A SURVEY OF SOME OF THE ALGAL EPIPHYTES ON THE SHELLS OF FRESHWATER MOLLUSCA.

Thesis presented by

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Abstract of thesis.

The epiphytic chlorophycean algae have been studied from different species of freshwater mollusc, including a variety of gastropods living within a few feet of the water surface and a number of lamellibranchs found usually either in or on the water bed.

The animals were killed with chloroform, the bodies removed and the shells, after washing, were cultured in Soil Solution in glass dishes. The algae were present in a very reduced state - due probably to their being browsed by other snails, but after several weeks in the laboratory they grew to maturity and could be subcultured and identified.

Over one hundred forms of algae were isolated the majority of which were thought to be separate species; most of them belonged to the Ulotrichales, the Chaetophorales and the Oedogoniales.

The population in general seemed to bear very little relationship to the species of mollusc. The size of the snail, its speed of movement, the shell surface and shape did not appear to affect the species of algal epiphytes.

Apart from one species the epiphytes on the bivalves were very similar to those on the snails although there were differences in the frequency with which each occurred.

Empty snail shells had populations almost identical with those on living specimens if the shells remained in the same environment.

As far as could be discovered no relationship existed between the composition of the shell and the algal epiphytes.

The cells of a species of Oedogonium were larger on snails in a pond than on adjacent stationary objects.

A number of algae have been shown to pass almost unharmed through a snail's gut.

Inter-relationships of snails and algae are discussed.

CONTENTS.

Introduction ---------- 1

Material used ---------- 4

Methods ---------- 13

The Algae ---------- 24

Experimental Work :-

A comparison of the algae on the shells of various species of mollusc all collected at the same time from a single pond ------- 35

The relationship between the colonising algae and the substratum ---------- 46

A comparison of the algae on the shells of a single species of snail collected from a number of different places ---------- 52

The lamellibranchs ---------- 62

Roughness of the shell ---------- 69

The algal population considered in relation to the age of the shell ---------- 72

The algal populations on empty shells compared with those on living snails ------ 80

The snail shell as a substratum ---------- 85

Experiments considering shell composition ---- 91

Inter-relationships between snails and algae ------- 95

Discussion ---------- 100

Summary ---------- 106

Bibliography ---------- 108

Drawings ---------- 111

Introduction.

It is perhaps surprising that molluscan shells have been given so little consideration as substrata for epiphytic algae; the literature contains numerous references to the presence of algae in such situations but almost invariably no more than brief mention is made.

West, (1904) and Heering,(1914) record Chaetophora pisciformis, Delf,(1915) mentions Draparnaldia and Stratton,(1946) an alga probably a species of Gongrosira on the shells of aquatic snails; while Boycott,(1926) describes a Chlorella-like form embedded in the soft parts of specimens of Limnaea peregra used for breeding experiments.

Brief references are made to the feeding habits of these animals, usually that they devour bacterial slime, epiphytic algae and any other organic matter adhering to stones, water-plants and similar substrata. Delf,(1915) however states that Oedogonium, Ulothrix and various other green algae are readily eaten although Spirogyra is left alone.

There are sixty two species of freshwater mollusc in Britain of which twenty six are lamellibranchs and thirty six are gastropods. Most of the bivalves spend their lives partly embedded in the material at the

bottom of the water although certain species may be found on plants, while snails spend most of their lives nearer the top of the water usually on rocks, or weed, but sometimes floating freely on the surface film.

They vary in size from Limnaea stagnalis to Planorbis crista among the pulmonates; from Viviparus viviparus to Bithynia leachii among the operculates; and from Margaretifera margaretifera to the small species of Pisidium among the lamellibranchs.

The speed of movement likewise shows considerable variation. The large bivalves do not move very fast although the smaller ones may be quite active. However, the pulmonates may travel at anything up to about six centimetres a minute and some of these, for example L. stagnalis, may show peculiar jerky motions during which the visceral hump is moved through a considerable angle.

The shell which in Limnaea peregra tends to be thin and smooth is thick and coarsely ridged in Limnaea palustris.

Some of the larger lamellibranchs live up to about fifteen years, a few gastropods exist for four or five but in general snails and small bivalves are annuals - the eggs being laid or the young produced during the summer after which the adults gradually die off leaving only the offspring to overwinter.

With these differences in view it was decided to make a survey of the algae living on the shells of various freshwater molluscs; to see if certain algae were associated with certain species of snail, and if the habits and speed of movement were determining factors. The size and age of the shell, its surface characters and shape were considered as possible factors affecting the algae growing on them.

Specimens were collected of a number of species from a wide range of localities. It was found necessary to remove the bodies of the animals and culture the shell alone and for this reason since the complete removal of the body without seriously damaging the shell in some of the smaller forms is well nigh impossible thus causing the results to be somewhat unreliable, attention was concentrated on some of the larger species of mollusc.

Material Used.

Seventeen species of freshwater Mollusca were investigated. These included four prosobranchs:-

Bithynia tentaculata Linn.

Viviparus viviparus Linn.

Valvata piscinalis Müll.

Hydrobia jenkinsi Smith.

ten basommatophoran pulmonates:-

Limnaea stagnalis Linn.

Limnaea peregra Müll.

Limnaea palustris Müll.

Planorbis corneus Linn.

Planorbis spirorbis Linn.

Planorbis albus Müll.

Planorbis carinatus Müll.

Planorbis complanatus Linn.

Physa fontinalis Linn.

Ancylus lacustris Linn.

and one stylommatophoran pulmonate:-

Succinea putris Linné.

Two species of large lamellibranch were also studied:-

Margaretifera margaretifera Linnaeus

Anodonta cygnea Linnaeus

also several small bivalves belonging to the genera Sphaerium and Pisidium. At the beginning of the work the similarities of these two genera and their species, and in particular their variability under different conditions was not realised, and it has since been thought best to consider all the specimens together as "small bivalves"- their habitats overlap considerably and their shells appear to form a very similar substratum for algae.

Shells of dead specimens of five of these species were also investigated.

The places from which these Mollusca were collected include:-

A number of small ponds in the grounds of Royal Holloway College, Englefield Green, Surrey.

The Village Green Pond, Englefield Green, Surrey, a small pond used by cattle.

Langham Pond, near Egham, Surrey, a deep pond with very rich flora and fauna.

The Lower Pond, Stanmore, Middlesex.

Fishponds at Hounslow, Middx., North London, Reading, and Orpington, Kent.

The River Stour near Wye, Kent - one region where wide and deep and another where shallow and narrow.

A small stream running down to the beach at Barmouth, Merion.

A concrete-sided, small reservoir at Port Erin, Isle of Man.

Malham Tarn, Yorks.

Various lakes, tarns and ponds in the Lake District.

Altogether two hundred and thirty nine live specimens and thirty one shells were studied beside a considerable number used for preliminary investigations.

The gastropods and small bivalves were collected using a fine mesh gravy strainer attached to an extensible pole, or a large bolting silk fishing net. They were brought back to the laboratory in jars with weed, and inspected under the binocular microscope straight away.

Sometimes a shell appeared green under natural conditions, occasionally algae could be seen with the unaided eye growing on the shell - either as mucilaginous masses of Chaetophora, as unbranched filaments of Oedogonium or Ulothrix for example, or as branched filaments of Cladophora.

Under the binocular microscope flat patches

of green could be seen scattered over the surface, which on further inspection were clearly one cell thick and showed a branched, filamentous structure to a greater or lesser extent - they were the prostrate systems of members of the Chaetophorales. Sometimes a few short, erect filaments, usually unbranched, protruded from these prostrate growths but only rarely was this erect system extensive.

The shells almost invariably showed ridges along the lines of growth and sometimes running along the coils as well, this sculpturing was most prominent in thick shelled forms such as Limnaea palustris, and was not very evident in the thin shelled Limnaea peregra.

The prostrate systems occurred more or less scattered over the shell surface, they were however quite often, though by no means always, more frequent in the sutures between the whorls, and sometimes, particularly in Planorbis corneus there were none or very fewnear the mouth - this being the youngest part of the shell, and it suggested that the motile spores could not settle and grow so easily on the newly formed surface; further inspection of very young specimens of other species e.g. Limnaea peregra showed that this was not true of all snails.

Quite frequently when the prostrate system showed a clearly filamentous origin it was elongated

in the direction of the ridges.

Occasionally on the edge of the shell's aperture specimens of Stigeoclonium sps. were found with fully formed erect systems, similarly at the apex of the spire of a form such as Limnaea stagnalis.

Coleochaete occurred on any part of the shell.

When Chaetophora was present as gelatinous masses it tended to be more at the apex of the spire, and in the sutures; it has only been recorded in large masses from Limnaea peregra although it must have been present as the prostrate system alone on far more species since it developed when the shell was cultured.

Cladophora was not very frequent on the shells investigated though it has been very common in one pond in particular; when present it existed in the typical form.

Oedogonium was common, and was sometimes so dense and long (up to three centimetres) as to obscure the shape of the shell. More often however its filaments were not more than a few millimetres in length and were far more scattered.

Mougeotia, Spirogyra and one or two other forms with unbranched filaments occurred occasionally, but rarely in any quantity, in fact, they were frequently overlooked in preliminary investigations. Members of

the Ulotrichales existed as very short filaments and could not be identified until later.

Such filamentous algae showed no zonation, occurring scattered all over the shell surface except that they were often not quite so abundant near the mouth of the shell. Sometimes a Chaetophoraceous prostrate system was found growing over an Oedogonium holdfast.

When filamentous forms were well developed erect systems of the heterotrichous algae growing among them were often quite well developed; under such conditions Chaetophora was occasionally evident as cushions of well-branched filaments not embedded in mucilage.

The appearance of the lamellibranch shells was very similar to that of the gastropods, but usually more algae were present towards the umbo and hinge area. Anodonta and Margaretifera, the two large forms studied, move very little and spend most of their time with the lower part of the anterior region of the shell buried in the substratum and as may be expected this had less algal growth than the emergent part.

The smaller bivalves - Sphaerium and Pisidium are more active and although they may be partly submerged in the same way as Anodonta, or even completely buried, they may also rest on the surface of the substratum

under calm conditions. Some species may even climb up the stems of water plants. Their algal cover was fairly evenly distributed but tended to be altogether less than on the larger forms.

Other algae - diatoms, bluegreens and Xanthophyceae, as well as water moulds and Protozoa were frequently encountered, but it was decided to limit these observations to members of the Chlorophyceae.

Almost all gastropods are herbivorous. Those investigated live on the algae and other microscopic organisms epiphytic on water plants, stones, tree-trunks and similar substrata in their habitat. However, another source of supply is found on the shells of the snails and one quite often observes in nature and frequently if specimens are kept in an aquarium that they do in fact eat the epiphytes from each other. A large snail may be found to have two or three smaller ones feasting upon the material on its shell, but it is not necessarily the smaller ones that feed upon the larger specimens.

A snail obtains its food by rasping off any material in its path with its radula. In the laboratory this process can readily be followed under a binocular microscope and it is evident that although the erect

systems of Chaetophoraceous forms may be completely devoured only rarely is the prostrate growth totally removed. Since almost all shells are ridged and these prostrate systems often develop along these ridges even if part is removed by the scraping of the radula it is not likely that those parts in the hollows will also be completely rasped away since the radula cannot be protruded and can only remove that with which it actually comes into contact. These algae have remarkable powers of regeneration from vestiges of the prostrate systems and this, coupled with the easy removal of the erect systems explains the appearance of the shell on collection. Similarly, plants growing in the sutures between the whorls tend to be protected. An invading snail would find it more difficult to devour plants growing on the apex of the spire or around the edge of the shell's mouth, and this accounts for the presence of fully developed algae in these regions.

Filamentous algae in the path of a snail's radula are frequently likewise eaten, their holdfasts often remaining on the shell, or the whole plant may only be detached and left floating.

A snail does not seem to be able to penetrate a thick turf of Oedogonium and it will be remembered that quite frequently plants of Stigeoclonium and Chaetophora with well developed erect systems are found

growing under such conditions; clearly, the Oedogonium gives protection to these algae which can develop fully only when unattacked by other snails.

That the population tends to be thicker in the sutures may have two explanations. The algae reach the shell either as zoospores or as zygotes and in either case the holdfast first produced must be of a very flimsy nature. The movement of the snail, particularly the jerky, twisting motions shown by some species must tend to wash away anything not firmly attached. In a suture a certain amount of shelter would be obtained and here a delicate sporeling may be able to develop more easily than one on the surface of the whorls. Another factor is the rasping action of other snails - as has already been mentioned the radula would be unable to remove algae living in the sutures simply because it could not reach them, therefore plants in the sutures would be more protected than those on the flatter surface and would tend to grow more freely.

Methods.

Since so many of the algae existed on the shell in a far from normal condition it was necessary to culture them in order to identify them.

Attempts were made to keep the snails separate and culture the algae at the same time. Large, sterile boiling tubes plugged loosely with cotton wool were more satisfactory than dishes as the snails tended to wander so readily and the lids could not be sealed because a continuous supply of air was necessary.

It was found that a snail could live for several days without food and did not seem to mind being shut in a tube, and after four days or more according to the algae present and the time of year sporelings developed on the sides of the tubes thus forming a constant source of food.

However, it takes at least a fortnight for these particular species of algae to grow to their maximum size and the populationon a shell was so thick that after this time it was utterly impossible to separate the plants, and those entirely or mainly prostrate in form were certainly not visible.

The snail's faeces caused pollution of the liquid which needed frequent changing; even so, bacteria, Chlorella and other unicells multiplied at a great rate.

Coverslips introduced into the tube on which the sporelings could develop were cleaned by the snails in feeding.

Thus, this method proved unsatisfactory.

Live snails could not easily be kept in dishes and so it was decided to kill and remove the body and culture the shell alone. It was therefore necessary to find some method of killing the animal without harming the algae. Thus the whole snail could not be immersed in an anaesthetising solution or everything would have been killed, and if the shell was to be kept out of the water during the process of killing the animal it was important that death should occur quickly or the algae might die due to exposure.

Any solution capable of killing a snail may quite possibly harm or even kill algae.

To avoid contamination of the plants by these fluids the snail was propped against the edge of a petri-dish with the mouth uppermost, a sharp prod with a seaker in the head region caused the whole body to contract leaving a cavity above the head in the mouth of the shell. In this way a liquid could be dropped onto the animal without flowing over the algae.

Various solutions were tried, including chloral hydrate, chloroform, menthol, phenol, ether and

alcohol in various concentrations and it was finally decided that the most satisfactory one was neat chloroform. A small glass pipette containing a few drops of the liquid was brought near the head of the snail, the vapour causing it to contract well within the shell; one to three drops on the body according to the size of the species caused complete anaesthetisation, if not death, within a minute or two. An added advantage of this method is that since chloroform is only sparingly miscible with water it is unlikely that very much would spread over the algae in the surface film of moisture and as it is so volatile it is unlikely that much would still be in the liquid state after the few minutes during which it was left to take effect.

The foot was then grasped with forceps and the body screwed out. In most species of Limnaea it was thus possible to remove the whole body in one operation, but the tighter coils of the Planorbis shells offered some resistance and then they were broken to remove all traces of the body. After thorough washing in sterile distilled water each shell was put separately into a dish and cultured.

Various solutions were used in an attempt to find one which would give good growth, where reproduction could be readily and plentifully induced, and where none of the algae on the snail shell would be eliminated.

The following solutions were tried:-

Pearsall and Bengry 1940.

Godward 1942.

Beijerinck
Pringsheim
Knop's modified
Molisch
} Pringsheim 1946.

Reynolds 143 (Private communication):-

KNO_3	0.00008	gm.mols.
KH_2PO_4	0.00001	"
$CaCl_2$	0.00005	"
$MgSO_4$	0.0001	"
NaCl	0.00001	"
$FeCl_3$	0.0000002	"

Distilled water to 1 litre.

Soil Solution (For method of preparation see p 19)

Pond water, sterilised and in various concentrations.

Single, well-grown plants of Stigeoclonium 100 were put into 100ml. of each of these solutions in Pyrex crystallising dishes. The experiment was carried out in

triplicate and again with Stigeoclonium 300.

After a fortnight the original plant, the number and amount of growth of any sporelings produced were observed. Soil Solution gave markedly better results than any of the others.

Further, similar experiments were carried out using Knop's modified, Beijerinck and Reynold's 143 solutions in strengths of one tenth, one fifth, one half, times one, two, five and ten normal but again the results with Soil Solution were much better - the parent plants having grown much more and appearing more healthy than those in mineral solutions, and the sporelings were far more numerous and showed better growth.

Thus it was decided to concentrate on Soil Solution as it did not seem to inhibit the development of any of the algae encountered.

Similar experiments using Soil Solution in concentrations of one tenth, one fifth, one half, times one, two, five and ten normal were carried out and it was found that the range from one half to times two of the strength used gave the best growth and maximum number of sporelings, and that within that range there was very little difference. It was found that by renewing these solutions once a fortnight excellent growth was maintained, and that even after a month most algae were still alive although not actively growing.

Potassium nitrate is often added to soil solutions and an experiment was carried out using Stigeoclonium 100, again in triplicate, to see the amount needed. One, 0.4, 0.2, 0.1, 0.05 and 0.025 gms. per litre were used. Results showed that the 0.1 to 0.2 gms. per litre gave the best growth but the precise amount was obviously not of vital importance.

Various types of soil were tried, from a cultivated shrubbery, a field growing cabbages, an orchard, below turf, and leafmould. Apart from the leafmould which appeared to give a stronger solution there was very little difference. Hence most of the soil used in these investigations came from the cabbage field.

It seemed possible that the addition of yeast extract might improve the growth of these algae still further. This time Uronema gigas and Oedogonium 2 were used, each in duplicate. A 2% solution of "Difco" yeast extract was prepared and 1, 2, 5, 10 and 15 drops added to 100ml. of Soil Solution. Results showed that it was clearly not essential and even inhibited growth and so it was not used in futher work. Another advantage was that solutions containing it became more readily infected with bacteria than those without.

Similar experiments were carried out adding glucose to see if a carbohydrate was necessary, but that also was found to increase contamination without enhancing growth.

Thus, Soil Solution with potassium nitrate was used for all investigations.

There are various methods of preparing extracts from soil. The one found most suitable and which was used throughout this work was as follows:-

Approximately 1000gms. of soil were autoclaved at twenty pounds pressure per square inch for twenty minutes with about 1500ml. of distilled water. After cooling it was allowed to stand for several days and then filtered through a single and then double filter papers. The resulting liquid was made up to 1000ml.- this being known as the extract. One hundred millilitres of this were used with 900ml. of distilled water and about 0.1gms. of potassium nitrate to make up a litre of solution; this was then autoclaved as above before being used.

Since the precise strength had been shown to be unimportant no effort was made to weigh the soil accurately, if it was particularly wet slightly more than normal was used and vice versa if it was very dry.

It was realised that to identify the algae living epiphytically on a snail shell it would be not only necessary to produce well-grown, mature specimens but also to be able to subculture them readily.

It was thought that an ideal method would be

to culture the shell in a petri-dish containing a thin film of 2% agar covered with Soil Solution, the idea being that sporelings growing on this agar could be easily separated from one another and transferred to other vessels for further growth. However, when shells, or parts of shells were used as inmocula the bacteria present multiplied profusely on the agar thus ruining the results in spite of very careful washing of the shell before immersion.

In an effort to combat the bacterial contamination Rose Bengal was added to the agar, or to both the agar and Soil Solution in various strengths, but the concentration necessary to kill bacteria also proved lethal to tha algae.

Similar experiments using penicillin and streptomycin in various concentrations were tried, but again the strengths needed killed the algae. Attempts were made at immersing the shells in solutions of streptomycin of various strengths for varying periods of time before putting them into culture medium but this too gave unsatisfactory results.

It was found that the best method was to put the washed shell in 100ml. of Soil Solution in a covered, glass crystallising dish. All glassware was autoclaved at twenty pounds pressure per square inch for twenty minutes before use.

Both Pyrex and soft glass vessels were tried and the results seemed to be identical - evidently the algae encountered suffered no ill-effects from the soft glass; however, Pyrex dishes were used almost entirely for the rest of the investigation.

To enable the sporelings to be readily separated and subcultured a coverslip - flamed for sterilisation - was put into each dish when the experiment was begun; in this way unialgal cultures from single plants could be obtained by breaking up the coverslip and transferring the necessary parts to fresh dishes of solution. Unwanted species were removed by scraping the coverslips with small pieces of razor-blade inserted in a glass rod, and finally with small sewing needles likewise in glass tubing, both of which were flamed for sterilisation.

The most satisfactory position for the cultures was in a north-facing window in one of the laboratories.

During the winter the light intensity near the window was sometimes as low as twenty foot-candles, while in the summer it reached seven hundred. In an attempt to keep the illumination as constant as possible the window was covered with green distemper from April until September thus cutting down the light intensity.

The dishes were kept, never more than two deep on glass shelves several inches in from the window, and on glass plates, backed with white paper on the bench.

Air temperatures in the laboratory were only recorded from January 1952 onwards - this investigation was started the previous October; but from the records made the night temperature was never below 4.4°C, and very rarely under 7.5°C. During the November to April part of the year the minimum temperatures were between 4.4°C and 15°C, but during the other six months they were almost invariably over 10°C. Maxima over 30°C were recorded on several occasions. These summer temperatures were far from ideal as the culture liquids frequently reached 18°C and higher, and some algae were killed during this time.

Throughout these investigations the Soil Solution was changed every two weeks. The stock cultures were thoroughly washed with sterile distilled water about every six weeks when their liquids were being changed as well; from time to time they were subcultured by their coverslips to new dishes or some of the plants were removed by wiping them away with pads of sterile cotton wool followed by several washes in sterile distilled water before being refilled with Soil Solution. Filamentous forms were subcultured by transferring a single or a small group of filaments to a new dish of solution by a sewing needle held in glass tubing - this

being more readily visible under water and less fragile than glass needles, and much finer than ones of the normal dissecting type.

The Algae.

It is difficult to state just how many species of algae were found. To identify those of Oedogonium, Mougeotia and Spirogyra it is necessary to know the complete cycle of sexual reproduction. Quite a number of the species of Oedogonium began to develop oogonia but only three ever reached maturity and of these the complete process for both male and female was known for only one. A number of experiments were performed to try to obtain the necessary sex organs but these, in general, failed. Similarly with Mougeotia and Spirogyra which seemed even less inclined to do anything but grow vegetatively. For these reasons the various forms, separated by their cell size, chloroplast shape and number, pyrenoid content and appearance, most of which were probably distinct species, were known by numbers.

The characters separating the species and even the genera in the Ulotrichales are not very distinct and here too forms which did not obviously conform to any one description were considered as Ulotrichales 1, 2, 3, and so on.

Reynolds (1950), has shown that the form of Stigeoclonium varies considerably under different conditions and these investigations certainly showed this to be true. Consequently, it has been found

almost impossible to name species of this genus with any certainty and so again the forms encountered were known by numbers although it is thought that almost certainly some are duplicated. For the same reason Chaetophora and Draparnaldia were left unnamed.

The following list indicates the range of green algae found living epiphytically on freshwater molluscan shells.

Ulotrichales

Ulotrichaceae

Uronema confervicolum Lagerheim.

Uronema gigas Vischer.

Hormidium flaccidum forma aquatica Heering.

Ulothrix variabilis Kützing.

Ulothrix subtilissima Rabenhorst.

Ten unidentified forms listed on the next page.

Microsporaceae

Microspora stagnorum (Kützing) Lagerheim.

Cladophorales

Cladophora sp.

Rhizoclonium hieroglyphicum Kützing.

The ten unidentified forms belonging to the Ulotrichaceae:-

(All measurements to the nearest $\frac{1}{2}\mu$).

No.	Cell Breadth	Cell Length	Pyre-noid	Comments
1	6.0	12.0-14.5	none	Dark green
3	4.5-5.0	5.5-7.5	none	Bright green
5	6.5	8.0-12.0	none	Mid green
6	4.5	8.0-14.5	none	Bright green with mucilaginous sheath
8	4.5-5.0	20.0-24.0	1	Clear mid green
12	5.5	14.5-25.5	-	Pale green
13	Cells 8.0-10.0 +muc. 10.5-13.5	3.5-8.0	none	Some long. division mucilaginous sheath
15	7.5	4.5-12.0	-	-
16	Cells 9.0-10.0 +muc. 11.0-12.0	2.0-4.5	1	Some long. division thick muc. sheath
18	1.5-3.0	4.0	none	Cells often in pairs mucilaginous sheath

Oedogoniales

Oedogonium alternans Wittrock et Lundell sec. Hirn

The following thirty eight unidentified forms:-

(All measurements to the nearest $\frac{1}{2}\mu$).

R = Tip of filament round.

El= Tip of filament hairlike.

E = Basal cell elongated.

H = Basal cell hemispherical.

S = Basal cell almost spherical.

BC= Basal cell.

No.	Cell Breadth	Cell Length	Fil. tip	BC.	Pyrenoid	Comments
2	14.5-17.5	20.5-44.0	R	E	1-9	Pale green
3	13.0-19.0	23.5-44.0	-	-	1(2)	-
5	13.0-14.5	20.5-32.5	R	E	1(2)	Pale green
6	14.5-17.5	32.5-50.0	-	-	1	-
10	10.5-13.0	17.5-47.0	R	E	1 to sev.	Dark green
11	16.0-19.0	79.5-120.5	R	E	1 to few	Clear light green
12	20.5-26.5	32.5-50.0	-	-	-	-
13	29.5-38.0	79.5-100.0	-	-	-	-
15	22.0-23.5	61.5-82.5	R	E	4-8	Reticulum clear
22	42.5-44.0	216.0-285.0	-	-	-	-
26	14.5-19.0	20.5-59.0	R	E	1?	-
30	7.5-9.0	20.5-26.5	-	-	-	-

Unidentified Oedogonia continued.

No.	Cell Breadth	Cell Length	Fil. tip	BC.	Pyrenoid	Comments
31	9.0-14.5	23.5-35.5	El	E	-	Often sudden variations in filament width
32	17.5-20.5	50.0-100.0	R	E	4-6	-
33	10.5-13.0	23.5-50.0	R	E	1	Faintly capitellate
38	7.5-12.0	23.5-44.0	El	E	2-4	-
40	9.0	20.5-41.0	-	-	-	-
41	14.5-17.5	29.5-70.5	R	-	3-5 large	-
42	6.0-7.5	20.5-29.5	El	H	1	Faintly capitellate
44	10.5-13.0	26.5-61.5	R	-	-	Fils. twisted macroscopically
45	7.5-10.5	20.5-29.5	R	-	1	-
47	4.5-6.0	12.0-20.5	R	H	1 large	-
49	12.0	29.5-32.5	-	-	-	-
53	14.5-16.0	26.5-38.0	-	-	-	-
55	20.5-25.0	76.5-88.0	-	-	-	-
56	6.0-9.0	14.5-20.5	R	H	1 large	-
59	14.5-17.5	38.0-64.5	-	E	1-4	Fil. tip has cap with short hair
61	6.0-9.0	17.5-32.5	R	E	1 large	-
62	3.0-4.5	14.5-23.5	R	S	-	-
64	13.0-14.7	20.5-41.0	R	E	Several?	-
65	17.5-25.0	47.0-84.5	R	E	6-12	Close reticulum
70	17.5-22.0	50.0-73.5	R	E	4	Often sudden variations in filament width
71	6.0	9.0-14.5	-	-	1 large	-

Unidentified Oedogonia continued.

No.	Cell Breadth	Cell Length	Fil. tip	BC.	Pyrenoid	Comments
72	17.5	61.0-82.5	-	-	2-4 large	-
73	30.5-39.0	56.5-87.0	-	-	about 12 small	-
74	7.5-9.0	14.5-20.5	-	-	-	-
75	12.0	23.5-41.0	R	E	1	-
76	10.5-12.0	26.5-38.0	R	E	3-4 large	-

Chaetophorales

Coleochaetaceae

Coleochaete scutata Brébisson.

Chaetophoraceae

Microthamnion kützingianum Nägeli.

Microthamnion strictissimum Rabenhorst.

Draparnaldia sp.

Chaetophora sps.

Chaetopeltis orbicularis. Berthold.

"D" - like Stigeoclonium but with enlarged cells

Stigeoclonium farctum Berthold.

Stigeoclonium tenue Kützing (Sammerlart).

A large number of other Stigeoclonia:-

The forms of Stigeoclonium varied from those having a comparatively small prostrate system of a few, short moderately branched filaments with a small number, frequently about five, long erect filaments growing from them, and bearing primary and secondary branches which likewise grew to a considerable length; to others having a dense prostrate system which in older specimens only showed evidence of its filamentous origin round the edge, bearing short erect filaments which branched in mature specimens but were never extensive. In between were forms exhibiting all variations - some with very little prostrate had a few long, erect filaments bearing

short, primary branches, other similar ones showed secondary branching as well. Some with erect systems almost identical with these had a much more extensive prostrate system often of about five main filaments bearing large numbers of side branches giving a somewhat feathery appearance. Further development of this prostrate where the side branches were more extensive gave rise to a more dense system which again had well developed erect filaments. Others had very dense prostrate systems but also had very numerous erect filaments which were unbranched at first giving a somewhat cushion-like appearance.

So many varieties were encountered that it was found impossible to be certain which should and which should not be considered together as it was felt that either there were a large number of species having only slight morphological differences or there were fewer species each showing an unusually wide variation.

It was found after a time that two forms were fairly constant and agreed well with the descriptions for S. farctum and S. tenue, a few others were usually quite distinct but for the remainder it was quite impossible in the time available to be absolutely certain when a new form was encountered and so it was decided that for comparative purposes it would be best to consider them in groups separated as clearly as possible.

This obviously brings in considerable error as clearly different forms will not be exactly similar physiologically, but under the circumstances, and indeed to make any comparisons possible there was no alternative.

Conjugales.

Euconjugatae.

Spirogyra.

The following four unidentified forms:-

(All measurements to the nearest $\frac{1}{2}\mu$).

No.	Cell Breadth	Cell Length	Chloroplasts
1	67.5	117.5-235.0	8-9
2	23.5-26.5	56.0-82.5	1
3	13.0	70.5-138.0	1
4	13.0	117.5-279.5	1

Mougeotia.

Twenty one unidentified forms - see next page.

Unidentified species of Mougeotia.

(all measurements to the nearest $\frac{1}{2}\mu$).

No.	Cell Breadth	Cell Length
1	14.0	65.0-102.5
2	17.5	139.0-187.0
3	9.0-13.0	190.5-265.0
4	19.0	103.0
5	6.0	73.5
9	20.5	117.5-138.0
10	9.0	47.0-103.0
11	9.0	117.5-147.0
12	10.5	117.5
13	9.0	73.5-176.5
14	6.0	47.0-56.0
15	9.0	132.5
16	4.5-6.0	59.0-73.5
17	7.5-9.0	88.0-147.0
18	20.5-22.0	132.5-176.5
19	9.0	73.5-97.0
20	6.0	44.0-47.0
21	9.0	53.0-109.0
22	9.0-10.5	82.5-88.0
23	26.5	76.5-120.5
24	41.0	82.5-123.5

Also an alga agreeing well with Butcher's (1932b) original description of Sphaerobotrys fluviatilis except that under the cultural conditions used the cells of the middle part of the plant were 8.8-11.8μ in diameter while those towards the outside were sometimes narrower - to 4.4μ, and longer - to 13.2μ compared with Butcher's figures of 3-4μ broad by 3-5μ long.

The development of the whole plant, its overall size, and its cell structure agree very closely with the figures given for Sphaerobotrys fluviatilis and it will be referred to by this name throughout the following text.

A comparison of the algae on the shells of various species of mollusc all collected at the same time from a single pond -

Langham Pond, Egham, Surrey.

Specimens of eleven species of mollusc were collected from this pond on a single evening. On return to the laboratory they were left overnight in large dishes of water with quantities of weed and debris taken with them. The following day the shells of thirty three were prepared as previously described and the dishes, whose liquids were changed once, were left for twenty days.

The results were then scored.

Twenty three algal species altogether were found, including four of Oedogonium, one Mougeotia, one Spirogyra, one Rhizoclonium, one Microspora, one Chaetopeltis, one Chaetophora, one Cladophora, one Draparnaldia, Sphaerobotrys fluviatilis, nine forms of Stigeoclonium possibly all separate species, and one other type rather like Stigeoclonium but with structures appearing like sporangia and known here as "D".

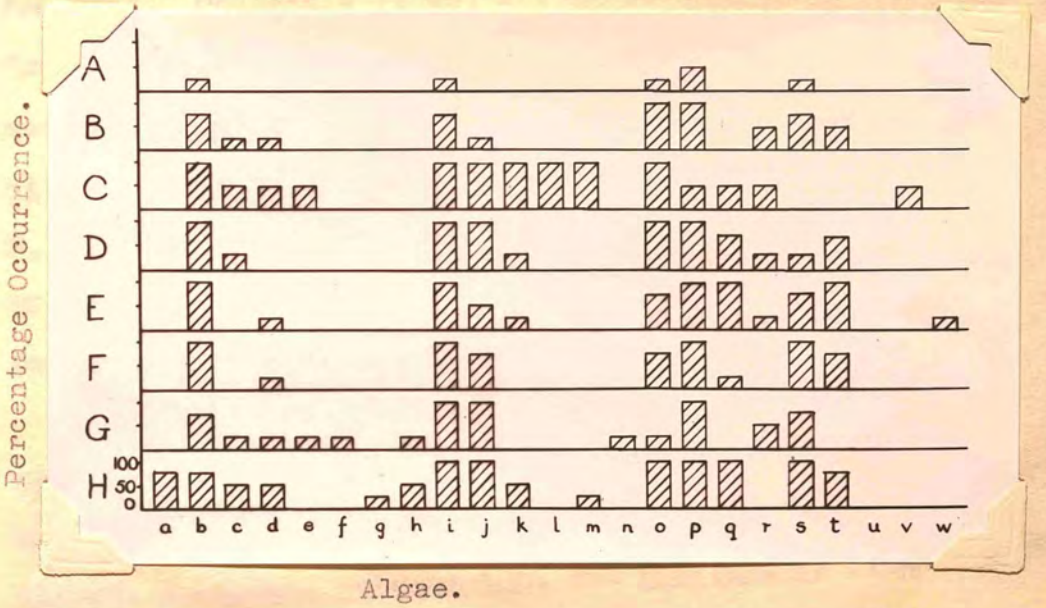
The occurrence of each on the various shells may be seen in the following table - x marking presence. Where both young and adult specimens of the same species of snail were taken the results were separated as shown.

Table showing the algae present on various species of mollusc from Langham Pond, Egham, Surrey.

		Oedogonium 70	71	72	73	Mougeotia sp.	Spirogyra sp.	Rhizoclonium h.	Microspora st.	Sphaerobotrys f.	Chaetopeltis o.	"D"	Draparnaldia sp.	Chaetophora sp.	Cladophora sp.	Stigeoclonium A	" tenue	B	" farctum	C	D	E	F	G
Limnaea stagnalis young	1	x		x						x	x	x				x	x	x		x	x			
	2	x	x							x	x	x				x	x	x		x				
	3		x	x	x			x	x	x	x			x		x	x	x		x	x			
	4	x	x		x				x	x	x					x	x	x		x	x			
Limnaea stagnalis old	1		x							x	x					x	x		x	x				
	2		x							x	x						x			x				
	3		x						x	x	x						x			x				
	4			x	x	x	x			x	x				x		x		x					
Limnaea peregra	1		x							x	x						x			x	x			
	2		x							x	x					x	x			x				
	3		x							x	x					x	x	x		x	x			
	4		x		x					x						x	x			x	x			
Planorbis complanatus	1		x							x	x					x	x	x		x	x			
	2		x							x	x	x				x	x	x		x	x			x
	3		x							x						x	x	x		x	x			
	4		x		x					x							x	x	x		x			
Planorbis spirorbis	1		x		x					x	x					x	x	x			x			
	2		x							x	x	x				x	x				x			
	3		x							x	x					x	x	x	x		x			
P.corneus	1		x							x		x		x		x				x				
Viviparus viviparus	1		x	x		x				x	x	x	x	x		x	x	x	x				x	
	2		x		x					x	x	x	x	x						x	x			
Bithynia tentaculata	1			x						x						x	x			x				
	2		x		x											x	x		x		x			
	3		x							x	x					x	x		x	x				
	4		x							x						x	x			x				
Physa fontinalis old	1																	x						
	2																x							
	3									x														
P.f.young	1		x							x								x	x	x	x			
Ancylus lac	1		x							x	x							x						
Sm.bivalve	1									x						x	x	x			x	x		
Succinea sh	1		x																					

The results indicated in terms of percentage occurrence may also be seen from the following histograms, only those from species of snail where more than two were investigated are shown - as the table shows the usual number considered was four.

Histograms showing the percentage occurrence of different algae on various snails all from Langham Pond, Surrey.



A Physa fontinalis
B Bithynia tentaculata
C Viviparus viviparus
D Planorbis spirorbis
E Planorbis complanatus
F Limnaea peregra
G Limnaea stagnalis, old
H Limnaea stagnalis, Young.

a Oedogonium 70
b Oedogonium 71
c Oedogonium 72
d Oedogonium 73
e Mougeotia sp.
f Spirogyra sp.
g Rhizoclonium hieroglyphicum
h Microspora stagnorum
i Sphaerobotrys fluviatilis
j Chaetopeltis orbicularis
k "D"
l Draparnaldia sp.
m Chaetophora sp.
n Cladophora sp.
o Stigeoclonium A
p Stigeoclonium tenue
q Stigeoclonium B
r Stigeoclonium farctum
s Stigeoclonium C
t Stigeoclonium D
u Stigeoclonium E
v Stigeoclonium F
w Stigeoclonium G

It is quite clear that the algae abundant on one snail species tended to be similarly abundant on others in the same pond. Thus Oedogonium 71 occurred on seventy five or more per cent of seven out of the eight groups of snails shown on the histograms, similarly with Sphaerobotrys, Chaetopeltis, Stigeoclonium tenue, A, B, and C which were likewise consistently frequent. Oedogonia 72 and 73, Mougeotia, Spirogyra, "D", Chaetophora, Stigeoclonium farctum and D were more variable, while Oedogonium 70, Rhizoclonium, Microspora, Draparnaldia, Cladophora, and Stigeoclonium F, and G occurred on only one species and in the case of Draparnaldia on 100% of those investigated of that particular species.

Material bearing epiphytes was also taken from the same pond and cultured - including pieces of bark from a submerged tree, stem and leaves of Hippuris, Alisma, Nymphaea, Potamogeton, and Hydrocharis from different levels. It was necessary to culture them so that the algae had the same growth form as those cultured on the shells and could therefore be compared.

It was found that nineteen of the twenty three types of algae present on the shells were growing in the pond on other substrata - only one species of Draparnaldia and three forms of Stigeoclonium which occurred most rarely on the shells were absent.

However, another species of Oedogonium, one of Zygnema, and one of Bulbochaete were present in the pond although not on the snails. It was not surprising that Zygnema did not occur on the shells as although it is epiphytic in the early stages its attaching organ is soon lost. Neither the Oedogonium nor the Bulbochaete occurred in great quantity and it is possible that their absence from the snails was due to the smallness of the numbers taken.

These results also indicated that there was very little difference between the algal populations on young and adult specimens of Limnaea stagnalis, the immature ones tending to have a larger number of species but this problem of age is dealt with more fully later (See p 72).

Small snails such as Planorbis complanatus and P. spirorbis had just as many species of algal epiphyte as large specimens of L. stagnalis and the types found were very similar.

It was thought that the rate at which an animal moved might have an effect on the epiphytes on its shell, because not only would the algae receive more aeration on the fast moving types than on those that travel more slowly, but their waste products - and those of the mollusc, would be removed more rapidly. Thus one might expect to find algae capable of living

under moderately poor conditions on both fast and slow moving types while less tolerant species may be found only on the fast moving animals.

Only one specimen of P. corneus was found and this bore eight species of algae. The average of a number of measurements taken indicates that P. corneus moves at about 8.6 cms. per minute yet each of these species was found on the shells of much slower moving snails - all were found for example on L. stagnalis which moves at about 5.5 cms. per minute.

The same is true of P. complanatus moving at 2.3 cms. per minute and P. spirorbis at 2.6 cms. per minute, except that one species is present on one specimen of P. complanatus which was found nowhere else.

Thus it seems that the rate of movement of a snail is not of vital importance in determining the species of algae epiphytic on its shell, but for further discussion see p76.

Added evidence is given by an empty shell of S. putris found in the pond together with the live snails. When cultured seven species of algae were obtained from it. (The average number from the live specimens was also seven). Of these algae one species of Stigeoclonium was found on this shell alone and not on any others, but the remaining six were quite common on the live animals. Since the Succinea shell was obviously

stationary and the live snails were moving this supports the view that the speed of movement of snails has very little effect on the epiphytes on their shells.

P. corneus and Bithynia tentaculata live two to three years and Viviparus viviparus also lives for several, the other species die after about fifteen months; yet, except for Viviparus the algae present are remarkably similar. The population tends to be more constant in Viviparus seven of the species of algae found being present on both specimens investigated.

It can be seen from both table and histogram that the algal population on Physa fontinalis tends to be very small - one specimen having three different species, another two each had only one, the young specimen likewise had a single species. This result is supported by other specimens too - of six from Loughrigg Tarn in The Lake District four had no epiphytes at all and the remaining two had only one species each, another from Wharton Tarn also in The Lake District, had no algae on it at all.

These results are explained by the fact that this is a species of snail having a very thin and smooth shell which, when the animal is expanded, is almost completely covered by the mantle which spreads in blunt finger-like processes almost to the apex of

the shell. This mantle is continuously gliding over the shell surface as the snail moves and very few motile spores would be able to lodge and develop on the shell.

Thus it seems that the algal population on a freshwater snail shell in general bears no relation to the species of snail - that on the shells being an unselected sample of the population in the pond itself.

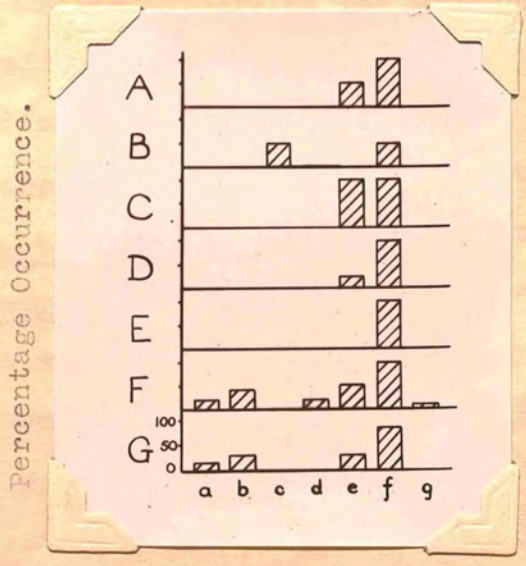
Similar results were obtained from material collected from a stretch of the River Stour near Wye in Kent, although only eight species of mollusc were collected and only seven species of algae were found on them.

See next page for table and histograms.

Table showing the percentage occurrence of the algae found on molluscs from the River Stour, in Kent.

Algae:- / Molluscs:-	Oedogonium A	B	C	"D"	S. farctum	S. tenue	S.A
L. stagnalis	14.3	28.6	-	-	28.6	85.7	-
L. peregra	22.7	40.9	-	22.7	50.0	100	9.1
B. tentaculata	-	-	-	-	25.0	100	-
P. corneus	-	-	-	-	-	100	-
Ancylus lacustris	-	-	-	-	100	100	-
Anodonta cygnea	-	-	-	-	50.0	100	-
Small bivalves	-	-	50.0	-	-	50.0	-

Histograms from the above results.



Key.

A. = Anodonta cygnea
B. = Small bivalves
C. = Ancylus lacustris
D. = B. tentaculata
E. = P. corneus
F. = L. peregra
G. = L. stagnalis

Algae in same order as table.

Succinea putris had no epiphytes. Succinea is not a water snail, it lives on the plants at the edge of the water and thus in a damp habitat but is not a submerged form. The shells were cultured in soil solution in the usual way but nothing appeared. Inspection under the binocular microscope of the freshly collected shells did not reveal any epiphytes, but it seems likely that some were present though they did not develop because of the cultural methods used. (No other live specimens of Succinea were taken but an empty shell was found in Langham Pond, Surrey and that had a wealth of epiphytic material - cf. p40. - and this suggests that it was not the surface or composition of the shell which prevented the algae from growing on the live snails).

Otherwise the most common alga was S. tenue and it is clearly evident that it occurred on all types of mollusc - on the large slow moving L. stagnalis, the large fast moving P. corneus, the smaller fast moving B. tentaculata, the almost stationary freshwater limpet Ancylus lacustris, and both the small and large bivalves. It was no more frequent on the rough-shelled P. corneus than on the smooth-shelled L. peregra, nor was it more frequent on the Anodonta which were probably at least six years old than on the Limnaeas that could only have been one year.

Similarly, S. farctum was on the sedentary Anodonta and Ancylus as well as the more mobile Limnaeas yet it was absent from P. corneus (though present in other habitats), and the smaller bivalves, this latter is probably explained by its inability to withstand silting up - but see page 65 (Note also that S. tenue was only present on 50% of the specimens and experiment shows that S. tenue can withstand silting more readily than S. farctum).

The other species of algae occurred so rarely that it is perhaps better not to attempt to draw conclusions from them.

Unfortunately the molluscan species from the river were mostly different from those in the pond but B. tentaculata, L. stagnalis and L. peregra show comparable results both in Langham Pond and the River Stour.

Thus both in Langham Pond in Surrey and the River Stour in Kent the algae living epiphytically on the shells of the freshwater molluscs seem to bear very little relation to the species of the mollusc; the populations tending to be similar regardless of speed of travel, habitat, shell form and size of the animal.

The Relationship between the Colonising Algae and the Substratum.

This experiment was carried out in an attempt to support the conclusion made from the previous investigation - namely that the epiphytic algal population does not vary with the species of mollusc.

Shells of adult snails collected from Langham Pond, Egham, Surrey and a fishpond at Orpington, Kent were scrubbed with a toothbrush to clean away the algae as far as possible, and then separately suspended by pieces of fine wire in wide-necked conical flasks plugged with cotton wool. In this state they were sterilised by autoclaving them for twenty minutes at twenty pounds pressure. After cooling, three shells of each species of snail were suspended at the same depth in each of two small ponds in the grounds of Royal Holloway College, Egham, Surrey, from pieces of string stretched across the ponds. The species used were P. corneus, P spirorbis, and L. stagnalis - a large fast moving type, a small, closely coiled, fast moving type and a large slowly moving species. At the same time three three-by-one inch glass microscope slides, sterilised in the same way as the shells, were immersed.

The shells and slides were left in the ponds for two weeks, after which they were taken back to the

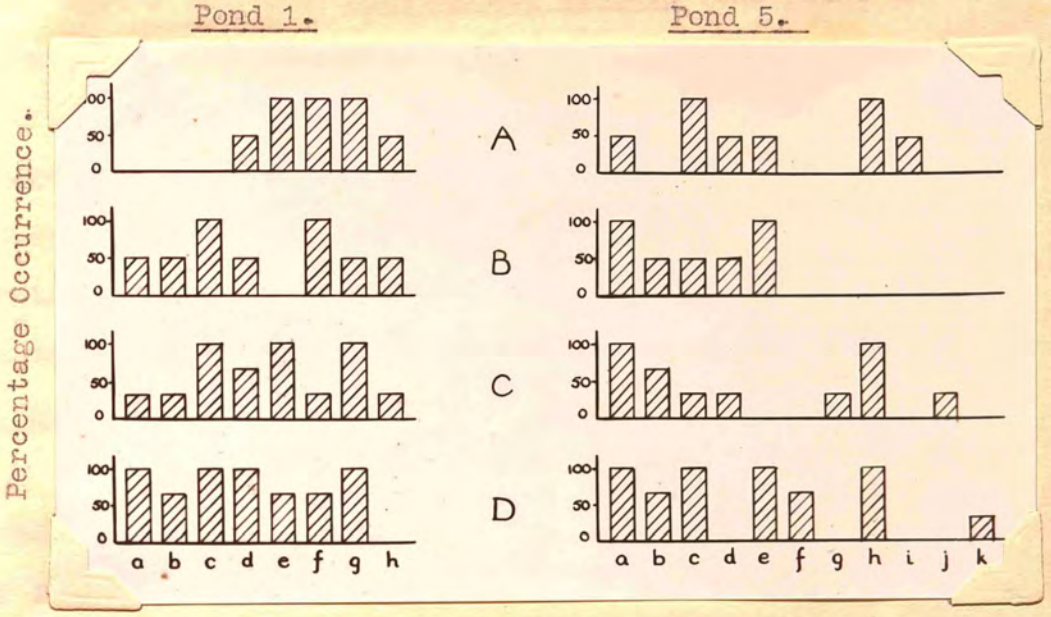
laboratory, carefully rinsed in sterile distilled water to remove any algae not actually attached, taken off the wire suspensions and cultured in soil solution for two weeks. In both cases one of the L. stagnalis and one of the P. spirorbis became broken in the ponds so that results were obtained for only two specimens of each of these species.

The results were as shown on the next page, an "x" marking presence. Histograms follow on the succeeding page.

Table showing the algae which colonised sterilised slides and shells immersed in two small ponds in the grounds of Royal Holloway College.

		Pond 1.									Pond 5.									
		Oedogonium 80	" 81	Spirogyra 20	" 21	Mougeotia 30	S. tenue	Stigeocl. 90	Sphaerobotrys		Oedogonium 80	" 81	Spirogyra 21	" 22	" 23	Mougeotia 30	S. tenue	Stigeocl. 90	" 91	Sphaerobotrys	Coleochaete
Slide	1	x	x	x	x	x	x				x	x	x		x		x				x
	2	x	x	x	x	x	x		x		x	x	x		x	x	x				
	3	x		x	x		x		x		x		x		x	x	x				
Planorbis corneus	1	x	x	x		x	x				x			x			x		x	x	
	2			x	x	x	x				x	x	x				x				
	3			x	x	x	x	x	x		x	x					x				
Limnaea stagnalis	1	x	x	x			x		x		x	x			x						
	2			x	x			x	x		x		x	x	x						
Planorbis spirorbis	1					x	x	x	x		x		x	x	x		x	x			
	2				x	x	x		x				x				x				

Histograms showing the percentage occurrence of algae which colonised sterilised snail shells and slides suspended in the ponds at Royal Holloway College.



A Planorbis spirorbis shells
B Limnaea stagnalis shells
C Planorbis corneus shells
D Slides

Pond 1.

a Oedogonium 80
b Oedogonium 81
c Spirogyra 20
d Spirogyra 21
e Mougeotia 30
f Sphaerobotrys fluviatilis
g Stigeoclonium tenue
h Stigeoclonium 90

Pond 5.

a Oedogonium 80
b Oedogonium 81
c Spirogyra 21
d Spirogyra 22
e Spirogyra 23
f Mougeotia 30
g Sphaerobotrys fluviatilis
h Stigeoclonium tenue
i Stigeoclonium 90
j Stigeoclonium 91
k Coleochaete scutata.

It can be seen that eight species of algae belonging to five genera appeared on the material collected from pond 1, and ten species from the same five genera and also Coleochaete scutata (one plant only) were found from pond 5.

Taking pond 1 first, it can be seen that all eight species occurred on three if not all four of the substrata provided, moreover, those occurring on all the shells of one species investigated tended to be frequent on the others - the S. tenue occurred on all P. corneus, P. spirorbis, and slides and on 50% of the L. stagnalis.

Similarly, those not so common were less frequent on all substrata - thus Oedogonium 81 occurred on 50% of L. stagnalis, 33.3% of the P. corneus, 66.7% of the slides and not at all on P. spirorbis.

Thus it seems that if an alga is common in a pond it will frequently be found on the shells of the freshwater snails living in that pond, and conversely, if it is not very common it will be equally rare on the snail shells regardless of their species.

In pond 5 the same occurred - Oedogonium 80 was common on all species investigated and on the slides whereas four of the algal species occurred on one shell or slide only and another on two slides only.

Thus, again, a common alga tends to be frequent on all species of snail and one more rare occurs much less.

It was noticable in pond 1 that Mougeotia was found on all the Planorbis shells and on 66.7% of the slides but not on L. stagnalis however, this hardly seems to be significant as the same species of Mougeotia in pond 5 occurred only on the slides.

Sphaerobotrys fluviatilis and Stigeoclonium 90 each present on only one shell in pond 5 were both common in pond 1.

If a shell offered a particularly good substratum for a certain species of alga one would expect that alga to be present in a high proportion of those shells investigated and to be absent or much less common on the slides which offered a very different substratum. In fact the populations on both shells and slides were remarkably similar suggesting that the shells exerted no particular attractive forces on the algae.

These results indicate that the species of algae on a shell depend not on the species of snail but the frequency with which they occur in the pond. It should be pointed out however that these observations were carried out on dead shells - not live animals - and although the conclusions are similar to those from the live snails in Langham Pond they are not strictly comparable.

A Comparison of the Algae on the Shells of a Single Species of Snail collected from a number of Different Places.

The most readily procurable and widespread freshwater snail in England is L. peregra and this has been studied from nineteen situations, these include:-

Windermere.

Brotherswater.

A number of tarns and pools in the Lake District varying from a small one with steeply sloping sides thickly populated with numerous species of water plant, to a much larger, more shallow one with far less vegetation.

Malham Tarn.

A small, much overgrown pond on the moor just south of Malham Tarn.

A pond whose water flowed very slowly in Surrey.

An artificial, but well-established, concrete-sided lily pond in the grounds of Royal Holloway College in Surrey.

A small although swiftly flowing stream near the seashore at Barmouth Merion. which was almost completely smothered with overhanging vegetation.

The River Stour near Wye in Kent, which was large and very deep.

Since the areas investigated were so diverse and widely distributed the species of algae present in one habitat were rarely the same as those in another, thus it was impossible to compare the results species for species. In view of this it was decided to consider the algae in groups in order to be able to compare the populations.

Thus, Oedogonium, Mougeotia, and Chaetophora are taken as generic groups, only one species of Coleochaete - C. scutata, was encountered so that is considered alone, likewise Microthamnion strictissimum occurred only once so that the Microthamnion here referred to is M. kützingianum.

S. farctum seems a fairly distinct species under the cultural conditions used and has been separated, the rest of the species of this genus are taken together.

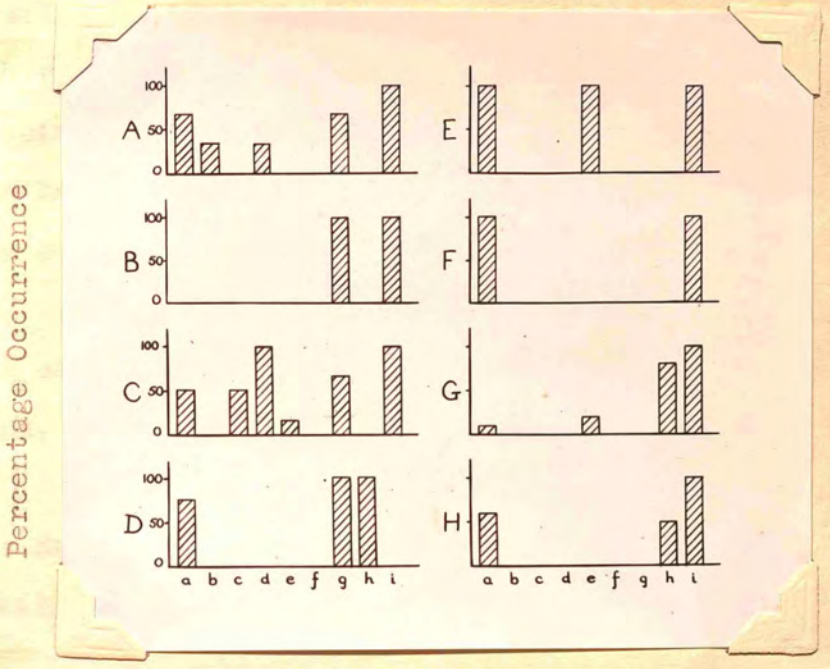
No one species of the Ulotrichales occurred frequently, and as the systematics of the group are not very definite the whole order is considered as one.

The reults may be presented in tabular form as on the next page, the figures representing the percentage of snail shells from that particular habitat bearing algae of that group.

Table showing the Percentage Occurrence of Algae on L. peregra collected from a number of habitats.

Origins \ Algae:-	Oedogonium	Mougeotia	Microthamnion	Ulotrichales	Sphaerobotrys	Coleochaete	Chaetophora	Stigeoclonium farctum	Other Stig.
Malham Tarn	100	-	-	-	100	-	-	-	100
Pond South of Malham Tarn	75	-	-	-	-	-	100	100	-
Knipe Tarn, Westmorland	-	-	67	33	33	-	33	-	67
High Burrows Tarn,,	33	33	-	67	67	-	33	-	67
Brotherswater, West.	67	67	-	67	-	-	33	-	100
New Tarn, West.	-	-	-	-	-	-	100	-	100
"Margaretifera pond"	67	67	67	-	33	33	-	-	100
Wharton Tarn, Lancs.	33	33	67	67	-	-	-	-	-
Rother Heath I,West.	100	100	-	67	-	-	33	-	100
Windermere, West.	67	33	-	33	-	-	67	-	100
Batenfold Tarn, West.	23	-	-	50	-	-	-	-	25
Clay Pond, Lancs.	50	-	50	100	17	-	67	-	100
Loughrigg Tarn, West.	-	-	-	100	-	-	-	-	-
Langham Pond, Surrey 1953	100	-	-	-	100	-	-	-	100
Langham Pond, 1952	-	-	-	-	75	-	-	25	75
R. Stour nr. Wye,Kent	59	-	-	-	-	-	-	50	100
Pd. 4, Royal Holloway College, Surrey	-	-	-	-	50	-	100	100	-
Lily Pond, R.H.C.	100	-	-	-	-	-	-	-	100
Barmouth Stream, Merion.	10	-	-	-	20	-	-	80	100

Histograms showing the percentage occurrence of algae from some of the habitats studied.



A Windermere, Lake District
B New Tarn, Lake District
C Clay Pond, Lake District
D Pond South of Malham Tarn, Yorkshire
E Langham Pond 1953, Egham, Surrey
F Lily Pond, Royal Holloway College, Surrey
G Stream at Barmouth, Merionethshire
H River Stour, near Wye, Kent

a Oedogonium
b Mougeotia
c Microthamnion kutzingianum
d Ulotrichales
e Sphaerobotrys fluviatilis
f Coleochaete scutata
g Chaetophora
h Stigeoclonium farctum
i Other Stigeoclonia.

Neither of the algae investigated is consistently dominant. The genus Stigeoclonium (excluding S.farctum) occurs more frequently than any other but if this group were split into constituent species no such correspondence would be found - in any one habitat only one or perhaps as many as eight species may occur on the shells.

If S. farctum is present it may occur on all the shells from that area as in the Pond South of Malham Tarn, or on only 25% as in Langham Pond in 1952.

Similarly with the other algae. none of them are present on a consistently high proportion of the snails studied.

Similar results are shown for L. stagnalis and P. corneus, and so far as can be seen from the work done this appears to be true of at least the majority of molluscs investigated. (As in L. peregra the Stigeoclonium represents a series of species).

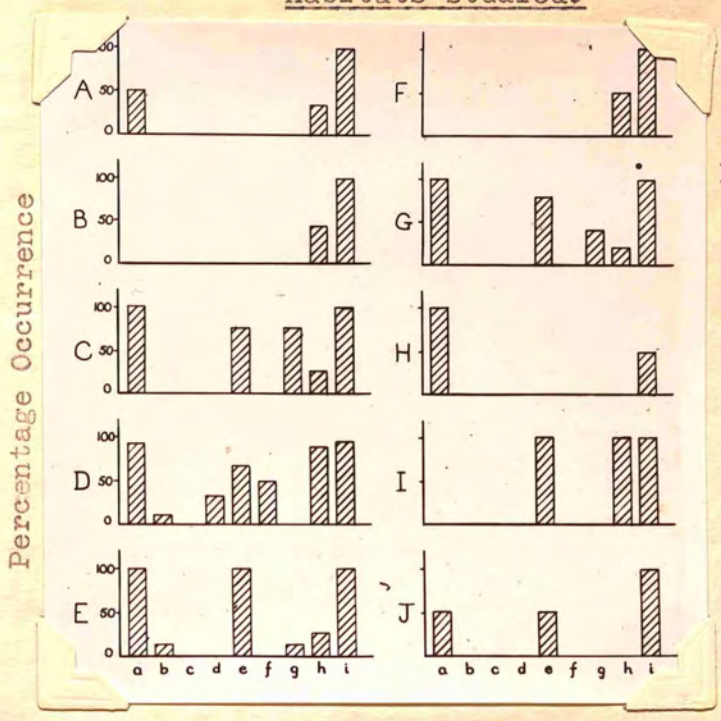
Tables of the results are on the next page.

Percentage occurrence of the algae on P.corneus (top table) and L.stagnalis (bottom table) taken from a number of different habitats.

Origins \ Algae	Oedogonium	Mougeotia	Microthamnion	Ulotrichales	Sphaerobotrys	Coleochaete	Chaetophora	S. farctum	Other Stigeo.
Langham Pond Egham, Surrey	100	-	-	-	100	-	100	-	100
N.London fishpond	-	-	-	-	-	-	-	-	100
Lily pond, Royal Holloway College	50	-	-	-	50	-	-	-	100
Fishpond, Hounslow Middlesex	-	-	-	-	-	-	100	100	100
Stanmore Lower Pond, Middx.	-	-	-	-	100	-	-	100	100
Pond 5, Royal Holloway College	100	-	-	-	-	-	-	-	50
Pond 1, Royal Holloway College	100	-	-	-	80	-	40	20	100
R. Stour, nr. Wye Kent	-	-	-	-	-	-	-	50	100

Malham Tarn	89	11	-	33	66	50	-	89	95
Langham Pond, '53 Egham, Surrey	100	13	-	-	100	-	13	25	100
N.London fishpond	100	-	-	-	75	-	75	25	100
Stanmore Lower Pond, Middx.	-	-	-	-	50	-	100	50	100
Englefield Green Pond, Surrey	-	-	-	-	-	-	-	43	100
Langham Pond '52 Egham, Surrey	7	-	-	-	50	-	14	57	100
R. Stour, nr. Wye Kent	50	-	-	-	-	-	-	33	100

Histograms showing the percentage occurrence of the algae on P. corneus and L. stagnalis from some of the habitats studied.



Limnaea stagnalis
A, B, C, D & E.

Planorbis corneus
F, G, H, I & J.

A River Stour
B Englefield Green Pond
C A north London fish-pond
D Malham Tarn
E Langham Pond 1953, Egham
F River Stour
G Pond 1, Royal Holloway College
H Pond 5, Royal Holloway College
I Stanmore lower pond
J Lily Pond, Royal Holloway College

a Oedogonium
b Mougeotia
c Microthamnion kützingianum
d Ulotrichales
e Sphaerobotrys fluviatilis
f Coleochaete scutata
g Chaetophora
h Stigeoclonium farctum
i Other Stigeoclonia.

It was considered possible that some association might exist between certain algae and certain species of snail. An association because the shell offered a particularly well suited substratum for the settling of the zoospores or because the plants on the snails received something beneficial to their growth seemed quite possible. In this case, assuming that there is normally a large mortality rate of the young stages of the algae, one might expect that those species especially adapted for living under these conditions would have a higher survival rate and tend to remain on the shell, whereas, other less suited algae would be more likely to die before reaching maturity.

Therefore the number of zoospores of these particular species which must settle on a shell in order to ensure that one at least grew to maturity would be much smaller than for the other algae; which means that even if the population of that species in the pond was comparatively low the likelehood of its being present on the snail would be fairly high. Thus one might expect that the proportion of snails bearing this alga would ~~be~~ not be directly related to the frequency with which it occurred in the pond, in other words, if this particular alga is present in the same region as the particular snail then one would expect a

large proportion of these snails to bear this plant regardless whether it was common or rare in the pond as a whole.

The percentage occurrence of such algae would tend always to be high and show far less variation than species which had no particular association with the snails.

From the results obtained no such link appears to exist - S. farctum was present on all specimens of L. peregra from the Pond South of Malham Tarn, yet on only 25% of those from Langham Pond, Surrey in 1952; similarly Chaetophora may occur on all or only on one third of the snails taken.

It is quite likely that the pond near Malham Tarn had more S. farctum than Langham Pond, but if any association existed the percentage occurrence figures would more likely have been closer than 100 and 25. Corresponding results were obtained for other algal species.

This suggests that the shells exert very little or no effect on the algae and may explain why the populations on the shells from the various habitats are so different.

Similar results were obtained for L. stagnalis and P. corneus and further investigations of other species suggest that this may be true of at least a

large number of freshwater molluscs.

The Lamellibranchs.

Only two species of the larger lamellibranchs have been investigated - Anodonta cygnea from the River Stour near Wye in Kent, and Margaretifera margaretifera from a stream near Windermere. Since their shells were so large they were cultured in lipless beakers sterilised in the same way as the crystallising dishes and covered with glass plates.

Only two species of algae were found on the Anodonta - S.tenue and S.farctum, but eleven species were present on the Margaretifera - three species of Oedogonium, Microthamnion kützingianum, a member of the Ulotrichales, Sphaerobotrys fluviatilis, Coleochaete scutata, S.tenue, S.farctum and two other forms of Stigeoclonium.

Percentage occurrence of algae on the large bivalves.

Algae / Molluscs	Oedogonium	Mougeotia	Microthamnion	Ulotrichales	Sphaerobotrys	Coleochaebe	Chaetophora	S.farctum	Other Stigeoc.
Anodonta cygnea	-	-	-	-	-	-	-	50	100
Margaretifera margaretifera	100	-	12.5	25	12.5	12.5	-	12.5	75

The river where the Anodonta were taken was fairly slow-flowing and shallow, it had a bed of sandy mud and fine gravel and the molluscs were about half buried. On examination, although prostrate systems of the Chaetophorales were distributed all over the shell they appeared far more commonly and were better developed on that part of the shell which had not been buried. After culture the whole shell became covered indicating that the algae must previously have been restricted because only part of the shell was exposed.

The Margaretifera were found in a fast-moving stream about three feet deep with large boulders and a bed of small pebbles and larger masses of rock, they seemed less buried than the Anodonta being partly protected by the boulders. Here again the algae, particularly the Oedogonia were more dense nearer the hinge and umbo, but otherwise the distribution was fairly general.

The numbers studied are too small to draw any definite conclusions but they prove that Oedogonium, Microthamnion, Ulotrichalean filaments, Sphaerobotrys, Coleochaete, and several species of Stigeoclonium can and do live on the shells of these bottom-living bivalves.

Two other genera of lamellibranchs were also collected - Sphaerium and Pisidium, and as already pointed out it has since been realised that some of the original identifications were unreliable - the specific and in fact the generic differences between the shells being very small, so that it was felt better to consider these two as a single group. Their habitats and habits are very similar - they are both small - those collected never exceeding fifteen millimetres in length, their shells are usually thin although sometimes thick - a factor known to vary considerably, they live usually either in or on the bed of the lake, pond or river where found but sometimes they climb up the stems of water plants - at Malham Tarn specimens were taken on Chara.

Quite frequently these shells appear to have no epiphytes, although sometimes Oedogonium filaments can be seen near the hinge. The shells tend to be rather pale and clean-looking, although not smooth as the periostracum is sometimes raised in almost spinelike projections and the whole surface is usually ridged.

The analysis of all the specimens collected follows on the next page.

Table showing the percentage occurrence of algae on the smaller bivalves.

Origins \ Algae	Oedogonium	Mougeotia	Microthamnion	Ulotrichales	Sphaerobotrys	Coleochaete	Chaetophora	S.farctum	Other Stig.
Malham Tarn	67.5	25	50	-	-	-	-	-	12.5
Rother Heath I Westmorland	-	-	-	100	-	-	-	-	100
Langham Pond Surrey	-	-	-	-	100	-	-	-	100
R. Stour, nr. Wye, Kent	50	-	-	-	-	-	-	-	50

It can be seen that neither S.farctum nor Chaetophora occurred on the shells investigated, this may be due to the habits or habitats of the molluscs, but S.farctum was present on 50% of the Anodonta and 12.5% of the Margaretifera. However, Sphaerium and Pisidium may actually burrow in mud, and in any case, since they are small they would readily be covered by material floating in the water although in life they do not normally remain submerged (except perhaps during hibernation), and it seems possible that this may account for the absence of S.farctum and Chaetophora.

In connection with this an experiment was set up to see the effect of silt on S.farctum - a well-grown

culture of the alga in a crystallising dish with soil solution was held in a sloping position and some washed silt from the bottom of a pond was carefully put in so that it covered about half of the plants - in the same way that they would be covered in nature if the animal burrowed.

After a fortnight those plants in the uppermost part of the dish i.e. those not silted up, were healthy and growing well; but those covered were a very pale green, disintegrating and obviously in a dying condition.

It thus seems certain that S.farctum is sensitive to silting up and since Sphaerium and Pisidium readily become at least partly covered with silt in nature this may explain why S.farctum is not found on them.

Similarly it can be seen that other forms of Stigeoclonium were likewise far less frequent here than on the gastropods, only one third of the specimens collected showing any, and apart from Physa fontinalis which also had Stigeoclonium (excluding S.farctum) on only one third of its shells the next lowest figure is 75% for Margaretifera and Viviparus.

Again, a silting up experiment was performed with S.tenue and this showed that that alga too was sensitive to being buried although it did not look quite so unhealthy as S.farctum after a fortnight.

It is interesting to note that the Anodonta were found only a few yards away from two of the small bivalves - in the River Stour in Kent yet 50% of the Anodonta had S.farctum growing on them and they all had S.tenue; this presumably is because these larger lamellibranchs do not become covered with silt as readily as the smaller ones and once an alga gained an attachment it would tend to remain on Anodonta more easily than on Sphaerium or Pisidium. It has already been stated that the thickest population of algae on these larger bivalves occurred near the hinge - the part which would not be dragged through the silt during the animal's locomotion.

Sphaerium and Pisidium are annual molluscs whereas both Anodonta and Margaretifera may live for fifteen years. This is a possible reason for the difference between the populations, but the annual cycle of the smaller bivalves is very similar to that of the gastropods so one would expect their populations to be comparable. It would seem reasonable to conclude that the differences therefore are due to the abrasion of the shells of the smaller forms as they are drawn along the substratum during movement.

Thus, in general, bivalve molluscs have fewer epiphytes than gastropods and whereas algae present on the snail show little if any correlation

the population on the smaller bivalves tends to be different from that on the larger ones.

Roughness of the Shell.

Two species of snail having very different shell surfaces were collected together from Rother Heath I a tarn in the Lake District - they were L.peregra, whose shell is in general thin and smooth although faint growth rings may be present; and L.palustris which has a very much thicker structure with marked ridges both spirally and along the lines of growth, thus the whole surface is pitted.

The bodies were removed, the shells washed and cultured as before and the algae present were noted.

A table showing the results appears on the next page.

Sixteen species of algae were found altogether, of these only five occurred on both species of shell, seven were each recorded only once, the remaining four being present on two specimens in each case.

Of the five which occurred on both L.peregra and L.palustris two were Oedogonia and three were Stigeoclonia.

Mougeotia, Hormidium flaccidum, and another member of the Ulotrichales, Chaetophora, and one species of Stigeoclonium were found on L.peregra alone, while two species of Oedogonium, Microthamnion, and Sphaerobotrys occurred only on L.palustris.

Table showing the species of algae present on L.peregra and L.palustris taken together from Rother Heath I in the Lake District.

(The letters here refer to separate species within the genera, x marks presence in the other columns).

Snails \ Algae		Oedogonium	Mougeotia	Microthamnion	Ulotrichales	Sphaerobotrys	Coleochaete	Chaetophora	S.farctum	Other Stigeocl.
Limnaea peregra	1	a	a	-	-	-	-	x	x	a
	2	b	b	-	a b	-	-	-	-	a
	3	a	c	-	a	-	-	-	-	abc
Limnaea palustris	1	-	-	-	-	-	-	-	-	a c
	2	b c	-	-	-	-	-	-	-	a c
	3	b c	-	-	-	-	-	-	-	a
	4	d	-	-	-	x	-	-	-	a
	5	b	-	-	-	x	-	-	x	a
	6	a d	-	x	-	-	-	-	x	a

However, from other specimens studied from another habitat Mougeotia was present on L.palustris but not on L.peregra, and Microthamnion and Sphaerobotrys

have both been taken on L.peregra.

Of the other six algae found here only on either the smooth shells or on the rough three species were recorded only once and the remaining ones only twice so with the small numbers taken these results may not be significant.

As far as could be judged therefore it seems that the epiphytic algae bear very little relationship to the degree of smoothness or roughness of a shell.

The Algal Population considered in Relation to the Age of the Shell.

Specimens of *L.stagnalis* collected from Malham Tarn were used for this investigation.

The eggs of *L.stagnalis* normally hatch during June and July, the animals mating the following Spring, subsequently laying eggs; they die in their second Autumn, thus the maximum age of a specimen is likely to be about sixteen months.

This material was collected altogether from well out in the Tharn on *Chara* using a plant grab in late October and included snails hatched that year and hence about four months old, adults from the previous year which must have been nearing their sixteenth month and therefore the end of their life, and empty shells the same age as the adults but which must have been dead for some time since no body remains were present. Other similar empty shells were taken from the northwest edge of the Tarn where it joins the hanging bog. Eight or ten specimens were collected in each case.

The shells were cultured as in the previous experiments and the occurrence of the algae noted. The results are as follows:-

Here again all forms of *Stigeoclonium* are

considered together except S.farctum, as are the members of the Ulotrichales (Uronema confervicolum and another species). Draparnaldia and Spirogyra which were found only rarely are omitted here for simplicity.

Table showing the percentage of the snails in each group bearing each group of algae.

Snails \ Algae	Oedogonium	Mougeotia	Ulotrichales	Sphaerobotrys	Coleochaete	Chaetophora	S. farctum	Other Stigeocl.
Young L.stagnalis mid Tarn.	75	12.5	50	75	12.5	-	75	87.5
Adult L.stagnalis mid Tarn.	100	-	10	70	70	-	100	100
L.stagnalis shells mid Tarn.	100	-	12.5	100	75	-	100	100
L.stagnalis shells near bog.	80	20	20	60	-	30	10	70

In order to make these results more comparable each alga or group of algae has been calculated as a percentage of the total algal flora for each group of shells.(See over).

Table showing the algae expressed as a percentage of the total flora on each group of snails.

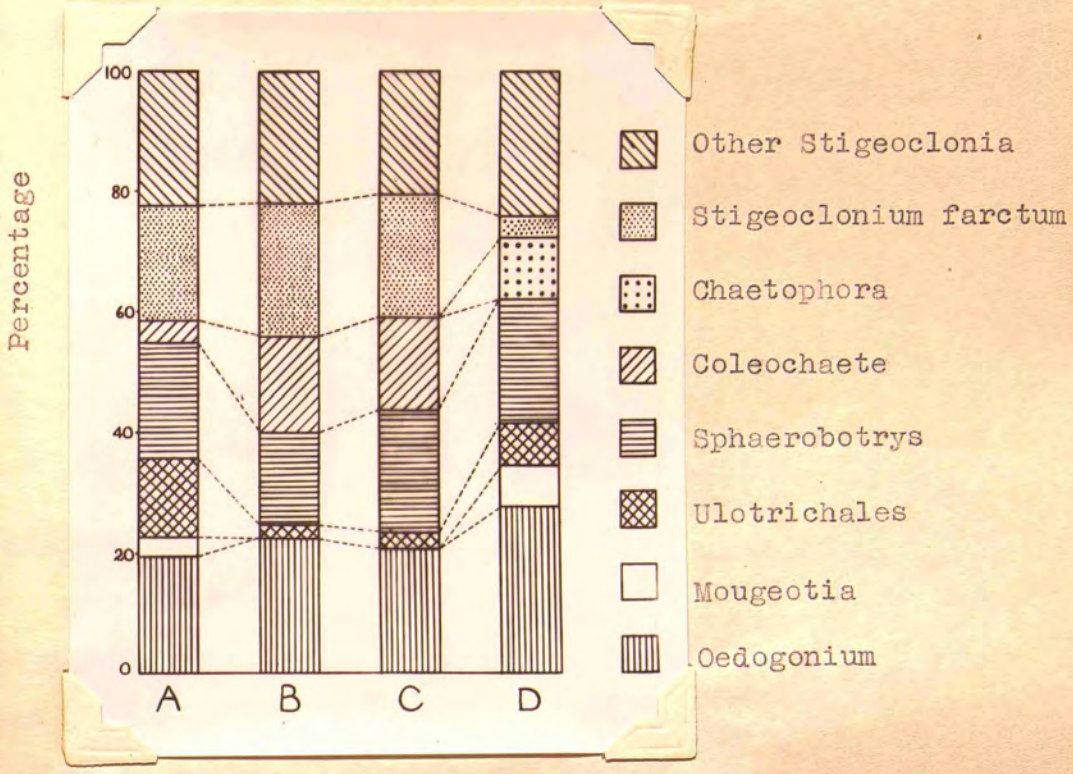
Snails \ Algae	Oedogonium	Mougeotia	Ulotrichales	Sphaerobotrys	Coleochaete	Chaetophora	S. farctum	Other Stigeocl.
Young L.stagnalis mid Tarn.	19.4	3.2	12.9	19.4	3.2	-	19.4	22.6
Adult L.stagnalis mid Tarn.	22.2	-	2.2	15.6	15.6	-	22.2	22.2
L.stagnalis shells mid Tarn.	20.5	-	2.6	20.5	15.4	-	20.5	20.5
L.stagnalis shells near bog.	27.6	6.9	6.9	20.7	-	10.3	3.5	24.1

From this table the following histograms were drawn:-

(See over).

Histograms showing the changes in the composition of the epiphytic flora on Limnaea stagnalis shells.

Each column represents the average population on shells of that particular group.



A Young Limnaea stagnalis, mid Tarn

B Adult Limnaea stagnalis, mid Tarn

C Limnaea stagnalis shells, mid Tarn

D Limnaea stagnalis shells, near bog.

From these histograms it can be seen that Oedogonium, Coleochaete and S.farctum increased, whereas Sphaerobotrys and the Ulotrichales decreased, and Mougeotia disappeared as the snail grew older.

The dead shells collected from the Chara with the live snails had almost identical proportions of algae except that Sphaerobotrys was much more abundant.

A number of factors must be considered here:-

1. An adult snail moves faster than a young one.
2. A considerable number of algae have their maximum periods of growth and reproduction in Spring - before the young snails were hatched.
3. The adult snails have obviously lived longer and therefore had more time for colonisation by the algae.

Considering the movement of the snail first:-

Limnaea stagnalis has a habit of progressing with jerky movements - the columella muscle contracting suddenly causing the whole shell and visceral mass to rotate through a considerable angle, and since these animals were taken from very dense beds of Chara the algae on the shells would be subjected to considerable abrasion. The young animals being smaller, could more easily pass through beds of weed than the larger specimens whose algae would be rubbed against the stems far more frequently. One might therefore expect that

algae only loosely attached to the shell would be more readily rubbed off the old than the young shells - and this may account for the disappearance of Mougeotia which is only weakly fixed, and the decrease of the Ulotrichales and Sphaerobotrys neither of which have very strong holdfasts. Conversely the more firmly attached Coleochaete scutata, Stigeoclonium farctum and Oedogonium would be less easily rubbed off and would be expected to increase with age.

After death of the animal only Sphaerobotrys shows any appreciable change - a marked increase, when it is remembered that this alga has a very weak attachment to the substratum and thus is readily rubbed off this result is quite logical.

The periodicity of the algae might be expected to exert an effect perhaps quantitatively considering each individual snail rather than qualitatively. Many algae, among them Mougeotia, Stigeoclonium and Coleochaete tend to have their maximum periods of growth and reproduction in Spring and early Summer - before the young snails had been hatched. Thus, other factors being equal the population on the older shells would be expected to have more of these algae, this may well account for some of the increase in Coleochaete although it is not responsible for the absence of

Mougeotia on the adult snails.

The dead shells were of the same age as the live adult snails and thus the periodicity of the algae would not be responsible for the differences between them.

The time during which the shell had been exposed to colonising algae may affect the population but only to a slight extent since, at least in the summer months, the time for one generation to develop and reproduce is very short. In fact a total of twelve different species of algae were found on the young snails compared with only eight on the adults.

The shells taken from the edge of the bog are in a way not very comparable as although of the same age as those from the Chara beds they were under very different conditions - the water had a pH of 7.0 (compared with 8.0 to 8.5 further out in the Tarn) because water of pH 4.0 was draining in from the bog. They were found with no weeds, in a very shallow region on a coarse sandy bottom where the water was being continually rippled by the wind causing the shells to be rubbed to and fro across the bed of the water. They were thus under more acid conditions, fully exposed to light and constantly abrased.

However, Chaetophora appeared there for the first time, and Mougeotia returned again (but it was a different species from that found before) whilst Coleochaete disappeared. Other small variations can also be seen from the histograms. Without further investigation it is difficult to say why these changes occurred. One would certainly have expected Sphaerobotrys to disappear under such conditions, it is considered that the pH of the water was probably the most important factor operating here although without more work it is impossible to say.

Thus, the populations of algae on a snail shell vary to some extent with the age of the snail, certain species increasing and others decreasing; while after death of the animal, if the shell remains under the same environmental conditions the population does not alter very much except in the case of Sphaerobotrys. It is suggested that the changes are correlated with the movement of the snails and the periodicity of the algae concerned.

Algal populations on empty shells compared with those on living snails - considering all specimens taken from several different habitats.

Shells of L.stagnalis, L.peregra and the small bivalves were collected from various habitats and their epiphytes scored in the usual way.

The figures compared with those for all the live specimens taken of these same species regardless of habitat are shown on the next page.

The table shows that Oedogonium, Sphaerobotrys, and Coleochaete increased markedly after death, while S.farctum constantly showed a decrease. The other algae varied from one species of mollusc to another.

These shells were collected from the same habitats as the live animals and were usually found under the same conditions - often entangled in weeds; the bivalve shells were mostly collected from the bed of the water. In general therefore the environment of these algae was very similar to that round the live animals. The most obvious difference between dead and live shells was that the latter were of course drawn through the water, admittedly at rather a slow rate but nevertheless at speed enough to wash the plants. When one considers that a shell may bear a dense turf

Table showing the percentage occurrence of algae on dead shells and those from live animals of L.stagnalis, L.peregra and small bivalves.

Snails		Oedogonium	Mougeotia	Microthamnion	Ulotrichales	Sphaerobotrys	Coleochaete	Chaetophora	S. farctum	Other Stigeocl.
A.	shell	88.9	-	-	16.7	77.8	33.0	16.7	50.0	83.3
	alive	55.0	5.0	-	10.0	54.0	15.2	13.6	56.0	98.0
B.	shell	71.4	28.6	14.3	14.3	28.6	28.6	14.3	14.3	85.7
	alive	47.1	11.8	10.6	22.4	18.8	1.2	22.4	30.6	81.2
C.	shell	75.0	-	-	-	25.0	-	-	-	100
	alive	50.0	16.6	33.0	8.3	8.3	-	-	-	33.0

A. = L.stagnalis.

B. = L.peregra.

C. = The small bivalves.

of algae producing relatively large amounts of waste products, including carbon dioxide, gentle agitation such as would occur when a snail moves may be just sufficient to remove these staling substances adequately enough to ensure good growth. A comparison therefore of the populations on dead and live shells may suggest which algae are tolerant of stationary conditions and which show a "preference" for a moving substratum.

These results indicate that S.farctum is affected by agitation of the water surrounding it since it showed a marked decrease on death of the animal, suggesting that the movement of the snail was beneficial in some way; whereas Oedogonium, Sphaerobotrys and Coleochaete showed an increase on death. It should be pointed out however that other factors may be active here - for example waste substances given out by the live snail may affect the plants.

It is useful here to refer back to the L.stagnalis material from Malham Tarn (P.72-79). There the S.farctum showed only a very slight decrease after death, and Oedogonium showed a similar one; Coleochaete was almost stable, but Sphaerobotrys had a marked increase. The only really constant result therefore is the increase in the population of Sphaerobotrys after death and possibly a decrease in S.farctum suggesting that Sphaerobotrys certainly does not benefit

by the snails movement, but that possibly S.farctum is helped by it.

Refering back to the lamellibranchs (P.62-68), S.farctum was noticably absent from the small bivalves while it occurred quite plentifully on both Anodonta and Margaretifera. It was shown by experiment to be sensitive to being buried by silt but it may also be that it was affected by the aeration since the small bivalves were, in general, taken from still waters whereas the larger ones were from a fast-flowing stream and a river.

Although Sphaerium and Pisidium move they are rarely as active as the snails and the algae on their shells therefore would not be so well bathed by water currents as those on L.stagnalis and L.peregra, whereas the fast-moving water from which the larger genera were taken would more than make up for their relatively slow rate of progression.

In general therefore the algal species colonising a dead shell are similar to those on a living animal except that it seems likely that S.farctum is more plentiful on moving shells than on dead, stationary ones and it is suggested that the removal of waste matter from this form - which is largely prostrate and which would tend to be rather smothered if much

filamentous material was present - may account for the changes in occurrence frequency.

The Snail Shell as a Substratum.

Since the previous work indicated that although the movement of a snail appears to exert very little influence in determining the species of algae colonising molluscan shells it may alter the frequency of a particular form an investigation was carried out to see if any difference existed between the size of an alga on a moving substratum and the same alga on a stationary one.

For ease of measurement it was decided to study an unbranched filamentous species and as a plentiful supply of Oedogonium alternans was readily available in a nearby pond containing snails this was selected.

This Oedogonium was growing in large quantities on the shells of Planorbis corneus, and several of these were taken. The filaments, which were up to about three centimetres long, were cut off with scissors, some being taken from each snail.

A few filaments from each were measured using a micrometer eyepiece. Readings of length of one hundred and breadth of fifty cells were made - some from each shell, carefully neglecting the five cells at either end of the filaments.

At the same time filaments of the same species

of Oedogonium were collected from the same pond attached to Lemna trisulca and Stratiotes plants. These plants shared the same conditions as the snails, in fact the normal habitat for P.corneus in this particular pond was either on the Stratiotes or floating on the water surface among the Lemna. However, the snails offered a moving substratum whereas the plants of course were stationary.

Histograms have been drawn from the measurements -

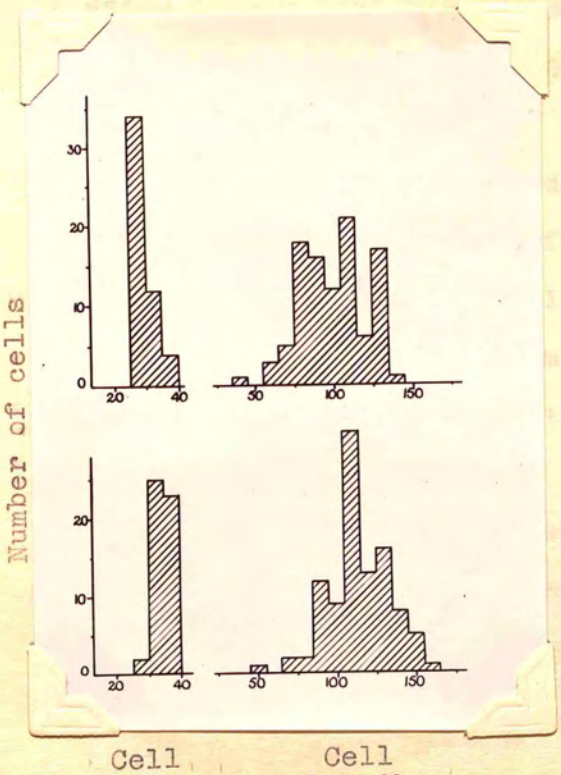
see over.

From these histograms it can be seen that both the average length and breadth of the cells were greater in the filaments attached to the snails :-

Average length of cell of filament attached to plants = 96.5μ
" " " " " " " " snails =114.0μ
" breadth " " " " " " plants = 27.5μ
" " " " " " " " snails = 34.0μ
" volume " " " " " " plants=57,335cu.μ
" " " " " " " " snails=103,512cuμ

The speed of movement of P.corneus was measured and an average of the readings for several mature specimens shows that they move at approximately 8.6 cms. a minute - a pace which would seem to be sufficient to

Histograms showing the varying cell size of Oedogonium alternans on stationary and moving substrata in the same pond.



On Lemna trisulca and Stratiotes plants

On Planorbis corneus.

wash away waste substances formed by the alga - and the snail too.

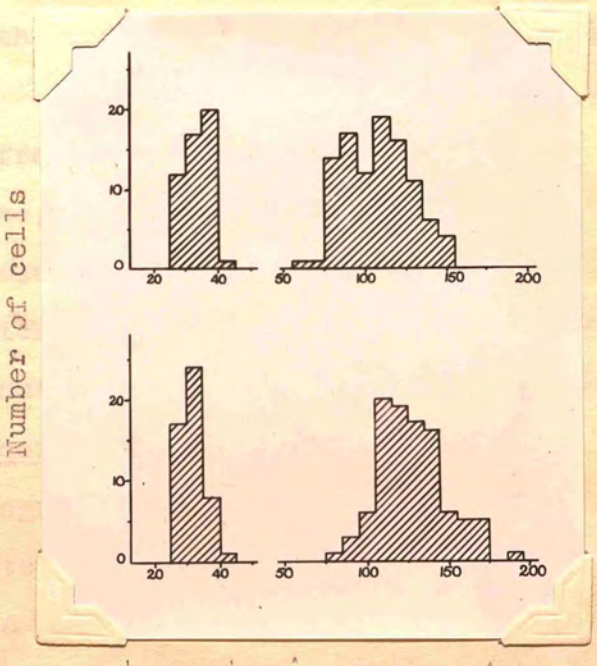
Since the Oedogonium filaments on the plants were under the same conditions as those on the shells it seems likely that the increased size of the latter was due to the movement of the snail which although rather slow would probably be sufficient to cause constant removal of waste products. Other factors may also be important here too - for example the nitrogenous material excreted by the snail, but it is considered likely that this would be of minor importance since it would soon be washed away as the animal moved.

These results suggest therefore that a snail shell offers better conditions as a substratum at least for O.alternans than stationary objects.

A Comparison of the Size of this same Alga on two Species of Snail.

Several specimens of P.corneus and P.albus from the same pond as before had been kept together in an aquarium for five weeks. The Oedogonium on their shells was measured as before and the following histograms were drawn :-

Histograms showing varying cell size of O.alternans on two species of snail from the same pond and kept together for five weeks in an aquarium.



Planorbis albus

Planorbis corneus

These histograms show that the cells of the filaments on P.corneus were much longer than those on P.albus yet the cells were wider on P.albus. One hundred readings were made in each case.

Average	length	of cells of filament from	P.corneus= 127.6μ
"	"	" " " "	P,albus = 107.3μ
"	breadth	" " " "	P.corneus= 32.6μ
"	"	" " " "	P.albus = 33.8μ
"	volume	" " " "	P.corneus=106,530cu.μ
"	"	" " " "	P. albus = 96,275cu.μ

Here again the average volumes are different although not so markedly as in the last investigation.

As far as could be ascertained the main differences between these two snail species, apart from size, was in their speed of movement - no actual measurements were made for P.albus but P.complanatus which is very similar moves at the rate of 2.3 cms. per minute - much slower than the 8.6 cms. per minute of P.corneus. Even this comparatively leisurely rate of progression in P.albus would help removal of waste material from the water around the algae, but although the difference in size between those filaments on the faster moving snail and those on the other is small it does perhaps indicate that the movement of the animal has some effect on the algae.

Experiments considering Shell Composition.

The shells of molluscs consist almost entirely of calcium carbonate, the actual percentage varying with the species and with the habitat but it is usually considered to represent anything up to ninety-five percent of the shells in the form of calcite or aragonite. They also contain conchyolin and are impregnated with other compounds the most important of which are calcium phosphate - possibly as much as one percent of the total shell, magnesium carbonate which occurs in still smaller quantities, and occasionally a trace of silica.

Since the proportion of calcium carbonate in a shell is so high it was thought that it may possibly affect the zoospores, causing them to move towards it.

A glass ring, sterilised by being flamed, was put into each ot three large petri-dishes and fixed to the bottom with a little Vaseline. Powdered calcium carbonate was put into each ring and the dishes were carefully filled with soil solution so that the rims of the rings were just covered. Each dish was inoculated with a single plant of Stigeoclonium 100. Three similar dishes were set up, the only difference being that their rings contained no calcium carbonate.

All six were left undisturbed in a north-facing window for a fortnight after which time the distribution

and size of the sporelings was noticed. In every case the sporelings were clustered round the parent plant, being fairly evenly distributed although possibly more were present towards the lighter side of the dish. No difference at all could be seen between the two sets, the calcium and carbonate ions had not apparently had any effect in determining where the zoospores settled, some were grwing actually on the powder and the glass rings.

Neither could any difference be seen in the amount of growth made.

Similar experiments were carried out using Stigeoclonium 300 but instead of powdered calcium carbonate in a glass ring lumps of crude although autoclaved chalk from a cutting in the Chilterns were used. Again the sporelings developed around the parent plant and no difference in growth could be seen between the two sets of dishes.

Further trials using soil solution made up with fifty percent crude chalk compared with ordinary soil solution again gave no difference.

It might be expected that some pH effects might have been evident in these experiments but in fact the changes in alkalinity and acidity were very small as soil solution acts as a buffer. The greatest difference in pH after a fortnight was 6.8 for normal soil solution

compared with 7.3 for similar solution but with lumps of added chalk.

These results therefore suggest that calcium carbonate has no great effect on either the position of settling of the zoospores or the growth of Stigeoclonium.

At the same time an experiment was carried out in crystallising dishes using sporelings of Oedogonium 2 instead of Stigeoclonium, with normal and normal-plus-chalk soil solutions.

After a month there was no apparent difference but measurements of cell length and breadth were taken. There was very slight difference in cell size; those in the solution plus chalk being slightly longer than those without - average lengths being 38.4μ:37.9μ, but also slightly narrower - 14.7μ:15.0μ. The mean volumes however are very similar - the average of cells from filaments growing in soil solution containing added chalk being 6520 cu.μ compared with 6698 cu.μ for those having no extra chalk - a difference of under three percent.

Experiments using soil solution plus and minus a pinch of calcium phosphate and magnesium carbonate - constituents of the shell - also gave negative results.

Brushed and autoclaved shells of L.peregra, L.stagnalis and P.corneus were placed in petri-dishes of soil solution to each of which single plants of Stigeoclonium 100 were added. After a fortnight the distribution of the sporelings showed no relation to the position of the shells nor to any part of them - they had developed around the parent plant, neither attracted to the shell as a whole nor to any one part of it as they were found growing inside the whorls, and in the sutures just as frequently as on the outside of the whorls.

From these experiments it seems unlikely that the composition of a snail shell bears any direct relationship to its epiphytic algae.

Inter-relationships of Snails and Algae.

Gastropods have been observed eating *Chaetophora*, *Stigeoclonium*, and other members of the Chaetophorales, *Uronema*, *Hormidium* and some other Ulotrichales, *Oedogonium*, diatoms, desmids and unicells with which they come into contact. Casual observations of snails travelling over mixed cultures of algae in crystallising dishes suggested that they removed everything in their path although a certain amount of the material was not eaten but remained floating. On rougher surfaces, however, such as another shell, parts of or whole prostrate systems were left in sutures, near the mouth of the shell, and in other regions inaccessible to the snail's radula.

Further inspection however suggested that the density and composition of the algal population may influence the animal's feeding habits - a well-grown, although young culture of *Oedogonium* (cell size 20.6-23.3μ by 38.2-102.9μ) with filaments up to about half an inch long containing numerous unicells was exposed to a specimen of *L.peregra* which had been previously starved. The culture was not uniformly distributed in the dish, some parts being denser than others, and the snail was left for several hours during which time it passed over a considerable amount of the surface. It was noticed

that in the less dense areas the unicells had been removed, some of the Oedogonium filaments were bent, but on the whole they were intact; whereas in the denser regions some areas of the dish's surface had been completely cleared, not even leaving the Oedogonium holdfasts (whether or not these remain depends at least to some extent on the species of Oedogonium). As a rough comparison of the densities the number of filaments in single low-power microscope fields in areas adjacent to those grazed were counted in both thickly and thinly populated regions. These results indicated that where there were only 0.6-0.7 filaments per square millimetre in some way or other the snail ate the material between the Oedogonium but tended to leave the filaments intact, whereas regions supporting over 2.5 filaments per square millimetre had been completely denuded. This suggests that L.peregra may only eat Oedogonium, at least of this species, when, because of the density of the culture, it is unable to engulf the material inbetween without also taking the filaments. Under such conditions many filaments become removed from the surface but are left floating, once this has happened, even if the threads lie on the surface of the substratum they do not seem to be eaten.

Well-grown cultures of Stigeoclonium and Chaetophora are readily taken by snails and it is thought that because the filaments of these algae have thinner cell walls and are pliable compared with the stiffness of Oedogonium they can more easily be attacked by the radula and eaten.

These observations explain why the erect systems of members of the Chaetophorales are frequently found to be well-grown when among dense Oedogonium yet in more exposed positions only rarely are they developed to any extent.

In order to follow the path of the algae further, faeces from snails fed on pure cultures were inspected after being squashed between slide and coverslip. Oedogonium, Stigeoclonium, Chaetophora and Uronema were clearly distinguishable and very little changed from their normal state. For more controlled observations starved specimens of L.stagnalis, L.peregra and P.corneus were fed on pure cultures for an hour or two, their faeces collected, washed in sterile distilled water to remove any filaments adhering to but not forming part of the excrement, and cultured. At the same time plants from the culture which was used as food were subcultured under similar conditions. The algae grew out from the faeces, produced zoospores and rapidly formed a thriving

stock. It seemed, however, that reproduction did not occur quite as soon in the material that had passed through the snail as in that which had not been eaten.

Digestion of food in a snail occurs mainly in the digestive gland, the ducts from which pass into the midgut region. To ascertain if any clear change occurred to the algae after subjection to the digestive juices snails which had just been fed were dissected and various regions of the gut removed, washed and cultured as before. So far as could be seen the algae in the hind and midgut which must have been in contact with the snail's enzymes were no different from those in the foregut which, apart from passing over the radula, had received no further treatment. These animals therefore clearly could not break down these algae. The reason for this is that very few snails possess a cellulase and therefore they cannot digest plant cell walls; hence any cells or filaments passing intact over the radula remain unharmed in their passage through the gut. Only those cells whose walls are broken by the teeth in the buccal mass can be digested, explaining why such enormous quantities of algae are eaten by snails since only by taking in large amounts will sufficient become available for digestion.

It is possible that the faeces may exert some

influence as fertilisers on the algae; Chaetophora cultured with faeces appeared slightly more luxuriant than that without, and the longest Oedogonium filament from a subculture with faeces was longer than the corresponding one in a parallel subculture without.

On a small scale in the laboratory the pH of a culture with a snail is lower than one without, whether this is important in nature is difficult to say.

Undoubtedly too a snail's phototaxicity causing its epiphytic algae to be carried towards the light must influence the plant's growth, and the actual movement has already been shown to have an effect at least on cell size.

Thus, while freshwater snails are clearly dependant at least to a considerable extent on algae for their food, the plants themselves appear to derive a certain amount of benefit by being in association with these animals.

Discussion.

Gastropods are rarely found in any numbers below six feet from the water surface, the thickest populations tending to be within the upper two feet. It is a fluctuating community, moving up and down not simply with the rise and fall of the water level but with the growth of fresh food supplies, and varying with the age of the snail and with the season. Some species - for example Bithynia tentaculata are frequently associated with certain plants - in this case Fontinalis antipyretica, others may be found almost anywhere in the water. Usually, however, a large proportion of the animals is distributed either on water plants, stones, tree-roots, or other substrata fairly near the edge of the water and apart from such species as Hydrobia jenkinsi most occur on plants and solid surfaces rather than on a shingly or sandy bottom.

The operculates - Viviparus and Bithynia for example, need never come to the surface for air and can remain totally submerged. The rest of the gastropods however are usually considered to have air in their mantle cavities which must periodically be renewed by visiting the water surface. Recent work (Hunter 1953) has shown that this depends not merely on the species but on the depth at which it lives and also its age,

certain types remaining submerged for considerable lengths of time while others of the same species regularly surface.

Many, if not all, water snails are positively phototactic and move into bright illumination, even floating on the surface film in direct sunlight in quiet waters. Thus, algae on the shells will be carried from place to place in regions which, because of their shallowness are light - apart from the movements of the snails towards high intensity; will quite often have a high oxygen tension due to the proximity of green, water plants, and which will already bear a considerable epiphytic algal population providing the animals with their food. At the same time decaying matter, including snail faeces, will supply organic materials to the water.

Somewhat similar conditions exist for the bivalves. Frequently they are found at a depth of about three feet, although this varies with the habitat and also the time of year; but instead of being associated with plants the larger species at least may be found partly embedded in gravel some distance from the nearest macroscopic vegetation, but these types are forms living often in fast-flowing, and thus well aerated, streams and rivers. The smaller species, although they may be on the bed of the water, may be found on water plants such as Chara - under similar conditions to the snails.

These algae therefore live under light conditions where oxygen will often be plentiful, where algae already exist and hence provide a source of Zoospores for colonising the shells, where organic salts will be present, and because of the upward and downward movement of at least certain species during the year the temperature will tend to be more constant than for those algae constantly attached near the surface of the water.

One might therefore expect to find a rich algal flora on these molluscan shells and this has indeed been shown to be the case. Some of these plants at least must be living in near-optimal conditions. Apart from the larger bivalves and to a lesser extent the smaller species very little vertical zonation of species occurs, most snails appearing in similar habitats. The calcium content of the water limits certain species - L.stagnalis for example being quite frequently used as an indicator of hard water. Comparison between the epiphytes on the bottom living forms and those nearer the surface indicate that, in general, the same algal species occur although sometimes in fewer numbers. When one considers that these algae live very near the bed of the water which, where these animals are found, is frequently sand or sharp silt and that the water, at least where these specimens were taken, was travelling at a considerable pace causing constant abrasion of the shells by

particles carried in the currents, it is not surprising that less plants are present - and experiments on two of the algae showing a marked decrease on these bivalves have shown that they are indeed adversely affected by silt.

Since, on the whole, snails of different species live under similar conditions in any one pond or stream this may explain why the algae tend not to vary much with the species of snail as has been shown during these investigations.

To look for variation in the epiphytic flora therefore it is necessary to consider the microhabitat in greater detail; some snails have a particularly rough shell, others a smoother one, some exist as flattened coils, others as elongated spires and so on; yet in each case investigated the algae have remained largely the same, varying hardly at all as far as can be ascertained from the work done, with the shell surface, size and shape. On further thought this is perhaps not surprising as a snail's progress is not a very smooth one, most species tend to twist the shell to a greater or lesser extent during movement so that the algae on the concave surface of a slightly intorted spire would be subjected to water movements in the same way as those on the edges of the shell; similarly, ridged shells would give little protection once the sporeling had developed

to even a small extent. The sutures and clefts between deep ridges would however be slightly more protected than the surface of the whorls and this may be important in the settling of motile stages, indeed, the population in a suture can sometimes be seen to be more dense than on the more exposed parts, (yet this was not so when algae colonised stationary shells in the laboratory).

A dense turf of algae such as is sometimes found on a shell will produce comparatively large quantities of waste materials including a considerable amount of carbon dioxide. Such masses on stationary substrata must rely either on water currents or diffusion for the removal of these substances and in some conditions this may well prove a factor influencing growth. However, algae on a snail shell will be drawn through the water, not always in a single direction - the animals tending to travel somewhat haphazardly, and so waste products will be removed very much faster than those from similar nearby populations on rocks, plants or other positions which do not move.

It might therefore be expected that at least in bodies of still water the algae on the snails may be better developed than those elsewhere in the water, and this has been shown to be true by the experiments on O. alternans and two species of Planorbis - the cells

of the filaments on moving substrata being considerably larger than those which remain still.

The interdependence of snails and algae, although by no means obligatory, is certainly interesting. In spite of the fact that the animals will eat almost anything which they find in the way of bacterial slime, epiphytic Protozoa, fungi and algae in a stretch of water thickly populated with molluscs the consumption of algae must be considerable - one has only to think of the sides of an aquarium bearing algae into which a few snails have been introduced to realise just how much they can remove in a day. Since the experiments have indicated that only the contents of those cells which are actually broken can be digested enormous quantities must clearly be eaten for sufficient to be assimilated. At the same time the algae are benefitting by the transport they receive and probably also by the waste products of the snails; and since certain species of mollusc have been shown to migrate, even from pond to pond it is possible that this carrying of species may aid in the dissemination of algae in nature.

Summary.

1. A survey of the green algae living epiphytically on the shells of some freshwater mollusca has been made.

2. Eighteen species of snail and a number of bivalves were studied. The animals were killed with chloroform, the bodies removed and the shells, after washing, were cultured in soil solution.

3. Between one hundred and a hundred and twenty species of algae were found.

4. All species of mollusc from a single habitat tend to have similar algal populations.

5. The epiphytes on lamellibranchs are, in general, similar to those on the gastropods from the same habitat, but certain species of algae are less frequent on these bivalves - they were shown by experiment to be very susceptible to silting up.

6. No correlation was found between roughness of shell and the colonising algal species.

7. Material from Malham Tarn indicated that at least in that habitat the algal population on adult snails

was somewhat different from that on young specimens, but that after death of the animal, providing that the empty shell remained in the same environment, except for one species, the flora remained constant.

8. The movement of a snail, although not important in determining the epiphytic species, may be sufficient to cause the algae to grow larger than on stationary substrata.

9. The composition of the shell bears very little, if any, relationship to the algae.

10. The algae have been shown to pass almost completely unharmed through a snail's gut and faeces.

11. Inter-relationships between molluscs and algae are discussed.

This work was carried out during my tenure of a studentship from Royal Holloway College.

I should like to thank my Supervisor, Professor F. W. Jane for suggesting this problem and for many helpful discussions during the progress of the work; I am also grateful to Dr. M. A. P. Madge for the interest she has shown throughout this investigation.

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FIGURES.

All figures are from Camera Lucida drawings of mature plants growing in unialgal cultures on coverslips in Soil Solution.

Figure 1.

The bases of three filaments of Mougeotia 3 showing the poorly developed attaching bases.

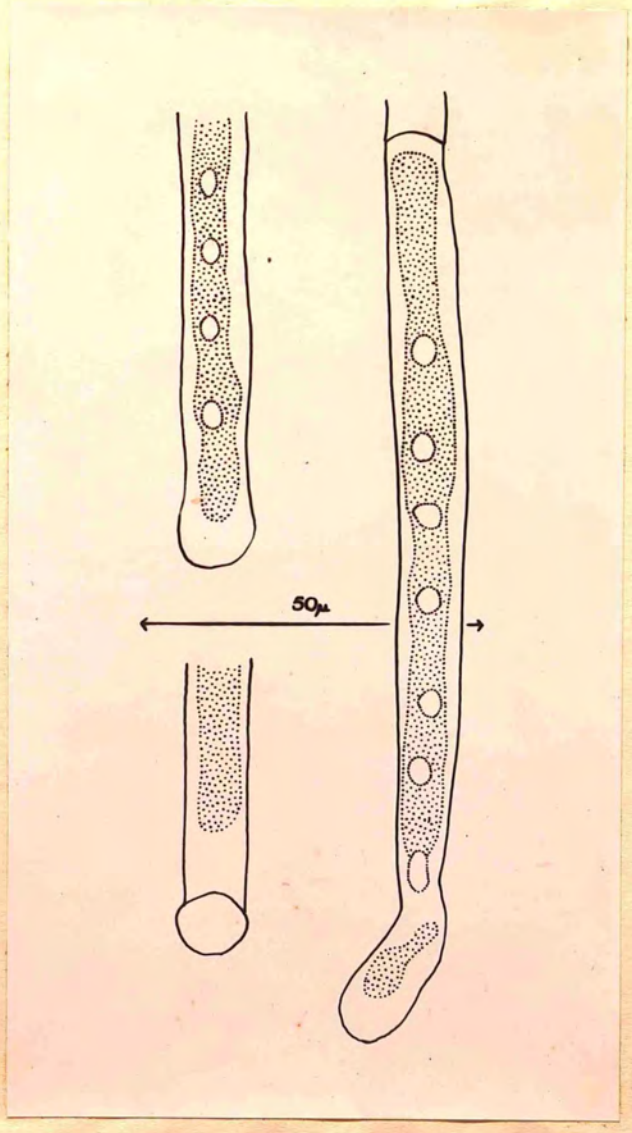

Figure 2.

The lower parts of the filaments of some members of the Ulotrichaceae showing the attaching bases.

The two at top left are of Ulothrix subtilissima.
Top right is of Uronema confervicolum.
The lower ones are of Ulotrichaceae 12.

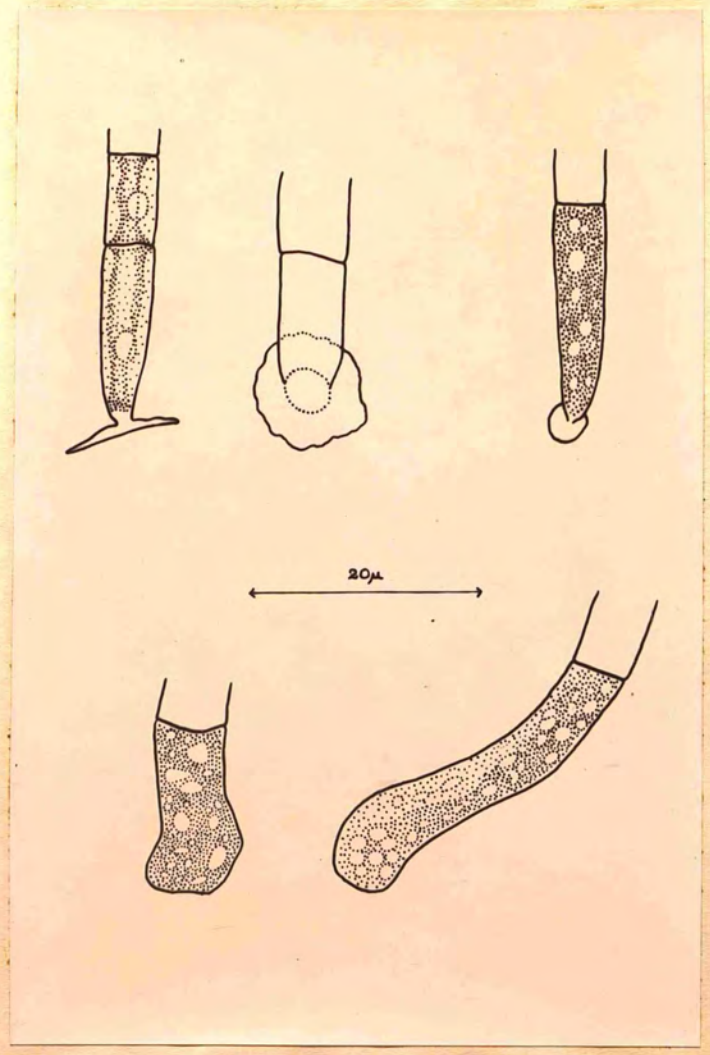

Figure 3.

The prostrate system of Stigeoclonium 300, one of the types used in the experiments on pages 16-17 and 92-93.
The prostrate part is shaded to distinguish it from the erect system.

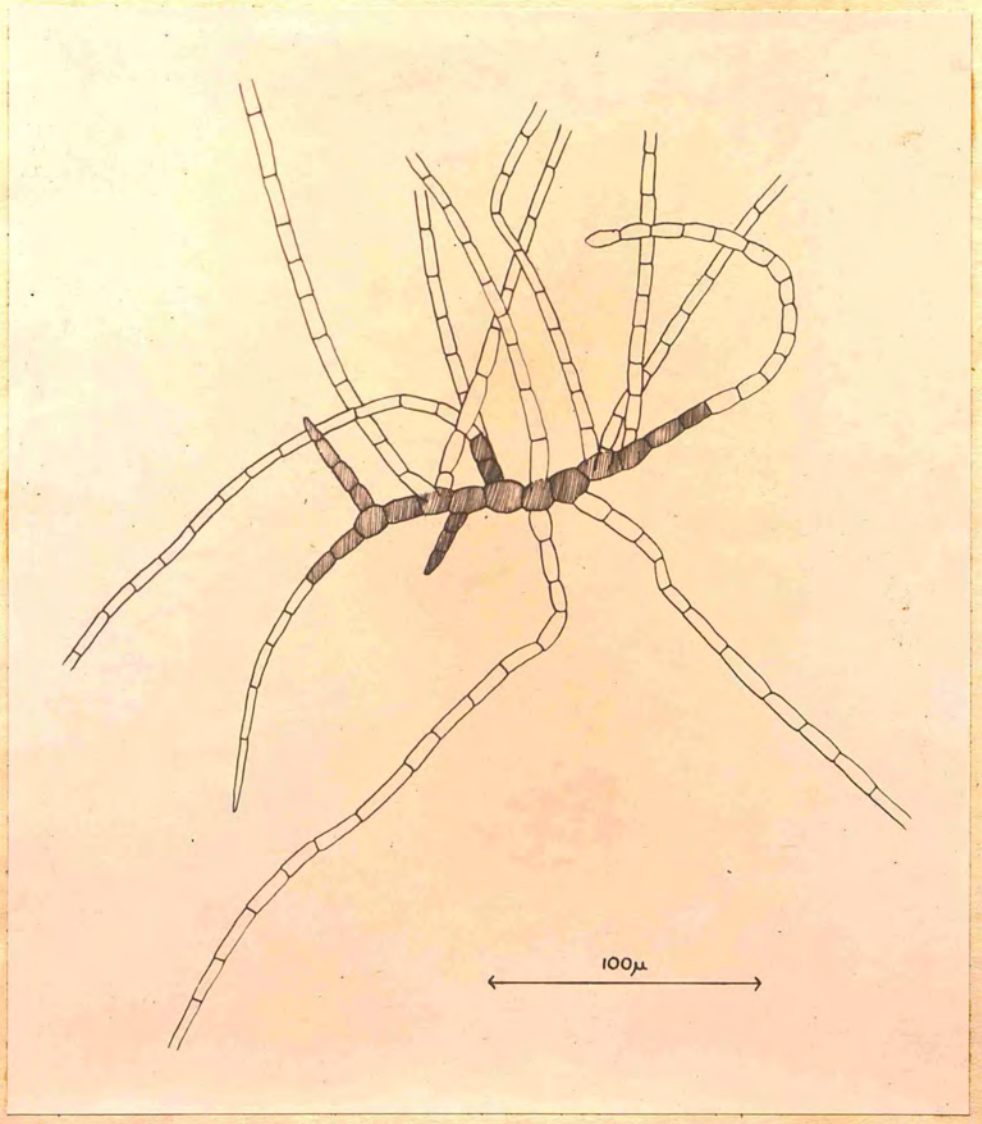

Figure 4.

The erect system of Stigeoclonium 300.

The apparently unilateral branching is a response to light - the secondary branches growing towards the highest intensity.

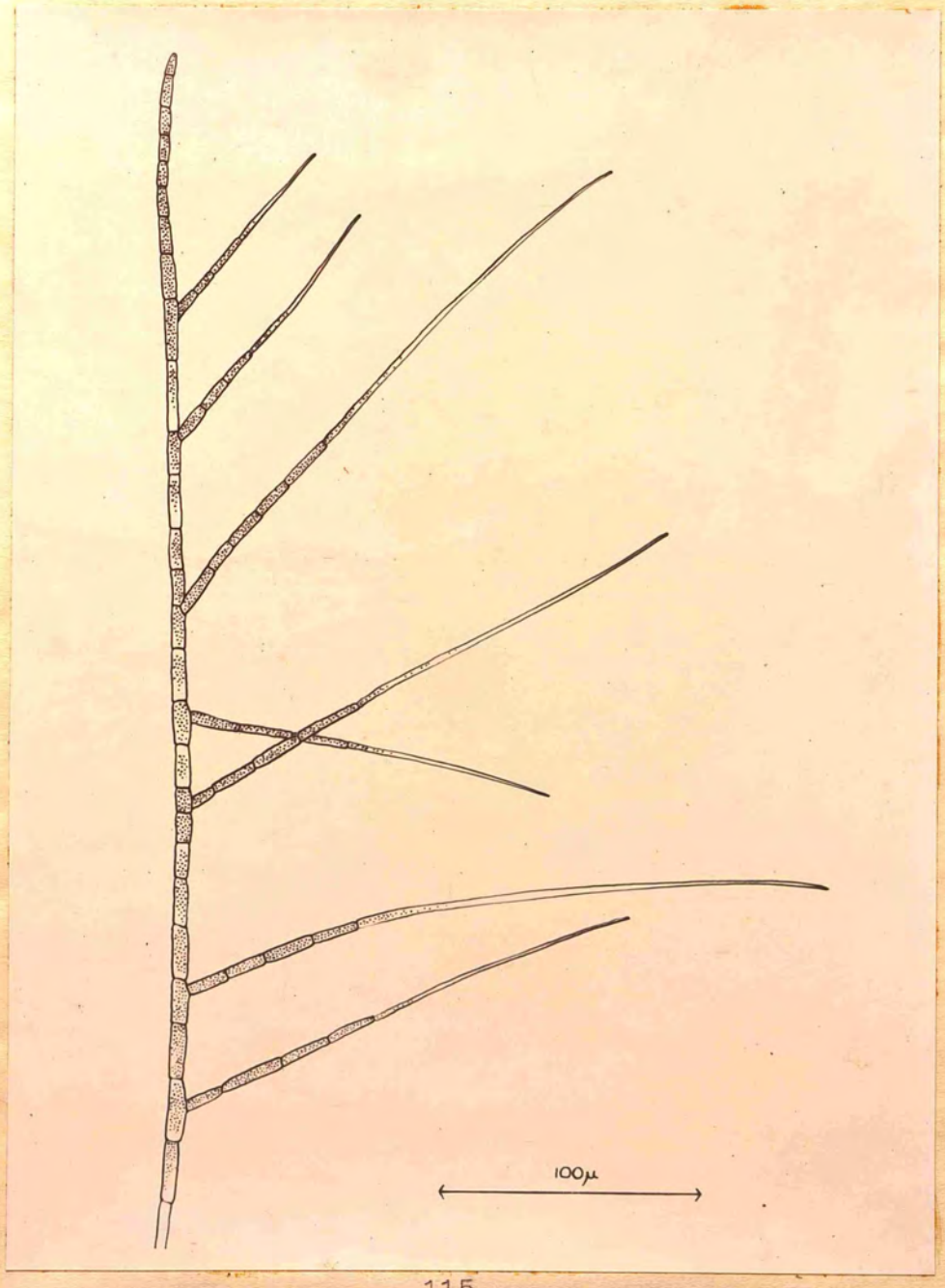

Figure 5.

The prostrate system of Stigeoclonium 100, one of the types used in the experiments on pages 16-18 and 91-94.
The prostrate part is shaded to distinguish it from the erect system.

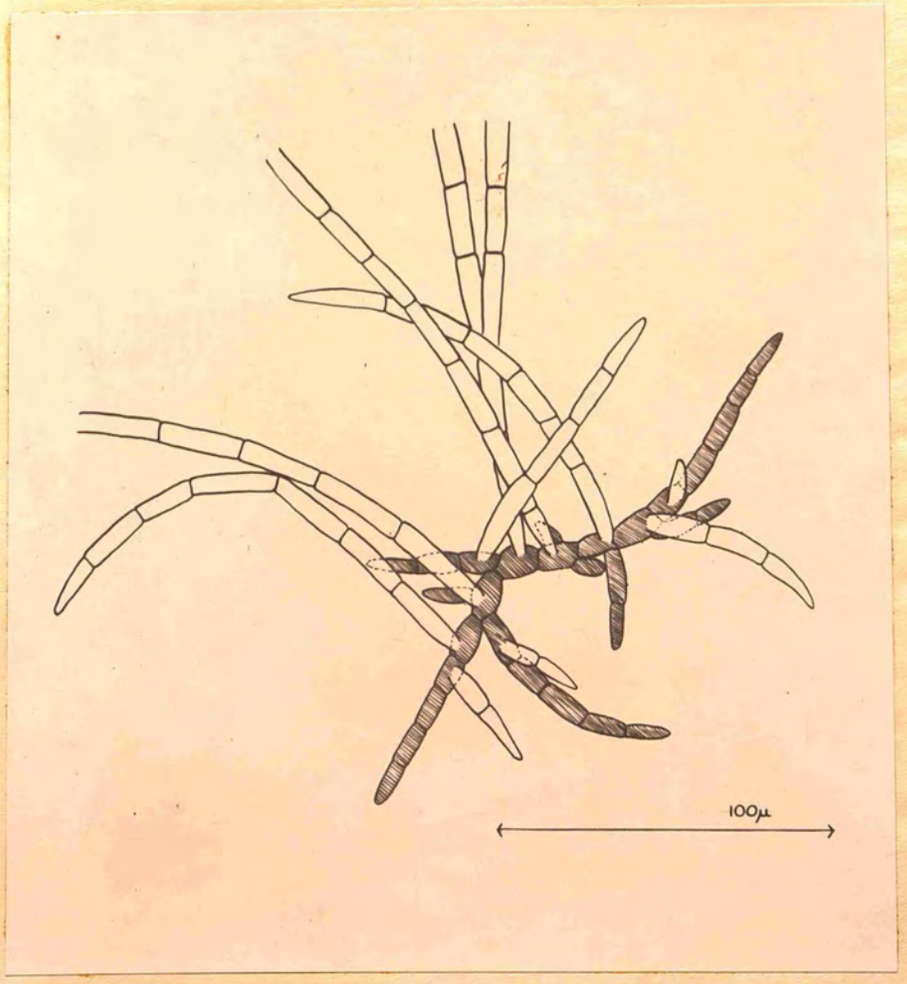

Figure 6.

The erect system of *Stigeoclonium* 100.