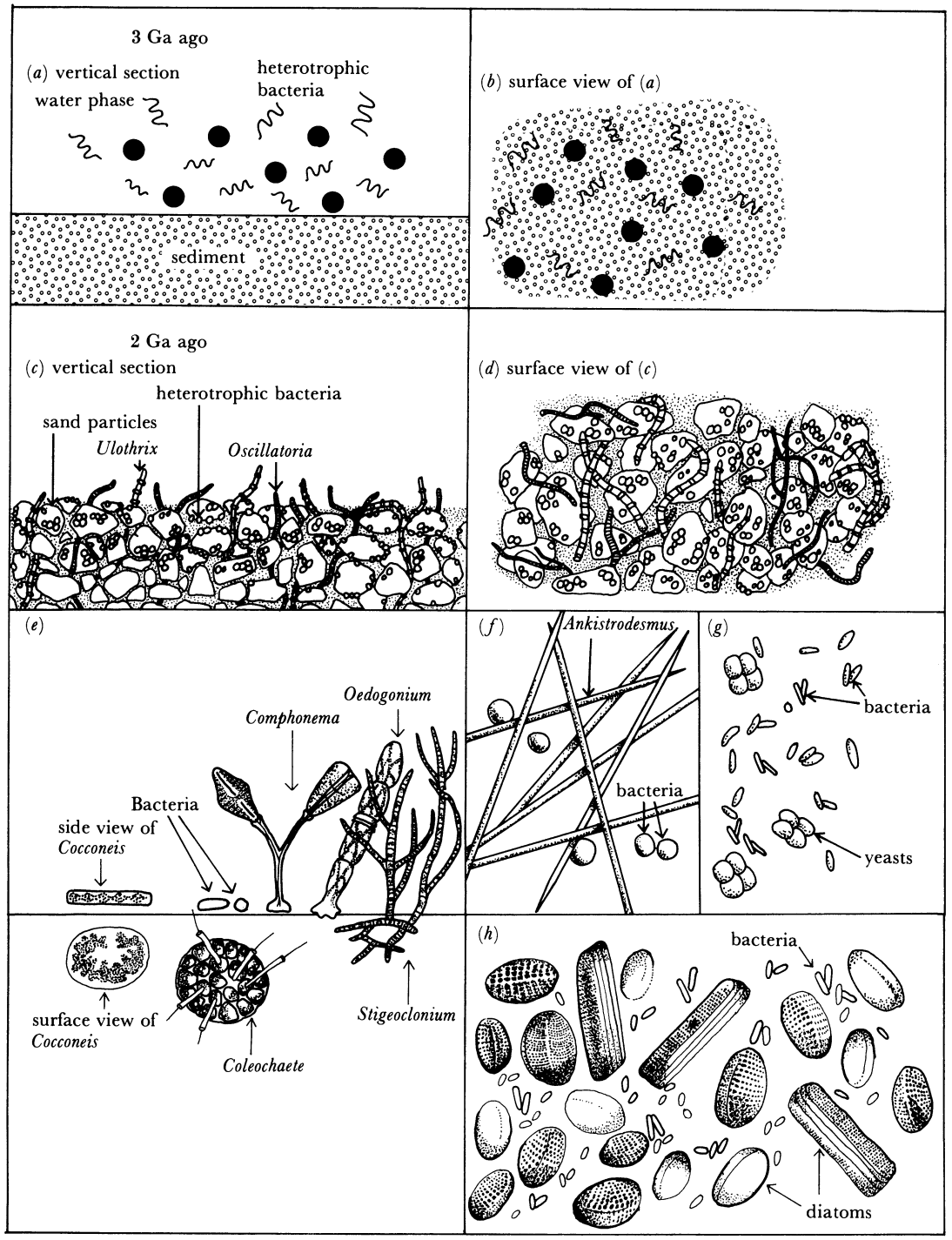

FIGURE 10. Evolution of heterotrophic bacteria and algae on surfaces: (*a*, *b*) Heterotrophic bacteria; (*c*, *d*) heterotrophic bacteria and algae on surfaces of sand particles; (*e*) micro-organisms on surface of macrophyte; (*f*) heterotrophic bacteria and algae (*Ankistrodesmus*) on surface of macrophyte; (*g*) heterotrophic bacteria and yeasts on surface of macrophytes; (*h*) diatoms and heterotrophic bacteria on surface of macrophyte.

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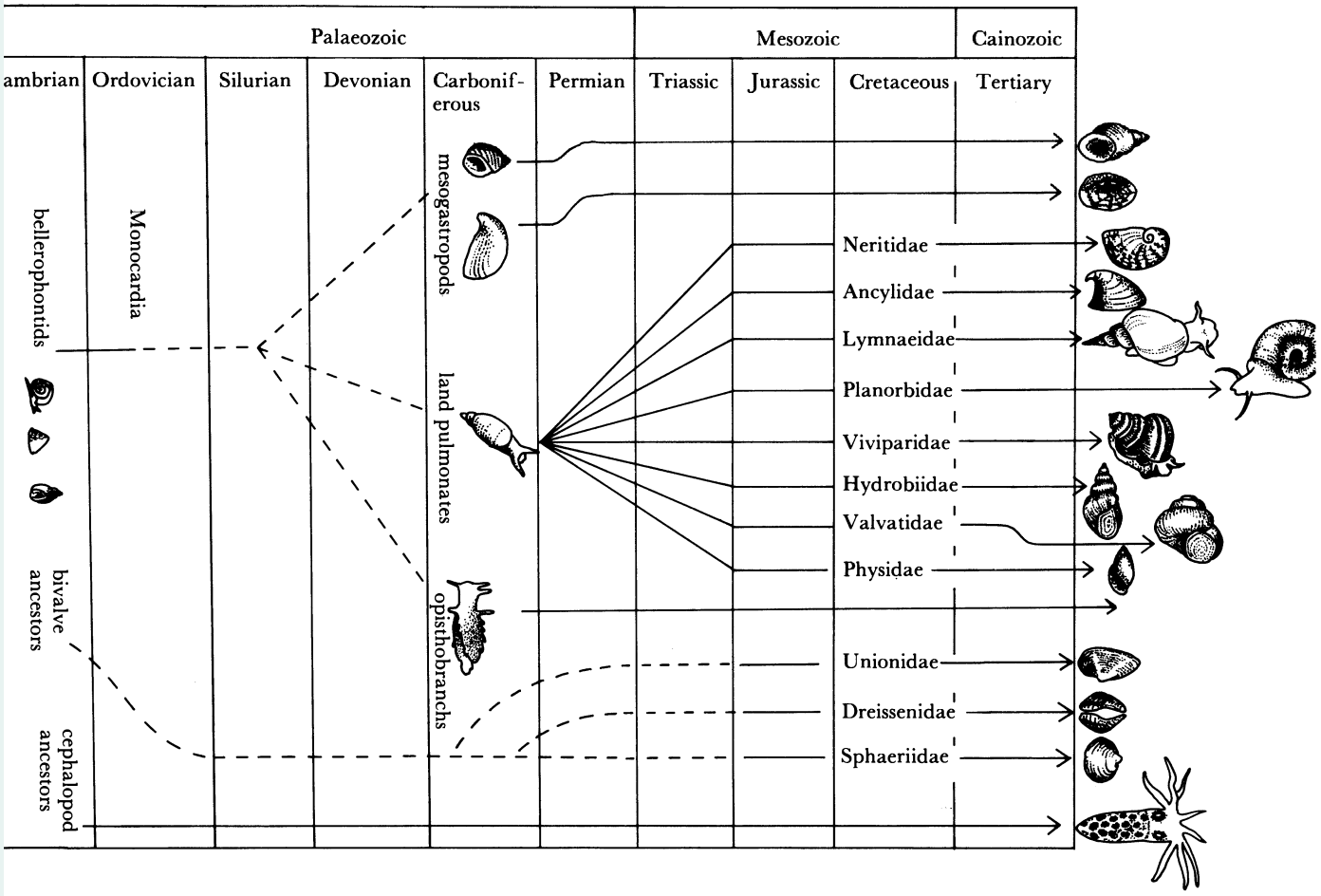

FIGURE 11. Evolution of pulmonate snails.

aquatic. However they retain a primarily aerial, pulmonary mode of gas exchange. The most advanced, purely aquatic groups, such as freshwater limpets and some of the smaller planorbidae, remain continually submerged. According to Russell-Hunter (1978) the invasion of freshwater by pulmonates occurred during the Jurassic, and is represented by many families, and in some cases genera, assignable to extant freshwater pulmonates (180±5 million years ago) (figure 11).

(iii) Phase 4 (figure 12). The radiation of the pulmonates in the Devonian and Carboniferous (440–400 million years ago) coincided with the appearance of the first vascular plants, the Psilophytopsida. It is possible that the Bryophyta (Hepaticae, Anthocerotae and Musci) may, like the ferns, horsetails and club mosses, have originated from psilophyte or prepsilophyte ancestors. Another view is that the Bryophytes may have evolved from green algal ancestors (Scagel *et al.* 1965). The angiosperms, which dominate freshwater bodies today, may have originated in the Triassic or even in the Carboniferous, but did not appear commonly in the fossil records until the Cretaceous (135±5 million years ago) (Scagel *et al.* 1965). By the mid-Cretaceous it was known that they were highly developed morphologically and that many modern families were clearly differentiated. Certainly a number of modern genera were recognizable. According to Moss (1980) the freshwater angiosperms are clearly a recently

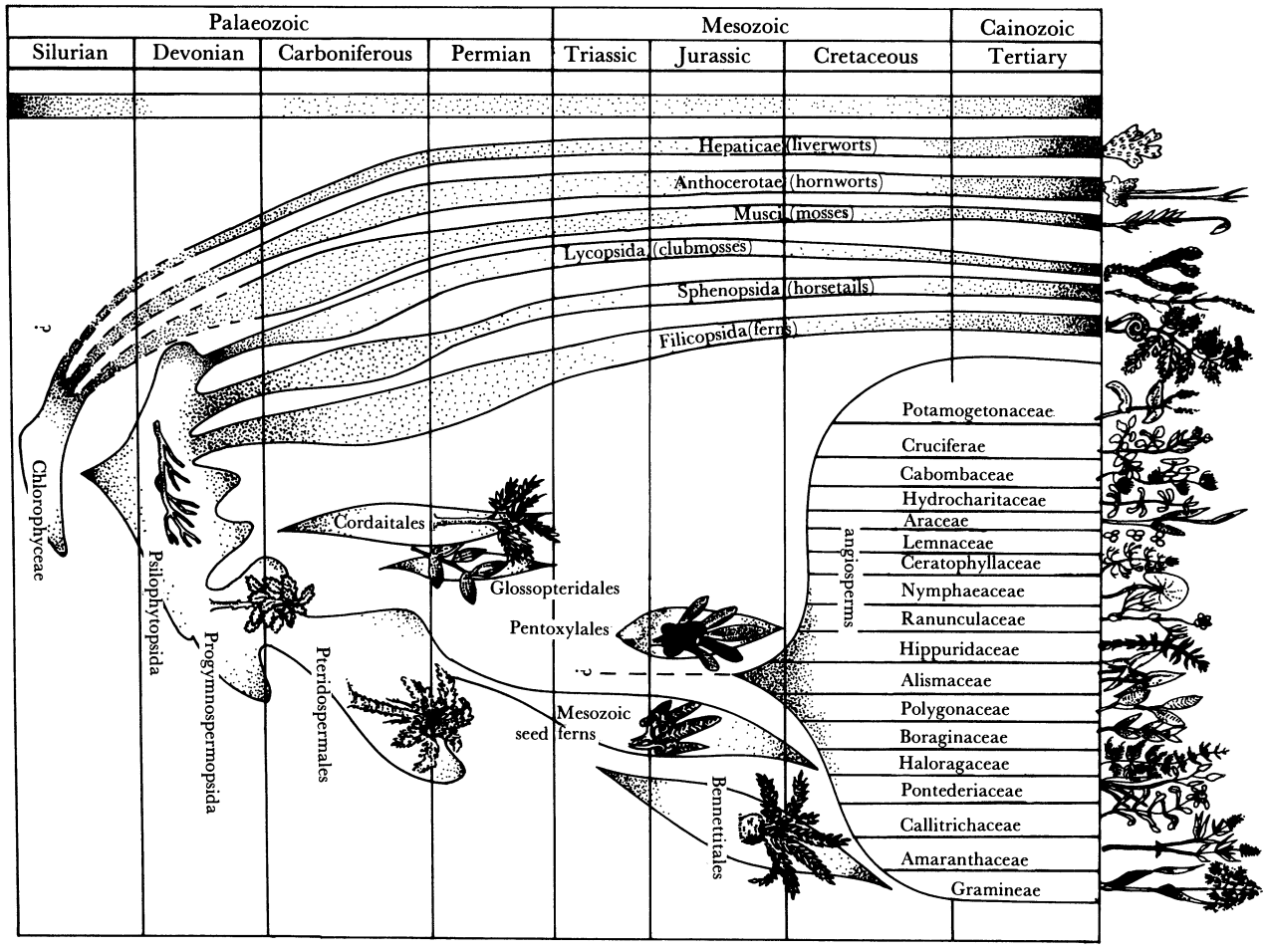

FIGURE 12. Evolution of freshwater macrophytes.

evolved group in which the general trend of plant evolution from water to land has been reversed, as was the case with pulmonate snails. The picture is one of an opportunistic colonization of fresh water by occasional species of predominantly land families. In only a few species, for example, *Ceratophyllum demersum*, has the transition been completed to the extent that the entire life history occurs under water. Most are obliged to produce flowers on shoots that emerge above the water surface and that are pollinated with the assistance of either wind or insects.

The surfaces of aquatic macrophytes become colonized by rod- and coccoid-shaped bacteria, which become involved in fermenting senescing plant material, as well as by epiphytic algae (Patience *et al.* 1983; Sterry *et al.* 1985). As a result the snails are provided with a vastly increased surface area of potential food compared with that which would be available on the sediments alone.

When these interactions between the various components of the module are considered on an evolutionary time scale the following propositions can be made. They concern the most likely adaptive strategies that might have been adopted by the snails and aquatic macrophytes when they began to overlap in space and time. These propositions may be evaluated by using empirical results obtained during the course of the present and related investigations.

4.2. *Propositions derived from the theory of phased coevolution of components of the module consisting of epiphytic bacteria, algae, snails and macrophytes*

4.2.1. *Proposition* 1. *That as the snails had become specialized to exploit surface communities of epiphytic algae, decaying plant material and dissolved organic matter* (DOM) *early in their evolutionary history, they would continue to exploit these resources when they later became associated with macrophytes*

The rationale for this proposition is that, as the snails had already become very specialized, feeding on living and decaying colonial algae and micro-organisms, both on surfaces as well as on DOM, the selective pressures would not favour mutations that might drastically alter this highly efficient feeding mechanism. In other words, the snails were preadapted to be efficient cleaning symbionts, and it might be expected that the selective pressures would favour the *status quo*.

This proposition is supported in general terms by the observations of a number of field and laboratory workers, as there is a general consensus that algae (both green and blue-green), as well as diatoms and decaying plant tissues, are the major food items of freshwater pulmonate snails, including those that serve as the snail hosts of schistosomiasis (Boycott 1936; Mozley 1954; McCullough & Duke 1954; Zakaria 1955; Watson 1958; Malek 1958; WHO Report 1957; Pimental & White 1959; Calow 1973*a*, *b*, 1974*a*, *b*; Scheerboom & Van Elk 1978; Ndifon 1979; Reavell 1980; Thomas *et al.* 1985). The epiphytic algae may help to shield the macrophytes from direct attack by acting as decoys, and they may also benefit the macrophytes by releasing antibiotics which protect them against attack by pathogenic bacteria and other herbivores (Hutchinson 1975; Round 1981). It would appear that decaying plant material is more important to planorbid snails such as *B. glabrata* than to lymnaeid snails (Heidermanns 1924; Gaevskaya 1969; Calow 1970, 1973*a*; Thomas *et al.* 1985) and is also a major item in the diet of most species of terrestrial molluscs (Mason 1970; Jenning & Barkam 1975; Wäreborn 1982).

The details of how detritivores such as *B. glabrata* obtain their metabolic requirements from decaying plant material and the DOM derived from it is still unresolved. In fact there is still much controversy as to whether detritivores obtain most of their nutrients from the detritus in the form of DOM or only after conversion of the DOM into bacterial protoplasm which is then digested (Pütter 1909; Krogh 1930; Stephens 1972; Jørgensen 1976; Sepers 1977; Calow 1975; Newell 1965, 1970; Baker & Bradman 1976; Ladle 1982). This problem remains unresolved although the general consensus favours the former possibility. This view receives support from the biochemical studies on decay of macrophytes and uptake of DOM in the form of short-chain carboxylic acids recently done by Patience *et al.* (1983), Sterry *et al.* (1985) and Thomas *et al.* (1984*a*). These studies show that the snails derive the following benefits from the processes involved in plant decomposition. First, the decaying material becomes flaccid and therefore more ingestible than the turgid living plant tissues, owing to the action of cellulolytic bacteria on the skeletal material in the outer envelope. Secondly, the microdecomposers involved in decay, which appear to be recruited mainly from rod and coccoid bacteria on the surface of living plant tissue, generate a biochemical environment which is favourable to the snails (Patience *et al.* 1983; Sterry *et al.* 1985). The outer leaf envelope tends not to break down immediately, as some of the more resistant polymers retain their integrity thus allowing the rod- and coccoid-shaped bacteria to invade the envelope and to grow exponentially by utilizing the plant biomass as a resource. These release energy by a process of anaerobic fermentation

with the production of C_2–C_5 short-chain carboxylic acids as end products (Patience *et al.* 1983; Sterry *et al.* 1985). When these acids diffuse out of the decaying envelopes they can be taken up actively through the body wall of the snail and metabolized with the release of CO_2 (Thomas *et al.* 1984*a*). These authors calculated that if the snails encountered the concentrations of 2250 μM and 400 μM of acetate and butanoate, respectively, found in the supernatant from laboratory-decaying *Lemna*, then each acid could provide the snail with more than 50% of its basal metabolic requirements. However, it will be necessary to measure the concentrations of these acids in the natural environment of the snails before their ecological importance can be evaluated. An alternative way of acquiring nutrients, such as short-chain carboxylic acids and amino acids present in the decaying envelope, is to ingest the envelope and assimilate the nutrient through the gut wall. On the face of it this might be expected to be more cost effective than assimilation through the body wall. The short-chain carboxylic acids and amino acids within the envelope would appear to provide the snails with the ideal form of energy currencies. Thus, the carboxylic acids can be readily incorporated into the Krebs cycle. It would seem probable that the amino acid pool in the decaying plant material would be less variable than would be the case if the snails were obliged to utilize the tissues of several different macrophytes, as the species of microdecomposers and the biochemical processes involved are likely to be similar irrespective of the macrophyte being decomposed. This could be to the advantage of the snail, as the amino acid supply–demand balance involved in protein synthesis and metabolic processes is a very delicate one, and could easily be disrupted if the snail was forced to switch repeatedly from one novel food source to another.

To utilize this energy source efficiently, it might be expected that *B. glabrata* would have developed an optimal foraging strategy which would enable them to identify senescing or decaying macrophyte material at the end of the acid-forming stage, when the carboxylic acids had reached asymptotic values, and before the onset of methanogenesis, which normally occurs after entry of the decaying material into the anoxic sediments. This view is supported by the observations that the snails normally occur on surfaces of macrophytes (Pimentel & White 1959; Thomas & Tait 1984*b*) and are therefore in a good position to locate decaying tissues and can detect sources of C_3–C_4 carboxylic acids at concentrations as low as 5×10^{-7} M over the pH range of 6–8 encountered in their environment (Thomas *et al.* 1980, 1983*b*; Sterry *et al.* 1983).

4.2.2. *Proposition* 2. *That pulmonate snails would tend to be feeding generalists, capable of adapting to food of varying chemical composition given sufficient time, particularly if it was either soluble, small or flaccid*

The rationale for this prediction is as follows. The snails living in associations with aquatic macrophytes encounter diverse biochemical assemblages containing nutrients and potential toxicants in the form of living macrophyte, algal and bacterial tissues, decaying plant material and DOM in the water column. Although the close association between the snails and macrophytes helps to reduce temporal variability in the biochemical composition, it should be borne in mind that the modular system is subject to diurnal, seasonal and sometimes catastrophic changes. It would be relatively advantageous for the snails to be versatile or generalist feeders. The purpose of the work described in the present section is to explore the influence of time and age on the adaptation process in snails.

It is possible to draw four conclusions from the present results, so far as the temporal aspects

of adaptation to a novel food resource is concerned. First, *B. glabrata* of various ages can become adapted to utilizing a variety of novel food items, including a number of species of freshwater macrophyte and even calcium alginate, a simple polymer of glucuronic acid. Secondly, the snails require a critical time to adapt to a novel food source. For example, the 4 mg snails required a period of approximately 14 days before growth achieved in the calcium alginate treatments became comparable to that of lettuce controls. Again, snails reared from the egg stage on a novel food such as watercress require from 50–60 days to enter the exponential growth phase, compared with 40–50 days by snails reared on lettuce, the staple food. Thirdly, the ability to adapt to a novel food is inversely related to age. An example of this trend is provided by the results obtained when snails were given watercress as a novel food. Thus, snails in the 4, 20 and 100 mg mass categories showed phases of accelerated growth, the onset of which was related to age, but only those reared from the egg were able to achieve the exponential phase of growth. Again, only the snails in the 4 mg size category were able to achieve growth comparable to the lettuce controls when provided with novel food items such as *Nasturtium* and *Callitriche*. Both the ability of *B. glabrata* to adapt to novel food items and the ontogenetic differences resemble those described for insects (Bernays & Chapman 1975; Chapman 1974; Chapman & Bernays 1977; Jermy *et al.* 1982), and for snakes (Burghart 1975). According to Chapman & Bernays (1977) the later larval instars of *Zonocerus variegatus* commonly reject cassava at first contact, but eventually defoliate the plant, whereas adult instars are unable to feed on it. Fourthly, there was a great deal of individual variation in the ability of *B. glabrata* to adapt to novel food items, such as watercress. This was reflected in the higher mortality suffered by the snails in the watercress treatments compared with those in the lettuce control. These results also resemble those described for *Zonocerus* by Chapman & Bernays (1977) and as suggested by these authors it is probable that individual differences in ability to adapt to a novel food is genetically determined.

Before considering possible explanations for the first three conclusions above, it is necessary to consider briefly the processes that are involved in adapting to a novel food. This would necessitate the sequential modification of the whole range of biochemical processes involved in detecting food, ingesting it and processing the assimilated material as well as learning to respond to a whole array of new chemical signals. Recent neurophysiological studies have shown that gastropod molluscs can learn as habituation processes (Lukoviak & Peretz 1977), and also more complex manifestation of learning have been demonstrated (Chang & Gelperin 1978). Croll & Chase (1977) have shown that snails such as *Achatina fulcata* can retain memories of food plants for periods of at least 120 days. It has also been demonstrated recently that certain phytophagous insects are capable of habituation to feeding deterrents, as well as aversion learning (Jermy *et al.* 1982). In view of the complexity of these processes it is not surprising that a snail such as *B. glabrata* requires a long period of up to 14 days to adapt to a novel food.

It is not unexpected that, over the time scale of the experiment, juvenile *B. glabrata* are better able to adapt to a novel food source than their adult conspecifics, as it is generally accepted that the ability of organisms to learn and adapt to novel situations tends to decline with age. In the case of *B. glabrata* the higher efficiency shown by juveniles in utilizing novel food items for growth, compared with their adult conspecifics, is correlated with their broader chemoreception niches for amino acids and related compounds (Thomas & Assefa 1979; Thomas *et al.* 1980, 1983*b*) and also with broader niches, as measured in terms of macrophyte species ingested when offered a choice (Cedeño-León & Thomas 1982). It can be postulated

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that the narrower chemical and feeding niches of the adult snails is a consequence of learning, as a result of specializing to feed on one or a small number of food plants. However, after the snails of various sizes become more experienced at handling food, the ontogenetic differences eventually disappear or become less well marked in the case of *B. glabrata* (Cedeño-León & Thomas 1982) (figure 16). It would appear that the only real difference between adult and juvenile *B. glabrata* is that the latter can adapt more quickly.

From the biochemical viewpoint, adapting to a novel food presents snails with two particular problems to solve, namely the detoxification of new SPC and the utilization of food such as calcium alginate which lacks a nitrogen source. Little is known about detoxification of SPC in invertebrates, but it is likely that it takes place mainly in the wall of the midgut by any one of four chemical pathways: oxidation, reduction, hydrolysis and conjugation (Smith 1962), a reduction in the sensitivity of enzymes (Hodgson & Motoyama 1984) and the possible involvement of the microbial flora (Fowden *et al.* 1967).

To explain the utilization of a pure carbohydrate food source, such as calcium alginate, it is necessary to invoke the involvement of micro-organisms to provide the snails with essential nutrients, particularly those containing nitrogen. There are two possible models that might be considered. First, the matrices could become colonized by micro-organisms which use nitrate present in the medium (0.05 mM of NO_3; see Thomas *et al.* 1975) as a nitrogen source. Secondly, the snails may have microbial symbionts present in their alimentary canal or tissues which are capable of fixing atmospheric nitrogen and of recycling sources of nitrogen derived from the snails, such as urea. Such micro-organisms have been shown to be present in other herbivorous organisms with a nitrogen deficient diet, such as plant-sucking insects (for example, aphids, scale insects and whitefly) (Büchner 1965), shipworms (Carpenter & Culliney 1975), as well as other molluscan species which filter cellulose-rich material from seawater or sediments (Morton 1978; Rosenberg & Breiter 1969). As a result of their symbiotic microflora such herbivores have very low requirements for specific amino acids or vitamins. However, the extent to which the microflora of *B. glabrata* is involved in supplying its nutritional needs remains to be investigated.

4.2.3. *Proposition* 3. *That although macrophytes and snails show a strong positive relationship, the living macrophyte tissue would be little utilized by the snails*

In view of the very strong positive associations between pulmonate snail and aquatic macrophytes, the latter may be described as apparent plants (Feeney 1976). Yet despite the fact that pulmonate snails live on the surface of an abundant potential food source, the present results and others show that the snails find it difficult to utilize young and mature leaves of macrophytes, particularly when they are inexperienced. Thus Cedeño-León & Thomas (1982) found that when inexperienced juvenile *B. glabrata* were offered a choice of novel food items, in the form of ten species of aquatic macrophyte, together with the staple experimental food, lettuce, in equal amounts, they consumed none of the *Eichhornia* and less than 7% of the other species (figure 13).

Inexperienced adult *B. glabrata* had even narrower food niches than their juvenile conspecifics, as they consumed none of the *Ceratophyllum*, *Potamogeton*, *Salvinia*, *Elodea*, *Eichhornia* and *Lemna*, and less than 8% of the remaining four species (figure 13). It is possible that these ontogenetic differences may have been due to the ability of the juvenile snails to exploit the small amount of decaying or senescent portions of plants that were present. In contrast *Marisa* is better at utilizing macrophytes than *Biomphalaria* and therefore has the potential to exclude

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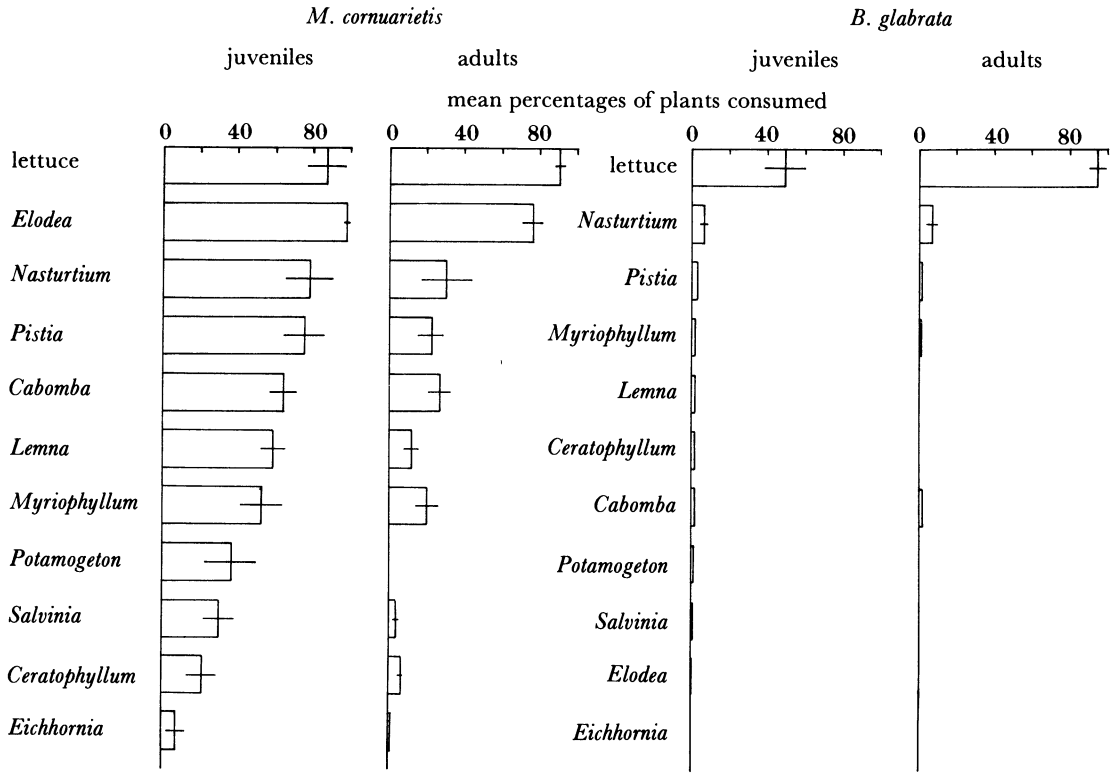

FIGURE 13. The mean percentage of various aquatic macrophytes and lettuce (as a control) consumed by inexperienced juveniles and adults of both *B. glabrata* and *Marisa cornuarietis*. (See Cedeño-León & Thomas (1982) for details.)

Biomphalaria in a competitive situation. However, as with *Biomphalaria*, the adult snails had a narrower niche than the juveniles. Similar results with other pulmonate snails described by Thomas & Tait (1984*b*) show that when *Biomphalaria pfeifferi* and *Helisoma duryi* were offered lettuce, *Acroceras*, *Azolla*, *Ceratophyllum*, *Lemna*, *Pistia*, *Salvinia* and *Utricularia* species in equal amounts, the only species to be eaten by *B. pfeifferi* in quantities greater that 30 % of that offered were lettuce, *Salvinia* and *Lemna* (figure 14). The pattern of consumption by *Helisoma* is similar, except that it tends to consume slightly larger amounts of each plant species than *B. pfeifferi*, which helps to explain why it is a better competitor (figure 14).

As might be expected, the pattern observed in the ingestion experiments involving *B. pfeifferi* and *H. duryi* is repeated in the growth experiments. Thus, in the case of *B. pfeifferi* the snail only achieved reasonable growth in the treatments containing *Salvinia* and *Lemna* and sustained a net loss in mass in those containing fresh *Acroceras*, *Azolla*, *Pistia* and *Utricularia* (figure 15). A small amount of growth was achieved in the *Ceratophyllum* and *Nymphaea* treatments. Presumably because they ingested more plant material than *B. pfeifferi*, *Helisoma duryi* achieved better growth, and they only lost weight in those treatments containing *Acroceras* and *Utricularia* (figure 15).

Ndifon (1979) also reached similar conclusions when assessing the food value of a number of aquatic plants presented as whole leaves: *Alternanthera sessilis*, *Commelina* sp., *Acroceras zizanoides*, *Ipomoea aquatica*, *Nymphaea lotus*, *Pistia stratiotes*, *Paspalum* sp., *Ludwigia* sp. and

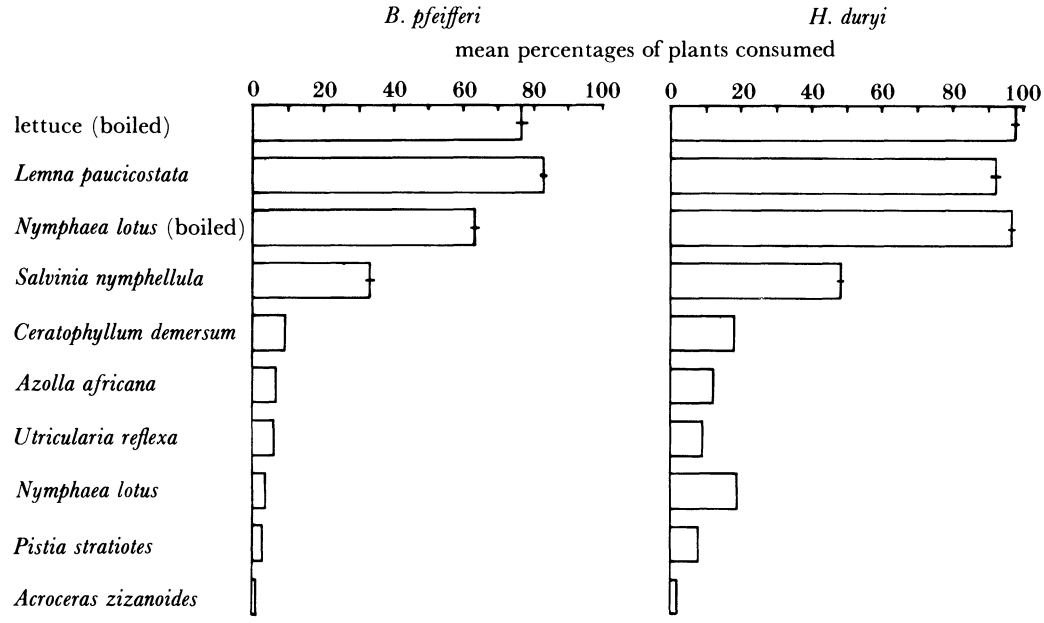

FIGURE 14. The mean precentage of various aquatic macrophytes consumed per week by *Biomphalaria pfeifferi* and *Helisoma duryi* compared with lettuce. Standard errors are shown.

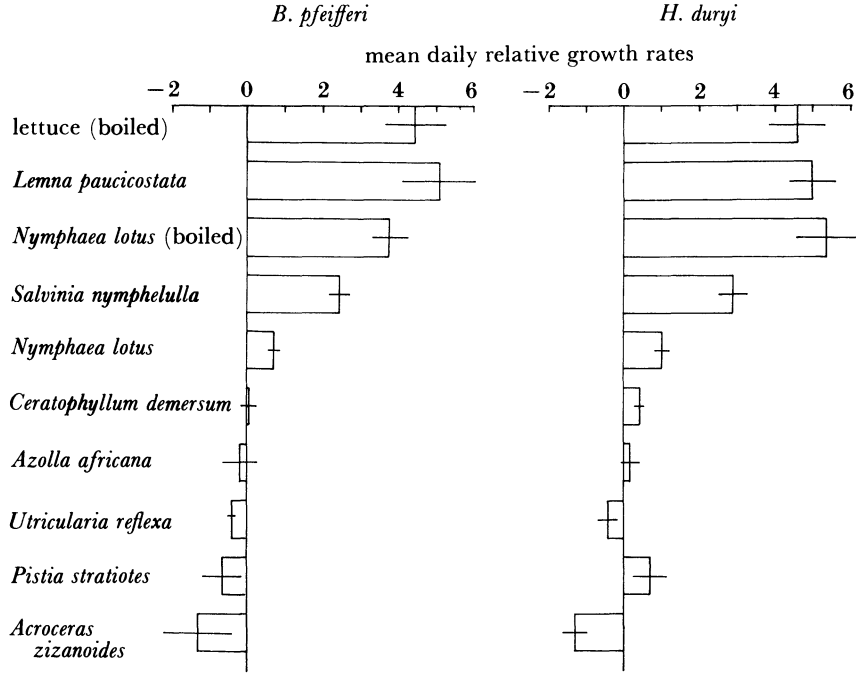

FIGURE 15. The mean relative growth rates $(W_n - W_0)\,100/W_0\,t$ achieved by *Biomphalaria pfeifferi* and *Helisoma duryi* fed on various aquatic macrophytes and lettuce as a control. (See Thomas & Tait (1984) for details.) Standard errors are shown.

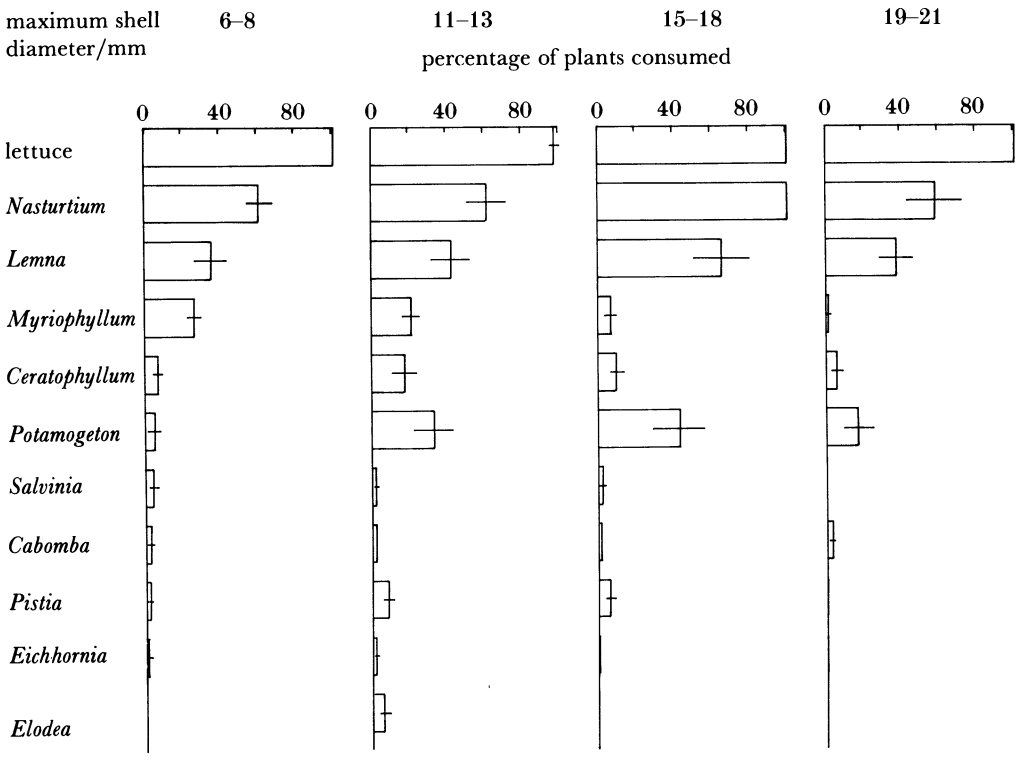

FIGURE 16. The mean percentage composition of the food ingested by experienced *B. glabrata* of various size groups ($\bar{x} \pm$ SE; see Cedeño-León & Thomas (1982) for details.)

Pithophora as food for another pulmonate snail *Bulinus* (*P.*) *globosus* under laboratory conditions. With the exception of *A. sessilis* and *I. aquatica* all the aquatic macrophytes proved to be inferior food material, as they resulted in much reduced survivorship, growth and fecundity, compared with snails provided with lettuce.

Recent studies in this laboratory, under simulated natural conditions in which *B. glabrata* of various ages were maintained in aquaria containing *Lemna paucicostata* and *Ceratophyllum demersum*, populations with a mixed age structure confirm that snails eat very little of the young and mature tissues (Thomas *et al.* 1985). Similar conclusions have also been reached by a number of field workers who have studied the feeding habits of pulmonate snails (Boycott 1936; Mozley 1954; MCullough & Duke 1954; Zakaria 1955; Watson 1958; Malek 1958; WHO report 1957; Calow 1973*a*, *b*, 1974*a*, *b*; Scheerboom & Van Elk 1978) and also by those who studied both the food and population growth of snails (Eisenberg 1966, 1970; Butler 1976; O'Keefe 1982). The latter authors have produced evidence to show that macrophytes cannot readily be utilized by the snails even when present in seeming abundance, and that both growth and reproduction of the snails could be enhanced by increasing both the quality and quantity of ingestible food.

4.2.4. *Proposition* 4. *That the hard outer envelope, inherited from their terrestrial ancestors, would remain as the major defence mechanism of aquatic macrophytes against attack by snails and other aquatic invertebrates*

The rationale for this proposition is as follows. To prevent or minimize damage from attack by herbivores, including pulmonates, aquatic plants could invest in the following defensive strategies: (i) a hard-textured outer envelope; (ii) nutrient deficiency; (iii) production of SPC which act as antifeedants or toxicants to the snail. Evolutionary considerations indicate that the snails were preadapted for feeding on the epiphytic organisms and decaying remains when they first encountered aquatic macrophytes. It seems probable that the hard textured outer envelope would have been an effective defence strategy at this time. If this was the case, it could be argued that it would be more cost-effective for the macrophytes to place most of their defensive investments into perfecting their epiphyte decoy and textural defence mechanisms, and less in those involving alteration of biochemical pathways to produce SPC or nutrient deficiency.

It is possible that this defensive strategy might also be very effective against the great majority of the other species constituting the guild of organisms associated with aquatic macrophytes, whether they utilize plant food material indirectly or directly. These include species of Annelida, Crustacea (Cladocera, Copepoda, Ostracoda, Isopoda, Amphipoda), Plecoptera, Ephemeroptera, Hemiptera (Heteroptera), Coleoptera (Hydrophilidae, Dryopidae, Curculionidae), Diptera (larvae), Trichoptera and Lepidoptera. The Cladocera and Copepoda have evolved mechanisms to utilize planktonic algae while the great majority of the other species have become specialized to feed on either detritus or on communities of micro-organisms. These include algae living on surface of living macrophyte tissue. These other invertebrates, like the snails, may benefit the plants by clearing them of epiphytic algae. A few species of Lepidoptera and Curculionidae appear to be exceptions to this general rule, as they appear to be directly phytophagous (Gayevskaya 1969). However, these species have a very limited distribution, as they both appear to be poorly adapted for life under water. A few species of the Limnephilidae (Trichoptera) also have strong mandibles, capable of cutting aquatic macrophytes for case building, but appear to obtain most of their food by scraping epiphytic algae or 'aufwuchs'. The other major taxonomic groups in freshwater, namely Tricladia, Hirudinea, Arachnida, Hemiptera (Heteroptera), Dytiscidae and Gyrinidae are represented entirely by carnivorous species.

In marked contrast, 34.5, 28.9, 90.7, 99.0, 100 and 25.0% of the species in the terrestrial Coleoptera, Diptera, Hemiptera, Lepidoptera, Orthoptera, Hymenoptera and other insect orders, respectively, are directly phytophagous (Strong *et al.* 1984). According to these authors, there are approximately 361000 species of insects in these taxonomic groups that may feed directly on terrestrial plants. It would appear that these plants are therefore subjected to much heavier pressures from herbivorous insects than is the case with aquatic macrophytes.

The dearth of insects in aquatic habitats compared with those on land has also been commented upon by others, including Cummins (1973), Lawton & Schroder (1977) and Lawton & Price (1979). This trend is not attributable to the relatively small surface area occupied by freshwater systems, as the above authors have found that aquatic dicotylendons have smaller insect faunas than terrestrial species with similar sized ranges. They concluded that the reasons for these differences were obscure. However, there are two plausible

explanations. First, the problem of obtaining oxygen in the water regimen has imposed serious constraints on the invasion of this habitat by insects that had evolved to exploit terrestrial plants. Secondly, the selective pressures that terrestrial phytophagous insects have been subjected to have resulted in their using evolutionary strategies, such as the detoxification of SPC in particular plants, high dispersionary activity and diapausing in soil, that have made them too specialized to exploit the aquatic habitat.

The majority of the results obtained in the short-term experiments, involving plant homogenates in calcium alginate matrices, support the proposition that the tough outer envelope is the major defence strategy developed by aquatic macrophytes. Homogenization of the plant material resulted in a softer, more uniform texture. This was followed by a marked improvement in the growth achieved by the assay snails in the majority of cases. For example, growth was faster in the lettuce control treatments than in those containing *Glyceria* and *Apium* and more than 90% of the lettuce controls in those containing *Chara*, *Groenlandia*, *Cabomba*, *Vallisneria*, *Elodea*, *Hydrocharis*, *Ceratophyllum*, *Ranunculus*, *Oenanthe* and *Callitriche*. In contrast many of these species, such as *Glyceria* and *Ceratophyllum*, could hardly be ingested when presented in the normal form, and as a result could induce very little snail growth.

However, there is an alternative explanation for these results as the enhancement in ingestibility and utilization as food by the snails following homogenization and matrix formation may have been partly due to dilution of loss of volatile antifeedants which might have been present in the waxy surface layers. Such factors have been demonstrated in terrestrial plants such as *Sorghum* (Woodhead *et al.* 1982; Woodhead 1982, 1983) and can be removed by treatment of the waxy coat with an organic solvent such as chloroform. However, before an aquatic macrophyte could be subjected to such a treatment to test for the presence of diffusible antifeedants, it would be necessary to work with sterile plants, as any treatment effect would be confounded by the influence of the organic solvents on the epiphytes which generally cover the leaf surfaces of aquatic macrophytes.

Exceptions to the general rule, that homogenization and incorporation in a matrix is followed by an increase in ingestion and snail growth, are provided by *Nymphaea*, *Polygonum* and *Fontinalis*. It can be concluded that, in these cases, even if the hard outer envelope is a barrier, it is not the only one. Possible alternative explanations for these results are discussed below (§4.2.6.)

Despite these exceptions and the possible alternative explanation for increase in snail growth following homogenization, it is safe to conclude that the hard outer envelope of aquatic macrophytes is one of their main strategies in preventing their young and functional mature tissues from being eaten by invertebrates such as pulmonate snails. The conclusion is in accord with those of other workers who have studied the interactions between terrestrial snails and plants (Dirzo 1980; Grime *et al.* 1970; Jennings & Barkham 1975).

4.2.5. *Proposition* 5. *That aquatic macrophytes would invest little in a nutrient deficiency strategy to reduce the probability of attack by aquatic invertebrates such as snails*

The rationale for this proposition has already been given in §4.2.4 above. In addition, however, there are two other considerations which would appear to make this an inefficient strategy to prevent attack by the snails. First, this defensive measure has only been exploited successfully in cases where insect herbivores, such as the terrestrial Hemiptera, attack specific tissues, such as the phloem (McNeill 1973; Moss *et al.* 1975; Hill 1975; Dixon 1976; Crawley 1983; Southwood 1984; Strong *et al.* 1984). As a result the plant has the option to

compartmentalize its resources and to store its nutrients in tissues which would not be accessible to the herbivore. If, however, the herbivore is obliged to use an inefficient multiple chain-saw system, rather than a rapier, as its offensive weapon, as would be the case with pulmonate snails, this method of defence would not be cost-effective. Secondly, it has been shown that the snails can adapt in time to a novel food source, even when it is deficient in nutrients, probably with the assistance of their gut symbionts. As a result it would be necessary for the plants to alter their biochemical milieu constantly. Clearly, this would be very difficult.

On the whole, the present results support the above proposition. First, the results in table 4 show that the species of aquatic plants used in the present experiments, or species closely related to them, contain appreciable amounts of proteins, carbohydrates, fats, cations and other nutrients, such as iron and phosphates. According to Boyd (1974) they compare quite well with high-quality herbivore food, such as alfalfa hay. On average they contain only slightly less protein and somewhat more ash and fat than alfalfa hay. On the debit side, aquatic plants tend to have a higher moisture content, and when the pH of the water exceeds 8.3, calcium carbonate may be deposited as marl on their surfaces. The latter factor would have an adverse effect on their utilization as food by snails, but does not apply to the plants used in the present experiments. On the credit side, as far as their potential food value is concerned, aquatic plants contain slightly less fibre and higher concentrations of cations, such as Ca^{2+}, Na^{+}, K^{+}, iron and phosphates than land plants (Boyd 1974; Hutchinson 1975). Submerged and floating plants, with which snails would be associated, have higher values for crude protein and ash than emergent and floating-leaved plants (Boyd 1974).

Secondly, the snails, particularly when young and provided they are given time to adapt, appear to be able to utilize novel foods, even very simple substrates such as calcium alginate, which lacks nitrogen. The possible mechanism involved in this adaptive response are discussed below. Thirdly, the present experimental results indicate that when aquatic macrophytes are incorporated as homogenates in calcium alginate matrices, they become more ingestible and support a growth rate which is comparable to that of the lettuce controls. However, there are exceptions to his general rule, namely the homogenates of *Nymphaea*, *Polygonum* and *Fontinalis*, which resulted in the assay snails losing mass. Homogenates of *Potamogeton natans*, *Nasturtium*, *Lemna*, *Sagittaria*, *Alisma* and *Azolla* species resulted in poor growth of less than 50% of that achieved in the lettuce controls. *Myosotis* proved something of an anomaly, as it induced very good growth when presented as a homogenate in the alginate matrix in the short-term experiments, whereas in the long-term experiments, snail growth in all three age groups was very poor. It is possible that these differences are the result of seasonal changes in the biochemical composition of this plant.

These growth inhibitory effects may have been caused either by nutrient deficiency, or by the presence of growth inhibiting or toxic secondary plant compounds. In the former event it is possible that the limiting factors were aromatic compounds, such as the amino acids phenylalanine or tyrosine and porphyrins. Some support for this hypothesis is provided by the following observations. First, it has been shown recently that some aquatic plants are deficient in amino acids, such as tyrosine or phenylalanine, whereas lettuce, which is a very adequate snail food, has measurable amounts of these amino acids (Janauer 1977; Bousfield *et al.* 1980, table 5). Secondly, when lettuce is dried with a consequent loss of the aromatic amino acids and porphyrins, it becomes a much poorer food for the snails. Thirdly, aromatic amino acids such as dopa are used for sclerotinization of the shell and skeletal elements, such as the radula

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TABLE 4. THE CHEMICAL COMPOSITION OF SOME OF THE SPECIES OF AQUATIC PLANT (OR RELATED SPECIES) USED IN THE PRESENT INVESTIGATION

aquatic plants family, species or genus	percentage dry mass: dry matter	ash	crude protein	fibre extract	fibre	P	S	Ca	Mg	K	Na	p.p.m.: Fe	Mn	Zn	Cu	calorific value: kcal g^{-1}	percentage dry mass: cellulose	fat
Potamogetonaceae																		
Potamogeton natans	—	11.1	8.6	0.9	18.8	0.17	0.27	10.5	0.4	1.25	0.24	1700	1100	79	19	—	—	—
Potamogeton crispus	11.8	16.0	10.9	2.4	37.2	0.37	0.10	2.10	0.29	2.51	1.00	4300	2600	97	46	3.61	—	—
Cruciferae																		
Nasturtium officinale	6.7	16.7	32.8	—	10.4	0.8	—	12.25	—	4.2	0.77	253	—	—	—	2.83	—	—
Cabombaceae																		
Cabomba caroliana	7.0	9.6	13.1	5.4	26.8	0.14	0.25	1.21	0.11	2.20	2.60	2900	4300	792	46	3.76	—	—
Hydrocharitaceae																		
Vallisneria spiralis	5.1	15.6	14.2	4.2	14.4	0.21	0.38	1.55	0.76	6.77	2.39	46	39	—	0.3	—	11.1	3.3
Elodea canadensis	7.6	21.9	26.9	3.5	15.4	0.57	0.27	2.80	0.65	3.65	0.27	4000	3310	—	—	—	33.9	2.8
Pistia stratiotes	5.9	2.2	14.0	3.8	26.5	0.30	0.56	2.36	1.0	3.51	—	—	—	—	—	3.49	26.1§	3.7§
Lemnaceae																		
Lemna sp.	—	12.3	34.4	2.6	10.4	0.71	—	1.55	0.25	2.07	—	—	—	—	—	—	—	—
Lemna minor	—	—	—	—	—	1.87	0.58	1.95	0.35	4.16	0.80	2900	4700	370	36	—	17.9	2.4
Ceratophyllaceae																		
Ceratophyllum demersum	5.2‡	20.6	21.7	5.9	27.9	0.49	0.31	1.37	0.87	4.79	0.49	3000	2900	164	15	3.91	27.9§	6.0§
Nymphaeaceae																		
Nymphaea odorata	13.7	9.2	16.6	5.4	20.7	0.31	0.16	0.83	0.12	1.30	0.67	600	125	22	36	3.95	—	—
Alismaceae																		
Sagittaria latifolia	15.9	10.3	17.1	4.7	27.4	0.30	0.15	0.55	0.18	4.04	0.14	460	355	46	57	4.12	—	—
Polygonaceae																		
Polygonum sp.	—	—	—	—	—	0.15	—	0.82	0.46	1.92	—	2074	599	142	65	—	—	—
Haloragaceae																		
Myriophyllum spicatum	12.6	40.6	9.8	1.81	18.8	0.12	0.43	2.77	0.71	1.87	0.75	2991	1185	120	108	2.47	28.1	2.3
Pontederiaceae																		
Eichhornia crassipes	5.9	1.7	16.0	3.4	28.1	0.18	—	1.99	0.40	4.16	0.10	250	3940	50	11	3.82	28.2§	3.6§
Callitrichaceae																		
Callitriche sp.	—	—	—	—	—	0.26	0.22	0.35	0.23	2.17	0.60	2900	1400	150	19	—	—	—
Amaranthaceae																		
Alternanthera philoxeroides	14.5	13.9	15.6	2.7	21.3	0.32	0.29	0.52	0.52	5.2	—	1385	376	114	96	3.46	21.3§	2.7§
Characeae																		
Chara	8.4	35.8	17.5	1.6	23.8	0.25	0.55	8.03	0.92	2.35	0.13	2520	2926	89	19	2.58	15.8§	1.8§
Cyanophyta																		
Lyngbya	3.0	17.2	31.3	—	22.2	0.31	0.28	0.45	0.14	0.42	0.06	3666	5320	161	101	—	—	—
Lettuce† (Butterhead cultivar)	4.9	20.4	24.4	—	10.2	0.53	—	0.71	—	5.39	0.18	408	—	—	—	2.91	—	—

† Based on Watt & Merril (1963); ‡ on Howard-Williams & Junk (1977); § on Boyd (1974). All other data based on Boyd & Scarsbrook (1975).

TABLE 5. CONCENTRATIONS OF AMINO ACIDS IN MICROMOLES PER GRAM OF DRY MASS FOR LETTUCE AND A SELECTION OF AQUATIC MACROPHYTES

	lettuce† $\bar{x} \pm$ SD	*Ranunculus*‡ *aquatilis*	*Elodea*‡ *canadensis*	*Potamogeton*‡ *natans*
taurine	—	3.0	—	—
aspartic acid	0.57 ± 0.09	3.0	4.5	3.5
threonine	0.86 ± 0.08	1.5	0.5	2.0
serine	1.73 ± 0.17	3.5	2.0	3.0
asparagine	2.16 ± 0.25	10.5	61.0	9.5
glutamic acid	23.46 ± 1.27	5.5	+	1.5
glutamine	8.65 ± 1.68	29.0	8.4	0.5
glycine	1.47 ± 0.10	0.5	0.5	1.5
alanine	2.94 ± 0.59	7.5	2.0	4.5
isoleucine	0.94 ± 0.05	0.5	+	1.5
leucine	1.58 ± 0.09	0.5	+	2.0
proline	1.96 ± 0.38	—	—	—
valine	1.74 ± 0.09	—	—	—
tyrosine	0.45 ± 0.11	—	—	—
phenylalanine	1.26 ± 0.21	—	—	—
histidine	2.57 ± 1.32	—	—	—
γ-aminobutyric acid	3.69 ± 1.52	—	—	—
lysine	1.45 ± 0.57	—	—	—
arginine	0.74 ± 0.07	—	—	—
unidentified	0.83 ± 0.005	—	—	—
Total	59.05	65.0	78.9	29.5

† Based on Bousfield *et al.* (1980). ‡ Based on Janauer (1977).

in molluscs (Wilbur 1964; Saleuddin & Petit 1983). As porphyrins are needed for the manufacture of respiratory enzymes and haemoglobin they may often be limiting factors. Bernays & Woodhead (1982*a*, *b*, 1984), have recently shown that these are generally present in terrestrial plants at suboptimal concentrations for the growth of insect herbivores. As a result of selective pressures some species of insects have developed the ability to use phenols, such as gallic acid and 3- or 4-dihydroxyphenols, which normally serve as defence chemicals for the plants, as alternatives to phenylalanine as a cuticular tanning agent (Bernays & Woodhead 1984).

Large aggregates of plant material (over 0.22 μM) also appear to be nutritionally important to the snails as filtrates (under 0.22 μM), promoted growth to a significantly lesser extent than the unfiltered homogenates.

4.2.6. *Proposition* 6. *That truly aquatic, submerged macrophytes would not possess secondary plant compounds* (SPC) *with molluscicidal properties, although they may act as antifeedants*

The rationale for this proposition is as follows. It has been shown that aquatic macrophytes can display two successful defence strategies against attack by aquatic herbivores, a hard outer envelope and a screen of epiphytic algae and bacteria, which may act as decoys or as systems for producing feeding deterrents. It could be argued that these defences could be further strengthened by the deployment of SPC with antifeedants or molluscicidal properties. However, two counter arguments can be advanced against the involvement of the latter strategy against snails. First, if macrophytes do derive a net benefit from the presence of snails, it would not be cost-effective for them to go to the extreme of using molluscicidal factors. Secondly, it seems probable that the SPC present in aquatic macrophytes evolved mainly as a result of the severe

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selective pressures imposed upon them by insect herbivores during the preaquatic phase of their existence. If this is correct, and the selective pressures imposed upon the plants by the snails were minimal, it might be expected that during the course of the co-evolution of the macrophytes and snails in both terrestrial and aquatic habitats, either the snails or their microbial flora would have evolved mechanisms for detoxifying the SPC. As a corollary to this thesis, it would be predicted that plants, or parts of plants, which the snails would not normally encounter, would possess SPC which might be toxic to them. Alternatively, if the snails do impose some selective pressures on the plants, it might be expected that they would invest in SPC with some antifeedant properties.

The information that is currently available regarding SPC in freshwater macrophytes (table 6) and the results of the present investigation appear to support the above proposition, and the arguments presented in the accompanying rationale. According to Hutchinson (1975) aquatic macrophytes appear to invest less in SPC than their terrestrial counterparts. Table 6 shows that some of the species of aquatic macrophytes used in the present study, or species closely related to them, contain a formidable array of SPC including alkaloids, glycosides, cardenolides, saponins, cyanogenic substances, lectins and tannins. Unfortunately there appears to be little or no information available regarding the qualitative and quantitative changes that might occur in the distribution of SPC as aquatic or subaquatic macrophytes grow, age, senesce or emerge from the water. It seems probable that, as with terrestrial plants, there will be more investment in the production of SPC, such as cyanogenic substances, in the young growing stems, and more tannins and related compounds in mature tissues (Crawley 1983). It has also been suggested by Hutchinson (1975) that the emergent, aerial parts, of aquatic macrophytes have higher concentrations of SPC than the submerged portions. This is to be expected on evolutionary grounds, as they are likely to be subjected to much more concerted attacks by herbivores than submerged parts of the plants for reasons already given. Much of the information on the distribution of SPC is anecdotal, and it is clearly necessary to obtain more precise quantitative data.

The present results show that with the exception of *Alternanthera sessilis*, a subaquatic or amphibious plant, none of the macrophytes investigated contained factors that were strongly molluscicidal. It must be concluded therefore, that as predicted, *B. glabrata* or its associated microbial flora, are able to detoxify the various SPC that are known to be present in these plants. There is also evidence that some aquaic macrophytes investigated contained factors that were having an inhibitory effect on the snails. First, snails fed on calcium alginate matrices containing homogenates of *Fontinalis*, *Nymphaea* and *Polygonum* grew at significantly slower rates than those fed with matrices containing lettuce homogenate. Secondly, it was found that the presence of filtered homogenates of *Lemna*, *Ceratophyllum*, *Myosotis* and *Callitriche* in media containing snails provided with standard food rations, resulted in significant growth inhibition compared with controls. Although in the former case the inhibitory effect might have been due to nutritional deficiency, this is unlikely, in view of the results of chemical analyses carried out on the plants. This possibility can definitely be ruled out in the latter case. The growth inhibitory effects may have been caused by antifeedants such as tannins. According to Boyd (1968) the concentrations of tannins may be as high as 10% or more of the dry mass in aquatic macrophytes, including species of *Myriophyllum*, *Cabomba*, *Ludwigia*, *Brasenia* and *Nymphaea*. These tannins may act as antifeedants by impeding both ingestion and digestion. Thus Boyd (1968) was able to demonstrate that the digestibility of protein in plant material was inversely related to tannin

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TABLE 6. SECONDARY CONSTITUENTS OF AQUATIC PLANTS USED IN PRESENT EXPERIMENTS AND RELATED SPECIES

(Based on McClure 1970)

family and species of plants	alkaloids	terpenoids	simple phenolics	flavinoids	others	references
Potamogonaceae						
Potamogeton crispus	—	—	—	rutin	—	Tjia-Lo & Hegnauer 1956
Potamogeton natans	—	—	—	present	steroids	Hultin & Torssell 1965 Su *et al.* 1972
Potamogeton sp.	present	—	—	—	—	Hutchinson 1975
Cruciferae						
Nasturtium officinale	—	—	—	—	glucosinolates	Hutchinson 1975 McLeod & Islam 1975
Cabombaceae						
Cabomba furcata	—	—	—	(none)	—	Kubutzki & Resnik 1966
Hydrocharitaceae						
Vallisneria spiralis	—	—	caff, pc	(no antho)	—	Bate-Smith 1968*b* Reznik & Neuhausel 1959
Vallisneria americana	present	—	—	—	—	Su *et al.* 1972
Elodea canadensis	—	—	caff, cg	Qu, Cy (leuco?)	—	Reznik & Neuhausel 1959
Hydrocharis morsus-ranae	none	(no saponins)	caff	Cy, Pg rutin	no cyanogens no tannins	Tjia-Lo & Hegnauer 1956 Hegnauer 1963
Limnobium stoloniferum	—	—	—	unknown anth	—	Bate-Smith 1954
Araceae						
Pistia stratiotes	—	—	caff (?)	Cy	—	Bate-Smith 1968*b*
Lemnaceae						
Lemna minor	present	saponin	S, Cg	orientin vitexin isoorientin isovitexin lutonarin lucenin vicenin saponaretin	much apiose steroid	Bate-Smith 1968*a*, *b* Van Beusekon 1967 McClure & Alston 1966 Su *et al.* 1973 Hutchinson 1975
Lemna paucicostata	—	—	—	present	—	Su *et al.* 1973
Ceratophyllaceae						
Ceratophyllum demersum	present	—	cg, caff, S, F, Cy (leuco?)	Mv (leuco?) Dp (leuco?) present steroid	— cyanogenic glycosides β-sitosterol	Bate-Smith 1962 Reznik & Neuhausel 1959 Su *et al.* 1972 Gibbs 1974 Hutchinson 1975
Nymphaeaceae						
Nymphaea lotus	present	—	—	—	tannic gallic acids	Hutchinson 1975

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TABLE 6 (*cont.*)

family and species of plants	alkaloids	terpenoids	simple phenolics	flavonoids	others	references
Ranunculaceae *Ranunculus* sp.	—	—	—	—	lactones	Hutchinson 1975
Hippuridaceae *Hippuris vulgaris*	—	no saponins	E, caff, F scopoletin(?) coumarins	Km	tannins aucubin catalpol cyanogenic glycoides	Bate-Smith 1962 Dekker 1913 Hegnauer 1967 Gibbs 1974
Alismaceae *Sagittaria* sp.	conflicting reports	saponins	—	Cy (leuco?)	no cyanogens	Tjia-Lo & Hegnauer 1956 Hultin & Torssell 1965 Altman 1954 Reznik & Neuhausel 1959
Alisma plantago-aquatica	conflicting reports	alisol A alisol B	— —	Cy (leuco?) rutin?	no cyanogens	Hultin & Torssell 1965 Massageltov 1947 Murata *et al.* 1968 Tjia-Lo & Hegnauer 1965 Reznik & Neuhausel 1959
Polygonaceae *Polygonum* sp.	—	polygadiol B			pseudocyanogenic glycosides	Dossaji *et al.* 1977 Hutchinson 1975
Umbelliferae *Oenanthe aquatica*	present	aldrol camphene crypton pinene phellandral phellandrene sabinene	— —	—	polyacetylenes oenanthetoxin	Guentter 1952 Gibbs 1974 Harborne 1977
Gramineae *Glyceria fluitans*	—	—	pC, F	Km	—	Bate-Smith 1968*b*
Boraginaceae *Myosotis scorpioides*	4 pyrrolizidine alkaloids	saponins	—	—	cyanogenic glycosides	Gibbs 1974 Resche *et al.* 1982

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Haloragaceae						
Myriophyllum	—	unknown	E	Qu, Cy (leuco?)	cyanogenic	Hegnauer 1964
proserpinacoides	—	saponins	—	—	steroids	Bate-Smith 1962
Myriophyllum	—	—	—	present	—	Reznik & Neuhausel 1959
exalbexens	—	—	—	—	—	Su *et al.* 1972;
Myriophyllum	cyanogenic	—	—	—	—	Hutchinson 1975
aquaticum	alkaloids					
Pontederiaceae						
Eichhornia	conflicting	triterpenes	caff, F	Dp, 3-diglu,	—	Bate-Smith 1968*a*, *b*
speciosa	reports	—	—	Cy(leuco?)	—	Hegnauer 1963
						Willaman & Schubert 1961
						Bate-Smith 1968*b*
						Shibata 1965
						Bate-Smith & Swain 1965
Callitrichaceae						
Callitriche	—	—	caff	Ap, Lu	aucubin	Hegnauer 1967
palustris					catapol	
Characeae						
Chara vulgaris	present	—	—	—	steroids	Su *et al.* 1973

Abbreviations: anth, anthocyanin; AP, apigenin; caff, caffeic acid; cg, chlorogenic acid; Cy, cyanidin; Dp, delphinidin; E, ellagic acid; F, ferulic acid; Km, kaempferol; Leuco, leucothyocyanin; Lu, luteolin; Mv, malvidin, pC, paracoumaric acid; Pg, pelargonidin; Qu, quercetin; S, sinapic acid.

concentration. It is possible that other SPC may also be implicated, as the inhibitory effects observed on snail growth when snails encountered plant material, might have been due to novel SPC, which were unfamiliar to them. Thus, *Fontinalis antipyretica* grows in fast-flowing water and would not normally be encountered by pulmonate snails. As a result they might not have evolved mechanisms to detoxify the SPC. This explanation may also apply to the results obtained with the species of *Polygonum*, *Myosotis* and *Callitriche*, as the material used in preparing the homogenate probably included emergent plant material which the snails would not normally encounter. Although the pulmonate snails normally meet the floating leaves of *Lemna* and *Nymphaea* it is possible that they would contain high concentrations of SPC for reasons already given and that these would impede snail growth. These hypotheses need to be tested empirically.

However, growth inhibition by homogenates placed in the medium is not the general rule, as in the majority of cases, including those treatments containing homogenates of *Myosotis*, *Groenlandia*, *Glyceria*, *Myriophyllum*, *Elodea*, *Hippuris*, *Alisma*, *Ranunculus* and *Oenanthe*, snail growth was significantly enhanced, compared with controls. Reasonable growth was also achieved in the majority of treatments in which the snails were provided with homogenates of aquatic macrophytes in calcium alginate matrices. It would appear safe to conclude that truly aquatic plants do not generally invest a great deal in SPC which act as antifeedants and have a growth-inhibiting effect on snails. None produced factors with strong molluscicidal effects.

4.2.7. *Proposition* 7. *Emergent parts of subaquatic or aquatic plants might be expected to be better sources of SPC with molluscicidal factors than either submerged aquatic plants or even terrestrial plants* (*figure* 17)

The rationale for this proposition is as follows. Emergent parts of subaquatic or aquatic plants, particularly those in draw-down areas are liable to be exploited by a highly efficient guild of terrestrial, herbivorous insects, including species of Coleoptera, Orthoptera, Lepidoptera and Hymenoptera. As such plants tend to invest in thinner outer envelopes than terrestrial plants, in consequence of their aquatic existence, it can be hypothesized that there would be continued strong selective pressures on them to produce new SPC with strong insecticidal properties. Aquatic pulmonates would not normally encounter these SPC. It might be expected therefore that they would not have evolved biochemical mechanisms to detoxify them. By the same token one would anticipate that fully terrestrial plants would also have molluscicidal SPC. However, this need not necessarily be the case, as the snails may have co-evolved with such plants during the terrestrial phase in their evolution, and thus acquired a measure of resistance to their SPC. It might be expected that there would be less pressure on fully terrestrial plants to produce new SPC as, unlike emergent aquatic plants, they can also invest in thicker outer envelopes as a defensive strategy.

The first proposition receives support from the observation that *Alternanthera sessilis*, the only plant found to have molluscicidal factors in the present investigation, is a subaquatic emergent plant occurring in the ecotone or draw-down areas of water bodies. Two other species which have also been shown to possess molluscicidal factors, namely *Polygonum senegalense* and *Canna indica* (Dossaji *et al.* 1977; Daffola & Amin 1979; Maradufu & Ouma 1977; Kloos & McCullough 1982) are also emergent ecotone species. It would be of interest to ascertain whether molluscicidal factors were concentrated in the emergent tissues of these ecotone species. In the present investigation, the second part of the hypothesis is supported by the observation that nearly all of the other plants which have factors inhibiting growth of the snails have either emergent or floating leaves (for example, *Nymphaea*, *Nasturtium*, *Lemna*, *Sagittaria*, *Alisma*, *Azolla*

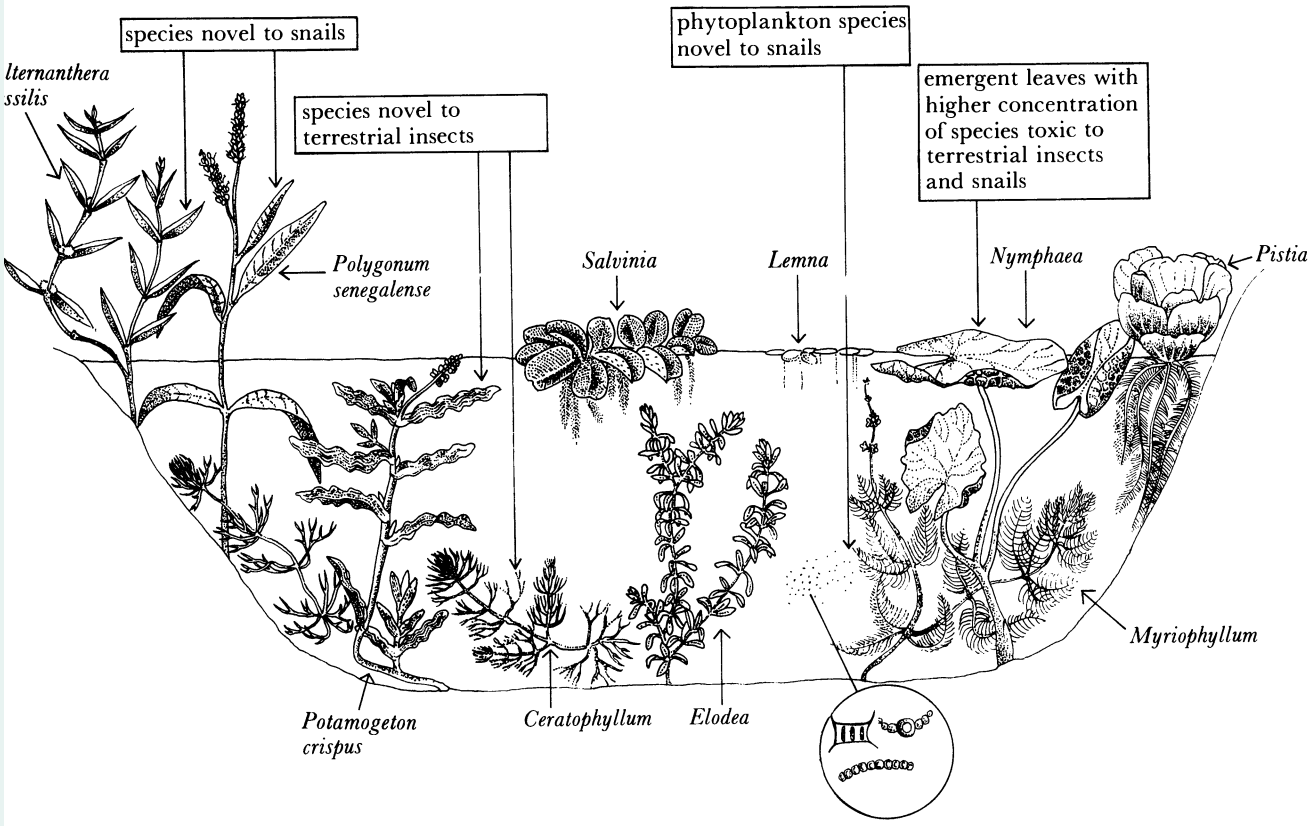

FIGURE 17. Diagrammatic representation of water plants showing likely sources of molluscicidal and insecticidal factors.

and *Myosotis*). As already suggested, these factors probably act by inhibiting ingestion or digestion.

The second prediction in this section is supported by the results of screening tests carried out on terrestrial plants which show that, from approximately 1000 species examined, only a few have shown any promise of having molluscicidal factors (Kloos & McCullough 1982). Some of these, such as *Ambrosia maritina* in Egypt (Sherif & El Sawry 1977), *Croton macrostachys* in the Sudan (Daffola & Amin 1979), and the desert palms *Balantides aegyptica* and *B. mauglami* (Wagner 1933), would appear to release molluscicides from living tissues. It has been suggested that they could be planted on the banks of riverine courses or ponds where snail control was needed, as either the leaves or fruit falling in the water would have the desired effect. However, in the case of other terrestrial plants with potent molluscicidal properties, such as *Phytolacca dodecandra* (Lemna 1970), the cashew nut *Anacridium occidentalis* and *Tetrapleura tetraptera*, it is necessary to develop a technology for extracting and concentrating the active factors before they can be used (Kloos & McCullough 1982; Adewumni & Sofowara 1980). This might prove difficult to implement in the developing countries. A further search for macrophytes with molluscicidal factors is therefore required. To make this more cost effective it is suggested that it might be directed at macrophytes occurring in draw-down areas of water bodies and at the small group of betalain-containing angiosperm families to which *Alternanthera*, *Polygonum* and *Phytolacca dodecandra* belong (Hutchinson 1959; Cronquist 1968; Smith 1976). The active factor in the latter species has been identified as saponin (Lemma 1970) and it is possible that this might be the active molluscicide in other members of this family as well.

4.2.8. *Proposition* 8. *Species of epiphytic algae might be better sources of* SPC *with molluscicidal effects than aquatic macrophytes* (*figure* 17)

The rationale for this proposition is as follows. As these are more vulnerable to ingestion by snails than macrophytes, by virtue of their small size and surface-living habits, it might be selectively advantageous for them to produce antifeedants or molluscicidal factors to discourage ingestion by the snails. Blue-green algae would appear to be suitable candidates for molluscicide screening in the algal community for the following reasons. First, it has been shown in the present study that *Lyngbya* causes the assay snails to lose mass, possibly because it contains a feeding inhibitor. Secondly, it is known that *Microcystis farlouriana* and *Pseudoanabaena franqueti* produce factors that are toxic to *Lymnaea* (Gevrey *et al.* 1976). Thirdly, various species of blue-green algae are rich sources of endotoxins (Carmichael 1980) whose possible effects upon molluscs have yet to be elucidated.

However, there are other survival strategies available to epiphytic algae. Thus, they may develop cell walls that are resistant to digestion by pulmonate snails, as is the case with *Chlorella* (van Aardt & Wolmarans 1981) and *Scenedesmus* (Thomas *et al.* 1985). Alternatively they may survive as small adpressed forms which are resistant to ingestion, as appears to be the case with *Cocconeis placentula*. Larger forms of this species can be readily ingested by the snails, and it may be advantageous for them to sacrifice some members of the clone to maintain sufficient herbivore pressure on other competitor species. It has been suggested that a similar strategy may be used by grass species in savanna communities (Owen & Wiegert 1981).

4.2.9. *Proposition 9. That the strategies developed by pulmonate snails for obtaining their energy supplies are not conducive to rapid speciation*

This proposition is supported by the following observations. First, it has been demonstrated that extant species of freshwater pulmonate snails are generalized feeders. They utilize a wide range of epiphytic algae, decaying plant material and DOM of varying chemical composition, rather than living macrophyte tissue as their food resource. It would be predicted, therefore, that they would tend not to select any particular macrophyte species if offered a choice. This hypothesis could readily be tested by choice experiments under field or laboratory conditions. Evidence has also been given that the early gastropods in the Cambrian or Ordovician probably used feeding methods that were basically the same as present-day snails. Secondly, when *B. glabrata* of various ages were forced to adapt to a novel food plant they showed no resistance to secondary conditioning. For example, even snails that had been reared from the egg on watercress reverted readily to feeding on lettuce when it was offered. Similar results have been obtained with other molluscs, such as the prosobranch *Marisa cornuarietis* (Cedeño-León & Thomas 1982), predatory snails (Wood 1968) as well as insects (Bernays *et al.* 1976) and snakes (Burghart 1975). It is highly unlikely, as pointed out by White (1978), that the feeding behaviour of such organisms could lead to reproductive isolation and sympatric speciation, as postulated by Thorpe (1930, 1940).

This view is supported by the following observations. First, the fossil evidence is indicative of a very slow rate of evolution. Thus, many of the extant families and even genera of freshwater pulmonates were already present in the Cretaceous. Secondly, the freshwater basommatophorans are represented by very few species today. For example, Thomas (1966) found only four species of basommatophoran snails in a West African lake, compared with 39, 33, over 30,

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and 68 species of Hemiptera, Odonata, Diptera and Coleoptera, respectively. Similarly, only eight species of basommatophoran snails were recorded by Ndifon (1979) in a 160000 km² area studied by him in Nigeria, and Brown (1980) lists only 92 species in the whole of the Afro-tropical region. This trend shown by the basommatophoran snails is paralleled in other taxonomic groups in which the species, for the most part, obtain their energy by ingesting detritus or colonies of surface-living micro-organisms, including algae, fungi and bacteria. Thus, the Ephemeroptera and Plecoptera are represented by only approximately 2000 and 1900 species, respectively. In contrast, the terrestrial Hymenoptera, Lepidoptera, Coleoptera and Diptera, which have more than 10^5 species in each case, are represented by a large number of species which have specialized to feed on particular plants.

4.3. *Predictions based on the theory of mutualism involving snails and macrophytes*

4.3.1. *Prediction* 1. *That truly aquatic macrophytes would release exogenous factors that will serve as attractants, arrestants and growth factors for the snails*

The rationale for this prediction is that if the macrophytes derive net benefits from the presence of snails, selective pressures would favour the evolution of mechanisms which would result in their producing exogenous factors that would attract and benefit the snails. An analogy is provided by the presence of extrafloral nectaries in plants that have a symbiotic relationship with ants (Strong *et al.* 1984). To test this prediction it is necessary to consider the possible interactions that may occur between the snails and the macrophyte on a spatiotemporal basis. The stimuli originating from the plant, the responses of the snails to them and kinds of assays that could be used to characterize the chemical factors involved, are summarized in table 3 and illustrated in a simplified form in figure 1. The precise definition of terms and the methods used to measure the responses to stimuli from the plants are given by Townsend (1973), Thomas *et al.* (1983*b*), Thomas & Benjamin (1974*a*), Bousfield (1978) and Thomas (1981).

The present results show that there is some basis to this prediction as the assay snails were observed on the leaf surfaces of all the plant species tested, including *Alternanthera sessilis*, which is strongly molluscicidal when presented in homogenized form. It is also supported by field observations which show that freshwater pulmonate snails, such as *B. glabrata* or *B. pfeifferi*, tend to be strongly positively associated with the surfaces of aquatic or subaquatic emergent plants, or their decaying remains (Ferguson 1977; Pimentel & White 1959; Thomas & Tait 1984*b*).

Although the latter authors also reported a few significant negative associations between the water fern *Salvinia nymphellula* and four snail species (*Bulinus forskali*, *Biomphalaria pfeifferi*, *Bulinus rohlfsi* and *Anisus coretus*) it is not necessary to postulate that this fern releases allelopathic factors to explain these results. The most plausible explanation is that snails tend not to occur in the large, tightly packed beds of this fern because they find it difficult to obtain oxygen for the following reasons. First, the water column underneath the beds would tend to become anoxic as the reduction in light intensity would have a detrimental effect on phytoplankton growth. Empirical evidence in support of this suggestion is given by Thomas & Ratcliffe (1973). Secondly, the tight packing of the fronds make it difficult for the snails to gain access to the air–water interface. This hypothesis is supported by the results of the present experiments and also by those of Thomas & Tait (1984*b*) which show that species of *Biomphalaria* can coexist with *Salvinia* and achieve some growth.

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However, the snails could be attracted to the surfaces of aquatic macrophytes by chemical factors released by epiphytic algae or bacteria, including those involved in decay processes, as well as by the plants themselves. To test the prediction, it would be necessary to show that axenically cultured macrophytes were releasing attractants and growth factors. That this is at least feasible is supported by the observations of Wetzel & Manny (1972), as they showed that axenic cultures of the water plant *Najas flexilis* released approximately 4% of their photoassimilates as organic matter, under natural conditions. As much of this organic matter was readily oxidized by ultraviolet light, it became metabolically available to other organisms. The compounds identified included glucose, sucrose, fructose, xylose and glycine. Of these compounds it is known that glucose and sucrose can serve as attractants to the pulmonate snail *B. glabrata* (Thomas *et al.* 1985) and that glucose can be taken up actively by this snail (Lewert & Para 1966).

Other chemical species that may be implicated as attractants and growth factors of plant origin are small molecular mass compounds, such as glycolic acid, glutamate, aspartate, proline, asparagine, glutamine, short-chain carboxylic acids and dicarboxylic acids, for the following reasons. First, they have been shown to be potent attractants and arrestants of *B. glabrata* (Thomas & Assefa 1979; Thomas *et al.* 1980, 1983*b*). Secondly, it is known that these chemical species are either present in substantial amounts within the plants or their decaying remains and, therefore, likely to be released, or else they have been identified as exogenous factors in the vicinity of the plants (Wetzel & Manny 1972; Hellebust 1974; Boyd & Scarsbrook 1975; Nalewajko 1966; Nalewajko & Schindler 1976; Patience *et al.* 1983; Sterry *et al.* 1985). However, it remains to be shown that they are, in fact, released by living macrophyte tissue. It seems probable that most of the short-chain (C_2–C_5) carboxylic acids, for example, are in fact released as end products of glycolytic fermentation by bacteria involved in utilizing senescent macrophyte tissue (Patience *et al.* 1983; Sterry *et al.* 1985). According to Bousfield (1978, 1979) aquatic plants such as *Groenlandia densa*, *Callitriche obtusangula*, *Ranunculus trichophyllus*, *Potamogeton crispus* and *Elodea canadensis* contain large molecular mass compounds, possible proteins, which elicit positive rheotaxis in *B. glabrata*, whereas homogenates from *Chara*, *Apium nodiflorum* and *Nasturtium officinale* failed to do so, possibly because of the presence of an antagonistic factor. However, it remains to be shown that these rheotaxis inducers are, in fact, released naturally by the plants.

Exogenous glycolic acid, amino acids, sugars and proteins, which are released by macrophytes (Wetzel & Manny 1972; Hellebust 1974; Bardach 1975) may provide the snails with a source of energy as well as being attractants. It is known that amino acids and sugars may be taken up actively through the body wall of *B. glabrata* (Gilbertson & Jones 1972; Lewert & Para 1966). However, it is necessary to relate the uptake characteristics to the naturally occurring concentrations before their importance can be fully evaluated. It has been suggested by Ogston (1976) that large cationic molecules of plant origin may be implicated in promoting growth of *B. glabrata* by facilitating a Gibbs–Donnan effect, thus enhancing the rate of uptake of Ca^{2+} from the medium by the snails. Such a process could promote growth as Ca^{2+} is a major component of the shell as well as being implicated in other physiological processes. The statistically significant growth enhancement effects observed when snails were treated with filtered homogenates of species of *Myosotis*, *Groenlandia*, *Glyceria*, *Myriophyllum*, *Elodea*, *Hippuris* and *Oenanthe* in the present investigation, may have been caused by factors that would be released exogenously, but this remains to be proven. Similar effects have been demonstrated

when snails were treated with lettuce homogenate, media heterotypically conditioned by snails feeding on lettuce and media conditioned by lettuce discs (Benjamin 1973; Thomas *et al.* 1975; Thomas *et al.* 1983*a*).

A similar mutualistic relationship may also occur between certain species of epiphytic algae and snails. It is known that such algae release considerable amounts of glycolic acid, amino acids, sugars and proteins (Fogg & Nalewajko 1964; Nalewajko 1966; Nalewajko & Schindler 1976; Jüttner & Matuschek 1978). Certain of these algae, such as *Chlorella* and *Scenedesmus* species may pass through the alimentary canal of pulmonate snails undigested, although the snails may be able to take up their exogenous factors through the gut wall. The advantages to the algae are not known, but it is possible that they might obtain nutrients from the snail during their passage through the gut.

It is evident that a great deal more work remains to be done before the functional significance of the exogenous substances released by the macrophytes and algae are fully elucidated.

4.3.2. *Prediction* 2. *That the snails might protect the macrophytes from attack by other herbivores*

Pulmonate snails release considerable amounts of mucus as they glide over the surface of macrophytes. The possible benefits that they may derive from this have been discussed by Thomas *et al.* (1985). There is also the possibility that chemical factors in the mucus might benefit the plant by protecting it from attack by other herbivores. This hypothesis does not appear to have been tested. However, it has been shown that mucus deposited by *Cepaea* species may cause plant food to be less palatable to conspecifics and related species (Cameron & Carter 1979). Although these effects were attributed to the presence of inhibitory pheromones in the mucus, and only their role in regulating population growth considered, the possibility that these chemical factors may have greater significance in protecting the plant against attack by other herbivores is worthy of investigation.

4.3.3. *Prediction* 3. *That truly aquatic plants would not contain or release factors that would act as repellents or molluscicides*

None of the plant species used in the present investigation released exogenous repellents into the medium. This was the case even with *Alternanthera sessilis* whose homogenized tissues are highly toxic to snails. Claims have been made that water plants release factors into the medium which act as repellents or toxicants to aquatic invertebrates. Thus, Pennak (1973) reported that species of *Elodea*, *Nitella* and *Myriophyllum* secrete substances into the water that repel *Daphnia*, whereas *Myriophyllum*, *Potamogeton*, *Ceratophyllum*, *Chara*, *Elodea* and *Polygonum* secrete factors that repel copepods. It is difficult to suggest a functional significance for these responses, as neither *Daphnia* nor the copepods can apparently harm these macrophytes. Perhaps the most plausible explanation for this phenomenon is that these members of the zooplankton use the chemical signals released by the macrophytes as an indication of the absence of phytoplankton on which they feed, and therefore move away from them.

The present results show that not only can *B. glabrata* coexist with *Alternanthera sessilis*, but that the snails can achieve some growth. Ndifon (1979) obtained similar results when *Bulinus* (*P.*) *globosus* was kept in aquaria containing *A. sessilis*. There is no evidence that this plant releases molluscicidal factors into the medium under laboratory conditions. This also appears to be the case in the field, as Thomas (1966) found *Bulinus* (*P.*) *globosus* coexisting with

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A. sessilis in a man-made lake in Ghana. In fact this plant is a good biotic indicator of the presence of the snail's hosts of schistosomiasis both in West Africa and in the state of Minas Gerais in Brazil (personal observation).

However, there have been some reports of water plants such as *Chara vulgaris* and subaquatic emergent plants, such as *Polygonum senegalense* and *Canna indica*, releasing molluscicidal factors into the medium (Renno 1972; Kloos & McCullough 1981; 1982; Dossaji *et al.* 1977; Daffola & Amin 1979). According to Maradufu & Ouma (1977) the molluscicidal factors produced by *Polygonum senegalense* is a phenolic glycoside, but these results appear to be at variance with the present ones, and those obtained by Thomas & Tait (1984*b*) under field conditions in a Nigerian lake. The latter authors found the snail hosts of schistosomiasis (*Bulinus rohlfsi*, *Bulinus forskali*, *B.* (*P.*) *globosus* and *B. pfeifferi*) coexisting with *Polygonum senegalense* and two other *Polygonum* species. There was no evidence that either the *Polygonum* or *Chara* species used in the present laboratory experiments were releasing molluscicidal factors.

There are several possible explanations for these apparent anomalies. First, the molluscicidal factors might have originated from epiphytic micro-organisms, such as bacteria, yeasts, fungi or algae that covered the leaf surface, rather than the macrophytes themselves. If that were the case then the observed differences might have been due to differences in the species composition of the epiphytic communities. Secondly there may have been some varietal differences between the plants. Thirdly, the plants may have differed in the pattern of release of exogenous factors. Fourthly, the assay snails used might have differed in their susceptibilities to the toxicants. Further work is needed to test these hypotheses.

There are two possible explanations for the ability of the snail hosts to coexist, both in the laboratory and in the field, with living plants of *Alternanthera* and *Polygonum* despite the presence of a molluscicidal factor. First, the snails may only feed on epiphytic algae, DOM and decaying tissue, rather than on the living plant tissue. It is possible that the decaying plant material might be safer to ingest than the living tissue, as it is probable that decomposition would be accompanied by microbial degradation of SPC and a loss of concentrated, potentially toxic metal ions by outward diffusion (Hentges *et al.* 1973; Boyd & Scarsbrook 1975). Thirdly, the snails might ingest the plant at a sufficiently slow rate to prevent toxicants accumulating to lethal levels.

It would appear from the present results that in natural systems, or simulated natural systems in the laboratory, that the growth of snails at the individual and population levels will be constrained to a considerable extent by the rate at which the decaying microhabitats and DOM become available. This will impose a relatively slow growth rate. This hypothesis is supported by population studies on pulmonate snails carried out by Eisenberg (1966, 1970) and O'Keefe (1982) under field conditions. The relation between pulmonate snails and macrophytes appears similar to that described by Southwood (1984) for apparent plants and terrestrial herbivorous insects, as the former imposed slow growth on the latter.

5. Conclusions

The analysis in the discussion shows that the propositions based on the hypothesis of phased co-evolution involving various components in the module: the epiphytic bacteria, algae, macrophytes and the pulmonate snails are supported by the present empirical data and those of other workers. There is good evidence that pulmonate snails, early in their evolutionary

history, developed specialised mechanisms to exploit DOM, detritus and communities of algae, fungi and bacteria living on the interfaces between the water and rocks, sand, silt or air. Consequently when freshwater macrophytes first appeared in the snails' environment the snails were already preadapted to be cleaning symbionts. Evidence is given that they have retained these feeding strategies to the present day. They appear to have remained feeding generalists and, given sufficient time, they are able to adapt to novel foods of varying chemical composition. Living macrophyte tissue is little utilized by pulmonate snails or by other members of the guild of invertebrates associated with aquatic macrophytes.

The relatively hard outer texture of the aquatic macrophytes, their large size and their ability to provide the snails and other aquatic invertebrates with alternative food sources in the form of epiphytic algae and decaying plant material appear to be their main strategies for defence. Submerged aquatic plants seem to have invested little in biochemical strategies which would result in them having nutrient deficient tissues or SPC which would be strongly toxic or lethal to the snails. Certain of the propositions, namely those that suggest that emergent portions of subaquatic or aquatic plants and epiphytic or planktonic algae might be better sources of molluscicidal factors, have practical applications and require further testing. Finally, it is concluded that the strategies developed by the snails for obtaining their energy supplies are not conducive to rapid speciation.

The predictions made on the basis that the relation between the snails and macrophytes is mutualistic receive some support. Thus, there is some evidence that the macrophytes release exogenous factors that may serve as attractants, arrestants and growth factors. However, the picture is further complicated by the fact that these exogenous factors may also influence other components in the module, namely the epiphytic algae and bacteria, which may also have a mutualistic relationship with the macrophytes. To test the theory of mutualism empirically it will be necessary to ascertain how the removal of individual components in the module, including the snails, the epiphytic algae and bacteria, will influence the stability of the system. This approach has already been used successfully to investigate similar modules in the marine environment (Paine 1980). In addition to this, it will be necessary to obtain more information on the costs and benefits of the interactions between the various components. As linkages are mainly of a chemical nature, this will necessitate the development of a new area of integrated research in biochemical ecology. It is only by developing a better understanding of the possible mutualistic interaction between the components of such modules that the question posed by May (1983), regarding the factors that determine the numbers and relative abundance of species in a community, may be answered.

The present results show interesting contrasts between the interactions between herbivores and plants in aquatic and terrestrial ecosystems. Thus, in terrestrial habitats there are a large number of species (approximately 361 000) belonging to the Coleoptera, Diptera, Lepidoptera, Hymenoptera, Hemiptera, Orthoptera and other orders of insects that are directly phytophagous (Strong *et al.* 1984). Certain of these can maintain population densities of their plant food species at low levels and have been used as agents for biological control. It follows that these herbivorous species are limited by their macrophyte food supply. That this appears not to be the case in freshwater ecosystems may be due to the following reasons. First, the dominant potential herbivores in freshwater, including both the snail and insect members of the guild, were preadapted to be detritivores or epiphyte consumers before macrophytes appeared and have retained this feeding strategy. The epiphytic algae provide them with an alternative food

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source. It would therefore benefit the macrophytes to maintain both these and the bacteria that will eventually facilitate the turnover of nutrients with assistance from macrodetritivores, such as the snails. The efficient utilizers of macrophyte tissues that have evolved in the terrestrial environment have as yet been unable to overcome the environmental constraints imposed upon them by the aquatic environment.

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