

FURTHER CONTRIBUTIONS TO

THE STUDY OF

BULINUS TROPICUS (KRAUSS)

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1. INTRODUCTION

Any organism is, in a sense, the morphological resultant of the physiological processes of which it is composed. Each of these processes is interrelated with the other functions of the organism, and the complex of processes cannot be separated from the environment. The functioning of an individual is determined by the relation of its constituent processes to the factors of the environment. Organisms often adjust their rate functions to the local conditions. Sometimes they are able to acclimatize if conditions are changed slowly, but frequently limits of tolerance have become genetically fixed in local races or strains, with or without morphological manifestation. It is, therefore, evident that taxonomists should not rely on morphological characters exclusively to distinguish taxonomic categories. Although morphological characters are still more useful than others, they should be supplemented to an ever increasing extent by physiological, ecological, ethological and geographical characters. This is not only necessary to permit the application of the biological species concept, but the great broadening of the use of new kinds of taxonomic character increases the reliability of classification.

It is becoming more and more evident that the very cursory identifications which have satisfied freshwater snail ecologists hitherto are not simply a minor incon-

venience but a serious source of error. Just as taxonomists should acquaint themselves with the ecology of the species that they are dealing with, the ecologist cannot escape being, to some extent, a taxonomist.

Populations of a single species that occur in different habitats in the same region are often visibly different and these local variants may either be ecological races or nongenetic ecophenotypes. The latter are particularly common in plastic species such as the freshwater snails. It is, therefore, obligatory that every possible character which could be used in discrimination should be investigated to furnish a sound basis for distinguishing the different categories below the generic level.

In the present study contributions are made towards establishing the taxonomic characters of Bulinus tropicus (Krauss) as regards the morphology, ecology and biochemical^{mical} composition.

Certain ~~facts~~^{aspects} of the morphology of this species have already been investigated by Stiglingh et al., (1962). There is great morphological similarity between members of the tropicus and truncatus groups. For example, agreement has not yet been reached concerning the group to which Bulinus sericinus Jickeli should be relegated. Watson (1925) lists it as a synonym of B. truncatus (Audouin) while Mandahl-Barth (1956) places it in the tropicus group, though he too, formerly regarded it as a member of the

truncatus group. Because of the similarity between members of these two groups any difference which can be detected between B.tropicus and B.truncatus is of importance.

Although the Planorbidae appear not to exhibit morphologically induced variations as clearly as in the case of the Lymnaeidae (Baker, 1928), they are not unaffected by ecological change. Therefore, as a secondary part of the project, an attempt was made to establish the extent to which certain abiotic and biotic factors may influence the snails. A fuller understanding of the range of ecologically induced variations should contribute to a more accurate delimitation of these polymorphic species.

Because of its extreme sensitivity chromatography has recently found a place among the techniques of chemical analysis employed in many fields such as industry, pharmacy, pathology, botany etc. The aid of this technique is now being increasingly sought also by zoologists. It has already been employed in various groups from the insects to the vertebrates, and in diverse fields including biochemistry, physiology genetics and taxonomy. It is therefore not surprising that the malacologist, with his not inconsiderable problems has also grasped at this technique. In the present instance therefore an analysis of the free amino acids in the haemolymph of B.tropicus was undertaken. The results could then be compared with the results obtained through similar analyses on other snails, and

any information obtained also has intrinsic value as another shred of information concerning the biochemistry of the organism.

2. MATERIAL AND METHODS

Breeding

For easy access to live material snails were maintained in the laboratory. The aquarium was a shallow wooden tray 1m 83cm long 61cm broad and 10cm deep. The tray was lined with a polythene material, coarse sand spread on the bottom, and then filled with water. Because the tapwater is heavily chlorinated it was passed through a beaker containing an aerator before being allowed to overflow into the aquarium. Slow circulation of the water was effected by means of an outlet at the end opposite to the inlet. The outlet was so placed as to ensure that the water level was maintained at 5cm. The snails were fed mainly on boiled lettuce but spinach was also readily taken. Occasionally a small amount of fish food was scattered on the surface of the water, and in addition the snails also browsed on the periphyton which developed in the aquarium.

Morphology and histology

Dissections were performed under a binocular stereo microscope while morphological detail, as also histology, were studied by making slides. The snails were narcotized in a 1% watery solution of nembutal and then, following the procedures of Pantin (1948), fixed in 5% formalin and embedded according to Peterfi's celloidin-paraffin method. A series of snails of various ~~series~~ ^{sizes} 1.5, 3.2, 4.7, 7.2, 9.4, 10.7, 12.0, 13.4, 16.0mm were sectioned at 4 micra and stained with Mal-

lory's triple stain (Pantin, 1948). It was found necessary drastically to cut the staining times to prevent overstaining, and this applied to snails of all sizes. Instead of staining for 15 and 75 seconds in acid fuchsin and Mallory's stain, the slides were stained for only 1 and 45 seconds respectively.

It seems from the literature, that Mann's methyl blue-eosin procedure (MBE) (Pantin, 1948) is but rarely used. In the present study the stain was used with good results. This stain is recommended by Pantin (1948) for the vascular and nervous systems and for insect sections. In the present instance the value of this stain for nervous tissue was appreciated, but it was also found to give very good results on striated muscle and to facilitate distinguishing between various types of gland cell.

For special histochemical procedures sections were cut at 4 or 8 micra. In the determination of carbohydrate containing proteins the PAS-technique was applied. Formalin-fixed material was used, as suggested by Campion (1961), but the fixation was performed according to the method of Pantin (1948). The McManus variation of the PAS procedure was employed as it is recommended by Pearse (1960) as generally being the most useful.

The presence of calcium, in the form of carbonates or phosphates was brought to light by treatment with an aqueous silver nitrate solution (von Kossa's method, in Pearse, 1960).

Campion (1961) advises brief immersion in the silver solution to prevent darkening of the background but this was found to be in no way a hindrance and, in some cases, proved useful in that certain von Kóssa-negative glands stained brownish and could thus be distinguished from the surrounding tissues.

Feeding trails

The tracks left by snails when feeding have long been observed on the alginated glass walls of aquaria but in the present project an attempt was made to find some artificial material as a substitute for the algal growth. The advantages entailed in using such a medium are a homogeneous coating on the slide, and the fact that a number of slides may be prepared and then stored till required. A gelatine solution was poured over the slides and then allowed to dry. The slides were subsequently placed on the bottom of small glass dishes containing sufficient water to cover a snail, and snails were then placed on the slides to feed. The gelatine surface proved too slippery; the snails toppled over or were pushed backwards at each rasp. A boiled starch solution ($\pm$ 1gm/200ml water) poured over the slides was found to be admirable, especially as the slides may easily be stained with iodine before examining the traces. By this means the light areas of the rasps are thrown into sharp relief against the dark background. The slides were then placed in an enlarger and direct prints were thus obtained.

Ecology

The snails studied were all obtained from habitats in or around Potchefstroom. Initially 21 habitats were visited but this number was later reduced because some proved unsuitable for further observation, and also, sampling became too time consuming for a project not primarily ecological. In the latter half of 1963 regular visits were paid to only five of the habitats.

For three years collecting trips were undertaken at intervals of roughly one month to form a general impression of the seasonal changes occurring both in the habitats and the snail populations. On these trips notes were made on the physical conditions in the habitat, the vegetation, the fauna, and samples of the snails, and sometimes also the associated fauna, were taken. In this manner over two hundred snail samples were collected.

Various aspects of sampling are discussed critically by Olivier (1956) and Muirhead Thompson (1958) and the subject is thoroughly reviewed by Hairston et al (1958). A number of difficulties including some of those mentioned by the above mentioned authors, were encountered, the chief being the enormous fluctuations in the size of the habitat and populations. Consequently the samples do not reflect the true density of the population and this should be borne in mind when studying the histograms which merely reveal the size range of the population and give some indication of its density.

The practice of measuring the snails in the field and then replacing them in the habitat was not followed as this would have been too laborious, especially during the first part of the study when the number of habitats visited was great. The apparatus used in sampling was that described by van Eeden (1960) and the snails were initially narcotized and fixed according to the methods recommended by the same author (1958). In the latter part of the investigation the snails were merely dropped into 70% ethanol containing 5% glycerol. The shells were measured under a binocular stereo microscope, using a micrometer grid eyepiece.

3. EXTERNAL MORPHOLOGY

Upon hatching the young snail is already housed in a tiny shell which is about 0.8mm in length. From underneath this shell the head-foot projects but slightly, in contrast to the condition in mature snails in which a relatively large part of the head-foot is normally exposed. The young snails differ from mature snails also in the proportions of the body. The head is relatively larger, the tentacles more squat and the visceral mass consists of a smaller number of whorls and these overlap each other to a greater extent than in fullgrown snails (see later).

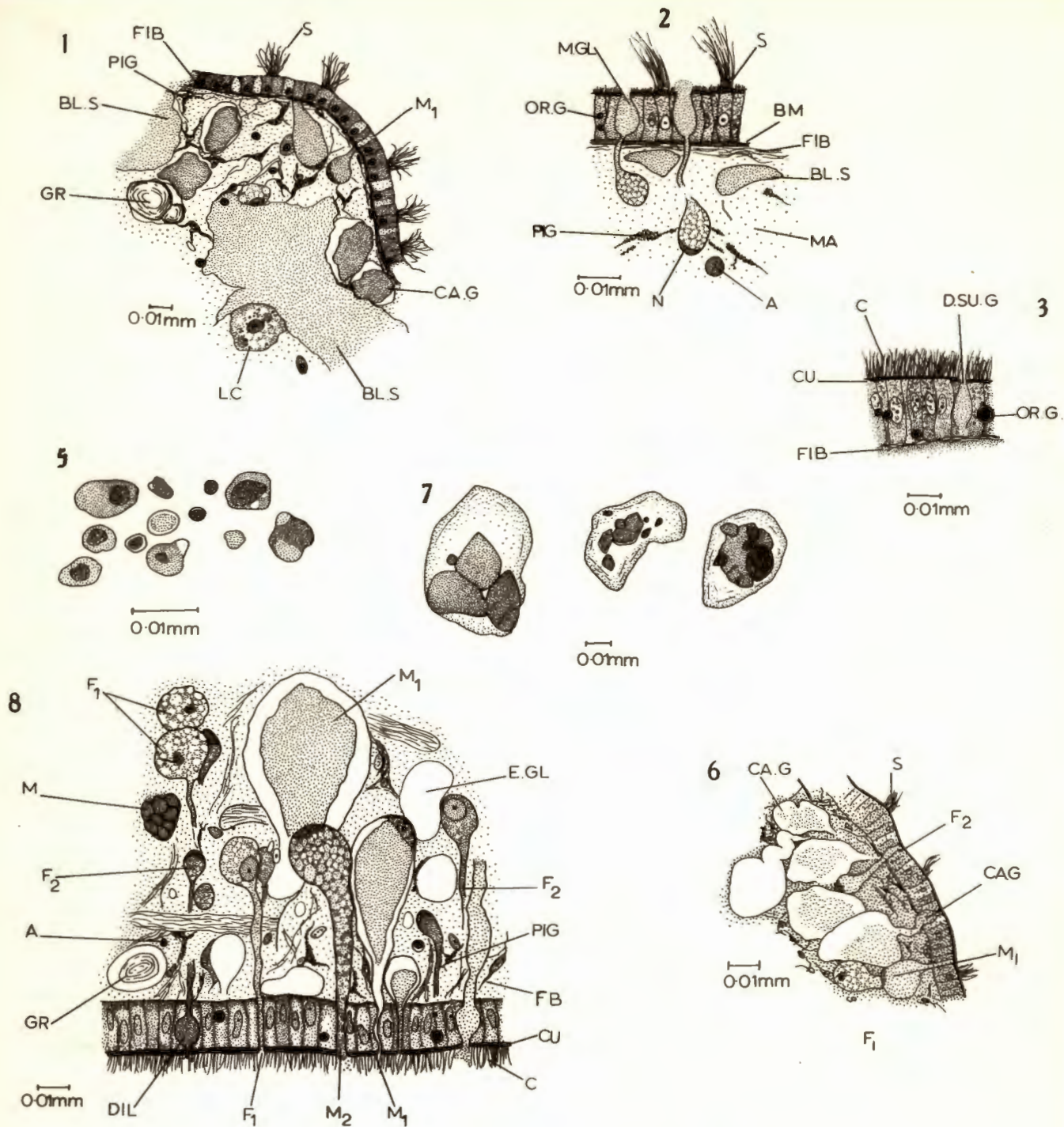


FIG. 1. DORSAL SURFACE OF TENTACLE, NEAR BASE.
 FIG. 2. CELLS OF TENTACULAR GROOVE SHOWING SUBEPITHELIAL GLANDS.
 FIG. 3. EPITHELIUM OF ORAL LOBE.
 FIG. 5. AMOEBOCYTES.
 FIG. 6. CALCIUM GLANDS FROM LATERAL BODY WALL.
 FIG. 7. GRANULES CONSTRUCTED AROUND CORE.
 FIG. 8. SOLE OF FOOT, TO SHOW VARIOUS TYPES OF GLAND.

3.1 THE HEAD

In the following chapters (unless otherwise stated) all descriptions of the colours assumed by the tissues refer to slides stained with Mallory's triple stain.

The most conspicuous feature of the head is the pair of tentacles. These are anterodorsally situated and increase greatly in relative length and slenderness during development. The tentacles of B. tropicus are in almost continual movement being slowly waved about in the water and thus testing a large part of the immediate surroundings of the snail. The function of these organs is two-fold. The greater part of the tentacle is sensitive to mechanical stimuli while laterally, the base of each tentacle is modified as previously described (Stiglingh et al., 1962) to receive chemical stimuli. The epithelial cells clothing the tentacle are columnar and possess large centrally to basally placed nuclei (fig. 1). Scattered among these cells are the sensillae. They protrude slightly beyond the other cells and are somewhat broader apically than basally. The nucleus of such a cell is larger than that of the other epithelial cells and is roundish. It is poor in chromatin but contains a conspicuous nucleolus. These cells possess a thick orange refractile border and bear numerous long cilia.

The proximal region of the tentacle differs from the distal in having slightly taller cells, and in having fewer sensillae. Such a distribution of the sensillae is in accordance with the functions of the tentacle, as it is the distal

end which is most exposed to mechanical stimuli and it should therefore be richly supplied with sensory cells.

Among the epithelial cells occasional clear spaces are visible. Initially these were taken to represent non-staining goblet cells but in PAS-stained material it is clear that some, perhaps all, the lighter areas are the distended ducts of sub-epithelial gland cells of the types M_1 and F_3 described later.

The unspecialised epithelial cells of the tentacles do not bear cilia and consequently the cilia of the sensillae must be held responsible for creating the water current surrounding the tentacles. The presence of this current is easily established by observing the movements of small particles suspended in the water for they are caught up in the currents and are swept down the length of the tentacle to its base where the olfactory cells are concentrated.

The cells lining this olfactory groove are columnar, have large centrally to basally placed nuclei, and the cytoplasm in the basal and apical parts of the cells stains differently (fig. 2). This is due to the accumulation of secretory material in the apical parts of the cells. The secretion is mucus, intensely basophilic, and gives only a light PAS-positive reaction. A cuticle is present in this region and it is probably the presence of this cuticle that imparts to the groove a velvety appearance. The cells bear cilia, though this is not clear in all sections. Scattered among these cells are sensillae.

The tentacular epithelium is underlain by a conspicuous basement membrane which, as found in the other basement membranes, stains blue and is therefore composed of collagen or some related substance. It was found that the basement membranes of Anadara trapezia (Deshayes) exhibit a strong PAS-positive reaction (Sullivan, 1961) but in B. tropicus this was not so. It was noted, however, that in many instances the basement membranes were rich in calcium which was present in finely granular form, as revealed by the von Kóssa technique.

The olfactory organ is underlain by an aggregation of nerve cells which form a ganglion surrounding the groove. There is apparently no special name for this organ but the use of the term rhinophore for this structure in B. truncatus (Audouin) (Demian, 1960) seems to me an unfortunate choice, as the term is strongly associated with the olfactory organs of the Opisthobranchia.

Transverse sections show that the tentacle has a central blood space surrounded by smaller spaces (fig. 15 of Stiglingh et al., 1962) of which there may be as many as eight in older snails. The central cavity is surrounded by longitudinal muscle fibres, connective tissue and numerous stout, longitudinal nerves. The blood spaces act as a haemoskeleton in these long structures which are otherwise without support. Besides lending rigidity to the tentacles, it is concluded that the spaces act antagonistically to longitudinal muscle fibres in effecting changes in the length and position of the tentacles.

Increase in the blood volume, accompanied by a relaxation of the longitudinal muscles should result in a lengthening of the tentacle, while a decrease in blood volume accompanied by contraction of the longitudinal fibres would have the opposite effect. Differential contraction of the scattered peripheral muscle fibres could be responsible for the bending movements.

Median to, and at the base of the tentacles, the pair of eyes is visible as two black spots. They are not situated on the surface of the body but are embedded in the tissue through which they are clearly visible. The tissue overlying the eyes is free of granules and almost devoid of pigment cells which would interfere with the incidence of light rays.

The mouth of the snail is not visible from above because it is overhung by the two oral lobes. These are two flaps which meet in the midline just dorsal to the mouth. Their lateral borders are attached to the body wall in the region between the head and foot. The flaps project anteroventrally, being long enough to overhang the anterior margin of the foot. During locomotion the lobes are lightly brushed over the substratum and assist the tentacles in keeping the snail informed on its surroundings. They are capable of a fair amount of contraction and expansion and, as in the case of the tentacles, this is brought about by the antagonistic effect of pressure in the blood spaces, and contraction of the muscle fibres.

The epithelial cells of the oral lobes are tall, have large centrally to basally placed nuclei and secrete a mucus

which is fairly strongly PAS-positive. Along their borders the lobes are very richly ciliated (fig. 3). This ciliation most probably represents a dense aggregation of sensillae in this area. This is deduced from the fact that the cilia are much longer than normal. According to Boettger (1944) the cilia of these cells differ, in the Basommatophora, from those occurring on the rest of the body, in that they are usually static and come into motion only when stimulated. This author is also of the opinion that the sensory cells are apparently the receptors for both mechanical and chemical stimuli. A similar condition was found to obtain in the prosobranchs some of which have tentacles with an exceedingly large number of immobile cilia (Fretter and Graham, 1962) which are considered to be touch-taste receptors (Burdon-Jones and Desai (in lit.) quoted from Fretter and Graham, 1962).

The outer surface of each lobe is clothed with a cuboidal epithelium possessing numerous sensillae. The inner surface of each lobe shows differences in the median and lateral portions. The median part of the lobe possesses a columnar epithelium and there are traces of a delicate cuticle as a protection against chafing during the prolonged movements of the lobes. As in the tentacular groove, the cells secrete mucus, the apices, and also the cuticle being weakly PAS-positive and the cells staining intensely with methyl blue. The usual sensillae do not occur in this region but there seem to be sparsely scattered sensillae which bear only a few cilia and which

differ from those of the other type in being shorter and straight. More laterally the cells of the inner surface of each oral lobe become cuboidal, leaving columnar cells only near the border of the lobe. In this region the border sensillae no longer form a continuous sheet but lie scattered among the columnar cells. This is to be expected as it is the median part of each lobe which is most exposed to stimulation. No clear indication of goblet cells was seen, and as in the tentacles, the clear spaces between cells represent the ducts of deeper lying gland cells (fig. 3). The epithelium rests upon a clearly visible basement membrane and the central part of the lobes is filled mainly with glandular cells, nerve cells, muscle fibres and connective tissue.

Through the thin inner epithelial surface of the oral lobes two sets of muscle fibres may be seen. Superficially there are a few delicate strands running parallel to the border of the lobe while in the deeper layers many radial strands may be seen extending to the edge of the lobe. As the snail moves forward a series of small local contractions may sometimes be seen in the oral lobe edge, suggesting that groups of radial fibres are stimulated to contract simultaneously.

The gland cells are similar to those found in the foot, differing only in the lighter purplish red colour assumed after PAS-treatment (fig. 4). This slight discrepancy in colour is the only difference detected between these oral lobe glands and the glands characteristic of the foot and

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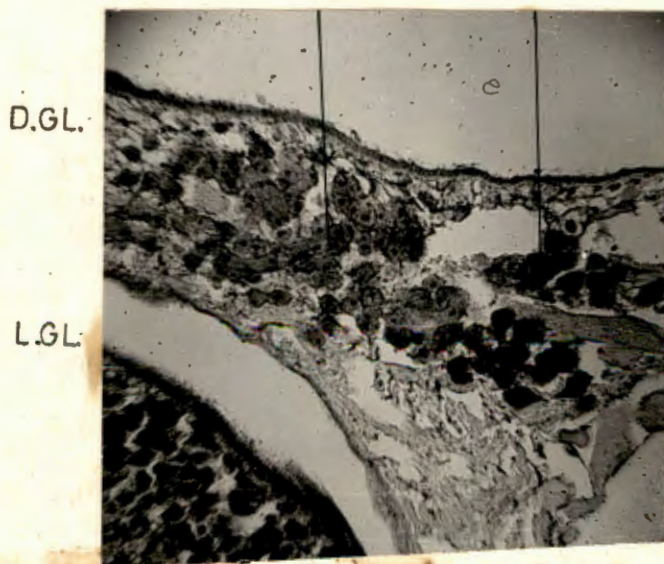


Fig. 4. Transverse section through oral lobe to show the two types of gland.

therefore both types are designated as F_1 . The body of the gland is large and, although irregular in form, is more or less round and communicates with the exterior by a long duct. The nucleus is large and centrally placed and in sections stained with PAS it is not clearly visible because of the heavily staining secretion. This mucoid substance stains only very faintly with Mallory's which is said to stain mucus blue (Pantin, 1948); the substance does not stain with MBE and it therefore cannot be described as either basophilic or oxyphilic. The von Kóssa test does not reveal the presence of calcium in these cells. The MBE technique reveals the large, basophilic, granular nucleus clearly outlined. The prominent nucleolus is purple, indicating that it is amphophilic. The ducts of the glands are narrow and of uniform thickness. They show up distinctly after PAS-treatment and are mostly to be seen passing singly to the exterior, discharging almost exclusively on the inner surface of the lobe. In some cases the glands are arranged in groups of varying degrees of compactness but in spite of this arrangement the ducts either pass to the exterior singly or as a thick rope, but no fusion of the ducts could be discerned. The many separate openings of these glands ensure a more uniform distribution of the secretion.

The glands stain intensely after PAS-treatment, those in the lower part of the lobe being lighter (an intense pink) than those higher up in the lobe which tend to be purple, though some pink glands still persist in the upper region.

Another conspicuous feature of the lobes is the nerve cells which are arranged rather loosely to form a ganglion along the border of the lobe. Associated with the ganglia are very stout nerves. Several blood spaces also occur in this region, probably playing a major role in the continuous movements of the lobes. The blood contains numerous amoebocytes (fig. 5) which, may also be seen amongst the connective tissue cells where they wander, performing their functions.

The muscle fibres are slender and the rest of the lobe is filled in by connective tissue containing pigment cells. More posteriorly there are gland cells which are found only a short distance below the epithelium and are designated type M_1 . These cells occur far less frequently than do the previous type and do not occur in loosely packed groups but are separate from one another while the body of the cell is club-shaped. These cells possess large nuclei which become displaced towards the base of the cell as the amount of secretion increases and in mature cells hardly any cytoplasm is left surrounding the nucleus so that, except for the duct, they rather resemble giant fat cells. These cells, in contrast to the F_1 type contain a substance staining blue with Mallory's (indicating mucus); yet they give a negative reaction to methyl blue which is also a mucus stain. They are not rich in calcium, but in some of them traces of calcium are visible. The glands stain a pale pink upon being treated with PAS.

The F_1 and M_1 glands agree with the unicellular glands described by Simroth and Hoffman (1928) who name them sole glands and mantle glands respectively owing to their dominance in those regions. These M_1 glands open on the outer surface of each lobe.

When viewed from the ventral surface of the body, the mouth may be seen on the anteroventral part of the head. It is a smallish longitudinal slit which has at right angles to it an anterior and a smaller posterior, slit. In certain snails a distinct yellowish border is visible along the mouth. Such yellowish, velvety areas are also visible along the internal surfaces of the oral lobes and olfactory groove at the base of the tentacle. I feel that the velvety appearance and the yellowish colour are due to the cuticle and underlying ganglia respectively.

Mottling

A feature of the ^egeneral surface of the headfoot is the occurrence of a fine white mottling over most of this region. The mottling is caused by small white irregular flecks of some opaque substance. As pointed out to me by Prof. Lever of the Free University, Amsterdam, there are notable aggregations of these flecks just median, and slightly anterior to the eyes where they form two conspicuous white patches. They are also densely packed in the dorsal tentacular process and appear more numerous on the dorsal side of the tentacle than on the ventral

surface of that organ.

The amount of flecks varies from snail to snail but there is usually a fair sprinkling over the general surface of the headfoot. The flecks are much reduced in number on the sole of the foot, decrease in the nuchal region, and disappear at the junction of the headfoot and visceral mass. They are rare in the oral region, are absent from the immediate vicinity of the eye and from the tentacular groove. The flecks are not restricted to the headfoot and occur, though generally in reduced numbers, in the collar and also the gill.

This white mottling has also been noted in other snails eg. the local L. natalensis Krauss in which the flecks are, however, much larger, and their distribution different. It is interesting that in this species too, two aggregations occur in the eye region, but in this instance just median to the eyes. No such white patches were observed in Physa mosambiquensis Clessin. White mottling has also been reported to occur in B. truncatus (Audouin) (Demian, 1960), the Planorbidae generally ^{Abdel-} (Malek, 1952) and L. stagnalis appressa (Say) (Carriker, 1945). The last mentioned author, however, does not describe the external parts of the snail but mentions the mottling over the liver and all the other organs of the body. This mottling he ascribes to the presence of calciferous concretions in the connective tissue and states that these were also found in Helix by other workers. He is

probably referring to the granules discussed below, which are not to be confused with the white flecks described in B. tropicus.

Sullivan (1961) records whitish patches in A. trapezia and states that the distribution of the mottling coincides with the occurrence of translucent granules found in the connective tissue. From the description it is not clear whether it is to be inferred that the granules cause the white mottling or whether the distribution of granules and mottling coincides. The mottling in B. tropicus is caused by opaque white flecks in the more superficial layers of the tissues. These flecks consist of irregular accumulations^u of a granular substance which is mostly surrounded by a small space (fig. 6). It was for a long time not realised that these are glands but I now feel convinced that this is the case, for sometimes a narrow clear space, the duct of the gland, may be seen leading to the body surface. Nothing is known concerning the nucleus of these glands and they do not have the well defined appearance of the other glands of the foot or mantle. That the contents of the glands is calciferous is obvious from the fact that after being stained with Mallory's, they assume the same colour as the calcareous concretions found in the tissues, and they react positively to the von Kossa test. They probably correspond to the calcium glands described by Simroth (1928) but are different in structure.

Visible through the semi-transparent tissues of the snail, and occurring in the deeper layers, are translucent granules which are easily distinguishable in the living snail, and also in sections. These granules are more plentiful than the calcium glands and seem to be especially numerous in young snails. They are clearly visible in the collar and in the connective tissue surrounding the stomach. They occur also in the gill. In sections they are seen to consist of a number of concentric layers, sometimes formed around a discrete core (fig. 7). Such calcareous granules, either single or compound, and consisting of several concentric layers, were also found in Helix pomatia by Kisker (Simroth, 1928). In contrast to the calcium glands the translucent granules are refractile and therefore sharply outlined. In some cases concentric rings may be seen around a single core while in others the granule may be composed of several small granules which are initially separate but later coalesce to form a large irregular granule. In sections the calcium glands are a pale yellowish colour and the translucent granules are often of the same hue. Sometimes, however, the granules stain differently, those of the foot being yellowish while those in the region of the alimentary canal are blue, and in the mantle region greenish; but granules of a certain colour are by no means restricted to the regions mentioned above. In certain sections stained with MBE some granules are blue and others white, while yet others are composed of

both these colours, the core being blue and the surrounding material white. In spite of this great range of colours, the granules seem to me to be composed of a calcareous substance and the various colours they may assume on a single slide are probably caused by the incorporation of various other substances in the granule.

As in the case of A. glabratus (Say) (Pan, 1958) these granules stain light pink with PAS, showing that they are probably of the same composition in the two species. The positive reaction to the von Kóssa test indicates that they are either calcium carbonate or calcium phosphate compounds. The calciiferous nature of these granules explains their occurrence in the connective tissue surrounding the alimentary canal, (whence the calcium is absorbed) and also in the collar where calcium is needed in the fabrication of the shell.

The granules dissolved in glacial acetic acid, with the evolution of gas, and this points to their being composed of calcium carbonate. By contrast the calcium glands disappeared upon being exposed to glacial acetic acid but no gas liberation was observed during this process. They may therefore be calcium phosphate or some other substance which is either dissolved or made transparent by glacial acetic acid.

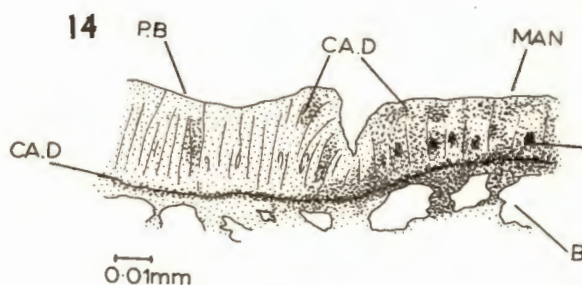
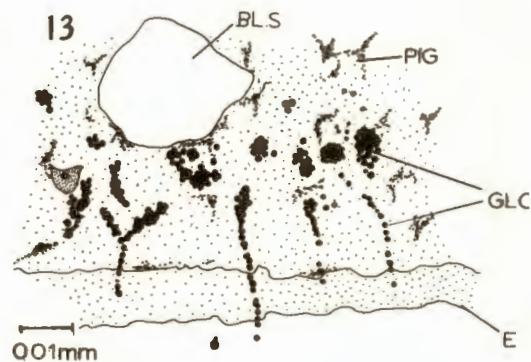
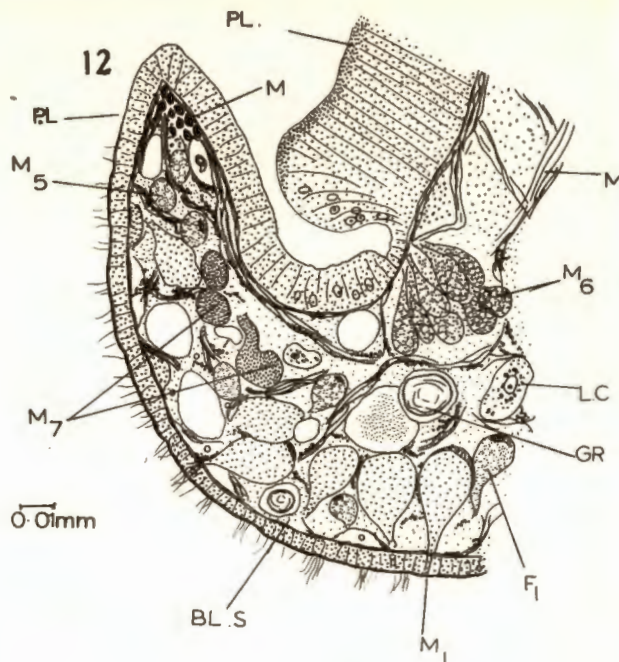
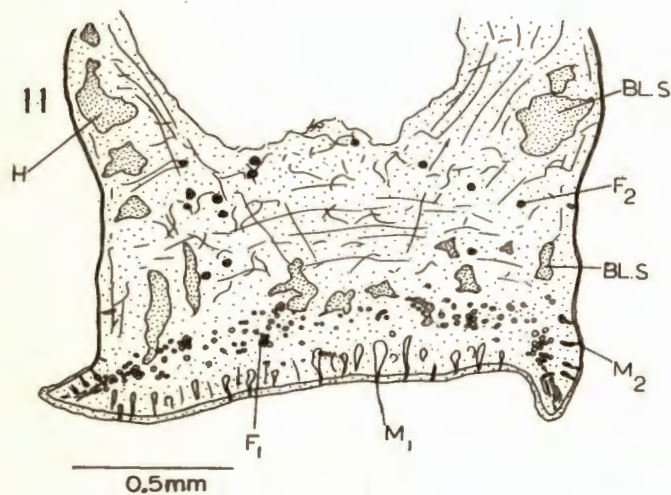
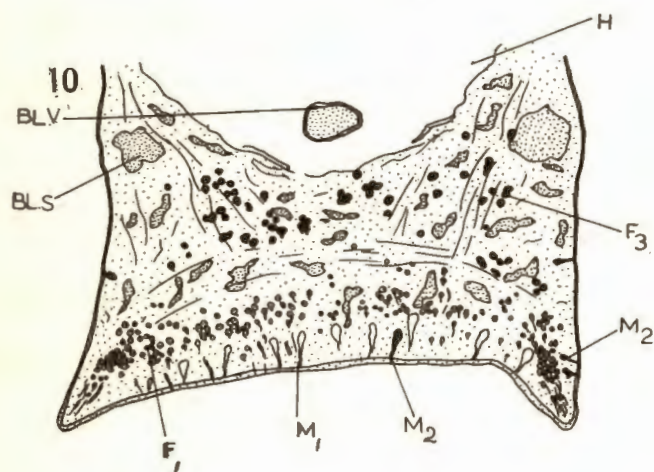
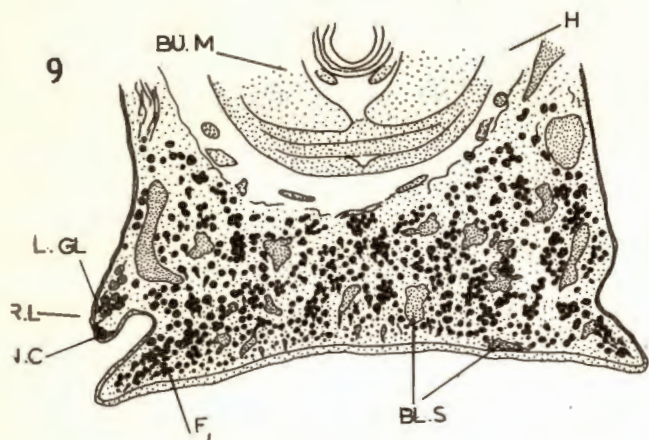
The translucent granules of B. tropicus are apparently not homologous with the translucent granules of A. trapezia as they are very much larger and are not formed in the manner described by Sullivan (1961). This author and Atkins (1936, quoted from Sullivan, 1961) who worked on Glycymeris glycymeris (Deshayes) (Pelecypoda) found the distribution of the granules, and mottling, to coincide with those parts of the animal exposed to light, and Sullivan (1961) suggests that they are formed in response to illumination. If this is partly true, (for there is a general agreement between mottling and areas exposed to light), the matter is not so simple in B. tropicus. The formation of the granules in response to light would not explain the conspicuous localisation of opaque patches at the base of the tentacle, or in the dorsal tentacular process. The formation of these granules as a light response seems to be precluded also by the fact that they are formed in the deeper layers, and are concentrated in the connective tissue surrounding the alimentary canal.

3.2. THE FOOT

The foot is an elongated organ, bluntly rounded anteriorly and more pointed posteriorly, though not as sharply so as in P. mosambiguensis. Histologically the structure consists of a framework of interlacing muscle fibres and connective tissue among which many unicellular glands and blood spaces occur.

The general appearance of the sole is the same as that of the rest of the body surface except for the fact that fewer white flecks are present. This part of the foot has an epithelium of columnar cells with a strongly refractile, orange-staining border and well developed cilia which also, to some extent, take up the orange stain. The nuclei are large and do not stain deeply. Their position in the cell seems to be variable. The cells are lightly PAS-positive and stain intensely with MBE. They are strongly basophilic. The highest degree of secretory activity occurs along the margins of the footsole, and also in a narrow band on the lateral surfaces of the foot, just dorsal to the sole. In the epithelium of the foot and other parts of the body eg. tentacular groove and oral lobes etc. there are orange staining globules of various sizes. Sullivan (1961) reported amoebocytes wandering in the epithelium of A. trapezia, but if these globules in B. tropicus are amoebocytes, they must be laden with some substance which take up the orange stain, for the amoebocytes normally stain lilac or light orange. In the globules no nuclei^s can be seen, but this is in any case difficult to observe in the amoebocytes of B. tropicus. These globules are thought to be large drops of some secretion, as the same kind of globule is observed also in the uterine lining which is described later.

The epithelium of the sides of the foot consists of cells which are less tall than those of the sole, The cuticle



FIGS. 9-11. DEPICTING CHANGES IN FOOT STRUCTURE IN AN ANTERO-POSTERIOR DIRECTION.

FIG. 12. STRUCTURE OF COLLAR.

FIG. 13. COLLAR IN PNEUMOSTOME REGION TO SHOW M_4 GLANDS (MEE STAINING).

FIG. 14. CELLS OF PALLIAL BAND AND GENERAL MANTLE SURFACE TO SHOW CALCIUM. (VON KOSSA STAINING).

is less well-developed and the cells are not uniformly ciliated, only scattered sensillae being present. The nuclei are conspicuous and, as in the epithelium of the sole, orange globules are to be seen in the cells. The epithelium does not rest on a well developed basement membrane.

In the anterior part of the foot which lies ventral to the snout, the epithelium is unciliated. The sides of the foot are ciliated in the area from the anterior margin posterior to the level of the attachment of the oral lobes. Posterior to that point only a very narrow strip of finely ciliated cells is found adjacent to the footsole. The more dorsal regions of the foot are unciliated and possess occasional sensillae. Dorsally the cells are no longer columnar but become cuboidal or flattened.

The narrow strip of ciliated cells along the edge of the foot becomes reduced more posteriorly till, in transverse sections at the level of the base of the tentacles, only the sole of the foot is ciliated.

The anterior part of the foot is filled mainly with gland cells packed by connective tissue (fig. 8 and 9). The glands in the anterior region of the foot are mainly of the type already described as occurring in the oral lobes, viz., F_1 . Occasional large club-shaped glands, M_1 , also occur, especially in the region posterior to the tentacles. These are the largest unicellular glands occurring in the body.

They are rather stout glands with robust walls, and are filled with a blue-staining secretion. Application of the PAS-technique reveals that the substance is lightly PAS-positive. This secretion sometimes appears to be homogeneous and in other glands it is a lighter blue and is finely granular. I am inclined to think that these may be merely sections cut through the central portion, and through the edge of the gland, respectively. Thus the sections of glandular secretion may vary from 4 micra to a fraction of a micron. The nucleus is basally situated and is very large. This nucleus is coarsely granular in appearance, rich in chromatin and stains purple except for the conspicuous nucleolus which stains orange.

Glands having the form of F_1 and M_1 are found also along the sides and upper surface of the foot but in these situations they are many times smaller than the types described above. The small M_1 glands are in other respects identical to those of the sole, but the small retort-shaped glands differ from the F_1 in three respects, other than size (fig. 8). PAS-treatment shows that these small glands stain a deep purple while the F_1 stain a reddish-purple. The nucleus of the small glands is basally situated and that of the F_1 glands centrally. The small glands also give a slight reaction to the calcium test. These three differences seem to warrant the little glands being designated F_2 . These F_2 glands are also encountered among the other glands of the sole where

they are present in great numbers.

Two additional types of gland are distinguished in the foot. More posteriorly a difference becomes apparent between the dorsal and ventral foot glands. The ventral glands are of the F_1 type while the dorsal glands (F_3) are larger than the F_1 , less closely packed together and stain more deeply with Mallory's and MBE. The latter staining method clearly shows up the larger and more deeply-staining nucleus. In contrast to the F_1 glands they are clearly calcium-containing.

The other type of gland encountered in the foot was initially overlooked because it resembles, in form, the clubshaped M_1 glands and the secretion is globular. In PAS-treated material these glands are a startling purplish red, being so dark as to appear almost black. When the other elements on the slide are suitably stained, these glands are always overstained and are thus easily distinguished from the M_1 glands which stain a pale pink. These intensely staining glands are termed M_2 and occur also on the sole, though in far smaller numbers than the M_1 type. They also occur on the sides of the foot, reduced in size, except in a band just above the sole, in which position they are of normal size and attain their greatest frequency (fig. 10).

Crofts (1929), quoted by Fretter and Graham, (1962), found the mucous secretions of Haliotis to be dissimilar in quality, and distinguishes between a tacky and a slippery mucus.

This invites speculation as to the possibility of the secretions of glands of the F_1 and M_1 type differing in texture. If such a disparity exists, the F_1 glands possibly serve to lubricate the sole while the M_1 glands secrete a more viscid substance for adhesion. For several reasons the secretion of a viscous slime is attributed to the M_1 glands rather than the F_1 . Because B. tropicus is an active snail it is supposed that the main function of the foot glands will be lubrication and not adhesion, and the F_1 glands by far outnumber the M_1 glands in the foot. Also, it is the F_1 type of gland which occurs in the buccal mass and oral lobes where one may suppose the function to be lubrication. Furthermore, the greater part of the glands in the collar are of the M_1 type and it is normally these glands which are responsible (in Pulmonata) for the formation of the epiphragm.

In snails retracted deeply into the shell, it is apparent that the rounded, inner part of the collar is greatly distended with blood and it is this swollen part of the animal which is exposed to the air. Between the swollen edges of the collar only a narrow strip of the foot is visible and it may be that in a snail still further retracted into the shell, the foot may be completely covered. The exposed surface of the collar is well-equipped to provide a copious mucous cloak for itself, and in instances of exposure of part of the foot, the sole glands could probably perform this function.

In the loose connective tissue which has a matrix staining light blue there are many amoebocytes but Leydig's (see later) cells are rarely encountered. From the anterior to posterior part of the foot the F_1 and F_2 types of gland cell are progressively reduced in an antero-posterior direction until, at the level of the female aperture, there is only a relatively thin layer of F_1 gland cells above the sole of the foot, and sparsely scattered F_2 glands in the central part (fig. 10). The connective tissue in this region is very loose, and amoebocytes, refractile granules, Leydig's cells, stellate cells and pigment cells occur. The M_1 gland cells are large in this more posterior part of the foot. The foot retains this structure more posteriorly till at the level of the cerebral ganglia, where the F_1 gland cells are still further reduced in number, the connective tissue still looser, and distinctly fibrous, and the F_3 gland cells of the central region have practically disappeared (fig. 11). The M_1 type of gland still persists, and there are numerous Leydig's cells and small blood spaces. At the level of the pedal ganglia the F_1 type of gland cell disappear almost completely above the sole, and the M_1 type is also diminished in number. Posterior to the pedal ganglia the structure of the foot remains more or less as described above. The M_1 glands occur along the whole length of the sole, also on the upper surface of the foot of the posterior region, and occasional F_2 type gland cells also occur. The upper sur-

face of the foot has an unciliated cuboidal epithelium with sensillae. The epithelial cells of the sole seem to remain the same in the posterior, as in the anterior region of the foot, except for the fact that the length of the cilia diminishes antero-posteriorly. In the posterior part of the foot the muscles are well developed, especially dorsally where longitudinal strands predominate.

3.3 MANTLE AND ASSOCIATED STRUCTURES

Lining the aperture of the shell and slightly overlapping its margin is a thickened mantle edge, termed the collar. This is fused to the body at the junction of neck and visceral mass. The dorsally situated chamber between the mantle and visceral mass is termed the mantle cavity, and the space between the collar and the neck has been termed hypopeplar cavity.

At the left hand side of the animal in the sharp angle of the shell aperture, the pseudobranch, anal lobe, pneumostome and pulmonary siphon are situated. The pneumostome leads into the mantle cavity in which the kidney, heart and mantle ridges may be seen. The structure and mutual relation of these parts have been described in a previous paper (Stiglingh et al., 1962) and only those aspects neglected in the previous paper, will here be touched upon.

3. 3 1 The Collar (fig.12)

That part of the mantle forming the roof of the mantle

cavity is relatively thin but distally it thickens to form the collar which is smoothly rounded on the surface not in contact with the shell. In the following account the term outer surface of the collar and mantle, will be used to denote the surface in contact with the shell; and inner surface of the collar and mantle will be used to denote the surface not in contact with the shell. In B. truncatus the edge of the collar closely follows the edge of the shell aperture and never extends beyond the inner or outer lips of the peristome (Demian 1960). In B. tropicus the extreme edge of the collar is attenuated and slightly reflected over the labrum (fig.12). Adjacent to the collar edge and lying on the outer surface, is an elevated ridge, the pallial band ("bandelette palléale" of the French authors). The simpler term mantle ridge is avoided to prevent confusion with the ridges occurring inside the mantle cavity. The term pallial lobe will be used to denote the reflected edge of the collar. That part of the mantle border which is in contact with the parietal "edge" of the aperture is flat as there is no edge over which it can be reflected. The pallial lobe and band are discernable but are not as well developed as at the opposite side of the aperture. The elements occurring in the columellar region of the mantle are, likewise, less fully developed. This is to be expected as shell growth around the umbilicus proceeds at a tempo many times slower than that of shell growth at

the labrum.

The inner surface of the pallial lobe has an epithelium of low columnar cells with large centrally to basally placed nuclei having clearly visible nucleoli. The cytoplasm does not stain as deeply as that of the general collar surface. In some cases there is a faint blue edge to the cells, suggesting that a delicate cuticle is present. These cells are in an exposed position and it is therefore not surprising to find sensillae scattered among the normal epithelial cells. The epithelium of the outer surface of the pallial lobe is composed of larger cells which stain more lightly than do those of the inner surface. The cells are also broader and possess large nuclei which are rounder than those of the inner surface. The cells are also more uniform in appearance and are not crammed together or distorted as those of the inner surface of the lobe often are.

The narrow space between the inner and outer epithelia is more compactly filled than the rest of the collar and contains pigment cells, amoebocytes, some small blood spaces and a diversity of gland cells. There are also conspicuous muscle fibres present for the retraction of the pallial lobe.

Besides a small number of F_1 , M_1 , and also even M_2 glands three other types of subepithelial gland, apparently restricted to the collar, occur. In the pallial lobe itself are

numerous small, roundish gland cells (M_5), filled with a hyaline substance which is not stained by any of the techniques employed. In the cytoplasm large secretory spherules may be seen filling the body of the cell. At the base of the pallial lobe, just below the outer epithelium, is a compact cluster of gland cells, M_6 , having lightly-staining nuclei and a hyaline, highly vacuolated cytoplasm. The glands discharge into the groove between the base of the pallial lobe and the pallial band (see fig. 12). The third type of gland occurring in the pallial lobe, differs from all other types of gland in the snail. The form of the body is long and tortuous and the secretion stains red with Mallory's. These red glands (M_7), discharge onto the outer surface of the pallial lobe.

On the outer surface of the mantle, and situated at the base of the pallial lobe, the pallial band is found. This consists of very tall unciliated epithelial cells with large, lightly-staining oval nuclei which are situated somewhat below the middle of the cell. The nuclei possess orange-staining nucleoli which are fairly conspicuous. The cytoplasm at the base of such a cell differs from that at the apex. Basally the colour of the cells is a brownish-pink while apically the cytoplasm stains a light blue, indicating the presence of mucus. This mucus is very faintly pink after PAS treatment. In the apical region the cytoplasm is highly vacuolated and the contents of these vacuoles does not stain so that the

nature of these spherules is unknown. In the more proximal region of the pallial band the cells contain a small amount of calcium, but this has not been noted in the distal cells. Distally, and sometimes proximally, slightly curved cells are transitional between the tall cells of the pallial band and the cells of the pallial lobe and the general mantle surface, respectively, especially in the former situation. The cells of the general mantle surface are cuboidal cells which scarcely stain at all, and contain large round nuclei. These cells, and those of the thin epithelium investing the visceral mass, are the only epithelial cells, in B.tropicus, to contain pigment granules. These are normally carried only by the subepithelial chromatophores, though a few, black granules are also usually present in Leydig's cells. In A.trapezia the pigmentation of the foot and mantle is also caused by granules located in the epithelial cells (Sullivan, 1961). The formation of these granules is said to be the result of light falling on those parts of the animal, a reaction which also takes place in other Pelecypoda (List 1899, quoted by Sullivan, 1961). In B.tropicus the pigmentation is not caused by illumination as the two most heavily pigmented areas, the mantle and the apex of the visceral mass, are not more exposed to illumination than other parts of the snail, and are, indeed, less exposed than the headfoot. The theory of pigment formation in response to illumination would

also not account for the dense aggregation of melanophores on the surface of the preputium, and certain specific areas in the haemocoelic walls. If the pigment patches of B. tropicus have any connection with light, it is possible that, as suggested by a certain author, they have a protective function. In this connection it is significant that they overlies the accessory sex glands and organs while the dense apical patch adumbrates the hermaphrodite gland.

The mantle epithelium of the hypopeplar cavity is composed of low columnar cells which become cuboidal towards the region in which the mantle is attached to the body wall. The collar is ciliated in parts and near the body wall the cilia of the sensillae are very long, being in some cases more than twice the height of the cell. The central part of the collar is spongy in appearance. A great many glands open onto the inner surface of the collar and the substance of the collar is composed of the bodies of these glands, pigment cells, Leydig's cells, other connective tissue and muscle fibres.

Conspicuous in the collar region are the large M_1 glands. These constitute a large part of the subepithelial glands and careful observation reveals variations in their contents. Besides the apparently homogeneous and granular contents, there are also certain gland cells which resemble the M_1 type in

most details, but contain a secretion which tends to be lilac. In this secretion are numbers of red and yellow particles which are spindle-shaped and seem to be crystals of some sort. It is possible that these gland cells are, in reality, only a specific secretory phase of the M_1 type of gland but, as they differ so markedly from the normal M_1 glands, they are provisionally designated M_3 .

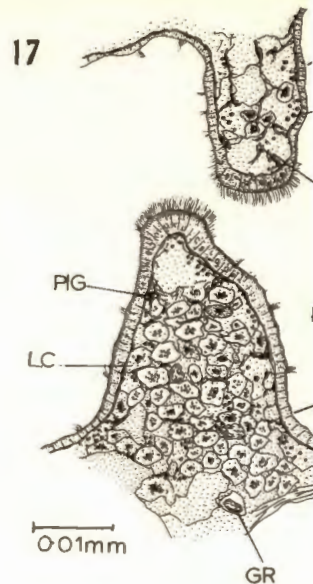
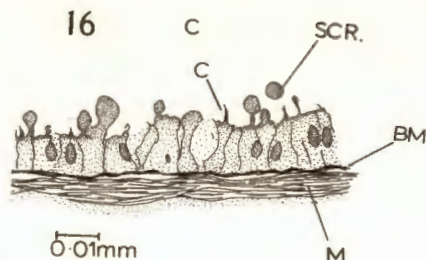
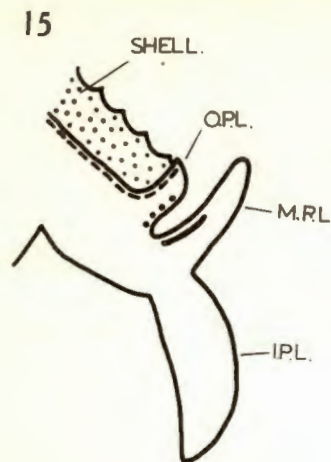
The inner surface of the collar receives the secretion of the M_1 and M_2 glands as well as a few of the M_3 type. Besides these glands there are special glands which are restricted to the collar. The outline of these glands M_4 is almost impossible to discern but may be gauged by the presence of the secretory droplets which stain well. The glands are considerably smaller than the M_1 , M_2 types and are slender clubs which are in many cases displaced by the pressure of the surrounding glands. The cytoplasm does not stain at all and appears completely hyaline but the globules which also appear hyaline, and almost fill the cell, stain intensely yellow with Mallory's and shocking pink with MBE, showing that they are strongly oxyphilic (fig. 13). These glands seem to occur most frequently in that region of the collar overhanging the pneumostome and do not extend as far as the collar edge. The nature of these glands is not known with certainty, but they possibly contain a protein secretion. Simroth (1928) states that protein glands occur in the

pulmonates; and as the protein gland is said to occur in the mantle and to be strongly oxyphilic, these little glands, being the only oxyphilic mantle glands may be protein glands. In this connection the various carbohydrate-protein glands (PAS-positive) must also be borne in mind.

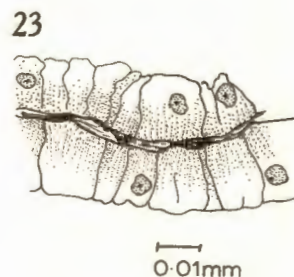
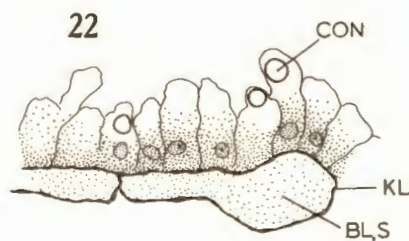
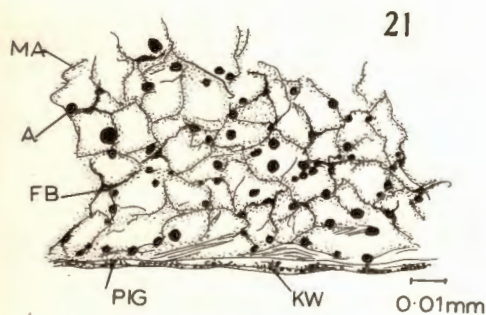
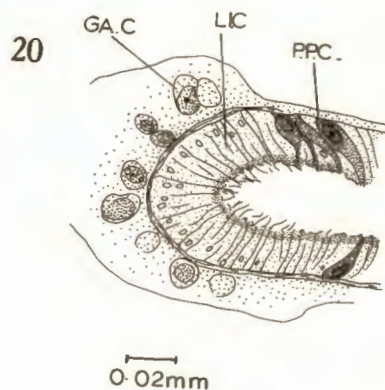
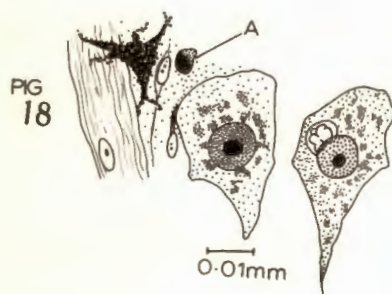
The spongy appearance of the collar is due to discharged gland cells, the numerous blood spaces, the loose connective tissue (which includes many amoebocytes) and in certain areas, the presence of Leydig's cells which are a characteristic feature of the mantle. Superficial muscle fibres run parallel to the epithelia of both mantle and collar, and on the left side of the animal is a sheaf of muscle fibres which run more or less parallel to the lateral edge of the mantle cavity and find their insertion in the collar above the pneumostome. These muscles might play an important part in respiration for in snails dropped into alcohol, these muscles are often found in a contracted state, causing the collar to be drawn back from, and so exposing the pneumostome.

3.3 2 Mantle and shell formation

The manner in which the shell is formed is not properly understood and as the histology of the mantle edge gives no clear indication of the parts played by the various structures (Fretter and Graham, 1962) we are forced to fall back on suppositions and consequently opinions on shell formation



- SITE OF CALCIUM FORMATION.
 — SITE OF PERIOSTRACUM FORMATION (OLDER WORKERS).
 ... SITE OF PERIOSTRACUM FORMATION (RECENT WORKERS).



- FIG. 15. MANTLE EDGE PALLIAL LOBES OF *DIODORA APERTURA* (AFTER FRETTER AND GRAHAM, 1962).
 FIG. 16. TYPICAL DROP-SECRETION FROM FLOOR OF MANTLE CAVITY.
 FIG. 17. SECTION THROUGH INTERMEDIATE MANTLE RIDGE AND LATERAL RECTAL RIDGE.
 FIG. 18. NERVIDIG'S CELL.
 FIG. 20. LONGITUDINAL SECTION THROUGH OSPHRADIUM TO SHOW LIGHTLY STAINING PROXIMAL, AND PAS-POSITIVE DISTAL, CELLS.
 FIG. 21. SECTION THROUGH SACCULAR PART OF KIDNEY.
 FIG. 22. SECTION THROUGH PROXIMAL REGION OF TUBULAR PART OF KIDNEY.
 FIG. 23. SECTION THROUGH DISTAL REGION OF TUBULAR PART OF KIDNEY.

differ widely.

All workers, however, seem to hold the general surface of the mantle at least partly responsible for the secretion of the calcareous layer and it seems that this is the case also in B.tropicus. In certain areas the apices of the mantle cells have been noticed to be uneven and small colourless flakes have been observed above these cells. Sometimes patches of these epithelial cells also contain much calcium and in both these instances the appearance of the cells is taken to indicate that the cells are in an active calcium-secreting phase (fig. 14).

The rest of the shell formation is undertaken by the edge of the mantle and for a better understanding of the process it is profitable to compare the mantle edge in B.tropicus with that of certain other molluscs.

In most Pelecypoda and some Gastropoda, eg. the Zeugobranchia, the mantle edge has three lobes termed the outer, middle and inner pallial lobes (fig. 15) though in most Gastropoda the outer lobe is reduced (Fretter and Graham 1962²). It is, perhaps, possible that the mantle lobes of B.tropicus are not at all homologous with the mantle lobes of Zeugobranchia. So great is the similarity between these forms, however, that it is more probable that such a correspondence does exist.

In the event of such an homology, the pallial band of B.tropicus corresponds with the outer pallial lobe of the Zeugobranchia. In B.tropicus, as in Siphonaria sp (Fretter and Graham, 1952) and Vitrina pellucida ^{(as seen in Eckardt's} ~~(author) (Eckardt,~~ ^{figure in} ~~quoted by~~ Simroth and Hoffman, 1928) the reduction of the outer pallial lobe has been carried so far that it has become vestigial and is represented merely by a band of tall epithelial cells. The "pallial lobe" of B.tropicus is homologous with the middle pallial lobe, and the fleshy part of the collar with the inner pallial lobe of the Zeugobranchia.

Fretter and Graham (1962), reviewing the literature, write as follows on the possible method of formation of the gastropod shell :

The periostracum is secreted in the groove between the outer and middle pallial lobes. Older workers held the lateral surface of the median lobe to be the seat of this secretion, but of late opinion has veered to favour the median surface of the outer lobe as the site of periostracum formation. Calcium is thought to be deposited by the lateral surface of the outer lobe, in conjunction with the general mantle surface. The conchiolin of the shell is thought to be secreted by glands discharging into the groove between the outer and middle pallial lobe (fig. 15).

In B.tropicus the general mantle surface is responsible

for at least some of the calcium secretion and the proximal region of the pallial band is markedly calcium-rich, while the more distal region, adjoining the pallial lobe, is not. The calcium secretion in B.tropicus thus seems to agree with the conditions generally obtaining in the gastropods.

The correspondence between the formation of the other shell constituents in the forms under discussion, is rather hazy. The inner surface of the outer lobe of B.tropicus secretes a calcium-poor substance which stains light blue with Mallory's and therefore is, or contains, mucus. This secrete is PAS-positive indicating a carbohydrate-protein complex. In other gastropods this is said to be the site of periostracum formation.

With regard to the secretion of conchiolin, it is probable, from their situation, that the hyaline glands M_5 , in the pallial lobe as well as the group of vacuolated, hyaline glands M_6 , at the base of the pallial lobe, perform this function. In oysters the conchiolin-secreting glands contain a protein and polysaccharide substance (Bevelander and Benzer, 1948, quoted by Fretter and Graham, 1962), but unfortunately the chemical nature of the secretions of gland types M_5 and M_6 in B.tropicus is unknown, owing to their having no affinity for the stains employed.

The most teasing question in this connection is the

function of the tortuous red glands M_7 . From their position, on the lateral surface of the pallial lobe, the red glands would appear to be part of the conchiolin glands complex, but there are other facts suggesting that this gland may play a role in periostracum formation. Firstly, in sections stained with Mallory's the red colour may indicate "chitin", and secondly, although the openings of the ducts are sometimes situated at the bottom of the groove between the outer and middle lobe, they are usually to be found on the outer surface of the middle lobe. It is important to bear in mind that during life the middle pallial lobe in B. tropicus is slightly reflected over the edge of the shell aperture, and comes to lie against the outer periostracum.

3.3 3 Remainder of mantle, and floor of the mantle cavity

The cells lining the mantle cavity vary from cuboidal to columnar. The columnar cells are found at their highest development in the anterior and left hand side of the mantle cavity, while the right hand side of the cavity is clothed with the cuboidal epithelium. The cells almost all show signs of secretory activity, this property being more highly developed in the columnar than in the cuboidal cells. The apices of the columnar cells staining orange with Mallory's, as do the epithelial cells of the sole of the foot. The appearance of the secretory epithelium of the mantle cavity

is highly characteristic and blobs of secretion may be seen being discharged from the cells. This gives to the epithelium the same appearance as that of the upper posterior part of the foot of Planorbarius corneus, Linnaeus as figured by Herfs (reproduced in Boettger, 1944). Many spherules may be seen above the epithelium of this region (fig. 16) and the droplets may be blue, purple, orange or a combination of these colours. The nuclei are oval, light-staining and clearly visible. Scattered among these cells are sensillae which are a little bigger than the other cells, have a large distinct nucleus, and cilia which may be much longer than the cell height. Large goblet cells, the contents of which do not stain, are also found among the normal epithelial cells. The epithelium is underlain by a very distinct basement membrane of considerable thickness, and under this, scattered throughout the extent of the mantle cavity, are tiny unicellular glands. They are intensely PAS-positive, apparently the only glands of the mantle cavity to have this property.

3.3 4 Leydig's cells

A very conspicuous feature of the mantle is the large number of Leydig's cells occurring in this region. They seem to extend throughout the mantle, occurring in the collar, in the roof of the mantle cavity where they are densely packed and may be seen even in the basal portions of the

kidney lamellae. They occur also in the ridges of the mantle cavity, especially the lateral rectal ridge (see later)(fig. 17). The cells (fig. 18) are large and though irregular in form, they do not exhibit long pseudopodia. The cytoplasm is hyaline, and not stained by any of the methods used. The nucleus is clearly visible in the cell, and varies in form from round to slightly oval. It is lilac in colour and coarsely granular in appearance. Against the lilac of the nucleus the large, round, orange nucleolus forms a striking contrast. The nucleus is generally somewhat obscured by an aggregation of lilac or pinkish particles which are irregular in shape, and vary in number and degree of compactness. Numbers of black granules are also to be seen in some of the cells, and though in certain cases some of the granules may belong to the cytoplasm of melanophores, the Leydig's cells, themselves also contain some granules.

The functions of these cells have been thought to be glycogen storage, phagocytosis^{is} and excretion (Cuènot 1892, quoted by Sullivan, 1961), the storage of food substances and calcium (Boettger, 1944), while Sullivan(1961) is also of the opinion that the cells are possibly in some way connected with excretion.

A small amount of lightly PAS-positive material was demonstrated in some of the cells but, from the sections, it does not appear to be a very important function. On

several occasions sacs containing a little PAS-positive material were found. It might be that these sacs are a part of sectioned Leydig cells. From their intimate connection with the mantle cavity and kidney it is thought possible that these cells may also play a part in the excretory processes of the snail.

In A. trapezia Sullivan (1961) found a large number of granule cells in the kidney region and thought it possible that these granule cells were concerned with storage and excretion. No Leydig's cells were found in any part of this species. It is thus interesting that in B. tropicus, many Leydig cells occur, especially in the mantle and thus also in the kidney region, where, as has been mentioned, they may even invade the basal parts of the kidney lamellae. No granule cells were found to occur in this species. In sections of Burnupia, (kindly lent by ^{Mr} Hamilton-Attwell of this Department) granule cells are conspicuous in the mantle region. They bear some resemblance to the Leydig cells of B. tropicus but are smaller and contain fairly large, light brownish granules. Only on rare occasions were clear cells observed. In these three species mentioned it therefore seems permitted to speculate that the same functions are performed by the granule cells or Leydig cells, either one type or the other occurring in any one species.

Cuénot (1892, quoted by Sullivan, 1961) states that aquatic pulmonates possess two types of Leydig cell, one concerned with glycogen storage and the other having phagocytic and excretory functions. As glycogen storage could not be clearly demonstrated in B.tropicus and no other differences in Leydig's cells were obvious, no distinction could be made. It seems desirable to repeat the PAS-technique on B.tropicus and also to experiment with other staining methods to determine whether there are in reality two types of this cell.

Two other types of vesicular cell are distinguished in in B.tropicus. They occur in the buccal mass and reproductive system and will be described under those headings.

3. 3 5 Mantle ridges

The position and form of the mantle ridges in B.tropicus have been described (Stiglingh et al, 1962) using the terminology suggested by Schutte and Van Eeden (1959). In B.tropicus both the intermediate mantle ridge and the lateral rectal ridge are well-developed, as in B.truncatus (Demian 1960). As in the lastmentioned species, the ventral part of the lateral rectal ridge in B.tropicus is much more pronounced, and usually inclined slightly to the left of the intermediate mantle ridge. In certain sections the intermediate mantle ridge may be seen inclined slightly to the animal's right. The position of these two ridges relative

to each other varies, the lateral rectal ridge may also touch its apex, or even lie to the right of the intermediate mantle ridge.

As can be seen in fig. 17, the crests of the ridges are well rounded. The sides of the ridges are clothed with a cuboidal epithelium which is of a secretory type. There is considerable difference in the secretory ability of the two sides of the intermediate mantle ridge, the lateral surface of the ridge apparently having this property better developed than the median surface. In the lateral rectal ridge the condition is just the reverse, there is a very marked difference, in the anterior part of the ridge, the median surface of the ridge showing a high degree of secretory activity, while this is not to be detected on the lateral surface. The secretion is fairly rich in calcium and stains a very light pink upon being treated with PAS. Scattered among the normal epithelial cells are sensillae and the crest of each ridge is crowned by tall columnar cells bearing cilia almost as long as the cells are high (fig. 17). The nuclei are situated below the middle of the cells and are large and round to oval in form. The body of the intermediate mantle ridge contains only little connective tissue - amoebocytes, pigment cells and a few leydig's cells. The space between the two epithelia is taken up mainly by numerous large blood sinuses. The body of the lateral rectal

ridge is much more compact and is almost entirely filled with Leydig's cells, consolidated by the necessary connective tissue elements. The significance of this dense aggregation of Leydig's cells in this region is unknown, but it is possible that they, as in the case of other vesicular cells, (see below) function as supporting cells.

Recently Harry (1964), in the course of his studies on the phylogeny of the Basommatophora, also investigated the occurrence of ridges in the pulmonary cavity of several of these forms. He employs the term pulmonary lamella to denote the intermediate mantle ridge and the lateral rectal ridge which he regards as a single structure because, although located on the roof, and on the floor of the mantle cavity respectively, the two ridges become continuous in the posterior region of the pulmonary chamber.

Such a continuity has long been noted in various snails, and was observed, more recently, in B.tropicus (Stiglingh et al, 1962), B.truncatus (Demian, 1960), Chilina fluctuosa (Harry, 1964) and B.(P)africanus (Kraus) (Van Aardt unpublished thesis). The lastmentioned author, quoting Schutte and Van Eeden (1959) states that such a junction of the intermediate mantle ridge and lateral rectal ridge does not take place in B.pfeifferi (Krauss). Actually, Schutte and Van Eeden (1959) do not express themselves at all on this point, and my dissection of B.pfeifferi showed that the two

ridges do, in fact, merge posteriorly.

Harry (1964) puts forward the suggestion that in those Planorbidae and Bulinidae in which the intermediate mantle ridge (dorsal part of the pulmonary lamella) is conspicuous in the region of the pneumostome, the ridge becomes continuous with the lateral rectal ridge.

The simplicity of the term "pulmonary lamella" as opposed to the cumbersome intermediate mantle ridge and lateral rectal ridge, is appreciated but not yet adopted as the term may not suffice in all instances. However, I find the author's use of the term dorsal lamella objectionable, in the same way that I find the use of the terms anterior renal, and posterior renal ridge by Schutte van Van Eeden (1959), to be so. Such abbreviated usage (omitting the insertion of the words "part of") may mislead the reader to believe the author is referring to two structures instead of one. Awareness of this might have prevented the last mentioned authors from misinterpreting Boettgers' (1944) description of the mantle cavity lamellae in Planorbarius corneus (Din-naeus). They took the anterior dorsal fold (renal ridge) and posterior dorsal fold (intermediate mantle ridge) to be anterior and posterior parts of the dorsal fold and stated that Boettger (1944) does not mention the intermediate mantle ridge which he in fact described and figured.

The extent to which the median rectal ridge is developed in the Basommatophora is not clear. ^{Schutte}~~Schutte~~ and van Eeden, (1959) applied this term to the continuation of the lateral edge of the siphon in B. pfeifferi, though Harry (1964) considers the small structure unworthy of a special designation. Also Watson (1925, quoted by Connolly, 1925) does not refer to the median rectal ridge in B. pfeifferi, though he described the other ridges occurring in the cavity. In B. tropicus the lateral edge of the siphon is prolonged for a short distance along the rectum and though this was figured (Stiglingh et al., 1962), this puny structure was not specially named. Histologically this ridge is found to differ from the intermediate mantle ridge and lateral rectal ridge. Although the prolongation of the siphon also has columnar epithelial cells along its crest these in no way approach the highly developed epithelial cells along the crests of the two other ridges.

A median rectal ridge has been reported in P. globosa (Watson, 1925, quoted by Connolly, 1925) and P. africana (van Aardt, unpublished thesis). Watson (1925) regards the absence of the median rectal ridge and renal ridge in the more typical species of Isidora (= Bulinus) eg. I. forskali (Ehrenberg), I. contorta (Michaud), I. tropica (Krauss) and I. lamellata (Smith), as the main criterion for distinguishing the subgenus Bulinus from the subgenus Physopsis. Although Watson (1925) distinguishes the median rectal ridge in P. globosa he comments that though the renal and median rectal

ridges correspond to the two ridges occurring in the same positions in P. corneus, they are relatively much smaller in P. globosa.

In Australorbis glabratus (Say) ~~three~~ mantle ridges have been described, (Pan, 1958), no mention being made of a median rectal ridge. From the figures 6, and 7, (Pan, 1958) it seems that there are indeed two ridges present on the rectum, so that in this form, all four ridges may be present. In both figures the intermediate mantle ridge and lateral rectal ridge are in such close proximity that they could apparently touch each other, and in figure 6 this is seen to be true also in the case of the other two ridges.

The ridges occurring ⁱⁿ the mantle cavity of the snail P. corneus have been described by Simroth and Hoffman (1928) and Boettger (1944) (who is, however, apparently only quoting Simroth and Hoffman (1928)). Figures of the mantle cavity in this form are to be found in Simroth (1928), one by the author himself and the other after Pelseneer. In P. corneus there is a well developed renal ridge and median rectal ridge, both extending far towards the posterior part of the mantle cavity, and both being considerably more robust than the intermediate mantle ridge and lateral rectal ridge. Whether the renal ridge and the median rectal ridge are posteriorly continuous is not known. If this be so, then the term pulmonary lamella would be insufficient as, in certain forms two pulmonary lamellae may exist. It is thus desirable that

someone study the pulmonary cavity lamellae in P. corneus and related forms and, if necessary, devise a suitable terminology which is applicable to all these forms.

The function of these ridges must be related to their arrangement in the mantle cavity, but what the precise relation of these ridges is, is not yet a settled question. There is disagreement as to whether or not these ridges, divide the mantle cavity into two compartments. According to Demian (1960), Boettger (1944) states that the ridges cannot completely divide the mantle cavity into two chambers. However, Boettger (1944) and Simroth and Hoffman (1928) (whom Boettger is probably quoting) state that in P. corneus the renal ridge and median rectal ridge are large and so placed that their crests are contiguous, thus dividing the mantle cavity into a spacious anterior (right) chamber and a narrow posterior (left) chamber. In this narrow cavity the smaller intermediate mantle ridge and lateral rectal ridge project freely. From this it may be inferred that the renal ridge and median rectal ridge lie opposite each other, at least in the anterior part of the mantle cavity. Also in P. globosa these two ridges are said to oppose each other (Watson, 1925). It is thus possible that, in forms having four mantle cavity ridges, the renal ridge and median rectal ridge may constitute a partition in the mantle cavity.

In the other forms mentioned above, and in several others mentioned by Harry (1964), it is the intermediate mantle ridge

and lateral rectal ridge which constitute the partition. Although these two ridges are situated opposite each other in B. truncatus Demian (1960) states that the ridges cannot serve to partition the mantle cavity, and declares that this was found to be the case also by Rao (1923) and Boettger (1944), the latter apparently being misunderstood by Demian (1960), because, when referring to the renal ridge and median rectal ridge, he specifically uses the word "abteilen" (p.321). Harry (1964) is also of the opinion that the ridges "constitute a real, if ineffective, partition" (p.370), and observations on live B. tropicus have brought me to the same conclusion.

These folds are not rigid structures, but are labile, so that although the lateral rectal ridge normally lies slightly to the left of the intermediate mantle ridge, as in B. truncatus (Demian, 1960), it sometimes lies directly opposite, or slightly to the right of the intermediate mantle ridge. This means that when the ridges are in an elevated position and the floor of the mantle cavity is raised, the two ridges can be apposed, thus dividing the mantle cavity into two unequal portions. Such variable positions of the ridges relative to each other may be seen both in serial sections, and by careful observations of live specimens held in such a way as to expose the pneumostome during respiration.

Concerning the functions that these ridges may perform there has also been much conjecture. Rao (1923, quoted by

Demian, 1960), Boettger (1944), Hubendick (1955), and Demian (1960) all suggest that they aid in respiration. That this is the primary function in B. tropicus seems to me unlikely. The additional respiratory surface afforded by these ridges is so little as to make the effect upon the respiratory rate negligible. Besides the rather small size of the ridges, especially in the posterior part of the mantle cavity, there is, as in most (all?) the species possessing these ridges, already a pseudobranch present as respiratory organ. In instances where a ridge is modified in a complicated manner, as in B. pfeifferi, it is possible that it may aid in respiration.

Recently it has been suggested that the function of these ridges is to promote the circulation of the water in the mantle cavity (Harry, 1964). That one of the functions of the ridges is the creation of a current is obvious from the long cilia along the crest of each ridge. The advantage to B. tropicus, of a current of water in the mantle cavity is debatable for the mantle cavity is normally employed in aerial respiration. The copious secretion of slime in certain areas of the mantle cavity, and the sheet of mucus which is quite frequently to be seen overlying the cilia of the intermediate mantle ridge and left hand chamber of the mantle cavity suggests that in B. tropicus one of the main functions is the creation of a current to prevent stagnation and accumulation of the mucus secreted in the mantle cavity. Although there is no

direct proof, the direction of the cilia give the impression that the mucus is being moved in the direction (fig. 19) of the left hand chamber, the floor of which is especially well ciliated and shows intense secretory activity. The continuous movement of a mucous sheet in the mantle cavity would serve to prevent fouling of the chamber. The danger of silting up is often mentioned in connection with marine forms, and though not as great in freshwater forms, the water is on occasions laden with varying amounts of silt and organic particles which might accumulate in the mantle cavity. It may be supposed that the mucus sheet is drawn over the ridges into the narrow left hand chamber from where the superfluous mucus may be extruded from the cavity, but such a supposition wants experimental confirmation.

It also seems to me, that in B. tropicus a further possible function of the ridges is the retention of the bubble in the mantle cavity. This is deduced partly from watching live B. tropicus and also from a comparison with L. natalensis and Physa mosambiquensis. The crux of the matter seems to be the position of the anus. In all three forms the anus lies lateral to the siphon, in such a position that closure of the pneumostome does not, in any way, interfere with the expulsion of the faeces. In both L. natalensis and Physa mosambiquensis the siphon extends the length of the pneumostome so that the anus also lies lateral to the pneumostome. In B. tropicus however, the position is different. The siphon extends across

only half of the pneumostome, and the anus is situated more or less half-way along the ventral margin of the pneumostome. Closure of the median half of the pneumostome thus presents no difficulties, but the lateral half of aperture has no siphon and might provide a conduit through which air could escape while the snail crawls over inclined surfaces or hangs inverted from the underside of leaves or other objects. The strategic placing of the intermediate mantle ridge and lateral rectal ridge render possible a sealing off of the large chamber on the right side of the animal from the narrow chamber on the left, so that the large chamber is in communication with the surrounding water only through the pneumostome which is guarded by the siphon.

Retention of the air bubble in the mantle cavity of B. tropicus is thus probably brought about by the siphon and lateral rectal ridge. When the snail is in such a position that the pneumostome may be clearly seen, and the aperture is wide open, the lateral rectal ridge is visible as a conspicuous vertical crest extending into the mantle cavity and lying beneath the intermediate mantle ridge. The ciliated edge of each ridge is visible as a lighter streak. To the right of the rectal ridge the siphon lies reflected into, and pressed onto the floor of, the mantle cavity. To retain the air in the mantle cavity it is apparently not necessary to close the pneumostome. Normally the air bubble may be seen lying near the posterior part of the pulmonary chamber. When

the animal, in crawling actively about, changes its position from the horizontal, the bubble may be seen to be displaced to another part of the chamber, but I have never observed the escape of such a bubble from an undisturbed snail. Even in narcotized snails the bubble is retained in the lung. Normally this retention could be ensured by the elevation of the pulmonary siphon into a more vertical position, but it also seems as though the pneumostome is sometimes temporarily occluded by the lateral rectal ridge which lies more or less diagonally across the pneumostome, to which it may act as a barrier when in ^{an} elevated position.

From the description of the mantle cavity in B. truncatus (Demian, 1960) it is clear that though certain differences occur, the structure closely resembles that of B. tropicus. Some of the observed differences may, possibly, be attributed to subjectivity on the part of the observers. Others may be caused by the specific respiratory conditions under which the snails were observed, for it is known that basommatophoran species exhibit great plasticity in their respiratory behaviour, in adaptation to varying conditions (Hunter, 1953).

Demian (1960) declares that the mantle cavity of B. truncatus is never filled with water. He submerged the snails in a dilute solution of methylene blue and found that only the ridges in the cavity stained blue. From this he deduced that, though the ridges are kept moist by a current, probably created by their cilia, the rest of the mantle cavity was

never filled with water. I suggest that the mucin in the secretory cells may have been stained by the methylene blue which did not "take" on the rest of the epithelium. In B. tropicus the mantle cavity is by no means air-filled, a bubble of air, about a quarter of the size of the cavity usually being found in the right hand compartment of the pulmonary chamber. Air was never observed in the narrow left hand chamber, and from the difference in the degree of vascularization of the roof of these two chambers, this is to be expected. Harry (1964) suggests that the partitioning of the mantle cavity may be the beginning of a division into a right hand cavity containing more air than water, and a left hand water filled cavity. No suggestion is made as to the possible significance of such a division.

Another difference between B. truncatus and B. tropicus is that in the former the siphon is rolled up and pressed against the upper lip of the pneumostome, thus bringing about a tight closure of the aperture while the animal is under water (Demian, 1960). In L. natalensis and P. mosambiquensis such a closure of the pneumostome was also observed by me, ⁱⁿ both cases the siphon bulged convexly over the pneumostome and the free edge of the siphon lay against the dorsal edge of the pneumostomal aperture. The pneumostome of B. tropicus was however, seen to be open under water, though the size of the opening varied.

The amount of air in the mantle cavity depends on conditions in the habitat and also, on the condition of the snail. The size of the snail is another factor which influences the respiratory behaviour, it having been established that small pulmonate snails come to the surface for air less often than the adults (Hunter, 1953). In fact Boettger (1944) states that young Basommatophora have no air in the mantle cavity, the rest of the body surface sufficing to comply with the animals respiratory demands. In larger snails the mass of the organism is relatively so much greater that it becomes necessary to take air into the mantle cavity. In winter, however, even large snails have a water-filled mantle cavity, (Boettger, 1944).

It was frequently observed in the laboratory that snails which are apparently suffering from lack of oxygen, may be recognised by their appearance. In such snails the greatly distended pseudobranch is extended well out from underneath the shell, and the shell itself is not borne in its normal horizontal position, but is slightly uptilted on the left side, so that the whole pneumostomal area is more clearly to be seen.

The complicated and varying structure of the mantle cavity of the Planorbidae shows that this is an important region of the snail and Morton (1960) is certainly at fault in stating that the pulmonary cavity^{is} disused in this family.

The structural and behavioural variations encountered must be seen as an important factor in their general adaptability, enabling them to survive respiratory hardships, and inhabit a wide variety of habitats.

3.3 6 The Siphon

The substance of the siphon consists of a variety of elements. The most outstanding of these is muscular tissue. There is an intricate system of muscle fibres arranged to allow great lability during respiration. Among these fibres the rest of the elements are packed. Numerous glands are found, and these are of at least two types. The form of these glands is the same, consisting of a smallish rounded body. One kind of gland possesses a hyaline cytoplasm which sometimes stains a very light greenish colour, in which large colourless ^{sph}~~sph~~erules may be discerned, almost filling the cell body. The other type of gland possesses spherules which stain purple. The colourless type of gland may be the same as those found in the pallial lobe, but the second type of gland is unique in the snail. The purple glands stain intensely with MBE, showing that the spherules are basophilic, but beyond that, their chemical nature is unknown. Also present in the siphon are numerous amoebocytes, pigment cells, and, towards the base of the siphon, Leydig's cells. In some snails translucent granules are found in great numbers in this region, and may also be observed in the living snail.

3.3 7 The Osphradium

The presence of this organ in B. tropicus has already been reported (Stiglingh et al., 1962) and a sketch made of a longitudinal section through the organ. In that paper, however, practically nothing was said concerning the structure or function, and it seems appropriate now to do so.

The organ is elongated and hollow and it is embedded in the tissue connecting the body and the base of the collar. At its blind end the cavity is surrounded by a mass of ganglionic cells constituting the osphradial ganglion. The ganglionic cells are large and contain very large nuclei which almost fill the body of the cell. These nuclei stain darkly and possess many closely packed chromatin granules which are rather coarse in texture. In PAS-stained material some of the ganglionic cells may be seen to have nuclei which are clear and contain scattered aggregations of granules. It is not known whether or not these cells are normal. On occasions a number of intensely basophilic granules ^{were} ~~are to be~~ seen in the nucleus. Normally a conspicuous orange nucleolus is to be seen though this structure is indistinct in PAS-stained material. These nerve cells are in communication with the left parietal ganglion, so that the innervation is in reality by the parietal ganglion, an important fact in the consideration of the homology of this organ with the olfactory organs of other molluscs.

The lumen of the osphradium communicates with the ambient water by means of a small aperture which lies just median to the pneumostome. The epithelial cells surrounding the osphradial aperture are tall epithelial cells which bear long cilia. The nuclei are oval in form, situated near the base of the cell and do not stain well with Mallory's. Near the apices of the cells the cytoplasm stains blue and a large number of vacuoles of various sizes is visible. The apical parts of the cells stain orange with Mallory's, and this colour is taken up also by the cilia. The secretion of these cells is PAS-positive, staining a light pink, and contains a large amount of calcium. A few rather squat goblet cells occur on the periphery of this circlet of tall cells, and a few goblet cells which are more slender than the first type occur nearer to the aperture. They are not outstanding and their secretion is not stained by any of the techniques employed.

Just posterior to the osphradial aperture the lumen is lined by tall cells which also bear well developed cilia and seem to be restricted to this part of the body. The cells are goblet cells and contain darkly staining granular nuclei which are considerably larger than those of the other osphradial cells. These goblet cells contain numbers of rather small secretory droplets which are PAS-negative, and, when stained with MBE, a deep blue, and therefore strongly basophilic (fig. 20). In the posterior part of the osphradium the cells consist of columnar glandular cells bearing short cilia.

Round, lightly staining but granular nuclei are present at the base of the cell and the cytoplasm resembles that of the cells surrounding the osphradial aperture. The cytoplasm stains blue and is highly vacuolated, so that a frothy appearance is given to the cells. The apices of the cells do not stain as deep an orange as those surrounding the aperture, the secretion being more yellow. It is also PAS-positive so that it is probably identical with or, closely akin to, that of the anterior cells.

Olfaction in the Gastropoda

The function of the osphradium in B. tropicus is olfaction but it is thought probable that this is a secondary function in the gastropods, the primary function having been the detection of silt (Yonge, 1960 (in litt.) quoted by Kohn, 1960). There are certain molluscs which make use of their eyes in obtaining their food, but this is unusual. Of all the Mollusca it is only the cephalopods, pelagic heteropods and the Strombidae (Morton, 1960) which have well developed eyes used in the detection of their food; the other members of the phylum must rely mainly on the sense of smell, (Cooke, 1913). As indicated by Kohn, (1960), reliance on the olfactory sense is important not only in connection with feeding, but also in avoiding enemies and finding mates.

Copeland (1918,^{quoted by Kohn, 1961}) was the first to prove the olfactory function of the osphradium in a prosobranch and three later

papers have since confirmed this function, two of the papers dealing with prosobranchs and one with a pulmonate. This literature and that on chemoreception in gastropods in general is very ably reviewed by Kohn (1961).

Before discussing the osphradium of B. tropicus it is interesting briefly to digress, and consider the condition of the osphradium and other olfactory organs of the gastropods in general. It is at once clear that these organs are not homologous throughout the class, or even necessarily in one order, eg. in the landliving and aquatic pulmonates. In an attempt to form an impression of the situation in this class, not only the morphology, but also the intimately connected situation and function of these organs will be discussed for each of the three orders.

In Octopus vulgaris the chemical sense seems spread over the whole body (Scheer, 1948) but in most of the other Mollusca the chemical sense is localised in certain areas of the body, a number of organs acting as the seat of the olfactory sense. In the prosobranchs the siphon, epipodial and pallial tentacles, and the anterior and posterior portions of the foot have been suspected of having, and in some cases have been proved to have, an olfactory function (Yonge, 1947; Kohn, 1961). The special chemoreceptor is, however, the osphradium which occurs inside the mantle cavity. Generally speaking the form varies from simple ridges in the Archaeogastropoda, and most Mesogastropoda, to a complex bipectinate struc-

ture in the Neogastropoda (Kohn, 1961). The innervation of this organ is by a nerve arising from the parietal ganglion.

The osphradium is always placed inside the mantle cavity, close to the ctenidia where it remains even in forms which have lost their ctenidia, as in *Patella* (Borradaile et al., 1959). Because they are associated with the gills they are liberally bathed by the surrounding water. According to Yonge, (1947) they are always in the direct line of the current or currents produced by the gills. Kohn (1961) on the other hand, states that the osphradium resembles mammalian olfactory organs in that (according to Prosser et al., 1950) a great deal of the sensitive area is not exposed to the main current but to eddy currents. The significance of eddy currents passing over respiratory epithelium of Mammalia is easy to comprehend, one of the advantages being the prevention of desiccation of the delicate membrane. The significance of such eddy currents in the case of an aquatic form remains, to me, unclear.

For many years there has been speculation as to the real function of the osphradium. It is generally held to be an olfactory organ but there are also some workers who regard it as a mechanoreceptor, while yet others are of the opinion that it may perform both of these functions. The osphradium is extremely large in carnivores (Morton, 1960) and that it acts as an olfactory organ in the detection of prey has been proved for a number of carnivorous gastropods eg.

Bullia (Brown and Noble, 1960) and Nassarius (Copeland, 1918).

It is easy to see the importance of such an organ to predators and carrion feeders. The water is continually tested for the presence of food and according to Kohn (1961) the inhalent siphon, which is swung from side to side, is used for the accurate localization of the prey or carrion.

Hulbert and Yonge (1937) were of the opinion that the osphradium could be used as an olfactory organ only by carnivorous gastropods and not by browsing herbivores such as Littorina or Pterocera, which however, both possess large osphradia (Yonge, 1947). Their work on a number of gastropods brought them to the conclusion that the osphradium should be thought of rather as a tactile organ concerned with estimating the amount of sediment in the water. This conclusion is based on the observation that there is usually a correlation between the size of the osphradium and the amount of sediment in the water. The potential value of such a sediment tester in preventing the clogging of the delicate lamellae of the ctenidium is obvious. This neat division of osphradial function into olfaction in the carnivores, and sediment testing in the herbivores is very pleasing but, unfortunately, an oversimplification.

It has since been proved that the osphradium is indeed used by certain herbivores for the detection of food eg. the prosobranch Littorina (van Dongen, 1956 quoted by Kohn, 1961) and the pulmonate Australorbis (Michelson, 1960). Littorina

moreover, exhibits a highly developed olfactory sense in decidedly preferring certain algae above others. Another finding which is inconsistent with the carnivore/olfactory organ, herbivore/sediment-tester theory, is the fact that the pelagic heteropods possess an osphradium. In these forms there is not the remotest danger of the mantle cavity becoming silted up. As the eyes are well developed and used in the capture of prey, the necessity of a chemoreceptor seems dubious. Morton's (1960) assertion that the osphradium of these forms is vestigial, seems the only explanation of its occurrence.

In the opisthobranchs the osphradium and rhinophores act as chemoreceptors. Osphradia are always present in the Tectibranchia (Yonge, 1947), while in the Nudibranchia the olfactory organs are rhinophores. The term rhinophore has been employed for various organs in the Mollusca. It is used by Pelseneer, (1906) to denote structures occurring on tentacle-like processes or, in the cephalopods, for the ciliated pit above the eyes, while Parker and Haswell (1940) also use the term in connection with the cephalopods, but apply it to the process occurring on the aboral side of the eye. According to Jahn and Wulff, (1950) the ciliated papillae found on the cephalic tentacles of many landsnails (~~should read opisthobranchs~~) are known as rhinophores. The term is now generally understood to indicate the cephalic processes of opisthobranchs. The rhinophores are innervated by

the cerebral ganglia (Pelseneer, 1906), but no direct reference to the innervation of the tectibranch osphradium has been discovered.

The relatively large rhinophores are well situated to act as olfactory organs as they are in an exposed position, continually bathed by the surrounding water, and they are anteriorly situated where they would soonest encounter a change in the environment. In the pulmonates the osphradium is lacking in adult Stylommatophora (Yonge, 1947) and the olfactory organs are tentacular, while in the ^{Basommatophora} they are osphradia, though in the latter group both types may occur simultaneously.

Tentacular olfactory organs are of two types; patches of ciliated cells along the tentacle as found in Stylommatophora or an olfactory organ at the base of the tentacle as in Basommatophora (Simroth, 1928). In Simroth (1928) there is some discussion as to the question of possible homology of tentacular olfactory organs in the Pulmonata. It is asserted by Sarasin (quoted by ^{Simroth, 1928} ~~S. 1928~~) that the invagination at the base of the tentacle in the Basommatophora serves an olfactory function and is homologous with the patches of chemosensory cells in the tentacles of the Stylommatophora. Both are innervated by the cerebral ganglia, so on these grounds nothing could be said against the theory.

The mantle may have sensory ridges in the Stylommatophora, or a pit called the osphradium (organ of Lacaze-Duthiers) in

the Basommatophora. Sarasin (in Simroth, 1918) working on a certain Helix sp. came to the conclusion that in that ⁵Stylommatophoran, there were remains of an osphradium. Pelseneer (1906) found in embryonic Helix aspersa osphradial ridges as in adult Testacella and this brought him to the conclusion that all Stylommatophora initially have the "Anlage" of an osphradium but that later in the ontogeny it is lost. According to Thiele (quoted by ^{Kükenthal} K.) the homology of the osphradial ridges of Testacella with the osphradium of Basommatophora is improbable. The tentacles and the organ at the base of the tentacle are both innervated by the cerebral ganglion.

Although the basommatophoran osphradium seems to be tucked away under the collar it is in fact, in the path of the strongest current of water passing over the animal, namely the current which passes posteriad along the left side of the animal, presumably drawn mainly by the cilia of the body wall and pseudobranch. This current is easily demonstrated by placing drops of carmine suspension over the anterior region of the snail. The sensory organ in the tentacular groove is also admirably placed. It is in a protected position yet, as a result of the currents created by the tentacular cilia, which beat towards the base of the tentacle, the organ is provided with a continuous stream of water. B. tropicus usually bears the tentacles extended in the direction of locomotion, and pointing slightly outward. At intervals the

tentacles are lowered to touch the substratum and small particles may be seen moving toward the tentacular groove. In this manner both the layer of water in intimate contact with the substratum, as well as the volume of water surrounding the animal, are tested chemically. At the same time the tentacles receive mechanical stimulation from the substratum or surrounding objects, thus performing a two-fold function.

Piéron (1908) found that in certain *Lymnaea* species the anterior part of the foot was the main site of contact chemoreception, and it may be that contact chemoreception plays an important part in other pulmonates as well. Michelson (1960) has proved that in Australorbis the osphradium is a chemoreceptor and it may be assumed that this is its function throughout the Basommatophora. So far known the shape of the organ in this group is a cup-shaped or slightly elongated hollow. Why the sensory epithelium should be "hidden" in a pit is not known, yet this is the case also in other forms eg. the ciliated pit of the dibranch cephalopods and the olfactory sacs of fishes.

From this very brief survey it becomes apparent that the olfactory organs are very variable in the gastropods. Since the time of Spengel (1881) there has been a trickle of experiments and observation on the chemical sense and organs but, apart from the literature review by Kolm, (1961) no attempt to form an integrated picture of olfaction in the class.

The first point which seems to want clarification is which of the range of olfactory organs or sediment testers are homologous, and what names should be employed to denote them. In doing this the innervation must, naturally be taken into account. The osphradia of prosobranchs are innervated by the parietal ganglia and though no reference to the situation in tectibranchs has been found, one may guess that the innervation is also by the parietal ganglion. In the nudibranchs the rhinophores are innervated by the cerebral ganglia while in the pulmonates the tentacular organs are innervated by the cerebral ganglia and the osphradia by the parietal ganglia. Although it is thus clear that certain of these olfactory organs are not homologous with others, the fact that the osphradia of both prosobranchs and pulmonates are innervated by the parietal ganglion cannot without further ado, be taken to imply that they are homologous. Although this may be the case, the difference in form and situation suggest rather, that they are merely analogous.

The osphradium of the Mollusca, is thus a protean organ of uncertain function, which is, in many cases and which, is lost or retained by certain forms in a rather inexplicable manner. For example, the bottom-dwelling Nautilus, (Tetra-branchia) which has a relatively feeble respiratory current, and might thus be expected to lose its osphradia, has retained them, while the Dibranchia, which have a strong respiratory current which flushes the mantle cavity, have lost them

(Yonge, 1947). The functions of the osphradium seem, on the whole, to be rather closely correlated with the mode of life. Thus in the prosobranchs where the organ is intrapallial and has been proved to have an olfactory function, it is not completely excluded that the organ may function also as a sediment tester. This would be a decided asset to an almost exclusively marine order in which many members are exposed to the danger of silting up of the mantle cavity or ctenidia. The osphradium of the Tectibranchia is thought to resemble that of the prosobranchs, their close relatives. In the terrestrial pulmonates the osphradium is lacking, but in the aquatic Basommatophora this organ is once more to be found (as a completely "new" structure?). The osphradium is situated, now outside the mantle cavity, a functional necessity as, even when the mantle cavity is not completely air-filled, the siphon may occlude the pneumostome, rendering impossible the testing of water by an intrapallial organ. On the basis of their habitat and mode of life, it seems as though olfaction is the function of the osphradium in this group.

3.3 8 The Kidney

The kidney is elongated and curved so as to be roughly U-shaped. The two extremities lie more or less parallel to one another, diagonally across the body whorl, while

the central part lies more or less parallel to the posterior border of the mantle cavity. The ureter is reflected a short distance along the lateral border of the left limb of the kidney.

The saccular part of the kidney lies to the right of the pericardium which it partly enfolds. Histologically this part of the kidney differs from the rest of the organ, in contrast to the kidney of B. boissyi in which the saccular and tubular parts of the kidney are histologically similar (Abdel-Malek, 1955). In B. tropicus the saccular region is lined by a layer of tissue which consists of a network of stellate cells and amoebocytes which are apparently embedded in a matrix which stains light blue (fig. 21). The transition from saccular to tubular part of the kidney is gradual, stellate cells initially occurring among the cells typical of the tubular region, and then, more distally, disappearing.

In the trochids (Prosobranchia) that part of the kidney lying against the pericardium and partly enfolding the ventricle, is called the nephridial gland. Diverticula from the lumen of the kidney push into a cushion of connective tissue, blood spaces and muscle fibres and in this region amoebocytes abound (Fretter and Graham, 1962). There are thus certain similarities between the proximal region of the kidney in B. tropicus and the trochids, viz. the position, partly enfolding the pericardium of the ventricular region of the heart, and the component connective tissue and blood spaces

abounding in amoebocytes. In B. tropicus, however there is no trace of glandular tissue. Fretter (1953) found that in the monotocardian Calyptraea chinensis these wandering cells seem to be concerned with the uptake of ions from the blood, so preserving the ion-balance. It is not impossible that they have the same functions in B. tropicus although they do not ~~have~~ contain granules and inclusions. Because it is a freshwater animal the problems of osmoregulation are more acute than those of marine forms, and what is more, the osmotic pressure fluctuates during drying up of the habitat, or during the rainy season.

The rest of the kidney is lined by an epithelium which changes towards the kidney aperture. In the previous paper (Stiglingh et al., 1962) it was erroneously stated that the kidney lamellae are never solid, for, in fact, more of them are solid than have sparse connective tissue. The latter condition may be artificially brought about by bad narcotization resulting in damming up of blood in the kidney.

Proximally the cells of the kidney are large, and have a granular, reddish-orange cytoplasm basally (fig. 22). Inside this or just above it, round nuclei may be seen. The nuclei take up the blue dye, staining but lightly, and they possess a yellowish nucleolus. The apical part of the cells is clear, probably a vacuole, because in young snails in which the membrane is delicate it is often ruptured during sectioning. In this clear space irregular spherules, the urinary

concretions, occur. These spherules may be seen under low power and if the kidney is damaged during dissection, they may be seen pouring out of the damaged part in great numbers. These granules sometimes stain yellowish or reddish but may remain colourless. The concretions are set free into the lumen of the kidney. Distally the cells are yellow-staining and the cytoplasm is no longer granular but fibrillar in appearance (fig. 23). The nucleus is situated in the middle of the cell and is irregular in shape. Concretions do not occur in this region. The kidney is usually visible through the shell and mantle of the living snail and the proximal and distal parts are easily distinguished, the former being much more conspicuous as a result of the yellow concretions which are visible under low power.

In the proximal part of the ureter the cells resemble those just described, but distally the cells become much smaller, later resembling a columnar epithelium and the nuclei are also basally placed. They are smaller than in the previous section and are also irregular in shape. The cells stain a purplish shade and there is an orange border and scattered ciliated cells and goblet cells occur. A few white gland cells also open into the lumen of the ureter. The last part of the ureter is lined by an epithelium which is very well ciliated. These cilia are apparently responsible for driving the urine to the exterior.

4. THE ALIMENTARY SYSTEM

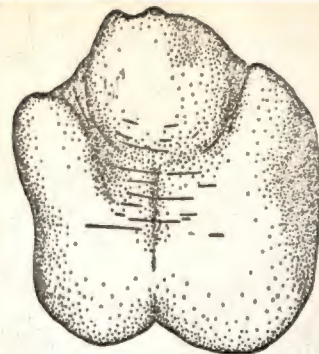
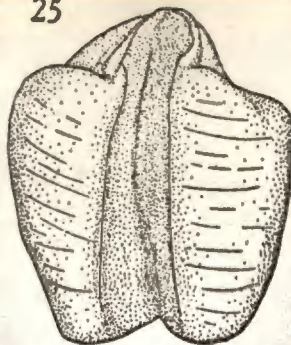
The appearance and situation of the mouth have been described above. The mouth opens into the buccal mass from where the alimentary system is continued posteriorly as the oesophagus. This runs on the right side of the animal, passes through the oesophageal iter (~~see later~~) to enter the anterior part of the stomach. This is a large muscular organ lying ventrally in the visceral mass, slightly on the right side of the animal. The alimentary canal is continued from the posterior part of the stomach, and in this region it receives ~~the hepatic duct~~ from the digestive gland which occupies almost all of the visceral mass posterior to the stomach. The intestine which leaves the posterior part of the stomach passes to the left of that organ and closely encircles it in a more or less horizontal plane running on the left, the anterior and then the right side of the stomach. When the circle is complete the canal makes a loop in the penultimate whorl, thus forming a figure 8 in conjunction with the first part of the intestine. From the centre of the 8 the intestine is continued forward on the left side of the animal, below the floor of the mantle cavity, to open via the anus situated on an anal lobe at the base of the pseudobranch.

24

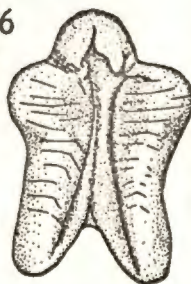


0.01 mm

25



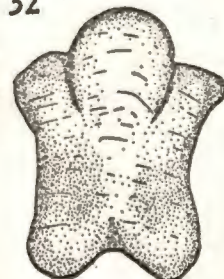
26



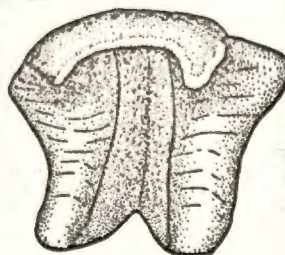
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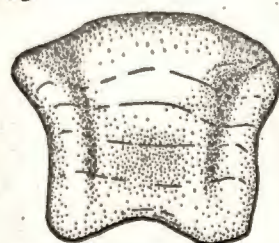
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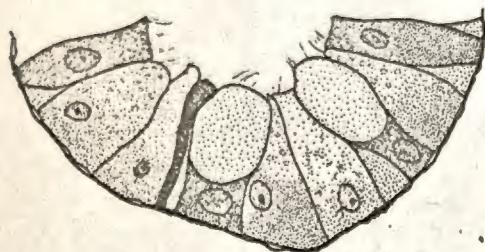
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33



36



0.01 mm

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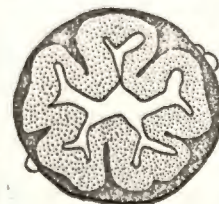


FIG. 24. MICROSCOPIC STRUCTURE OF LATERAL JAW.

FIGS. 25-27. DORSAL VIEWS OF STRAIGHT, SLIGHTLY REFLECTED AND REFLECTED ODONTOPHORAL CARTILAGE.

FIGS. 28-30. LATERAL VIEWS OF STRAIGHT, SLIGHTLY REFLECTED AND REFLECTED ODONTOPHORAL CARTILAGE.

FIGS. 31-33. VENTRAL VIEWS OF STRAIGHT, SLIGHTLY REFLECTED AND REFLECTED ODONTOPHORAL CARTILAGE.

FIG. 36. CROSS SECTION THROUGH THE SALIVARY GLAND TO SHOW THE THREE MAIN TYPES OF CELLS.

FIG. 37. CROSS SECTION THROUGH THE SALIVARY GLAND TO SHOW CALCIUM-RICH SUBSTANCE (VOLUME FORMS).

FIG. 40. DIAGRAM OF A CROSS SECTION THROUGH THE PROESOPHAGUS.

34



0.01 mm

Odontophoral cells.

24a



0.01mm

g. 24a. T/s through jaw.

4.1 The Jaws

Although the structure of the superior and lateral jaws is basically the same, the former is much more robust and heavily sclerotised than the lateral jaws. The superior jaw seems to be formed from its two extremities and possibly also from its dorsal margin. At the two ends of the superior jaw the epithelial cells stain a purplish colour and are so greatly elongated that they appear as loosely packed, fibrous material. The cytoplasm is granular and light, oval nuclei are to be seen a short distance above the basement membrane. The purplish region is succeeded by a region composed of yellowish brown rods embedded in a blue matrix (fig. 24). When these rods are so sectioned that they are seen end-on, they are irregular and geometrical in form, densely cemented together by the blue matrix. The central portion of each rod seems pitted, so that it is possible that these "rods" are in reality fibrillar in structure.

In the lateral jaws the structure is more clearly to be seen and it is evident that the jaws are only a specialised portion of the cuticle which lines the buccal cavity and part of the inner surface of the oral lobes. Lying as they do, at the entrance to the mouth, the lateral jaws separate the external epithelium of the head

from the internal epithelium of the buccal cavity. That edge of the jaw which lies against the outer epithelium is raised rather steeply above the epithelium while the inner edge of the jaw is smoothly continuous with the lining of the buccal cavity and is formed by a gradual thickening of the cuticle. The descending limbs of the lateral jaws become less and less well developed until, at the level of the ventral transverse slit of the mouth, they merge into the cuticle of this region. The lateral jaws are not thought to play a significant role in the feeding process, their main function being to protect the lateral lips which are in contact with the radula during the protrusion of the odontophore.

4.2 THE BUCCAL MASS

The buccal mass is specialized for rasping and consists of the radula, supporting structures and a large number of small muscles arranged in an intricate manner for the manipulation of these parts. The component structures of the buccal mass will be described, but little will be said on their general arrangement which is well known and essentially similar to that of L. stagnalis as described by Hubendick (1957). The reader is also referred to the admirable detailed account by Carriker (1945).

4.21 The radula

For a description of the radula, the reader is referred to the former study on B.tropicus (Stiglingh et al., 1962).

4.22 The Odontophoral Cartilage

The odontophoral cartilage is an elongated and turgid structure consisting of a central part projecting anteriorly beyond the two fleshy lateral parts. The structure is folded along its longitudinal axis so that there is a deep dorsal groove. The anterior part of the cartilage becomes dorsally reflected upon itself during feeding and it is the degree of folding in these two planes that determines the shape assumed by the cartilage. The following nine figures represent the dorsal (figs. 25 - 27), lateral (figs. 28 - 30) and ventral (figs. 31 - 33) aspects of the odontophoral cartilage in an extended, slightly reflected and reflected position, respectively.

At the level of the anterior borders of the lateral parts, the central part of the radula bulges ventrally, forming a gentle protuberance. The elevation of this protuberance varies with the shape of the cartilage and it is over this protuberance that the morphological tip of the radula is draped. The lateral pillars project laterally on either side of the protuberance and serve for the attachment of the muscles of the buccal mass.

As in B. truncatus (Demian, 1960) the posteroventral part of the cartilage has a deep notch but the anterior notch, found in B. truncatus, is absent. When the tip of the cartilage is slightly reflected it appears to form a notch but in the extended condition, or when the cartilage is well folded back, it is seen that the anterior border of the central part is straight.

The substance of the odontophore is composed of a mixture of muscle fibres and vesicular connective tissue cells. The tissue of the two lateral pillars is less compact than that of the central region and is also composed of much larger vesicular cells. These large cells are the elements which lend turgidity to the odontophore and because they are turgid and compressed together, they often appear polygonal in form. (fig. 34) as is the case in certain plant cells. The outline of the cell is distinct but the contents consist mostly of a colourless fluid in which a small cloud, or clouds, of granular cytoplasm may be seen. The nucleus is round, lightly staining and possesses a nucleolus which is not always distinct. Sometimes several small granules may be observed in the nucleus. Varying amounts of pigment granules, not forming aggregations, occur in the cells, but are rather rare in large cells. Calcium occurs only in small amounts in the odontophoral tissue and is present,

not in the cells themselves, but the intercellular substance where it is present in the form of fine granules. In certain of these cells clumps of PAS-positive granules are found, suggesting that these cells store a carbohydrate or carbohydrate-protein substance, probably glycogen.

In the central part, and along the edges of the odontophore the cells which are smaller, tend to be more rounded than the large cells and appear to be filled with a highly staining cytoplasm. Granules occur, generally in a greater numbers than in the large cells. .

In B. tropicus two main types of "vesicular" cell are distinguished, the vesicular cells described above, and Leydig's cells. The latter are termed "Blasenzellen" or vesicular cells by some German authors but it is obvious from the distribution and form of the "Blasenzellen" (see Simroth, 1928) that they are homologous with Leydig's cells rather than the supporting vesicular cells of the odontophore and anterodorsal part of the buccal mass.

In both L. stagnalis appressa (Say) (Carriker and Bilstad, 1946), and A. glabratus (Say) (Pan 1958) vesicular cells are distinguished but in both cases it is the vesicular supporting cells which are described, and no reference is made to Leydig's cells. These vesicular cells are, on authority of Baecker (1932), also termed "Blasenzellen" by Pan (1958).

There are certain similarities between these cells and in both types a considerable amount of variation may occur

but it is thought that in B. tropicus these should be regarded as two distinct types of cell. The similarities are the large size, irregular form and the hyaline appearance of these cells. The differences between the two are that, although the Leydig's cells may sometimes be compressed into a polygonal shape, the cells are, in the majority of cases, quite irregular in outline. In the cytoplasm of large cells of both types there is a marked difference, Leydig's cells containing a rather dense cloud of irregular pinkish staining matter around the nucleus, and sometimes throughout the cytoplasm of the cell. When pigment granules are present they are generally in clumps, as though they may be derived from pigment cells. The cytoplasm of vesicular cells is very delicate, and finely granular, never as dense as in Leydig's cells, and pigment granules are scattered singly over the cell. The third, and a most conspicuous difference between the two types is the structure of the nucleus. In Leydig's cells this is conspicuous, oval, coarsely granular and a large distinct, orange-staining nucleolus is present; while in the vesicular cells the nucleus is smaller, round, lightly staining and the nucleolus is not always easy to discern. A further type of vesicular cell, also functioning as a supporting cell, occurs in the genital system and will be described under that heading.

In preserved material the anterior tip of the cartilage is generally much lighter in colour than the rest of the cartilage and has a whitish sheen. The connective tissue cells are compact and small, and are not vesicular as the others, but apparently filled with a lightly staining cytoplasm. It is assumed that this is an early stage in the development of the vesicular cells.

Externally the transverse fibres of the cartilage are visible. They are especially prominent in the lateral pillars of the structure and also in the posterior half of the ventral surface. Also present is a network of fibres which run at right angles between the anterior and posterior surfaces of the odontophoral cartilage. Contraction of these fibres is partly responsible for the changes of shape which the cartilage undergoes during feeding.

When the radula is protruded the odontophoral cartilage is in an extended position (fig. 25) and the anterior tip lowered beyond the horizontal. The two halves of the cartilage are folded dorsad so that they lie fairly close to each other and, when viewed from the anterior end, the tip of the cartilage may be seen to form a V. When the median transverse fibres contract, as also the antero-ventral fibres of the anterior part of the cartilage, the cartilage becomes flatter and that part of the cartilage lying anterior to the lateral pillars becomes reflected against the dorsal surface of the pillars. (Fig. 27).

This results in the anterior end of the cartilage becoming broad and blunt and during this period the cartilage is returned to the vertical starting position.

The odontophoral cartilage may be considered the "backbone" of the buccal mass for without its support, functioning of that organ would be impossible. It is the cartilage which is directly responsible for the protrusion of the radula, and which thus enables the feeding process to take place.

The radula is not in life a flat structure, but is folded on itself and fits dorsally over the cartilage so that it loosely envelopes the anterior and posterior surfaces of the cartilage. The posterior part of the radula is, in its turn, folded posteriorly to encircle a second supporting structure, the collostyle.

4.23 Collostyle

This is a rodshaped structure which lies posterior to the odontophoral cartilage, extending from the postero-ventral part of the buccal mass to the collostylar hood, which is, when the odontophore is in a resting position, located at the most dorsal point of the odontophore. Although the collostyle is rodshaped, the dorsal part has a posterior groove so that in this region it is also roughly U-shaped in cross section, though not as markedly so as the odontophoral cartilage. The posterior hollow is partly

filled by muscle fibres and vesicular cells of the same type as those occurring in the dense regions of the odontophoral cartilage. The supralateral radular tensors of the hood (see below) are attached to the collostyle, and ventral to that point the posterior hollow of the collostyle becomes obliterated by vesicular cells. These cells differ from those of the odontophore in being of a much more solid appearance. The cell contents stain lightly purple with Mallory's and the cells are cemented together by a blue substance. As in the case of the odontophoral cartilage, the collostyle is ensheathed by a layer of the same connective substance.

4.24 The Buccal Muscles

The radula and supporting structures, constituting the odontophore, are manipulated by a large number of tiny muscles constituting the bulk of the buccal mass. This organ increases slightly in width and greatly in height towards its posterior margin where it terminates abruptly in an oblique surface, so that when viewed from the side, the buccal mass is roughly triangular in shape. Anterodorsally, on the buccal mass where the organ comes into contact with the body wall, is a cushion of vesicular cells, here called the buccal shield.

The muscles differ slightly in a number of respects from those of Lymnaea stagnalis appressa, as described by Carriker (1945), but the similarity is so close that I

have not figured the buccal mass. The names and numbers employed by that author to denote these muscles will be adhered to in order to facilitate comparison of the buccal mass in the two species.

Externally the most conspicuous muscles are those which serve to move the buccal mass as a whole, and therefore connect the buccal mass to the body wall and jaw.

1. Dorsolateral protractors. Origin: These are two flat, thin bands which have their origin as several strands arising from the antero-lateral part of the body wall. The strands are well spaced at the origin and pass between, and are connected to, the strands forming the origins of the preventral protractors. Insertion: They are attached to the buccal mass dorso-laterally, just posterior to the posterior margin of the anterior jugalis, but a few fibres are visible stretching posteriorly beyond this point, on either side of the salivary glands.

2. Preventral protractors. Origin: the anterior ventral body wall behind the mouth. Insertion: anterior to the buccal valve. This muscle consists of numerous fibres which converge at the insertion.

3. Postventral protractors. Origin: the ventro-lateral body wall a short distance behind the mouth, and also in the median fibres of the lateral muscle bands in the body wall. Insertion: Postero-ventrally on the buccal mass on either side of the odontophoral valve. These muscles

are also two thin bands.

4. **Dorsolateral retractors.** Origin: The dorsolateral body wall on a level with, and slightly median to, the eyes. The origin is sometimes at the level of the large membrane in the cephalic haemocoel, and sometimes just below it. This rather high origin makes it probable that the muscle serves not only as a retractor but also as a levator. Insertion: this is double, one of the strands having its insertion at the edge of the buccal shield, about one third of the distance from the front; and the other adjacent to the dorsomandibular dilator in the tissue just lateral, and slightly ventral, to the mandible.

5. **Buccal retractors.** Origin: the columellar muscle where the floor of the mantle cavity becomes applied to the columellar muscle. Insertion: externally and ventrolaterally just posterior to the anterior jugalis. The buccal retractors pass asymmetrically among the ganglia of the central nervous system. (see also 6.2).

6. **Preventral levators.** Origin: the dorsolateral body wall at a point median and anterior to the tentacles. Insertion: the anterior ventrolateral part of the buccal mass.

7. **Postventral levators.** Origin: the same as the preventral levators. (6). Insertion: on either side of the buccal artery between the odontophoral and buccal valves. These muscles are two flat bands.

8. Suboral dilators. Origin: ventral body wall posterior to the oral aperture. Insertion: the anterior ventral wall of the buccal mass a short distance behind the mouth. These are a number of fine bands which depress the anterior wall of the buccal cavity.
9. Dorsomadibular dilators. Origin: the dorsal part of the snout a short distance above the mandible. Insertion: dorsal mandible. The muscles are not attached directly to the mandible but to the tissue immediately overlying the mandible. These are a large number of fine bands which elevate the mandible and draw it posteriad.
10. Suspensor of the radula sac. Origin: the dorsal surface of the buccal pouch. Insertion: over the ventral end of the radula sheath. This is a thin and relatively short band of fibres lying beneath the posterior jugalis (13).
11. Labial retractors. Origin: the body wall in the vicinity of the oral aperture. Insertion: the lips. In the midventral position there are only a few small fibres but the fibres connected to the lateral lips are well developed and function in drawing the lips outward to open the mouth.
12. Labial sphincter. These are fine circular fibres located within the body wall. They encircle the mouth which they serve to close.
13. Anterior jugalis. Origin: the origin of this muscle is not clear as it is thin at its anterior end where it overlies the buccal sphincter and it is partly obscured

by the buccal shield. The muscle extends as far as the mandible which may be taken as its origin, but it is also attached to the underlying muscles of the anterior part of the buccal mass. Insertion: This is conspicuous and may be seen externally and ventrolaterally on the odontophoral cartilage. This muscle is the most conspicuous of the superficial muscles of the buccal mass and it is very roughly triangular in shape, the apex being the origin, anteriorly, and the two corners at the base of the triangle being the insertions of the muscle. Contraction of the muscle thus results in protraction, and also elevation of the odontophoral cartilage, bringing it into a more vertical position.

14. Posterior jugalis. Origin: the dorsal mandible, Insertion: over the ventral end of the radula sac and ventrolateral surface of the buccal mass. This muscle consists of a number of sparse and rather thick fibres which pass posteriorly to form a delicate sheet which envelopes most of the buccal mass and finds its insertion over the ventral end of the radula sac, and ventrolateral surface of the buccal mass. Contraction of this muscle protracts the rear end of the buccal mass resulting in the organ becoming shorter and assuming a more bulbous form. In the sheet, muscle fibres are better developed in some areas than in others, this being most clearly seen at the posterior surface of the buccal mass, where the fibres pass lateral to the salivary glands and then cross over the radula sheath.

This crossing of the fibres is not mentioned by the other authors and yet is of great importance in an organ with this type of supporting system. Such a crossing of fibres or muscle bands occurs not only in the buccal mass but also, to a considerable extent in the body wall and the foot.

15. Buccal sphincter. This is a thick muscular ring visible through the anterior jugularis. It lies just posterior to the mouth and encircles that part of the buccal mass which acts as a passage between the mouth and the bulbous part of the buccal cavity into which the odontophore fits. Contraction of the muscle results in a narrowing and lengthening of the passage. The buccal sphincter lies sandwiched between the anterior and posterior jugalis.

16. Mandibular approximator. This is a U-shaped muscle, the ends of which are attached to the extremities of the mandible. This is a well developed muscle lying anterior to the buccal sphincter, and acting antagonistically to the dorsomandibular dilators (9); contraction results in the lowering of the mandible and closing of the mouth.

17. Dorsal odontophoral flexor. Origin: the extremities of the dorsal mandible and the antero-dorsal part of the buccal mass. Insertion: along the dorsal third of the lateral pillars of the odontophoral cartilage. The muscle lies under the anterior part of the posterior jugalis and buccal sphincter and partly adjacent to the buccal epithelium. Contraction of this muscle results in a protraction

of the cartilage and also a lowering of the anterior part of the structure so that it lies in a more horizontal position.

18. Ventral odontophoral protractor. Origin: the lateral mandibles and possibly, the ventral lip. Insertion: laterally towards the base of the lateral pillars of the odontophoral cartilage, and extending dorsally as far as the dorsal odontophoral flexors. In Lymnaea this muscle is a single sheet which is thicker at the sides than in the ventral part but in B. tropicus only lateral bands are clearly seen so that a pair of muscles may be present.

19. Infraventral odontophoral protractors. Origin: immediately behind the oral aperture. Insertion: the ventral base of the cartilage. They are a pair of flat bands lying in the ventral surface of the buccal mass and laterally they abutt against the anterior jugalis.

20. Supramedian radular tensors. Origin: the ventral posterior extremity of the cartilage. These muscles form two conspicuous knobs at the posterior ventral end of the buccal mass. Insertion: at the dorsal end of the radula sac. These are two distinct and well developed muscles which, directly after their origin, curve into the buccal mass where they lie between the cartilage and the anterior surface of the radula sac. At their insertion they are, in B. tropicus, broad and thin and lie contiguously, so that they may be fused.

21. Supralateral radula tensors. Origin: the outer ventrolateral extremities of the cartilage. The insertion is threefold. (1). Some of the fibres are attached to the middle of the posterolateral surface of the radula sac. (2). The second insertion is at a point more or less where the radula emerges from its sheath. This muscle has an origin apart from the rest of the fibres which are united to form the bulky mass of the muscle. Its origin is posterior to that of the rest of the muscle, and it stretches from the posteroventral part of the cartilage, running dorsally over the shoulder of the lateral pillar of the cartilage, to become attached to the subradular epithelium. Although this little muscle is distinct from the rest of the supralateral radula tensor it performs the same function and does not seem to merit a special name. (3). The ventral surface of the buccal cavity epithelium anterior, lateral and just posterior to, the dorsal part of the cartilage. These form the two large muscle masses which constitute the posterolateral part of the buccal mass. They are by far the largest muscles of the buccal mass and are easily seen through the thin posterior jugalis.

22. Inframedian radular tensors. Origin: the anterior face of the base of the cartilage. Each of these muscles seems to be one wide band which later splits, one part becoming attached to the underside of the subradular epithelium along the border of the radula. The other narrower part becomes attached to the subradular epithelium

at the transverse fold of the radula, just anterior to the insertion of muscle no. 2 of the supralateral radula tensors, so that the two act antagonistically. The latter muscle exerts a backward pull on the radula, while the inframedian radula tensors exert a forward pull on the radula.

23. Infralateral cartilage tensor. This is an arciform muscle similar to the mandibular approximator, and similarly situated, but posterior, and adjacent to, the buccal sphincter. The ends of the muscle are inserted on the lateral edges of the cartilage and contraction causes a flattening, and therefore a broadening of the cartilage.

24. Intracartilage tensors. (see description of the odontophoral cartilage).

25. Lateral suspensors of the radula sac. Origin: the posteroventral extremities of the cartilage median to the origin of the supramedian radular tensors (20). Insertion: the ventral base of the radula sac. The muscles are fine and short and horizontally placed.

26. Inferior suspensor of the radular sac. Origin: the posteroventral extremities of the cartilage just anterior to the origin of the supramedian radula tensors (20). Insertion: the distal base of the radula sac.

27. Superior suspensor of the radula sac. Origin: the dorsal surface of the buccal pouch. Insertion: the distal end of the radula sac. These three muscles (25 - 27), are fine and seem to form a sheath. They are not sturdy and their function seems to be the stabilizing and binding of the radula sheath and the adjoining cartilage.

28. Tensor of the hood. Origin: midway on the posterior surface of the radula sac on a level with the insertion of the supralateral radula tensors (21, No. 1). Insertion: the under surface of the collostylar hood. This is a small X-shaped

muscle, also of stabilizing function.

In considering the functioning of the buccal mass one cannot ignore the importance of the associated glands which serve both to lubricate and probably to consolidate the food; and to set into motion the processes of digestion. The glands here relevant are the buccal glands embedded amongst the muscles, and the salivary glands, which though not situated in the buccal mass, pour into it their secretion.

4.25 Buccal Gland

This differs from Pan's (1958) description of the buccal gland of A. glabratus, both in the distribution of the gland cells and in the manner of secretion.

In A. glabratus the gland is situated mainly on the dorsal wall of the buccal mass but extends also to the ventral wall, but in B. tropicus the gland is composed of many cells of the type found in the oral lobes and these cells occur either singly or gathered into small clusters, but mainly clumped together to form two large islands which are dorso-laterally situated. (fig. 35). These islands occur in the anterior narrow part of the buccal mass between the mouth and the radula, and are embedded in the muscles which probably help, by their contractions, to force the secretion out of the gland cells into the buccal cavity. Scattered gland cells are found also over the roof of the buccal cavity and, as in A. glabratus, in the proximal region of the oesophagus. These glands are filled with PAS-positive material which stains a deep purplish pink, and in sections at a certain

level, are seen to be the same colour as the glands of the oral lobe, both types being lighter in colour than the anterior foot glands which are of the same type, but stain a darker hue.

The most important difference between the buccal glands of B. tropicus and those occurring in A. glabratus, lies in the manner whereby the secretion is extruded. The buccal glands of A. glabratus are reported ^{by Pan (1958)} ~~to be~~ ^{to be} ductless, the secretion being forced into the buccal cavity by contractions of the muscles of the buccal mass. This seems to be an unusual arrangement especially for a gland of the alimentary canal. In B. tropicus the ducts of the glands are clearly visible, especially in PAS-stained sections.

4.26. Salivary Glands

These are tubular glands consisting of a single layer of very large gland cells. The epithelium rests upon a fine, but distinct, basement membrane and the whole structure seems to be bounded externally by sparse longitudinal muscle fibres and connective tissue including pigment cells. The cells lining the tube are ciliated and are all secretory in function, though naturally not simultaneously in the same phase. Three types of cell are distinguished. (fig. 36).

The most conspicuous type of cell, after MBE staining, is

also the largest. In sharp contrast to the rest of the cells which are lighter in colour, these cells are reddish purple and they contain conspicuous nuclei which stain a deeper purple, and seem, on the whole, larger than the nuclei of the other cells. It appears that the nuclei are initially centrally placed, but as the secretion gathers near the apex of the cell, that region becomes bulbous and the nucleus displaced towards the cell base. Also the secretion of these cells stains purplish with MBE, indicating that it is amphophilic, but whereas the cytoplasm is finely granular, the secretion is homogeneous. All that is known further of this secretion is that it is calcium rich, (fig. 37) and PAS-negative. These cells differ from those of L. stagnalis in having, at this stage, the large chromatin rich nucleus which is figured, by Carriker and Bilstad (1946), for the type of cell containing the alveolar cytoplasm. Further, it differs in the situation of these enormous nuclei which are always near the centre of the cell, just below the homogeneous secretory mass.

The second type of cell occurs far less frequently than the first. This type of cell is clubshaped and narrow, (fig 36), the secretion stains very faintly blue with Mallory's, and an intense purplish pink with PAS. The third type of cell is apically large and possesses coarsely reticular to alveolar cytoplasm. In these cells the nucleus is much smaller than in the first type of cell,

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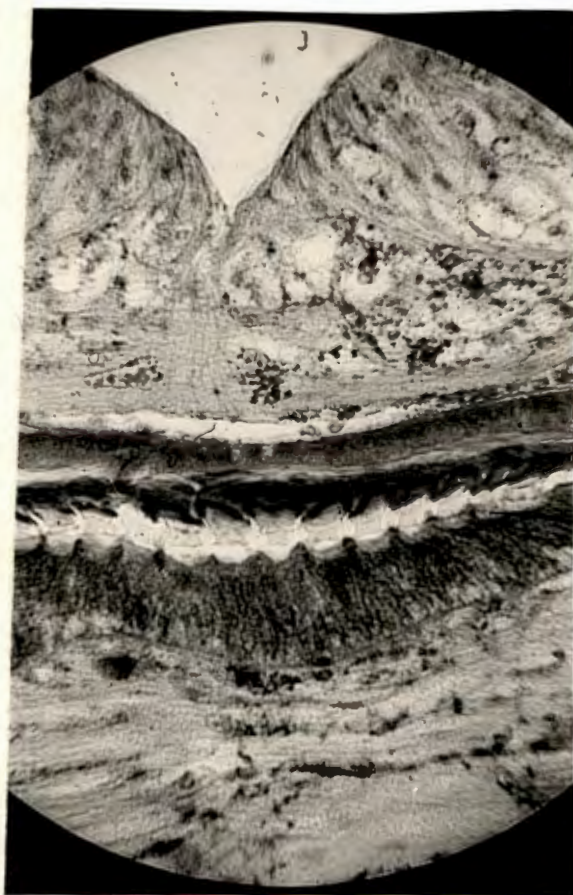


Fig. 38. T/s through buccal mass to show denticle etchings in cuticle.

and is always basally placed. In this third type of cell, two varieties are distinguished. The most commonly occurring type is yellowish in colour after MBE staining, while the other type, which is basally narrow, contains a coarsely alveolar basophilic cytoplasm. In these cells the nucleus too, is stained blue though the nucleolus remains reddish, and this blue nucleus is taken to indicate that the cells are degenerate.

4.27

Buccal Cavity

This chamber is largely filled by the odontophore which is situated in its posterior region. The lateral walls of the buccal cavity, anterior to the odontophore, are swollen and constitute two cushions which can be apposed so that the odontophore is snugly surrounded. These cushions consist of the same type of vesicular cells as those found in the odontophore and collostyle. The posterior extremities of these cushions are continued as dorsolateral ridges which later merge into the dorsolateral ridges of the oesophagus.

The walls of the buccal cavity are clothed by a tall columnar epithelium in which no goblet cells were discerned. In this absence of goblet cells the buccal epithelium of both A. glabratus (Pan, 1958) and B. tropicus differ from that of the prosobranchs, in which most of the glands opening into the buccal cavity are goblet cells. (Fretter and Graham, 1962).

B.P.

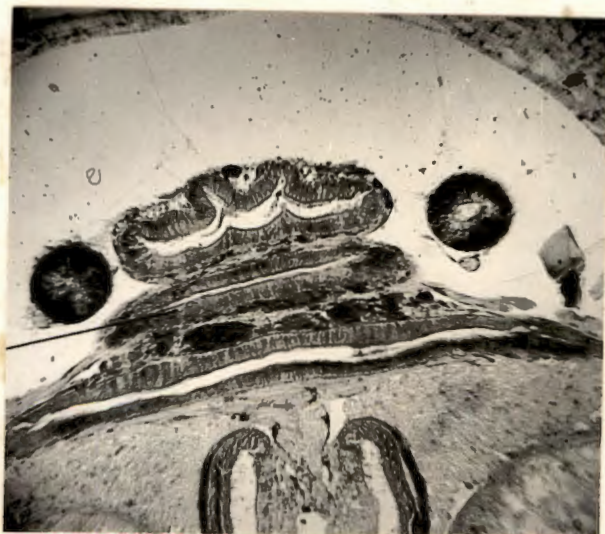


Fig. 39. T/s through head region showing situation of buccal pouch.

The buccal ^{cell}~~epithelium~~ of B. tropicus exhibits secretory activity. The PAS technique reveals that the apical regions of these cells contain a lightly PAS-positive substance. The cells are protected by a cuticle except in the posterolateral regions where the cells become cuboidal or flattened. It is noteworthy that this cuticle remains blue upon staining. As it merges into the modified "cuticle" forming the jaws, it takes on an orange-brown colour, and in the cuticle of the external surface of the organism is a bright orange. It differs also in appearing opaque blue whereas the orange cuticle appears refractile. Cuticular protection is necessary in those areas exposed to the abrasive action of the denticles during protraction, and especially during retraction, of the radula. The cuticle is thus strongly developed in those areas most exposed to abrasion. Because the crest (tip) of the radula is elevated during the retraction stroke, the roof of the buccal passage is also exposed to the tips of the sturdy denticles of the lateral teeth, and this region of the buccal cavity has consequently the best protection, the anterior part of the dorsal food groove being lined for a considerable distance by an unusually thick cuticle. The surface of the cuticle has fine longitudinal grooves etched into it by the denticles of the teeth, (fig. 38). Similar grooves are also evident in the cuticle of lateral parts of the buccal cavity which come into contact with the radula.

When the cuticle is sectioned in a plane parallel to its surface the structure is seen to consist in a large number of short rods which are geometrical in cross section. Each rod is the product of a single epithelial cell, and the geometrical shape is the result of the close packing of the cells forming the jaw.

The oesophagus opens dorsally into the posterior part of the buccal mass and the buccal cavity is continued posteroventrad for a short distance beyond this point. Lying ventral to the oesophagus, against the dorsal surface of the buccal mass, is a flat posteriorly directed pouch which is a continuation of the buccal cavity. (fig. 59). This pouch seems to be unnamed though it is figured by Carriker (1945) and Morton (1960) and other authors. This pouch will be referred to, in B. tropicus, as the buccal pouch. It has delicate walls which consist almost entirely of columnar epithelial cells which possess a cuticle which stains blue with Mallory's, and is PAS-positive. The nuclei are roundish and stain lightly and possess clearly visible nucleoli. These epithelial cells are ensheathed by a few muscle fibres. Gland cells which are part of the buccal gland occur on both the dorsal and ventral surfaces of the pouch, those ventral to it opening into the buccal cavity while those dorsal to it open into the pouch itself.

The buccal pouch of B. tropicus appears to differ

from the pharyngeal evagination of B. mooiensis which extends posteriad into the buccal mass (Oberholzer, unpublished thesis).

A dorsal pouch occurs also in the Pyramidellidae (Fretter and Graham, 1949) and an evagination of the dorsal wall of the pharynx is found in the Goniodoridae. These pouches are both functional in the specialised feeding methods, (sucking) of these animals and bear no relation to the buccal pouch of B. tropicus.

In this form it is thought to be merely a mechanical device permitting the crest of the radula to be lowered into a horizontal position. In dissections the extent of the buccal pouch is seen to vary greatly, sometimes being large and at other times narrow, depending on the muscular state of the animals. The crest of the radula and the buccal pouch walls may be compared to a drawbridge and its ropes. When the cartilage is in a horizontal position the posterior wall of the buccal pouch is drawn forward and the walls become taut, and the pouch small and narrow. When the cartilage is elevated to a vertical position the slack of the walls is taken up in a posterior folding of the epithelium, which so forms the buccal pouch.

4.3

OESOPHAGUS

This consists of two parts for which Carriker's (1945) terms prooesophagus and postoesophagus

will be used. The proesophagus is the narrower of the two and it extends from the buccal mass as far as the ganglionic ring. The proesophagus is usually slightly flattened in cross section, and internally it has a number of longitudinal folds. In B.tropicus there are usually 5 large folds; two dorsolateral and one ventral and two ventrolateral, (fig. 40), but a number of tiny intermediate ridges also occur. The degree of development of the ridges varies so that there may be three well developed dorsal ridges. The epithelium of this region is so tall that the ridges are composed almost entirely of epithelial cells. There is, however, a small core of loose connective tissue containing pigment cells and muscle fibres. The fibres are arranged in an inner longitudinal and an outer circular layer and the musculature is better developed than that of the intestine. As in A.glabratus (Pan, 1958) the epithelial cells are supported by a well developed basement membrane. The cells are of variable height, are well ciliated and contain roundish basally placed nuclei. A lightly staining cuticle is present and in some sections a faint black line is visible near the apex of the cell, and this probably represents the basal granules of the cilia. The cytoplasm of these cells is secretory, numerous globules which stain orange with Mallory's are seen lying in spacious vacuoles. The same stain indicates that the apices of the cells are filled with a mucous secretion. This secretion

is lightly PAS-positive, and was found to be rich in calcium.

As in A. glabratus (Pan, 1958) goblet cells (in the usual sense of the word) are not found among the epithelial cells. Mucous glands, of the same type as those constituting the buccal gland, are attached to the external surface of the oesophagus in the region of the buccal mass, but occur throughout the length of the oesophagus in the connective tissue of the grooves. Pan (1958) refers to these cells as goblet cells, a term which seems to me rather inappropriate.

The cells are not of uniform height and there are patches of low, unciliated cells in the oesophagus. These cells may represent a certain phase of the secretory cycle. Baecker (1932, in Carriker, 1946) observed a similar phenomenon occurring in the ciliated cells of the gut of the Stylommatophora. There is little histological difference between the cells of the pro- and postoesophagus. The latter originates at the point where the alimentary canal passes through the nerve ring. At that point it usually becomes wider and the walls thinner. The longitudinal folds become much less pronounced, though externally about 8 black longitudinal lines are still to be seen. In some cases the lumen was found to be almost smooth, probably when the oesophagus was in a greatly distended condition. In the posterior part of the postoesophagus the internal ridges are again

pronounced but never as well developed as those in the proesophagus.

The cells of this region are lower than in the proesophagus, the cytoplasm is more homogeneous and the apices of the cells tend to be rounded. In the posterior part of the oesophagus clear spaces are seen which may be non-staining goblet cells, but it is also possible that these are merely the empty distended ducts of the subepithelial PAS-positive glands. These often appear to be PAS-positive goblet cells but in the preceding or following section, the body of the gland may be discerned. Carriker and Bilstad (1946) also mention that the ducts of the buccal glands may become distended. They found this enlargement to occur just beneath the distal end of the cell, but in B. tropicus the swollen portion is of a much greater size and generally extends throughout the length of the cell.

4.4 STOMACH

The stomach is a large rounded organ with thick muscular walls and, as in other planorbids, consists of two lateral halves, the gizzard pads, connected dorsally and ventrally by the gizzard tendons. In B. tropicus these structures are not as distinct as they seem to be in B. truncatus (Demian, 1960). No hard cuticular lining to the pads is visible, all that is apparent is a tough silvery dorsal and ventral zone joining the two lateral halves of the gizzard.

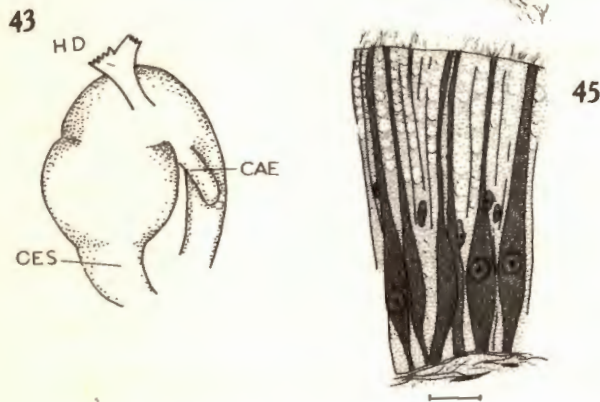
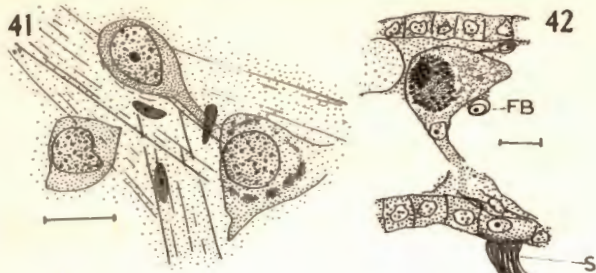


FIG. 41. GIANT CELLS OF THE STOMACH WALL.

FIG. 42. A GIANT CELL FROM THE MANTLE.

FIG. 43. FIGURE SHOWING RELATION OF STOMACH, CAECUM, INTESTINE AND HEPATIC DUCTS.

FIG. 45. CELLS OF THE CAECAL RIDGE.

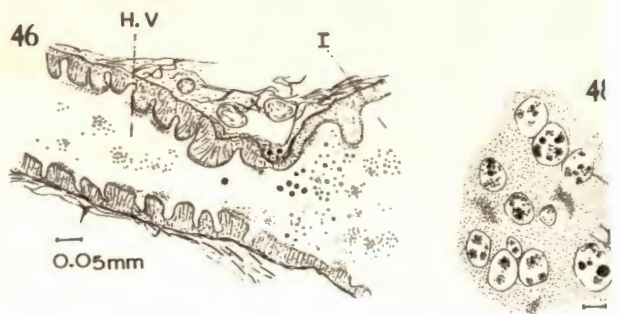


FIG. 46. FIGURE SHOWING CORPUGATIONS OF THE HEPATIC VEST.

FIG. 47. DIGESTIVE GLAND

FIG. 48. FAECAL VACUOLES IN INTESTINAL CONTENTS

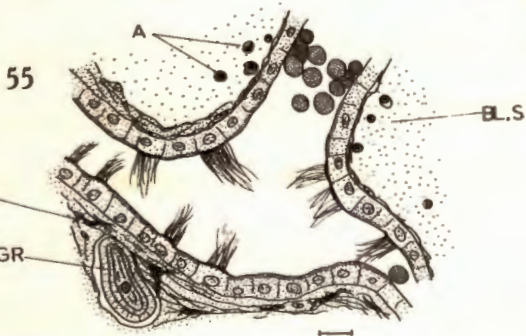
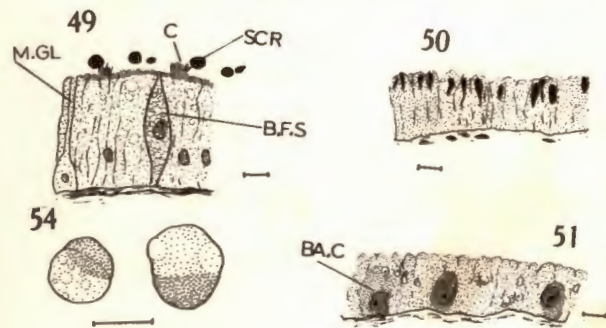


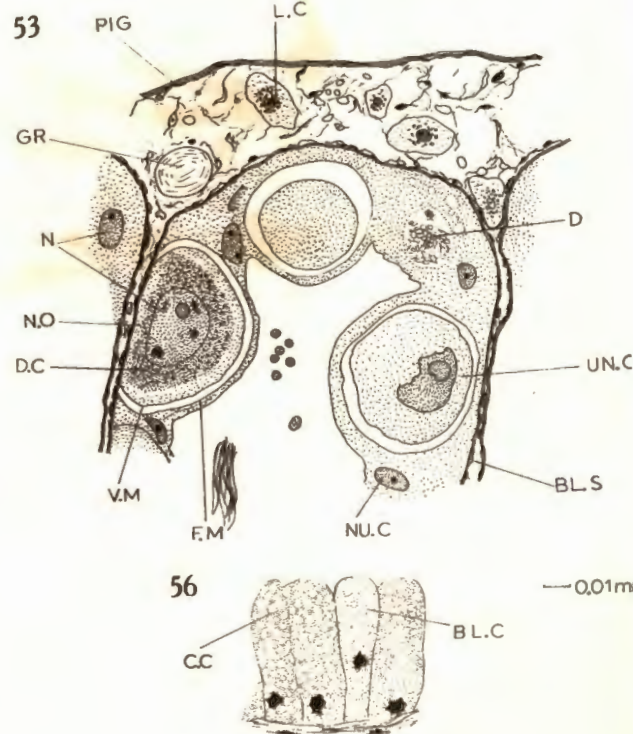
FIG. 49. PROINTESTINE.

FIG. 50. CALCIUM RICH CELLS IN PROINTESTINE (VON KÖSS).

FIG. 51. BASAL CELLS OF MIDINTESTINUM.

FIG. 54. DOUBLE NUCLEOLI

FIG. 55. COMMON ATRIUM



FIGS. 52, 53. HEPATIC VEST; DIGESTIVE GLAND

The stomach epithelium is no way remarkable. It rests upon a thick basement membrane and consists of tall, columnar cells which bear stiff, moderately long cilia. The cells are, as usual, secretory, staining lightly PAS-positive, while vacuoles are sometimes to be seen. On the whole, the cytoplasm appears to be granular and there is a thick PAS-positive cuticle which serves to protect the cells from the abrasive effect of the sand which is ingested by the snail. The nuclei are round to oval, possess a distinct nucleolus and are situated towards the base of the cell. The subepithelial gland cells possess fine ducts which may be seen passing to the lumen of the stomach, and the bodies of the glands themselves are small and intensely PAS-positive. The muscular walls of the gizzard are composed of 8 or 9 layers of fibres which are arranged at right angles or obliquely to one another. It is interesting to note among the cells of the stomach wall giant cells (fig. 41) which are irregular in shape and contain relatively huge nuclei, thus bearing a superficial resemblance to ganglionic cells. The nuclei are rather poor in chromatin which is gathered into loose aggregations. The nucleolus is discernable but ill-defined and seems to consist of a similar but denser, aggregation of chromatin granules. The cytoplasm of these cells seems to contain tiny vacuoles. Such cells were also found in the gastric tissue of A. glabratus (Pan, 1958) and, as in

44



that snail, these cells sometimes contain a small amount of PAS-positive material. No suggestion is made by Pan (1958) as to the function of these cells but he regards them as being connective tissue elements. Carriker and Bilstad (1946) on the other hand, regard them as giant nerve cells, and on the basis of their nuclear structure, I incline to the latter view. These giant cells have also been observed in other parts of the body of B. tropicus, eg. in the sparse connective tissue between the stomach and the liver, throughout the length of the alimentary canal, and also in the mantle, (fig. 42). In the latter situation it is difficult to be certain about their occurrence as they may be confused with developing gland cells (M_1) which have enormous nuclei.

The pyloric region of the stomach is similar to the anterior region.

At the distal end of the pylorus the alimentary canal is in communication with two structures, the caecum and the digestive gland. The mutual relations of these parts are best understood by referring to figure 43.

4.5

CAECUM

The caecum is a cylindrical, an elongated sac which has two large internal ridges situated opposite each other dividing the lumen into two unequal spaces, the small incurrent and larger excurrent channels (fig. 44).

The caecum wall is composed of a single layer of epithelial cells resting upon a well developed basement membrane and ensheathed by muscle fibres and sparse connective tissue. The incurrent channel is lined by columnar epithelial cells while the excurrent channel, though lined by the same type of cell, differs in that cells lining last mentioned region are more than twice as tall as those lining the incurrent channel. The cytoplasm of the excurrent channel shows signs of much secretory activity, and large secretory drops can be seen in the lumen of the caecum lying above the epithelial cells. The cilia of the caecum are stiff and short, giving the impression of sturdiness. The nucleus in the cells of the two channels is oval, lightly staining and situated anywhere in the cell from above the middle to the base. Occasional goblet cells seem to be present in this region. The nucleolus is prominent.

The cells of the caecal ridges resemble those so far described but are extremely elongated (fig.45). The cytoplasm contains so many secretory droplets that the cell sometimes appears to contain a row of vacuoles. Two types of cells are distinguished in the ridges, The majority of the cells is of the highly vacuolated type and the nuclei are elongated and situated near the centre of the cell. The other type of cell is a narrow goblet cell which has a round nucleus situated near the base of the cell.

The latter cells are PAS-positive. Neither of these cell types gives a positive reaction to the calcium test.

4.6

DIGESTIVE GLAND

The digestive gland consists of a great many more or less similar lobules. The gland is connected to the alimentary canal by means of the hepatic ducts. These ducts have smooth, thin walls except in the region adjoining the hepatic vestibule. This portion of the duct, as in L. stagnalis appressa (Carriker and Bilstad, 1946) and A. glabratus (Pan, 1958) is corrugated (fig. ⁴⁶~~36~~) and, in this, differs from B. mooiensis (Oberholzer, unpublished thesis).

Histologically this gland agrees closely with the description of Pan (1958) and Carriker and Bilstad (1946) for the livers of A. glabratus and L. stagnalis appressa respectively.

There is general agreement among authors that the digestive gland of the Pulmonata, is composed of two main types of cell viz, the digestive cells, which are present in various phases, and the lime cells. As is the case in both L. stagnalis and A. glabratus, mucous cells are also present in the liver of B. tropicus.

The digestive cells are the most commonly occurring, (fig. ⁴⁷~~37~~), in fact they constitute the greater part of the gland. They may be regarded as large, modified columnar epithelial cells.

In the description of the digestive gland of Burnupia (Oberholzer, unpublished thesis), no mention is made of the presence of cilia, but in B. tropicus these cells bear short cilia. The cytoplasm is full of vacuoles and inclusions of various forms so that the cells have a highly heterogeneous appearance. The nucleus is situated towards the base of the cell, is oval, rather poor in chromatin and possesses a distinct nucleolus. The cytoplasm in the basal region of the cell is grossly alveolar and stains orange, while that of the swollen apical part of the cell stains blue, indicating the presence of mucus. This region of the cell stains a very light pink upon being treated with PAS.

The secretory activity of these cells is evident from the numerous large globules which may be seen passing from the cells to the lumina of the tubules. From the appearance of the slides, it seems as though patches of cells often tend to be in the same phase simultaneously. Groups of cells are often observed to be either in the secretory phase (when the lumen becomes rich in secretory globules) or in the "digestive" phase (when the cells contain many inclusions and faecal vacuoles). The middle region of the cell is taken up by vacuoles and inclusions which are various shades of light yellowish-green and blue-green. Masses of material having these colours are also reported as occurring in Burnupia mooliensis (Oberholzer, unpublished).

From the work of certain authors quoted by him he designates the yellowgreen bodies enterochlorophyll. I find this designation unacceptable as it seems unlikely that chlorophyll would occur in that form in an intracellular position. Furthermore such vacuoles are also formed in the ovotestis, and the finely particulate matter is therefore thought to be debris. This interpretation is also in agreement with the work of Carriker and Bilstad (1946) who designate such vacuoles as faecal vacuoles, and the compact masses (generally blue-green in B. tropicus), excretory bodies. As was found in L. stagnalis appressa (Carriker and Bilstad, 1946) these vacuoles are to be seen in the contents of the intestine (fig. ⁴⁸~~38~~) possibly through a sloughing off of the distal (faecal vacuole containing) portion of the digestive cells.

A puzzling point in connection with the liver is the fact that in B. tropicus the only PAS-positive material seen in the liver was that of the mucous cells, yet the digestive cells are supposed to store glycogen (several authors including Hurst, 1927^{quoted by} ~~in~~ Carriker and Bilstad, 1946).

The digestive gland of B. tropicus differs markedly from that of Burnupia mooiensis as described by Oberholzer (unpublished), in possessing lime cells. The lime cells of B. tropicus show general agreement with those described by Pan (1958) and Carriker and Bilstad (1946) except that the large, bodies described by Carriker and Bilstad (1946)

do not occur. These lime cells do not give a positive reaction to the calcium test, although several authors (quoted by Carriker and Bilstad, 1946) have found that they are responsible for storing calcium. This raises the question of the true function of these cells.

It is perhaps possible that the cells are not at all important in calcium metabolism. The other alternative is that the cells are important but that they contain calcium which is chemically bound to some other substance which prevents the von Kóssa reaction from taking place. If these cells indeed play a role in calcium metabolism a further question is posed, viz. how does Burnupia manage without them?

The mucous cells of the liver are sparsely scattered and smaller than the other types of cell found in this organ. They agree with the description of the liver mucous cells of B. mooiensis (Oberholzer unpublished). They are flask-shaped cells having a fairly broad base and a nucleus moderately rich in chromatin granules. The neck of the gland is narrow and the whole is striking in PAS-stained sections but less obvious when stained by other techniques, and could easily be overlooked. They are, nevertheless, also to be seen in sections stained with Mallory's and in such slides assume a light blue colour. The mucoid secretion of these cells is in the form of large globules.

4.7

INTESTINEThe Prointestine (fig. ⁴⁹~~39~~)

This consists of a single layer of epithelial cells surrounded by circular and longitudinal muscle fibres and an external layer of pigment cells. The epithelial cells are fairly slender and possess a well developed cuticle. They bear short, sturdy cilia and the nucleus is situated between the middle and the base of the cell. The cytoplasm is granular and in the apical part of the cell secretory spherules may be seen. The second type of cell is a large goblet cell with a rounded body and fairly narrow neck region. These cells stain a very light blue and are filled with a globular secretion. The nucleus stains purple and possesses an orange staining nucleolus which is clearly visible. It is thought that three types of cell occur though details of the structure of the third type are wanting. On the whole the epithelial cells stain very lightly pink with PAS, but rarely small accumulations of intensely PAS-positive material may be seen near the lumen of the intestine and in certain slides it can be seen that this is derived from cells with rounded bodies and long narrow ducts. The nuclei of these cells are round, basally situated and fairly rich in chromatin. Such cells become visible after applying the von Kossa technique. (Fig. ⁵⁰~~40~~). The cells then appear jet black, showing that they are very rich in calcium. Throughout the epithelium

vacuoles and inclusions may be seen and there is a high degree of secretory activity.

The Midintestine (fig. ⁵¹~~41~~)

The muscular layer is poorly developed in this part of the intestine. As in the former region three types of cell are distinguishable. The most outstanding of these are the very large cells which Carriker and Bilstad (1946) call basal cells, after Howells (1942), quoted by ~~in~~ Carriker and Bilstad, (1946) who described such cells in Aplysia. These are large, squat cells. They are initially mound-shaped and do not extend as far as the lumen of the intestine, but they later grow to such a size that they do extend that far. The cytoplasm is alveolar, stains blue with PAS and there is a huge, chromatin rich nucleus. In PAS-stained sections the nucleolus is indistinct, but in MBE sections, the chromatin granules of the nucleus are clearly resolved and there is a large homogeneous nucleolus. When these cells are mature they communicate with the lumen by a rather narrow neck, so that the cell is flask shaped. This is best seen in Mallory-stained sections. In these, there is present in the neck region an accumulation of smallish, deep orange secretory droplets. The second type of cell is a very compressed, lightly staining cell which has a nucleus situated high in the cell body. These nuclei are chromatin poor and there is a distinct nucleolus. The third type of cell occurring in this region is a PAS-positive club shaped cell in which the globular secretion

obscures the nucleus.

The Postintestinum (fig. 42)

The musculature of this region is also weakly developed. The cells lining the postintestinum are tall columnar cells containing granular cells with swollen apices. The nuclei are found to be lightly staining and contain distinct nucleoli. The second type of cell is rather slender, being clubshaped, and stains a very light blue. A third type of cell in this region is PAS-positive, and is a stout goblet shaped cell in which nuclear detail is masked by the dark colour assumed by the cell after staining.

The Rectum

In the rectum two regions are distinguished. In the more proximal of these the lumen of the rectum resembles that of the rest of the intestine. There is a well developed basement membrane and in certain of the sections the cells of this region are very irregular, being compressed and distorted and having a very uneven height. The nuclei too, show signs of compression and are irregular in form. This is perhaps to be attributed to the state of contraction of the rectum. The secretion of these cells is globular and the apices of the cells are distended. A sparse and delicate ciliation seems to occur in this region but no trace of a cuticle could be found.

PAS-positive goblet cells also occur in this region.

The second part of the rectum is thrown into a number of longitudinal folds as in the oesophagus. About 7 or 8 large folds occur in this region, and lying between these, are a number of smaller folds. The cells are columnar with large round to oval nuclei which stain but lightly. Scattered chromatin granules occur in these nuclei. The apical cytoplasm of the cells is dense, and a cuticle is to be found in this region. No cilia were observed. The contents of the cells does not give any reaction to the von Kóssa test, but the margin of the cells in contact with the lumen, is shown to be rich in calcium. It is not known whether this calcium is located in the cuticle or not, but more probably it is in the mucus secreted by the cells.

5. FURTHER NOTES ON THE REPRODUCTIVE SYSTEM OF B.TROPICUS

The gross morphology and certain aspects of the histology of this system have previously been described (Stiglingh et al., 1962). Therefore in this study, I have concentrated mainly on the lining of the genital tract.

5.1 Hermaphrodite gland and its duct

The gland is situated at the apex of the visceral mass and is overlain by the epithelial sheet investing the whole of the visceral mass, (fig. 53). The cells of this layer are cuboidal and resemble those constituting the

external surface of the mantle. The thin basement membrane rests upon a delicate layer of muscle fibres. Between these fibres and the fibrous layer and investing the acini of the hermaphrodite gland, is connective tissue which acts as a packing for the gland as a whole. This connective tissue consists of Leydig's cells, vesicular cells, fibroblasts, pigment cells, and among these are refractile granules, and also blood spaces rich in amoebocytes. Some of the vesicular cells of this region contain calcium concretions and it seems that these grow to form the large refractile granules. Certain Leydig's cells contain PAS-positive material localized at one point against the cell wall as in the collar. A feature of the Leydig's cells of this region, is that they are heavily charged with granules and also substances which are lightly PAS-positive; the latter characteristic differentiating them from the Leydig's cells occurring in other parts of the body. From this may be inferred that they probably have a nutritive function. Pan (1958) found that in A. glabratus the female elements occurred in the apex of the acinus while the male elements were to be found along the side walls. This is not true of B. tropicus in which it may be stated that the younger stages of gametogenesis both male and female, are to be found along the side walls, and the more mature, at the apex. Bundles of maturing sperm are frequently to be seen attached to Sertoli cells, apparently resting on the follicle walls

of ova. These may be either young ova along the side walls, or maturing apical ova.

Each ovum is surrounded by a follicular membrane which is relatively thick. The follicular membrane may be contiguous with the ovum, but is generally separated from the latter by a narrow follicular cavity. I do not know

whether this is to some extent the result of shrinkage during processing of the slide, or whether the space is a true follicular cavity. In some sections the follicular membrane appears to be a double structure.

The double nature of this membrane is most clearly seen in follicles which have ruptured during sectioning, but it is also apparent in some normal follicles. Both layers of the membrane may contain nurse cells. Large nurse cells are frequently to be seen lying against the apex of the acinus, and these cells are in intimate connection with the ovum. The nurse cells as such, are not visible in B. tropicus, only their nuclei which are relatively much larger than those occurring in B. mooiensis (Oberholzer unpublished thesis), and are not polymorphic as in that species.

In B. tropicus the nuclei are more or less oval.

Where they occur in the follicular membrane between ovum and acinus lumen, they are generally slightly flattened on one surface; fitting snugly against the ovum.

A large orange nucleolus is present and sometimes two of these seem to occur in a single nucleus. In most instances

these are seen to belong to overlapping cells. In the cytoplasm forming the follicular membrane, rounded masses of a greenish substance, enclosed in a vacuole, have been encountered (fig. 53). Occasionally they are situated in the membrane where it overlies an ovum, but more often these bodies are to be found in the cytoplasm lining the apex of an acinus. Frequently they occur in ruptured follicles from which the ova have been liberated, and therefore seem to be debris which is being extruded. This supposition is strengthened by the fact that the colour, greenish or blue-green, of these masses, agrees very well with that of the "faecal vacuoles" (debris) found in the digestive cells of the liver.

The ovum itself is roughly round to oval in shape, but slightly flattened where it abutts against the acinus wall. The outer membrane, vitelline membrane, is visible only as a refractile line surrounding the cytoplasm. Below this thin membrane the cytoplasm of the ovum is fairly clear and stains bluish, while the rest of the cytoplasm is brownish and dense. The colour and texture of the ova changes during development. Initially the cytoplasm is coarsely granular and stains a purplish colour but during development it becomes extremely coarse and heterogeneous and contains many small vacuoles. This coarsening of the texture is due to the accumulation of nutritive materials in the cytoplasm, and some of this material is PAS-positive.

The nucleus of the ovum is large and roundish or slightly oval. In some cases the margin is irregular and undulating but this seems to me to be an abnormality resulting from chemical treatment, and the normal nucleus is smoothly rounded. The nucleus is coarsely granular and stains a very light purple. The nucleolus is a large orange spherule, dense and homogeneous. In sections stained with MBE it is only the sperm heads and the nucleolus of the ovum which stain intensely, both being amphophilic.

In a previous paper (Stiglingh et al. 1962) the occurrence of double nucleoli in the ovum was reported. Nucleoli of this type have been recorded in a number of other pulmonate species, (Simroth, 1928 Raven, 1948, Pan, 1958, van Aardt and Oberholzer unpublished theses). In the present study nembutal instead of menthol was used in narcotization and double nucleoli were rarely encountered. Even when the nucleolus has an equatorial indentation, this is relatively slight, and there is no differentiation of colour in the two parts; the nucleolus remaining orange.

Furthermore, the presence of double nucleoli seems to be correlated with the use of van Kóssa and PAS-techniques, so that possibly such nucleoli are a chemically induced phenomenon. The findings of Kisker (1924) on the effect of cocaine on the cell nucleus further seem to make this point worth investigating.

In PAS-stained slides double nucleoli were found, one part staining blue, and the larger portion remaining

unstained (fig. 54). In such nuclei vacuoles occurred, sometimes one large vacuole, and at other times, a number of smaller vacuoles being found in one half of a nucleolus. Oberholzer (unpublished thesis) also figures such nucleoli as occurring in B. mooiensis.

In the nuclear plasm irregular masses of a substance staining in the same manner as the cytoplasm of a mature ovum, are found. These masses are usually smallish and compact, often located near the periphery of the cell. In no instance was more than one nucleolus found in any ovum of B. tropicus.

The lining to the common atrium (fig. 55) shows considerable variation. In certain sections the cells are columnar and in others they are of a squat, cuboidal form.

In all cases cilia are present on some of the cells; in the squat type of cell they are longer than the cell, and in the columnar type, relatively shorter. The ciliated cells possess a cuticle which is well developed, and they probably correspond to the mushroom-shaped cells described by van Aardt and Oberholzer (unpublished theses) as occurring in the atrium of P. africana and B. mooiensis respectively. The ciliated cells of B. tropicus do not seem to differ from those occurring in other regions of the body where scattered ciliated cells occur, eg. the tentacles or sides of the foot. The nuclei of the atrial cells are oval, and fairly light, those of the ciliated cells being

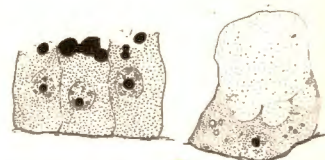
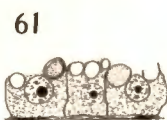
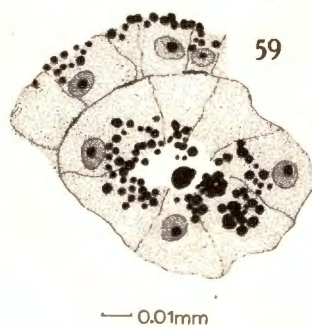
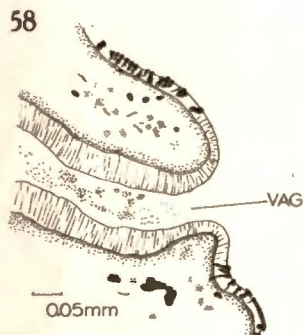
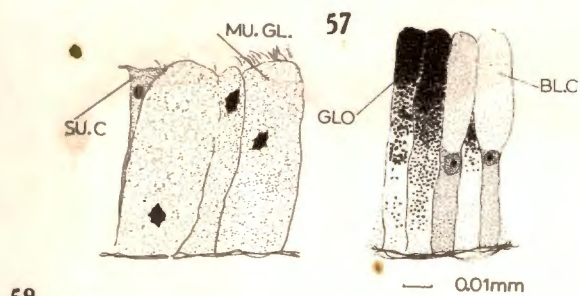


FIG. 57. FIGURE DEPICTING VARIOUS TYPE OF UTERINE CELL.

FIG. 58. GLANDS SURROUNDING THE FEMALE APERTURE.

FIG. 59. ALBUMEN GLAND.

FIG. 60. SPERM TRACT.

FIG. 61. PROSTATE.

FIG. 63. VAS DEFERENS.

FIG. 65. LONGITUDINAL SECTION OF PART OF THE PREPUTIUM.

lighter than those of the unciliated cells. The cytoplasm of these cells stains but lightly and in the apices, except in the ciliated cells, there is an accumulation of lightly PAS-positive material.

5.2

FEMALE SYSTEMOviduct (fig. 56)

The cells lining the proximal part of the oviduct are rather large, and are filled with an alveolar cytoplasm. The nuclei are roundish, situated near the base of the cell and possess a fair amount of chromatin and a conspicuous nucleolus. Some of these cells give a PAS-positive reaction while others remain negative. It is thought that the latter type of cell is merely a later phase of the former. In the cells thought to be in the younger phase, the cytoplasm stains (light) blue whereas in the latter, it is white, or greyish and contains scattered yellow-staining material. The nuclei of the younger cells are roundish and lie further from the base of the cell while those of the older cells lie nearer the cell base and appear to be irregular as a result of secretory globules which surround them. Scattered among these are certain very slender cells which are notable for having nuclei which may occur so high up in the cell body that they are situated near the apex. This type of cell occurs throughout the female system and is merely a supporting cell. A very

sparse ciliation seems to occur in this region.

Distally there is an increase in the number of squat, lighter cells which are typical of the muciparous gland.

There is a relatively well developed muscular coat of circularly arranged muscle fibres.

Uterus (fig. 57)

The histology of the sac surrounding the distal part of the oviduct is simple. The lining is composed of tall epithelial cells, slender and highly vacuolated in appearance. The cilia and the apices of the cells stain a dark brickish colour. This property is a feature characteristic of the cells of the reproductive system generally, in contrast to external cilia, eg. those of the tentacles, body wall and sole of the foot. The external cilia appear much lighter and stain orange. The cytoplasm of the cells is blue and orange. Oval, lightly-staining nuclei with distinct nucleoli are to be seen lying between the middle and base of the cells.

The muciparous gland is composed of large cells which bear some resemblance to those found in the oviduct. The ciliation of the cells in the muciparous gland is dense, staining a dark brickish colour. The cytoplasm is grossly alveolar and the cells differ slightly from those of the oviduct in that the reticulum between the globules of the muciparous gland stains very slightly red. Amongst these cells slender

sustentacular cells are also to be found.

Many of the cells occurring in the dorsal uterine wall are muciparous gland cells. Besides these, several other kinds of gland cell are distinguished. Very striking amongst these are certain cells which are basally slender and apically greatly distended. These cells are filled with red or yellow globules of a brilliant hue and they constitute the nidamental gland. A second type of cell is a ciliated one in which the base is broad and the apex narrow so that the cell in shape resembles an inverted trumpet. The cells broaden apically as the cell secretion accumulates in this region. The nucleus of these cells is noticeably larger than that of the usual alveolar cell of the uterine wall, and it is situated in the lower half of the cell.

Another kind of cell which stains blue and is therefore also a type of mucus cell, is basally narrow and apically swollen. The latter part is distended with a homogeneous blue secretion (as in the sperm duct).

The alveolar cells are squat in form, but may be small or large in size, and the nucleus is situated towards the base of the cell. Ciliation in the dorsal wall of the uterus is slight. The ventral uterine wall is composed mainly of columnar, vacuolated cells with oval nuclei situated towards the base of the cell. The apex of such cells contains secretory material which stains lightly PAS positive. Also found in the ventral uterus wall are cells which do not

stain, and are apically distended, with the cytoplasm in that region being alveolar. Trumpet shaped cells which stain bluish are also found in this region.

The dorsal and ventral walls of the uterus do not differ qualitatively. Slight cytological differences between the dorsal and ventral cells are the generally smaller size and greater density of the cytoplasm of the ventral cells. In the larger dorsal cells the cytoplasm tends to be more alveolar indicating greater secretory activity of the dorsal uterus wall. It also seems as though the nuclei of the ventral cells are situated below the middle of the cell, while in the dorsal cells the nuclei are basally placed. If it is true that the dorsal wall of the uterus exhibits greater secretory activity than the ventral wall, then this might be an explanation for the phenomenon of differing nuclear position; for, as mentioned previously, gathering secretion in the apical part of a cell tends to force the nucleus nearer the cell base.

Spermatheca

The lining of the spermatheca is simple and consists of unciliated columnar cells in which the apices remain light. Over the apices of the cells a thin pale blue layer is to be seen. This does not appear to be a cuticle and is probably the secretion produced by the cells, because the sperm contained in the spermatheca, is consolidated by

a substance which is also light blue. The nuclei differ only very slightly from those described by Pan (1958) who states that in A. glabratus the nuclei are round and basal whereas in B. tropicus they are roundish and central.

Vagina

The vaginal cells are columnar and have round to oval nuclei. The apices of the cells are PAS-positive. The female aperture is surrounded by unicellular subepithelial glands (fig. 58) which at first sight appear to be goblet cells but are in fact of the same kind as the preputial glands which they resemble in all respects.

It is possible that these small glands around the aperture facilitate copulation by secreting a substance which serves to guide the male organ to the female aperture.

Albumen gland (fig. 59)

The albumen gland is composed of cells which are well characterised by the word "massive". These cells are quite unmistakable for the secretion accumulates in globules of various sizes and these globules coalesce to form very large secretory globules. The secretion stains intensely with both Mallorys and PAS; a startling orange and pink respectively. Judging by the negative reaction to the von Kossa test they seem to be poor in calcium. Both the nucleus and the nucleolus are relatively enormous. The nucleus is round, basally placed and very rich in chromatin. This de-

scription tallies well with that of Pan (1958) for the albumen gland of A. glabratus.

Sperm duct (fig. 60)

The sperm duct consists of an epithelium of rather large cells resting on a basement membrane surrounded by sparse muscle fibres and a small amount of connective tissue. The sperm duct is yet another organ lined by cells which are quite characteristic for that region. The cells present an unusual appearance in that the nuclei may occur anywhere from the apex to the base. Two types of cell are distinguished. There are tall slender cells bearing short dense cilia which stain intensely. The nuclei of such cells are ovate, stain lightly and are to be seen near the apex of the cell.

The secretory cells are large and possess a coarsely granular cytoplasm. Near the apex of such a cell the large secretory mass may be seen. This stains blue with Mallory's and is PAS positive. There are a remarkable number of large orange-staining secretory droplets (probably protein) in the apical part of the cell and so it appears that these cells are capable of producing two types of secretion. The nucleus of such a secretory cell is large, basally situated and moderately rich in chromatin.

Prostate (fig. 61)

The cells of the prostate gland likewise, are large squat cells containing large accumulations of secretory material. The cytoplasm is highly vacuolated and the large secretory material is finely granular and stains various shades of blue. Sometimes small amounts of refractile, orange globules occur as in the sperm duct. These globules may be surrounded by a vacuole and the globule may stain bichromatically, a peripheral blue zone encircling a central orange area. In PAS stained sections the base of the cell is light coloured, the middle region is intensely PAS-positive and the apical portion only weakly positive.

Further staining techniques reveal other interesting features of these cells. MBE staining demonstrates the presence, in the middle region of the cell, of numbers of small, refractile acidophil droplets (fig. 61). Some of the cells bear cilia and these are thought to represent the younger stages of the secretory cells. The cytoplasm of the ciliated cells is alveolar and stains a purplish colour, that of cells thought to be mature . . . while in cells which have discharged their secretion, the cytoplasm is reticular and the nucleus enlarged. Among these large secretory cells supporting cells occur.

The von Kóssa technique proves that there is yet another type of cell present in the prostate, a cylindrical cell



Fig.62. Section through prostate gland to show calcium-rich cells.

which is very rich in calcium, fig. 62. Unfortunately the cell structure is obscured by the calcium so that no details of cell structure are available.

As an evagination of the sperm duct, the prostate of B. tropicus closely resembles that duct. Cytologically the prostate of L. stagnalis appressa bears no resemblance to the sperm duct because in that species the prostate is derived from a vas efferens (sperm duct) consisting of columnar cells (Holm, 1946). The glands of these two snails resemble each other only macroscopically, in each being composed of a translucent and opaque area.

Vas deferens (fig. 63)

The vas deferens of B. tropicus resembles that of L. natalensis (Pretorius unpublished thesis) ^{and} B. jousseumei (Wright, 1957) in having the epithelium ensheathed only by a layer of circularly arranged muscle fibres while the vas deferens of B. mboiensis is reported, (Oberholzer unpublished thesis) ^{seems} to be more complicated, having a layer of longitudinal fibres interpolated between the epithelium and the circular fibres. The epithelial lining consists of cuboidal ciliated cells with round nuclei. These cells do not react with either the von Kóssa or PAS tests.

The epiphallus is lined proximally by cuboidal, and distally by columnar cells which have basally situated light, oval nuclei. There is an accumulation of calcium material at

the basement membrane as is commonly found throughout the tissues of the snail, and calcium granules are also sparsely scattered throughout the cells.

In a previous paper (Stiglingh et al., 1962) the presence of special cells surrounding the distal end of the epiphallus was noted. The nature of these cells was not clear, but from their macroscopic appearance it was suggested that this tissue was of a glandular nature. Careful study of these cells has convinced me that they are vesicular cells. These vesicular cells differ from other vesicular supporting cells in being very rich in calcium (fig. 64). Demian (1960) mentions crystalline calcareous concretions in the connective tissue of both divisions of the ultra-penis and figures those surrounding the epiphallus. He makes no mention of their occurrence in vesicular cells, or their possible function in this position. When one considers the functional aspects of the male copulatory organ, it is easy to appreciate the importance of epiphallic supporting cells. When the penis is partially everted during the initial stages of copulation, the penis sheath is surrounded by the preputium in such a way that the stout muscular layer at the distal end of the penis sheath is at the foremost point of the everting organ. When full eversion has taken place the epiphallus and penis are in a position analogous to that just described, and in this instance it is the vesicular cells surrounding the distal end of the epiphallus which lend turgidity to the male organ.

64.

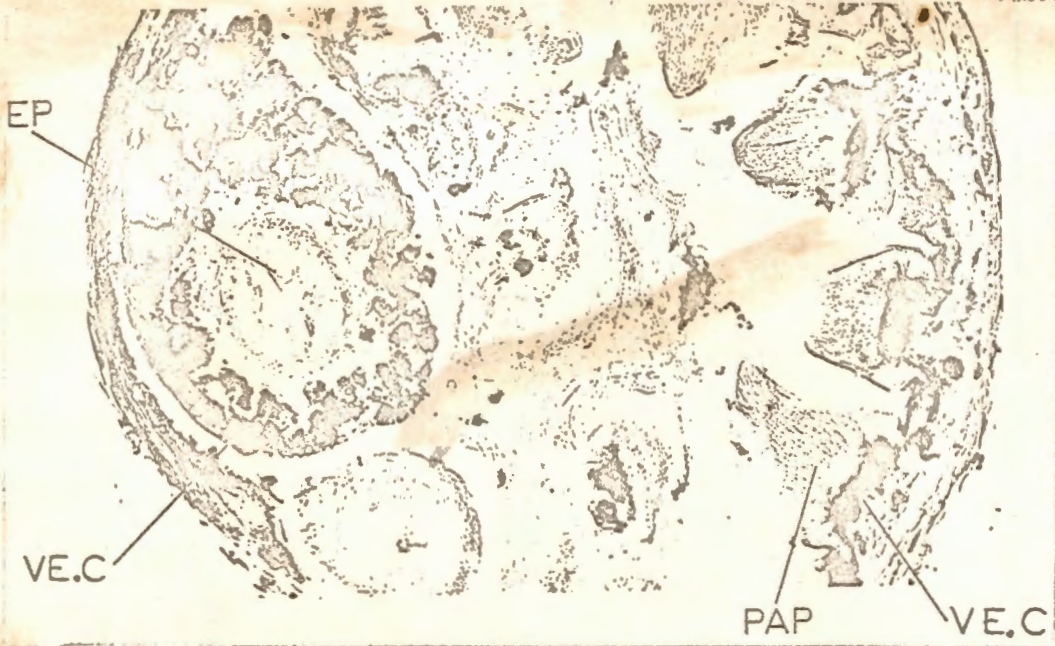


Fig.64. Transverse section through penis sheath surrounding penis and epiphallus.

Penis and Penis sheath

To the previous description of the penis and penis sheath it is necessary to add but little, and these organs are figured below. The tall apical cells of the papillae exhibit a high degree of secretory activity (fig. 64) and thus the cytoplasm and the controlling nucleus differ from the surrounding cells. The secretion of these cells is distinctly, though not intensely, PAS positive, and is strongly basophilic, when stained with MBE. Both these stains demonstrate that the nuclei of these apical cells stain much more darkly than the lower columnar cells. It should be mentioned that the cells which were in the previous paper (1962) named vacuolated cells, after Larambergue (1939) are now also considered to be vesicular cells. The lumen in the distal tip of the penis of B. tropicus is triradiate, as is that of B. jousseaumei, Wright (1956).

Preputium (fig. 65)

The lining of the preputium is composed of lightish, columnar cells of medium height. Many of the cells are ciliated, the cilia being about $1/3$ of the height of the cell. The ciliation is restricted to the proximal region of the preputium and the pilasters appear to be more heavily ciliated than the thin regions of the preputium.

It is the tip of the everted preputium (proximal part) which must "find" the female aperture. In this connection

it is perhaps significant that it is precisely this proximal region which is ciliated and it is here suggested that at least some of these cilia are sensillae, a phenomenon which is encountered in other parts of the snail, and also in various other invertebrate groups. Distally the cells are much shorter and instead of the cilia there is a thin blue cuticle. The nuclei of the preputial cells are light, oval and are basally situated.

Certain regions of the preputium are well supplied with gland cells. Gland cells have been recorded in the preputium of several molluscs, B.(P.) africanus (van Aardt, unpublished thesis), B. truncatus (Demian, 1960), L. natalensis (Pretorius, unpublished thesis) and authors quoted by him, as occurring also in Physidae and L. stagnalis appressa. All these authors state that the mucous cells occur in the epithelium and this was also stated to be the condition obtaining in the preputium of B. tropicus (Stiglingh, et al., 1962). Special staining methods by which the gland cells stain darkly (fig. 65) reveal that the cells are subepithelial as described by Wright (1957) and Pan (1958). Pan (1958) goes so far as to say there are no mucous cells present in the epithelial layer and this seems to be true also for B. tropicus. These gland cells are PAS positive and very rich in calcium.

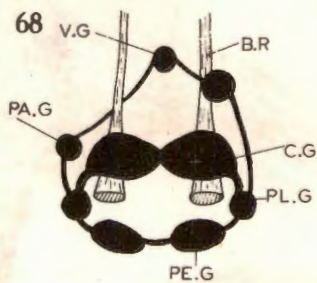
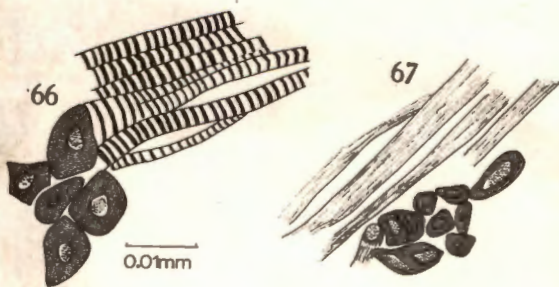


FIG. 66. INTERMEDIATE MUSCLE FROM THE BUCCAL MASS.

FIG. 67. GRANULAR MUSCLE FROM THE VENTRICLE.

FIG. 68. DIAGRAM OF THE ASYMMETRICAL PASSAGE OF THE BUCCAL RETRACTORS AMONGST THE GANGLIA OF *B. TROPICA*.

6. FURTHER NOTES ON THE MUSCULAR SYSTEM

6.1 COMPARISON OF VARIOUS TYPES OF MOLLUSCAN MUSCLE

At this point something should be said on the nature of the muscular tissue composing the buccal mass. For a better appreciation of its qualities the three different types of muscle found in B. tropicus will be described. All the muscle fibres of the foot tentacles etc. thus far described have been of the smooth type, and they constitute all the muscular tissue of the animal except that of the buccal mass and the heart.

Smooth Muscle

This tissue may consist of single fibres loosely interwoven to form a mesh, as in the foot, or a mass of fibres compactly grouped into bundles to form discrete muscles such as the columellar muscle or penis retractors. The fibres of these muscles are long, thinner than the other types of muscle fibre, and have a round or irregular appearance in cross section. Pan (1958) mentions that the muscle fibres of A. glabratus, and especially the smooth muscle fibres, occasionally take up stains after the manner of collagen-like substance. This affinity for basic stains such as aniline blue was found to be the rule in B. tropicus in which the fibres generally have a lilac or blueish colour and are only occasionally reddish.

Each fibre is composed of a number of fibrils which are easily discerned, as was also found in A. glabratus (Pan, 1958). In this respect the fibres resemble the chromatophore muscles of cephalopods but differ from the smooth adductor fibres of pelecypods as described by Prosser et al. (1950). The fibrils themselves stain pale lilac or blue and are closely packed so that the fibre usually has a homogeneous appearance in cross section. Sometimes the fibrils appear less densely packed in the centre of the fibre. Each fibre is invested in a clear blue sarcolemma.

The nuclei seem to be mostly elongated in form and although they have a slightly granular appearance they remain unstained. A yellow nucleolus is clearly visible.

It sometimes happens that the columellar muscle or the mantle may take on a reddish tinge but this occurs rather seldom. Scattered among the smooth fibres are occasional narrower fibres which are also invested in a clear blue sheath. They occur sparsely in the foot and in moderate numbers in the mantle. The fibres stain orange and are refractile and their function is unknown. Because of their size and colour reaction they are not thought to be collagenous in nature ; although no fibrils are apparent in the undamaged fibres, the broken ends of fibres sometimes have a frayed appearance and they are thus thought to be a special type of muscle fibre. In any case the apparent

absence of fibrils in undamaged fibres does not preclude the possibility of their being muscle fibres because, according to Prosser et al., (1950), certain of the smooth muscles of Pelecypoda have not yet been found to consist of fibrils.

Intermediate Muscle

Except for the buccal retractors, the muscles of the buccal mass are composed of a distinctly different type of fibre. The fibres are also long and in some cases very much thicker than the smooth fibres. They are angular in cross section, probably as a result of large fibres being compressed into bundles. Under low power the buccal mass muscles have a distinctly reddish appearance when compared with the adjacent foot muscles, and this is caused by the orange- to reddish -staining substance forming the granular core of each fibre. The core is surrounded, closely or loosely, by a moderately thick sheath of fibrils which sometimes stain in the same manner as the smooth fibres and are sometimes purple. The fibrils constituting the fibrillar sheath are closely packed. The nuclei of the fibres resemble those of the smooth muscle fibres, but seem more oval. Each fibre is surrounded by a blue sarcolemma which is in this case not well developed. Thus these fibres too, differ in their staining properties from those of

A. glabratus in which the periferal fibrils are said to stain intensely acidophilic and the central granular portion, less acidophilic (Pan, 1958). The fibres also differ from Pan's (1958) description of those of A. glabratus in an important aspect, viz. they are striated (fig. 66). This is seen to best advantage in MBE preparations.

Scheer (1948) mentions that in a few instances striated muscle occurs in molluscs and, as examples he cites the cephalopod mantle and part of the adductor muscle of pelocypods. In the gastropods striated muscles have been reported to occur in conjunction with smooth fibres, in land pulmonates (Baecker, 1932) and in A. glabratus (Marcuzzi, 1950). Unfortunately I could not consult this last mentioned paper and I am uncertain as to whether the striated fibres described by him were seen also in the buccal mass. According to Pan (1958) Marcuzzi did, however, observe striated cardiac muscle. Pan (1958) maintains that he was unable to find fibres with striations similar to those occurring in higher animals, and goes on to state that the granules of the central part of the fibre may become arranged "so as to show an irregular, cross-striation-like appearance" (p.250), and suggests that these are the striations seen by Marcuzzi (1950). It is true that in B. tropicus, in fibres obliquely sectioned, a pseudostriation is apparent but this is easily recognisable, for the striations are faint and far apart and occur towards the cut end of the fibre. It is also true that the striations

are often difficult to discern but in certain 4 micron sections the striations are distinct and prominent.

In this character they differ from Prosser et al's (1950) description of striated molluscan muscle, which is said to possess only faint striations.

That the striations are caused by a grouping of the granules is disproved by the fact that in longitudinally sectioned fibres striations are not visible in the central part of the fibre, but are distinct in the fibrillar sheath surrounding the core. The striations are narrow and closely set when compared with those found in the muscle of other animals. Arthropods have closely striated muscles and in several insects it has been found that generally, the more quickly the muscle can contract, the closer the striations so that the flight muscles have striations 2μ apart. It is therefore noteworthy that although the snail muscles are obviously much slower than those of insects, the striations are more closely set, being $0.6 - 0.9 \mu$ in length and only 1.2μ apart. The sarcomeres are small, being roughly $1.8 - 2.1 \mu$ long. It is interesting to note that of all the buccal muscles it is apparently only the buccal retractors that do not belong to this group. Under low power the buccal retractors appear bluish in clear contrast to the other, reddish muscles, and high power examination confirms that they are not of the intermediate type, but are composed of smooth fibres. This is interesting because it suggests that the

buccal retractors have not only their morphological, but also their ontogenetic origin in the columellar muscle. Although this type of muscle is highly characteristic of the buccal mass, it is not restricted to it. Fibres on which no striations could be seen, but which, nevertheless, had a very small orange core were found near the base of the tentacle and it is possible that they occur also in other parts of the snail.

Granular Muscle (Fig. 67)

Fibres of this kind are restricted to the heart. In the ventricle they are gathered into small bundles which are enmeshed to form a loose network among which other cells occur. Under low power two regions are distinguishable on a basis of colour. The periferal fibres are predominantly orange in colour and the inner layers bear some resemblance to the muscles of the buccal mass. Close examination reveals that the similarity is not only superficial.

The fibres also consist of a central granular core which stains orange and is surrounded by a purplish layer and the whole is invested in a blue sarcolemma. The nucleus also resembles that found in the two other muscle types.

The difference between the central cardiac fibres and the buccal mass fibres seems to be qualitative rather than quantitative. In cross section the fibres present the same irregular appearance but differ in the ratio of

the amount of granular substance to the surrounding purple sheath. In the buccal mass the sheath is generally thick and there is a relatively small core while in the central cardiac fibres there is a large central core and a relatively thin sheath. The appearance of the fibres is highly variable, so that some cardiac fibres resemble buccal mass fibres and vice versa. In agreement with Pan's (1958) observations on A. glabratus, I can find no trace of striations in these fibres, but on the other hand, I differ from him in that I consider the purple sheath to be composed of fibrils. These are not very clear and are best seen in ruptured fibres.

The condition of the peripheral fibres agrees more closely with the description of Pan (1958) in that the granular core is surrounded by a thin sarcolemma and there is no trace of a sheath. This accounts for the colour difference in the two regions.

The significance of the occurrence of striated and smooth muscle is generally understood even though, according to Prosser et al. (1950), the reason why striated muscle is the faster is not yet clearly explained. It is here suggested that the distinction to be made in molluscs is between granular and smooth muscle. The former type has been found by several authors in many molluscs (Pan, 1958) and from the literature it is clear that smooth fibres are

common. These two morphological types seemingly correspond to the clonic and tonic physiological types. If this supposition is correct it explains why in B. tropicus it is the cardiac and (buccal muscle) which work continually which are of the granular (clonic) type, and the intermittently working foot and tentacle muscles etc. which are of the smooth (tonic) type. However, as pointed out by Prosser et al., (1950) more data are needed before the precise relation between muscle histology and function can be established.

6.2 The asymmetrical passage of the buccal retractors through the circumoesophageal ring

Torsion has been most thoroughly studied in the dextral prosobranchs and though some work has been done on the Opisthobranchiata the subject of torsion in the pulmonates is usually evaded in standard works on Mollusca.

In B. tropicus the newly hatched snail has already undergone torsion within the egg and, as in the case in the rest of the pulmonates, evidence, in the form of chiasstoneury, for the occurrence of torsion is not clearly to be found (Morton, 1960).

During a dissection of B. tropicus it was noticed that the buccal retractors pass asymmetrically amongst the ganglia of the circumoesophageal ring (fig. 68). In an attempt to find out why this should be so, various other pulmonates were

dissected to establish the relation of buccal retractors to the circumoesophageal ganglia.

For the sake of clarity the compact ganglionic mass will be described as though still consisting of two distinct elements as in the more primitive condition eg. certain Prosobranchiata. These elements are the ganglionic ring (cerebral, pleural and pedal ganglia) and the visceral loop (parietal and visceral ganglia). Both retractors pass through the ganglionic ring but whereas the right buccal retractor, as also the oesophagus, passes dorsal to the visceral loop, the left buccal retractor passes ventral to the loop.

In Achatina the condition is not comparable to that obtaining in B.tropicus owing to the fact that the retractor muscles consist in a broad ventral sheath and broad lateral bands which do not, however, pass through the nerve ring. Of the Basommatophora, specimens belonging to the Lymnaeidae, Physidae and the Planorbidae were dissected.

Regarding the position in Lymnaea stagnalis Carriker (1945) mentions only that both retractors pass to the right of the oesophagus and considers this a consequence of the position of the salivary glands. In Lymnaea natalensis the relation of the buccal retractors to the ganglia are precisely a mirror image of that found in B.tropicus, the

retractors

^ passing through the circumoesophageal ring, the right buccal retractor passing ventral to the visceral loop, while the left buccal retractor passes dorsal to the same.

In all the other specimens dissected viz. Physa mosambiquensis, . . Physopsis (africana?) and Biomphalaria pfeifferi, the arrangement of the structures was found to be the same as in B. tropicus.

This was figured, but not mentioned by Schutte (1962) for B. pfeifferi. Demian's (1960) description of buccal retractors in B. truncatus is unclear, both being said to pass above the pedal ganglion but as the right buccal retractor is stated to pass freely with the oesophagus through the nerve ring, it is assumed that the condition is identical to that obtaining in B. tropicus.

The difference between Lymnaea and the other specimens dissected is that, while the former is dextral, the other forms are sinistral and this fact sheds a ray of light on the question of the direction in which torsion takes place.

The standard description of torsion is of the process as it occurs in a dextral form, and in such forms torsion occurs in an anticlockwise direction (when viewed from above). No specific statement has been encountered stating that torsion takes place in a clockwise direction in sinistral forms and, in at least one instance it is stated (Fretter

and Graham, 1962) that torsion takes place during the developmental stages of all gastropods and that the twisting is invariably in an anti-clockwise direction, when viewed from above. That the arrangement of the buccal retractors in dextral forms is the mirror image of that found in sinistral form, seems explicable only by assuming that torsion takes place in an anti-clockwise and clockwise direction in the two respective groups.

The other thought that arises from a consideration of the asymmetrical arrangement of the buccal retractors, is the teasing question of how torsion is able to affect this region of the snail at all. Because the actual twisting of the visceral mass occurs in the neck region, and the ganglia of B. tropicus are consolidated into a ring, the ganglionic area should, in theory, be unaffected by the process. A possible explanation of the condition in B. tropicus is that the ganglia are in fact not compactly arranged at the moment of torsion.

If the "Anlagen" for the ganglia of the visceral loop are situated in a more posterior part of the snail, and later shift to the adult position, the arrangement may be explained in the following manner:- It is necessary to assume an initial asymmetry of the nervous system, so that the parietal ganglia are not on the same level. In dextral forms it is the right parietal ganglia which becomes the

"supraintestinal" and it is therefore more dorsally placed than the left parietal ganglion. In sinistral forms the condition is the reverse. If, at this stage, the buccal retractors arise symmetrically they will be situated ventral to one, and dorsal to the other, parietal ganglion in the snail. Arrangement of the ganglia in their final compact form will result in the buccal retractors being trapped by the connectives of the visceral loop and the relation of the various elements will remain constant.



Fig.69. Geological map of Potchefstroom area, showing showing the location of certain habitats.

An investigation was carried out on some of the ecological factors affecting snails in, and around Potchefstroom. The town is situated in the Transvaal province of the Republic of South Africa. It is located at latitude $26^{\circ}44'S$ and longitude $27^{\circ}05'E$ and lies among shallow plains constituting the High Veld region of the great inland plateau. The altitude (4436' or 1352m) above sea level is partly responsible for the considerable fluctuation in temperature experienced during the course of the year. The rainfall is closely correlated with the seasons, there being a sharply defined summer rainfall season, and generally a very dry winter.

7. GEOLOGICAL FEATURES

The geological formations in the area represented on the accompanying map (fig. 69) consist of the following rock groups. Alluvium occurs along the Loopspruit and the Mooi River. With regard to the latter, alluvium is, however, conspicuously absent from the Potchefstroom Lake and further upstream.

The alluvium is flanked on both sides by rather undulating plains in which diabase occasionally crops out.

Above these plains are found the various sedimentary and volcanic members of the Pretoria Series which represents the Transvaal System in this area. These rocks form the high ground to the west and north-east of Potchefstroom.

From the site of the Potchefstroom Lake further upstream

along the banks of the Mooi River and to the west thereof, the rocks consist of dolomite in which occur numerous veins of chert.

These geological strata give rise to the following soil types:

1. The alluvium along the rivers gives rise to a) heavy black clays which are fairly impervious to water and crack upon drying; and b) fine loamy clays which are mainly utilized in the extensive irrigation schemes around Potchefstroom.
2. The soils which develop on the diabase consist of a mixture of sedentary soils and colluvial drift. The sedentary soils are typical red clays when well drained, but become darker when the drainage is impeded. The colluvial soils which occur typically along the upper slopes of pediments, are rather similar to those which develop on the miscellaneous sedimentary and volcanic rocks which comprise the Pretoria series.
3. The soils which occur upon the Pretoria series may be described in general terms as consisting of shallow lithosols and fersiallitic soils. The latter are associated with mass wasting and aeolian drift from the arenaceous rocks of the Transvaal System.
4. The area in which the Dolomite Series crops out is covered by fersiallitic soils and lithosols. The fersiallitic soils are associated with aeolian drift while the lithosols comprise



the sedentary soils of the Dolomite Series.

The geological and climatological features so far mentioned are largely responsible for the type of vegetation occurring in this region, viz. a pure grassveld of the Cymbopogon-Themeda type. A glance at the countryside shows that considerable intrusion of thorn trees (Acacia karroo) has taken place, partly as a result of overstocking. This pure grassveld is surrounded on three sides by Bankenveld which is a pseudo-grassveld, and trees from this area have also encroached on the pure grassveld of the Potchefstroom region.

8. DESCRIPTION OF SOME SNAIL HABITATS

In this region B. tropicus were found existing in a number of different types of habitat. The snails found occurred in the Mooi River, Loopspruit and many other smaller streams, and in pools of both a temporary and a permanent nature. Certain of these habitats supported well-established colonies of B. tropicus and for this reason will be described in more detail. Furthermore, they serve to illustrate the various types of habitat well-suited to B. tropicus in this area; and they possess some features of special interest eg. temporariness, specific floral elements etc. which renders them worthy of description.

Habitat 1 (fig. 70)

The stream in which this habitat occurs is named Wasgoed-spruit. It derives its water partly from the Hunt's Poort (Picnic Poortjie) Dam and, after flowing through the town,

TABLE 1. ' List of associated flora.

Habitat 1

Rumex lanceolatus
Polygonum salicifolium
Veronica anagallis-aquatica
Berula thunbergii
Scirpus inclinatus
Typha australis
Cyperus longus tenuiflorus
Asparagus sp.
Mariscus congestus
Spirogyra
Ranunculus pubescens
Oenothera rosea
Senecio

Habitat 6

Gomphrena celosioides
Scirpus paludicola
grass
Lagarosiphon
Filamentous algae

Habitat 8a

Thorn trees Acacia karroo
Liliaceae Asparagus
Aloe transvaalensis
Zinnia
Lagarosiphon
Opuntia
Kalanchoe
Marsilia
Spirogyra
grass

TABLE 1 (Contd)

Habitat 9

Veronica anagallis-aquatica
Carex glomerabilis (= Carex vulpina)
Rumex lanceolatus (= R.ecklonianus)
Polygonum lapathifolium (white) var. glabrum
Polygonum salicifolium (red)
Denekia capensis
Scirpus muricinux
Eleocharis palustris
Ranunculus pubescens
Marsilia
Scirpus inclinatus (= S.corymbosus)

Habitat 11b

Poplars
Paspalum distichum
Cyperus longus tenuiflorus
Scirpus paludicola
Cyperus marginatus
Carex glomerabilis

Habitat 21b

Marsilia macrocarpa (?) fruits wanting
Crassula natans
Cyperus longus var. tenuiflorus
Scirpus inclinatus (= S.corymbosus)
Scirpus paludicola
Potamogeton pusillus
Paspalum distichum
Leersia hexandra
Lemna
diatoms
Spirogyra
Cladophoraceae (Cladophora?)
Chara
Cyperus fastigiatus
Cyanophyceae

TABLE 1 (Contd)

Habitat 21c

Spirogyra
Lemna 6.12.63
Berula thunbergii
Cyperus longus tenuiflorus
Cyperus fastigiatus
Marsilia

Table 2.

Fauna present in the described habitats

Habitat 1.

Decapoda; Potamon
Odonata : Zygoptera nymphs
Hemiptera : Micronecta
Coleoptera : Dytiscidae
mostly Laccophilus
Amphibia : Rana tadpoles

Habitat 6.

Branchiopoda : Anostraca - Streptocephalus
Conchostraca - Caenestheriella
Copepoda : Calanoida - Paradiaptomus greeni
Hemiptera : Micronecta
Coleoptera : Dytiscidae
B.forskali
Bos
Equus caballus

Habitat 8a

Mollusca : L.natalensis
Mammalia : Bos

Habitat 9

Nematoda
Annelida : Oligochaeta - Tubificidae, Limnodrilus, Branchiura
Sowerbyi
Hirudinea.
Branchiopoda : Cladocera - Simocephalus.
Collembola
Ephemeroptera : Baetidae - Austroclaeon virgiliae
Hemiptera : Sphaerodema, Tropocorixa, Micronecta dimidiata
Coleoptera : Gyrinidae, Dytiscidae - Laccophilus
Diptera: Chironomidae, - Tanytarsini, Chironomus
Culicidae
Ceratopogonidae
Mollusca : L.natalensis, Anisus, Gyraulus
~~Aves : (see table)~~
Mammalia : Bos

Table 2. (Contd.)

Habitat 21b

Nematoda
Annelida : Oligochaeta - Nais, Chaetogaster, Pristina,
Tubificidae, Branchiura sowerbyi
Hirundinea
Crustacea : Cladocera : Coelenterata : Hydra
Simocephalus vetula, Simocephalus sp.,
Chydorus
Scapholeberis, Ceriodaphnia,
Copepoda : Cyclopoida
Ostracoda
Odonata : Pseudagrion, Elatoneura, Trithemis, Anax,
Lestes, Orthetum, Crocothemis
Ephemeroptera : Baetidae - Caenis sp., Austroclon virgiliae
Hemiptera : Anisops, Tropocorixa
Coleoptera : Helmididae, Hydrophilidae - Berosus sp, Dytiscidae-
larvae, Laccophilus several spp. including
lineatus, Helmididae
Diptera : Muscidae, Culicidae, Chironomidae - Rheotanytarsus
Mollusca : Lymnaea natalensis, Physa mosambiquensis Anisus.
Gyraulus, Succinea
Pisces : Tilapia
Amphibia : Bufo tadole
Mammalia : Equus caballus, donkeys, Bos, Ovis

Habitat 21c

Protozoa : Vorticella
Annelida : Oligochaeta - Tubificidae
Hirundinea -
Branchiopoda : Cladocera - Simocephalus, Chydorus,
Scapholeberis
Ephemeroptera : Austroclon virgiliae
Hemiptera : Micronecta
Diptera : Muscidae, Chironomidae, Culicidae
Mollusca : Physa
Mammalia : Equus caballus, Equus asinus, Bos, Ovis
Copepoda : Cyclops

ultimately becomes confluent with the Mooi River. Habitat 1 lay immediately upstream of a small bridge. On the downstream side of this bridge the spruit is tortuous and the bed densely overgrown with rushes and other aquatic plants which cause a damming up of water above the bridge to form a pool 9.5 by 11m. The water in this pool was clear, generally about 76cm deep and in the centre of the pool it was stirred by a slight current. The bottom of this pool, as indeed that of most of the habitats studied, was of very fine black clay. These heavy alluvial clays have a good waterholding capacity. It has been reported that Lymnaea caillaudi (van Someren, 1946) and the South African bilharziasis vectors prefer habitats in which the substratum is not too soft (de Meillon et al., 1958). Several colonies of B. tropicus thrive in habitats having exceedingly soft clay bottoms. However, it is probable that this factor is of less importance to B. tropicus which is chiefly to be found crawling on the plants and other objects in the habitat, than to the bottom-feeding Physopsis species. Gordon et al. (1934) mention that P. globosa spend most of their time in the mud.

Sometimes masses of filamentous algae are present and a luxuriant algal growth occurs also on the stones, the plant stems and on the bridge supports. Marginal vegetation is well represented (table 1) and on the northern bank thorn trees (Acacia) "katbos" (Asparagus) and other plants are found. The shelter afforded by this vegetation, as also the high banks, about 91cm



Fig.71. Habitat 6, when full.



higher than the water level, must have been of considerable significance in reducing the evaporation rate in this habitat. Snails were generally an important component of the fauna (table 2). They attained large size and were sometimes present in great numbers. They were to be found grazing on the rich algal fields or floating upside down at the surface of the water.

Habitat 6

This temporary pool depended entirely upon rain for its water supply. The habitat consisted of a series of shallow pits dug by diamond prospectors. After very good rains the separate pools sometimes became confluent to form a small dam (fig. 71) but this occurred only once during the years 1961 - 1963. The pool was often found to be completely dry (fig. 72) or to be only a small puddle. (fig. 73). Because of variations in the size of this pool it is difficult to give representative values for the size and depth but after very good rains the water attained a depth of about 38cm. Normally it was 13 to 23 cm in the deepest part and 5cm or less, marginally. When the pool had retained water for a considerable time it became ecologically stable and the water was clear. Normally however, the pool did not have time to develop a balanced ecosystem before it was again overtaken by drought and the water was therefore usually turbid. The turbidity may be ascribed partly to the disturbance caused by animals using the pool as a watering place, but in certain other pools which were regularly visited, the turbidity was neither so marked nor of such common occurrence,



Fig.73. Habitat 6, when only a small pool.



and in this instance the type of substratum must be included as a factor influencing the turbidity. The pool, situated on a shale outlier had a bottom of fine, red clay which is typical of the sedentary soil developing on the diabase. Mixed with this red clay is a small amount of shale. This type of bottom has a considerably poorer water-holding capacity than the heavy black, or the fine loamy clays, both alluvial in origin, and the quicker seepage of water out of the pool must play an important part amongst the factors responsible for the temporary nature of the habitat. Another pool, habitat 13, which occurred in the same area and had the same type of substratum, was also characterized by a high degree of turbidity.

When the pool was in a stable condition it supported an abundance of Lagarosiphon and other aquatic plants. Normally however only a few straggling plants, mostly sedges, were to be found along the margins of the pool (table 1). The pool was not surrounded by shrubs or trees and the ground was covered by rather scanty grass and various small herbs so that there was no vegetation which could act as a windbreak. The extent of the depressions was so large that the banks could not reduce the force of the wind blowing over the pool and the nearest cottages and trees were so far removed (fig.73) that they may be disregarded as possible windbreaks for the pool which was therefore in a very exposed situation.

Besides the B. tropicus, also B. forskali was found in this pool. The associated fauna is interesting in that it

includes species characteristic of temporary habitats eg. Streptocephalus, Caenestheriella and especially Paradaptonus greeni; ^{Gurney} all of which occurred in such numbers after refilling of the habitat that the water was sometimes thick with Crustacea.

Habitat 8a (fig. 74)

This was a pool situated near the base of the wall of the Hunt's Poort Dam and it seems that the main source of water for the habitat was the leaks in an asbestos pipe conveying water from the dam. The pool was found to be dried up on only one occasion before the pipeline was removed and after this the habitat became almost permanently dry. The pool was a shallow depression roughly 23 - 46m and when full it was connected to other shallow, more ephemeral, pools beyond it. In depth the pool was only about 18cm at its deepest part. The pool lies on a chert band and the substratum is clayey and extremely soft. As in habitats 6 and 13 and in contrast to the those habitats in the heavy black clays, the water had a strong tendency to be reddish and turbid, and this was aggravated by the animals which wandered through the pool cropping the plants and using the pool as a watering place. Thriving vegetation occurred in and around the pool (table 1). The aquatic plants consisted mostly of grass, Marsilia and filamentous algae and the pool was surrounded by numerous thorn trees (Acacia) prickly pears (Opuntia) "Katbós" (Asparagus) and other plants among which was a small Zinnia. These plants are indicative of fairly dry conditions so that, although the habitat was protected from



strong wind by the vegetation as well as the high walls of the "poort" and dam, the depression in which the pool lay was hot and rather arid. The most conspicuous feature of the fauna was the dense population of tadpoles but the snails, which here grew to considerable size, were sometimes exceedingly numerous (table 2).

Habitat 9

This was a locus on the inlet side of Loxton Dam (fig 75) which is fed by the Loopspruit. Bordering on the dam itself is a large marshy area about 55m broad which was covered by only a few inches of water. Although this constitutes part of the dam, the habitat must be considered temporary as the shallow water, 8 - 15 cm deep, was greatly affected by fluctuations in the amount of water held by the dam, and large areas of the marsh were periodically laid dry. The bottom is alluvial, a very fine dark clay having a good waterholding capacity, and the water of the dam itself, even where not stirred up by cattle, was slightly turbid. In the marshy area, however it, was clear owing to the binding effect of the carpet of grass and other plants (table 1) of this zone. In the autumn when the grass died down the algae made their appearance on the surface of the dying plants or floated free in the water. Characteristic of the habitat was the presence of a large number of aquatic and semi-aquatic birds, especially in the marshy area which served as a nesting ground. The effect of these birds on the snail population was not established but they may be of



Fig.77. Habitat 21b.

considerable importance by acting as hosts to flukes transmitted by the snails, and/or utilizing the snails as food.

Habitat 11b (fig. 76)

This was a small temporary pool lying adjacent to, and fed by, an irrigation furrow. During the summer irrigation took place once per week and the pool then received water. The water was normally only about 23cm deep and the bottom composed of black alluvial clay covered by a dense growth of shortish grasses that the water was clear. On several occasions the water was coloured brown by the masses of decaying grass. Grass was almost the only vegetation occurring in the pool though some sedges and taller grasses grew around it (table 1). The pool was sheltered only on one side by a small grove of poplars through which the irrigation furrow flows.

Habitat 21b (figs. 77)

This was a permanent pool fed by water trickling from the Mooi River through a marshy area to the pool which was situated at a culvert. The habitat was about 30m long and 12m wide while the depth in the middle is roughly 75cm. The bottom consists of soft black alluvial clay. In spite of the nature of the bottom the water was fairly clear, probably because in the marginal zone frequented by cattle and horses, grass and Marsilia served to bind the substratum. In the centre of the pool the clay was very dark and smelt foul. At times the habitat as a whole smelt foul and there was often a film of oil floating upon the surface. In spite of these facts, the habitat



Fig.78. Habitat 21c, with Lemna-pool in background.



Fig.79. Habitat 21c.

supported well-established colonies of B. tropicus, L. natalensis Krauss and Physa mosambiquensis Clessin. The flora differed from that occurring in the habitats described above (table 1) and this could partly be attributed to the depth of the pool. Along the margins grass and Marsilia grew, while in certain areas there were also clumps of sedges. The true aquatic vegetation occurred only in the deeper parts of the pool, and consisted mostly of Potamogeton and Crassula which sometimes formed an almost solid mass in the water. Filamentous algae sometimes formed a dense mat in the water. The associated fauna is listed in table 2 .

Habitat 21c (figs. 78 , 79).

This was a temporary pool which was, nevertheless, fairly stable. It was found to be dry on only 2 /21 visits. The habitat was situated near to, but on the opposite side of the road from, habitat 21b. Like the previous pool, it was fed by the Mooi River but could receive water from two points. It was sometimes fed directly from the river by a short stream. After good rains, however, the pool became confluent with an adjacent pool which received water, via a culvert, from the marshy area surrounding habitat 21b. Normally habitat 21c seemed to be isolated and its constancy could be ascribed to its situation near the Mooi River in an area containing a dam, various small pools and a low-lying swampy area so that the environment was damp. It is further not to be excluded that the water table in this region may be high. In certain areas in the town it-

self the table rises to within 122cm. below ground surface. The pool was about 10.5m wide and elongate and irregular in shape. It was about 45 cm deep in the middle and the bottom consisted of black alluvial clay. This was carpeted by grass so that the water remained fairly clear throughout the period of observation in spite of much criss-crossing by cattle, horses and donkeys which visited the pool to drink water and to crop the lush grass. It also occasionally had a considerable amount of Lemna floating on its surface eg. in August 1962 the pool was found to be much smaller than usual and only a single shell was found. At this time the adjoining pool (visible in the background of fig. 78) was covered with Lemna. In September the pool was still smaller, had a thick growth of filamentous algae and the water was black and evil-smelling. At that time no snails, only three shells were found. Also in November the pool was small, no snails were recovered and a thick covering of Lemna was present. I conclude that conditions in such a senile habitat (see under flora) are inimical to a snail colony.

DISCUSSION OF ECOLOGICAL FACTORS

In order to avoid spending too much time on a subordinate part of the project it was decided to focus attention only on those features which were suspected of being specially interesting with regard to their effect upon the snails morphology and or mode of life.

THE AMOUNT OF WATER IN SOME OF THE HABITATS STUDIED

1961	J	F	M	A	M	J	J	A	S	O	N	D	1962	J	F	M	A	M	J	J	A	S	O	N	D	1963	J	F	M	A	M	J	J	A	S	O	N	D		
6		X			x			0	0	0	0	X		X		X						0	0	0	X			0	x			0	0	0	0	0	0	x	X	
11b									XB	XB	X			0		0						X	XB	0	XB			0	0		0		0	0	0	XB	0	0	0	
21c						X	X		X	x	x	X		x		x						x	x		x			0	x		X	X		X	x	x	0	x	X	
9																																								
21b						X	X		X	x	X	X		X		X						X	x	x	x			X	X		X	X	x	x	X	X	X	X		
8a						X		X		x		x		X		X						X	X	x	X			0	x											

KEY

0 - empty

x - a small amount of water

X - full to fairly full

XB - water brown in colour.

9. Desiccation and the factors controlling this condition

In this country many of the small or shallow water bodies are much reduced in volume or dry up each year because a considerable part of the Republic has a relatively low rainfall and the rain is confined largely to certain seasons. Many snail species have, in the course of time, become adapted to such conditions and even when most of the snails die, a few specimens survive and are able to recolonise such habitats in spite of the recurrent, enforced interruption of the snails' normal activities.

In habitats which are shallow, as are many of the pans, isolated pools or marshes and vleis in the interior, drying out of the habitat which is filled during the rainy season, is usual during the dry winter months, or even the greater part of the year. Such an isolated, raindependent pool was habitat 6 which was sometimes dry for most of the year (table 3). Also a large part of the marshy area surrounding the inlet of Loxton Dam (habitat 9) dried up because a slight variation in the level of the dam lead to the drying up of a considerable part of the very shallow marsh. It is, however, not only these types of habitat which are subject to drought, but even those which are dependent on constant rivers, eg. habitat 21c. As will be seen in the discussion of the life cycle of B. tropicus, the drying up of the habitat has a profound effect on the population curves. It also seems to be the main factor responsible for differences in the growth of populations in fairly closely

situated habitats.

Even in instances in which the water volume is merely reduced there are many changes in the ecological complex. This must of necessity affect the osmoregulation of the snails although it has been claimed (WHO, 1956) that the total amount of salts present is of less importance than the ratios of the various salts.

The sensitivity of the *Basommatophora* to changes in the salinity is stressed by several authors including Pilsbry and Bequaert (1927), Porter (1938), Boettger (1944) and Watson (1956). The effect of salinity on B. tropicus was not determined in the present study, but no evidence such as death resulting from higher salinity during hot weather, as reported by Porter (1938), was observed. From this I feel justified to conclude that B. tropicus is able to withstand a high degree of salinity, as witnessed by the fact that, in habitat 21b, a conductivity of more than 800 micromhos (Prinsloo pers. comm.) was on occasions recorded. This is in agreement with the salinity tolerance B. contortus, a species which has been found to occur in pools registering 2200, and even 5177, ohms per cc. (Marill et al., 1949).

Of the salts present in a snail habitat, calcium is one of the most important, influencing both the habitat and the snail's metabolism. By precipitating the suspended clay particles it serves to clarify the water. In this district the calcium content of the water is high, in the region of 300ppm. (Prinsloo,

pers. comm.) and the clarity of the waters, even in some of those habitats which have soft bottoms and are extensively used as watering places, could possibly be partly ascribed to the effect of the calcium.

The calcium also has a direct effect on the metabolism of the snail, notably in the role it plays in the fabrication of the shell. Calcium deficiency is claimed to result in the formation of thin, fragile shells (Boettger, 1944 and Abdel-Malek, 1956). Delicate shells were found in certain of my habitats, especially in habitat 2 and 9, but it is highly unlikely that this feature is the result of lack of calcium for the calcium content of local waters is very high and must be further increased by evaporation in these habitats. Besides, it has been found (WHO, 1956, and Deschiens, 1956) that some snail species survive successfully for long periods in waters containing less than 10 ppm calcium (WHO, 1956) because the snails utilize calcium ingested with the food, or even eaten directly. The cause of the formation of the thin shells in certain of the local habitats, was not established.

In addition to the fact that the salt concentration will increase in a drying pool there are also other effects such as the shallower water resulting in more extreme temperatures, and the accentuation of the effects of pollution as described in habitat 21c. It has been stated (WHO, 1956) that bilharzia vectors stranded by a receding water level make little or no attempt to regain the water even though they would appear physically capable of doing this. This may be true also in the case

of B. tropicus but the great number of snails found in pool 6 just prior to its drying up completely, suggests that this was not just the result of a decrease in water volume but that the snails tend to congregate in the deeper water, possibly in response to some stimulus such as the excessive heating of the shallow peripheral water. In much the same manner, but during cold weather, intertidal marine molluscs are often found in more favourable situations lower down the shore (Crisp, 1964). This is thought to be, in all probability, an active migration as a result of a low temperature stimulus (Janssen, 1960 quoted by Crisp, 1960).

In instances of complete drying up of a body of water, the snails are faced with the final obstacle for they must be able to withstand a measure of desiccation in order to survive.

A number of the habitats studied, dried up in the course of the three years during which the investigation was carried out. The frequency of drying up of some of these is reflected in table 3. It is of interest to note that though the rains occur during the summer (see below) drying up of the habitat may, under certain circumstances, occur also during the summer months. Unfortunately no data concerning these events can be given for habitat 9 as the collection sites varied considerably, owing to the fluctuations of the water level in the marshy area.

Observations on the drought resistance of local B. tropicus were made at several of the habitats, but chiefly at pool 6

because, of all the habitats studied, this was the habitat most often subject to drought. During the course of three years numerous visits were paid to this habitat, and on only 9/23 occasions the pool contained water, and on four of these it was only a few square metres in size, or virtually no more than a puddle. The nature of the population in such a small pool seems to depend on whether the pool is reduced in size, owing to evaporation, or whether it is a pool being refilled after a drought. This is best illustrated by an example taken from habitat 21c. In January 1963 the pool had dried up but in February it contained a small amount of water, the grass was beginning to grow again, and a fair number of adult snails had emerged after a period of anhydrobiosis. In September 1963 the pool was also small, this time as a result of drying up, and there were masses of the filamentous algae which are common under such circumstances. At that time both small and large snails were present in fair numbers. During the following month the pool dried up completely but by November it again held a small amount of water which was clear. Filamentous algae were absent and only a few snails were found, most of them being large.

Regarding the snail population it thus seems as though there might be a reduction in the number of small snails during a drought, and observations on habitat 6 seem to confirm this. In December 1961, after a drought of at least four months, small and large snails were found, and this was also the case in 1962

after a drought of at least three months. However, in November 1963 after a prolonged drought of at least six months only a number of large B. tropicus were recovered, suggesting that small snails are unable to withstand a prolonged drought. This opinion is also held by Deschiens (1956) and is in agreement with the general ecological precept that older stages of organisms are usually more resistant to adverse conditions than are the younger stages.

It is significant that after each refilling of habitat 6 egg masses, sometimes present in great numbers, were found. Frequent oviposition after a drought is a prime factor in the rapid reestablishment of a snail colony. (See also under oviposition).

The droughts experienced in habitat 6 are severe owing to its isolation from other waters, and consequent dependence on rainfall. This condition is, no doubt, aggravated by the pool's unsheltered situation which allows the winds, prevalent at the end of the dry winter months, to sweep across the habitat, desiccating it still further. Besides the fact that there was a dearth of surrounding vegetation there was generally also little or no aquatic vegetation, the pool having apparently dried up again before the flora had progressed beyond the primary seres. The significance of this must be viewed against the fact that, although emergent marginal plants to some extent increase the rate of evaporation and so aid in drying the habitat, true aquatic vegetation enriches the bottom with humus

which increases the water holding capacity of the pool (Boycott, 1936). The plant mass also acts as a protective blanket, keeping the mud moist after the water has evaporated. Furthermore, the vegetal blanket also affords excellent shelter for the snails and it acts as a thermal barrier, protecting them from extremes of temperature. During the very cold European winter of 1962/1963 the apparent insulating effect of a layer of seaweed on molluscs was reported (Crisp, 1964).

From the above description the severity of the droughts experienced by the organisms in this habitat will be appreciated, and simultaneously, the drought resistance of B. tropicus which is able to form a fluctuating, but tenacious colony in this generally inhospitable habitat.

The capacity for drought survival is well developed in many other Basommatophora. It has been found that under laboratory conditions certain bilharzial snails survived periods of disiccation of 3-11 mths (WHO, 1956), while Shousha (1949), Boettger (1944), and Watson (1956, quoting Barlow et al., 1945-1954) all report on Basommatophora surviving for twelve months. Survival for an even longer period is reported by Annecke and Peacock (1951) who claim to have raised colonies of B. tropicus from snails collected in pans which had been dry for 18 months. This capacity of many Basommatophora to exist out of water for long periods is perhaps, according to Boycott (1936),^a reflection of their ancestry. A certain degree

of isolation from the drying air may be obtained either by making use of the substratum or by closing the aperture of the shell with some structure formed by the snail.

Protection through blocking the shell aperture

Structures formed by the snail and used to seal the shell aperture may be of a permanent or temporary nature. In roso-branchs opercula serve this purpose. Thus Pila and Ianistes two fresh-water forms, are well able to tolerate desiccation (Pilsbry and Bequaert, 1927). As far as is known, an operculum is found among the Pulmonata only in the marine genus Amphibola (Morton, 1960). This means that fresh-water pulmonates must rely on some other means to avoid fatal desiccation.

The formation of epiphragms by planorbids has been reported by many authors. Among whom may be mentioned Simroth and Grimpe (1918), Barlow (1935), Boettger (1944), Annecke and Peacock (1951), Mozley (1954) and Barbosa and Olivier (1958). According to the lastmentioned authors A. glabratus does not form a true epiphragm though thin, membranous films are sometimes found in the aperture of individuals of this species aestivating in the field (Olivier and Barbosa, 1958).

The descriptions of the basommatophoran epiphragm vary and this is only to be expected as it seems probable that, besides possible species differences, the nature of the epiphragm may vary with the conditions under which it is formed. It may even vary in a single individual where successive epiphragms

are secreted during deep retraction into the shell, e.g. according to Boettger (1944) the epiphragms in any one snail may vary from chalky to membranous. It may be concluded that the structure of the basommatophoran epiphragm is generally not as robust as that occurring in the Stylommatophora.

In B. tropicus and Physopsis it is said to be composed largely of mud (Annecke and Peacock, 1951). In a few, approximately 10, specimens of the local B. tropicus examined, no trace of an epiphragm could be found. The shell aperture of these specimens was, however, caked with mud and it is possible that a delicate membrane could have been broken during removal of the mud. In some of the snails the mud clogging the aperture was in fact found to be greyish on the inside of the plug but it was not possible to decide whether the grey material represented the remains of an epiphragm or whether it was merely mould.

How successfully certain snails are able to cope with drought conditions, without the aid of an epiphragm is shown by A. glabratus of which 3/10 survived 129 days at 31% relative humidity (Olivier and Barbosa, 1958). It is noteworthy that these authors found aestivation at low relative humidities to be accompanied by deep retraction into the upper whorls of the shell.

Although retraction occurs also in B. tropicus, as also in the Egyptian vectors (Barlow, 1935), and probably in all anhydrobiotic snails, the retraction would seem to be a more

effective weapon against desiccation in planoid snails (small aperture and slowly increasing whorls) than in spiral shell eg. Lymnaea, which has a large aperture and rapidly increasing whorls, so that retraction of the massive headfoot into the smaller upper whorls is mechanically impossible. One might, on these grounds, consider the formation of an epiphragm to be less important in planoid forms than in spiral forms but there are two cases which seem to refute this supposition. I could find only a single example of a lymnaeid, L. palustris, forming epiphragm (Mozley, 1932 quoted by DeWitt, 1955); and Anisus which, besides being planoid, has a relatively small aperture, forms a chalky epiphragm (Boettger, 1944).

The whole question of epiphragm formation requires further study as little attention has been paid to the behaviour of different species and, as far as is known, no attention has been paid to epiphragm formation under differing conditions. Moreover, subjectivity in the description of epiphragms makes any comparison of the various epiphragms difficult if not impossible.

Protection through the substratum

Snails may also use the substratum or objects in the habitat to block the shell aperture. Thus B. tropicus which crawled out of the water and remained attached to the sides of the aquarium or fell onto the laboratory bench, were generally found to be sealed to the surfaces by mucus. This mucus could be effective in sealing off the surrounding air from

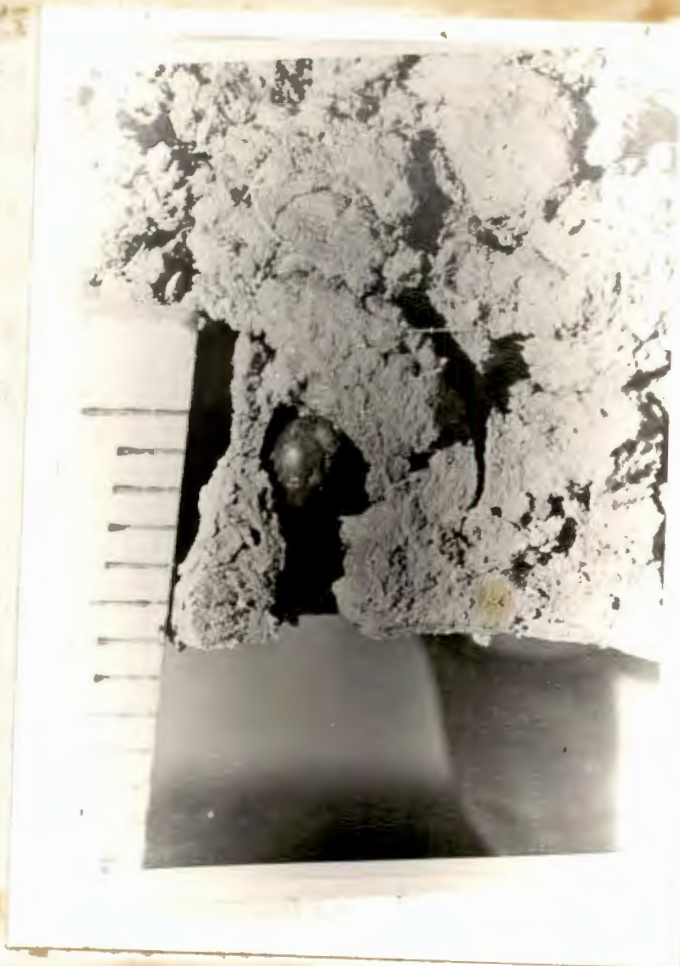


Fig. 80. Bottom of dried-up pool, showing burrows of *B. tropicus*.



the shell cavity, and also in effecting adhesion to the stones, plants etc. to which the snails may adhere in a dry habitat. The tackiness of this mucus was evident in the case of snails which crawled out of the water in the aquarium and became attached to the glass walls of the tank. Even when the snails crawled so high that only part of the aperture was still in contact with the glass, the mucus dribbling down from the aperture was sufficient to effect adhesion. Boettger (1944) states that the body is sealed off from the surrounding air by the snails pressing the newly-formed and still soft aperture edge against the substratum in much the same manner as described by Roberts (1950).

From the observations of Annecke and Peacock (1951) and my own observations during this study, I feel convinced that B. tropicus relies mainly upon burial in the mud to survive periods of drought. Opinions differ as to how this burial is effected. Some authors state that the snails burrow actively into the mud; others that they are accidentally buried during locomotion over a soft substratum, or fall into cracks in the drying mud. The WHO (1956) are of the opinion that the snails may bury themselves in the mud to escape the adverse effects of a flood, but seem to consider the burial during a drought to be accidental. It seems to me to be possible that a number of the snails might also be buried accidentally in silt washed down during a flood, and several authors describe deliberate burial during a drought.

Indications of the digging behaviour in B. tropicus were first obtained in habitat 21b which was usually fairly deep. On one occasion, however, most of the water had evaporated leaving only a soggy mass of aquatic plants on the surface of the mud. Specimens of pysa mosambiguensis, Lymnaea natalensis and B. tropicus were still alive and present in fair numbers. L. natalensis occurred mostly on top of, or amongst the plants but B. tropicus, significantly under the plant mass in the upper layers of the semi-liquid substratum. Some of the B. tropicus were brought to the laboratory, whereupon they tried to bury themselves in the sand at the bottom of the aquarium. The sandlayer was too shallow to allow this and after about five days most of the snails were crawling actively about and one egg mass had been deposited. Confirmation of the digging behaviour of the local B. tropicus was obtained by digging in habitats 11b and 6 when they were completely dry. In the latter clam shrimps (Caenestheriella) and snails were found in the mud, the snails at depths of as much as 8cm, leaving no doubt that these animals had buried themselves.

Further proof of the digging behaviour of B. tropicus during a drought is furnished by the admirable photographs sent by Mr. S. S. du Plessis (of the Division of Nature Conservation of the Transvaal) to Professor van Eeden who has kindly allowed me to publish them. These photographs (figs. 80 81) depict the surface of mud into which the snails have burrowed, and also a profile of the mud to show a snail at the bottom of its burrow. All these observations therefore seem to indicate

that though accidental burial (depending on the type of habitat) may play a small part in preserving some of the snails, the active self-burial must be of far greater importance.

Digging behaviour is, however, not exclusively associated with droughts but may be the response to other adverse conditions such as floods, as mentioned above, or unfavourable extremes, (either too high or too low), of temperature. Watson (1956) reports that B. truncatus burrows into the mud as a result of the low temperature of the water during the winter, and such behaviour was also noted in Lymnaea truncatula (Roberts, 1950 quoted from McClellan, 1948). In May at the onset of the cold weather locally, specimens of B. tropicus were also observed to dig themselves into the mud at the bottom of the aquarium. The water in the aquarium was rather foul at the time and it was initially thought that this was the cause of the burrowing. It was noticed however, that snails placed in water which they find in some way distasteful usually became negatively geotactic and left the water. Teesdale (1962) has a record of P. nasutus in Kenya crawling out of a dam a few days before it dried up and he cites other authors reporting the same behaviour in P. globosa. Van Someren (1946) also found that Lymnaea caillaudi will leave the water when conditions are unsuitable and will not return to it, rather dying of drought. I therefore concluded that the positive geotaxis exhibited by the B. tropicus in the present investigation was a response to the low temperature.

In the same way snails stranded in the shallow puddles left in irrigation canals burrow temporarily into the cooler mud when the water becomes very hot (Watson, 1956) . This seems to be characteristic only of the larger snails whereas egg clutches and very young snails are killed. Buttner (1958) also reports shallow burial in the mud when the heat becomes excessive.

Digging behaviour may thus, amongst others, be triggered off either by the drying up of the habitat or an unfavourable drop (or rise) in temperature. Potchefstroom lies in the summer rainfall area and it is thus possible that either the low night temperature at the onset of winter, or the diminishing water, may act as a stimulus to dig into the substratum.

Drought resistance is not just an isolated physiological attribute of the snails, but as in the case of the desert rat, which exhibits a beautiful combination of physiological and ethological adaptation, the adaptive behaviour of the snails is just as vital as, and intimately connected with, the physiological adaptation. This hardness must have great survival value for organisms in hot arid climates and it is therefore not surprising that this is a genetically controlled character. Olivier (1956) states that there is evidence of great variation occurring in the drought resistance of various geographical strains. Olivier (1956) and Barbosa and Olivier (1958) and Olivier and Barbosa (1958) have shown that strains from dry regions are more resistant to desiccation than those

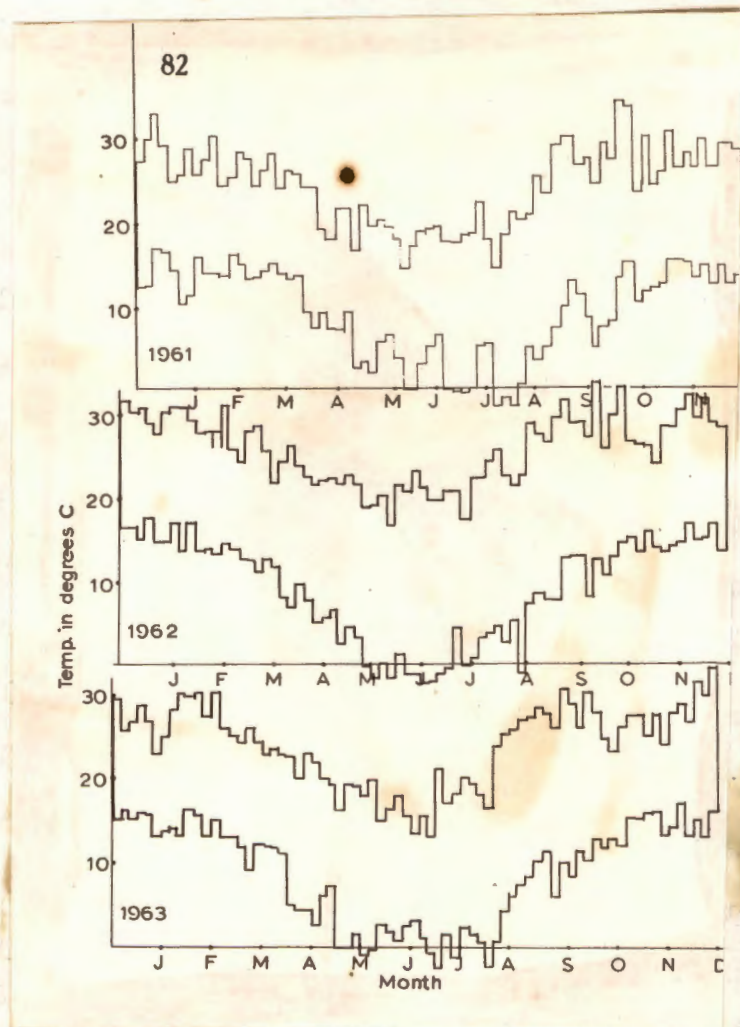
from moister regions.

DISCUSSION OF TERMINOLOGY

The terms aestivation and hibernation are commonly used in the literature when reference is made to freshwater snails undergoing a period of desiccation. It seems to me that in these instances the term anhydrobiosis is to be preferred.

In the first place this eliminates the problem of deciding whether to classify snails in such a condition during spring, as aestivating or hibernating individuals. Secondly it should be realized that though the terms hibernating and aestivating are unambiguous when applied to terrestrial pulmonates they lose their value when applied to freshwater species. In most overseas papers aestivation indicates a resting phase during the summer drought and hibernation a resting phase induced by very low winter temperature. It therefore so happens that aestivation has come to be associated with drought, and hibernation not. In the greater part of the Republic, however, the rainy months are those of the summer, and many snails "hibernate" under conditions of drought, which may be the actual cause of the diapause. It is therefore desirable that, wherever possible, one should ^{try to} distinguish clearly between aestivation, hibernation and anhydrobiosis.

A peculiar type of aestivation is reported to occur in Achatina fulica (Mead, 1961). In this species aestivation is said to be so common that the author never encountered a popu-



for Potchefstroom during the years 1961 - 1963.

lation in which there was not an aestivating individual, even when the external conditions were apparently optimal. A possible explanation for this frequent aestivation is offered by Howes and Wells (1934a and 1934b), who suggest that it is based on an intrinsic hydration cycle of the animal, dehydration resulting in aestivation. It seems to me doubtful whether this frequent cessation of normal activities of freshwater snails under favourable conditions should be termed aestivation, as by such usage, the meaning of the concept is lost.

10. CLIMATIC FACTORS AND POPULATION CYCLES

10.1 Temperature

On the whole this is probably the most-studied of ecological factors yet it remains difficult to assess the importance of this factor because of the fluctuations, (diurnal, monthly etc.) and the differing temperature of closely situated microhabitats. The case is further complicated by the fact that temperature is closely related to other factors such as light, dissolved gases etc.

That the local B. tropicus have a wide temperature tolerance is obvious when one considers the temperature range of this locality. The maximum and minimum temperatures in pentads (as measured in a 4ft Stevenson screen at the local agricultural college) for Potchefstroom for the years 1961-1963, are reflected in fig. 82. Although details of daily temperature fluctuations are sometimes required, the values in pentads

TABLE 4

ABSOLUTE MAX. AND MIN. AIR TEMP. (°C) FOR 1961 - 63			
MAXIMUM		MINIMUM	
Date	Temp.	Date	Temp.
22.10.1961	36.0	16.7.1961	- 6.7
16.10.1962	34.8	14.6.1962	- 8.2
26.12.1963	36.4	24.7.1963	- 6.1

suffice, in this instance, to reflect general temperature trends. The maximum and minimum temperatures are given, rather than the mean ^{temperature} because, as indicated by Louw (1951) with reference to other authors, it is the extremes of temperature which the organism must be able to endure if it is to survive. The wide temperature range of this area may be appreciated by referring to table 4 which reflects the absolute maximum and minimum air temperatures registered during the same three years. The records for the period 1906-1955 reveal that an absolute maximum of just over 39.2°C and an absolute minimum of -10.2°C were registered.

Freshwater snails are known to be able to survive high temperatures. Swart (pers. comm.) bred B. tropicus in shallow aquaria containing 3.8cm. water in a room in which the air temperature sometimes rose to over 30°C and from several countries have come reports of this capacity. In central Iraq populations reach a maximum in July when the water temperature has a minimum of 27°C and a maximum of 33°C (Watson, 1951 in Watson, 1956). In Puerto Rico A. glabratus was found in waters in which surface temperature was 34°C for several hours in the afternoons (Harry and Aldrich, 1958), though under the plants it would be cooler. Muirhead Thomson (1958) states that in general the maximum in snail habitats hardly ever lies beyond 35° or 36°C though he quotes Gordon et al's statement that 19/20 B. globosus survived 40°C for two hours though all of the B. pfeifferi succumbed.

The local B. tropicus became very active when placed in water of 25°C which is the temperature at which Standen (1949 quoted by Gaud 1956) found that B. truncatus exhibited the maximum activity. Gaud (1956) on the other hand, found that B. truncatus crawled out of the aquarium at 25°C. At 30°C the local B. tropicus became almost immobile and crawled around only very sluggishly. However the fact that Swart bred B. tropicus at a temperature which must have approximated 30°C indicates that the species is able to exist successfully at high temperatures. Also the maximum air temperature of 36.4°C that registered during this study and that in the shallow, small pools, sometimes no more than puddles in which B. tropicus occurred, the water temperature must rise considerably. In fact it has been found that shallow marginal water may have a much higher temperature than the surrounding air (Guppy, 1894, quoted by Boycott 1936). The fact that the snails became almost immobile in water of 30°C does not refute the statement that they possess a high degree of temperature tolerance because in the experiment they were suddenly transferred to this temperature without being allowed gradually to acclimatize themselves. The importance of acclimatization in resistance to extremes of temperature is generally recognized. It is illustrated by the reaction of Achatina to low temperature (Mead, 1961) and that author points out that the same principle is known to apply also in the case of certain insects.

It is of interest to learn that the snails are better able to withstand the very high temperature when they are in a desiccated or partly desiccated state. This was observed as early as 1935 (Barlow) and confirmed by Barbosa and Olivier (1958) and also by WHO (1956) who state that the beneficial effect of desiccation is applicable also to low temperatures. This fact was in reality established as early as 1930 by Duval who found the freezing point of hibernating Helix pomatia to be lower than that of normal individuals (Mead, 1961) and this was shown soon after by other workers, to be the result of the lower water content of the tissues of hibernating snails.

The Highveld winters are severe, an absolute minimum air temperature of -8.2°C having been registered during the years 1961-1963. Although some B. tropicus hibernate, at any month during the winter active individuals are to be found, and in the field notes, egg masses have been recorded throughout the winter. Even after the cold and snow of 1964 B. tropicus of all sizes were to be found in habitat 21b.

The diurnal fluctuation in temperature cannot be ignored though freshwater snails are able to withstand relatively great variations in temperature. I. caillaudi can withstand a diurnal fluctuation of 20°C apparently without ill effect (van Someren, 1946) and Abdel-Malek (1956) found B. pfeifferi and Bulinus species in the Sudan in a situation where he estimated the diurnal fluctuation in air temperature to be more or less 20°C . In Potchefstroom the diurnal variation in air

temperature may attain the magnitude mentioned above but it is liable to seasonal variation. On the average the smallest variation occurs in the summer, the February value being as small as 13.8°C while the greatest variation occurs during the winter, the June value being 19.7°C .

For two reasons temperature assumes even greater importance in small or shallow pools than it does in larger ones. Firstly the high temperature results in rapid evaporation because the surface/volume ratio is relatively large. In the second place the temperature range in such a pool at any given moment is narrower than in a deeper pool, offering less choice of microhabitat temperature. Besides this the shallow water also tends to become more easily overheated during the summer months, when it may even exceed the air temperature. In the same manner a small pool experiences greater cooling during the winter and the condition is generally aggravated by the winter recession of the waterlevel. Thus the snails are compelled to endure extremes of temperature which need not be encountered in deeper waters. The manner in which organisms avail themselves of a wide temperature range is admirably illustrated by DeWitt's findings (1955) that Physa gyrina remained in water 3-4 feet (91.5-122.0cm) deep while the temperature was low, but as the water temperature rose, they migrated to the shallower water 2-6 inches (5-15cm) deep.

Oviposition and Breeding Cycle

A spate of oviposition is not necessarily followed by a corresponding increase in the snail population, but no population increase is possible without a corresponding increase in the rate of oviposition. Although the oviposition rate is therefore basic to any population growth, , population dynamics are affected by numerous factors. Such factors include floods or the drying up of the habitat as shown by DeWitt (1955), Gadd (1956) and also in the present study. Because of the interruption of normal activities, desiccation sometimes leads to an extension of the life span of the animal concerned. This was found to occur in P.gyrina (DeWitt, 1955), Lymnaea peregra Müller and Planorbis corneus (Wilkins, 1948, quoted amongst other authors in Comfort, 1957).

Although a limited amount of oviposition takes place throughout the year in B. tropicus, this process is at certain times so stimulated as to result in a sharp population increase. Because of the interrupted sampling and the sampling difficulties, it was impossible to trace the entire annual cycle, there being insufficient data relating to the first few months of the year. It appears that in the local B. tropicus there are two breeding peaks, one of these occurring in October/November, the beginning of summer, and the other in June, midwinter.

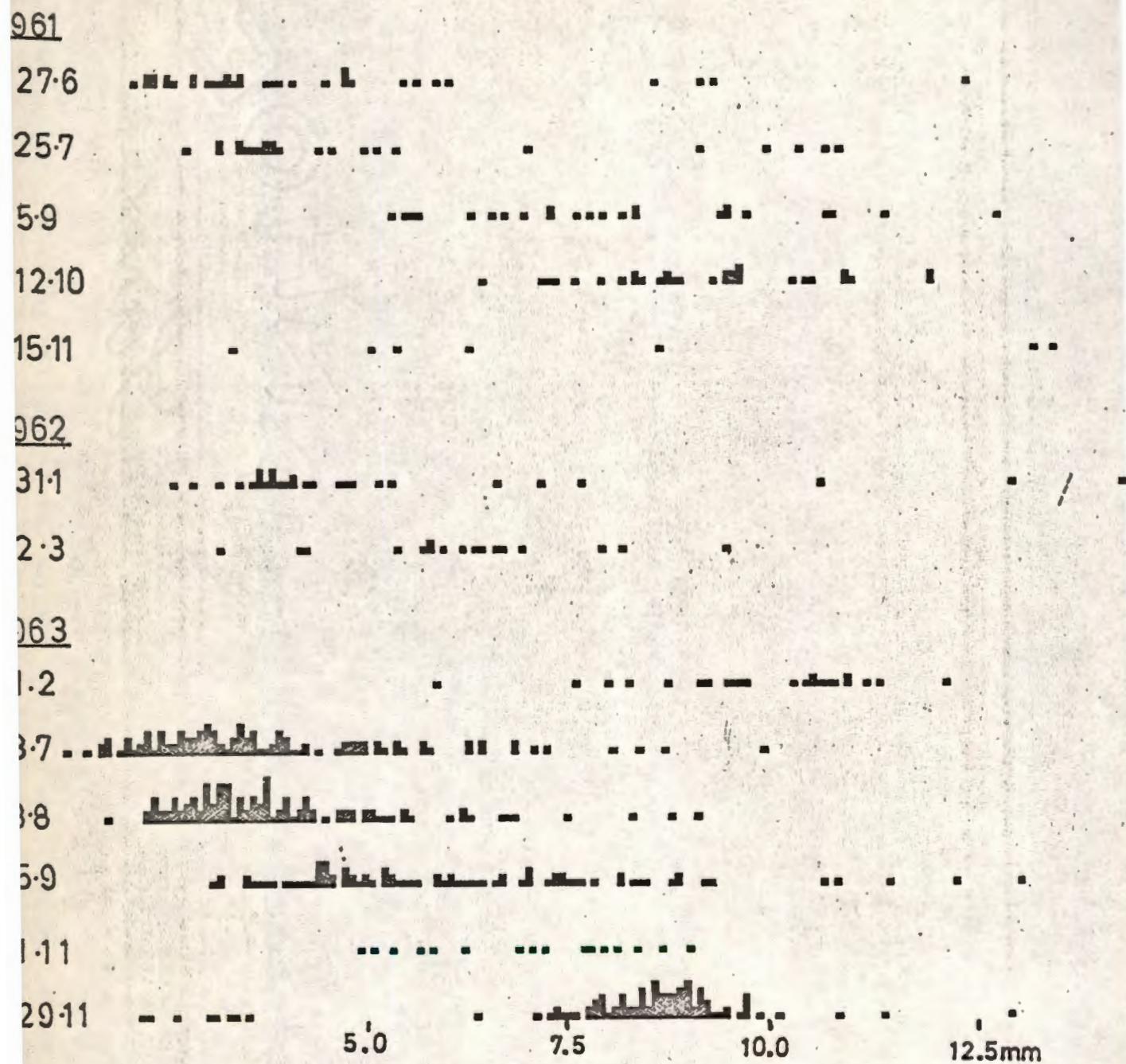


Fig.85. Size frequency distribution of *B. tropicus* in Habitat 21c for certain months during the years 1961 - 1963.

25.7 84

5.9

12.10

15.11

1962

31.1

2.3

16.8

7.9

4.10

6.11

1963

.1

.2

.6

.7

.8

.9

.10

1

9.11

5.0

7.5

10.0

12.5mm

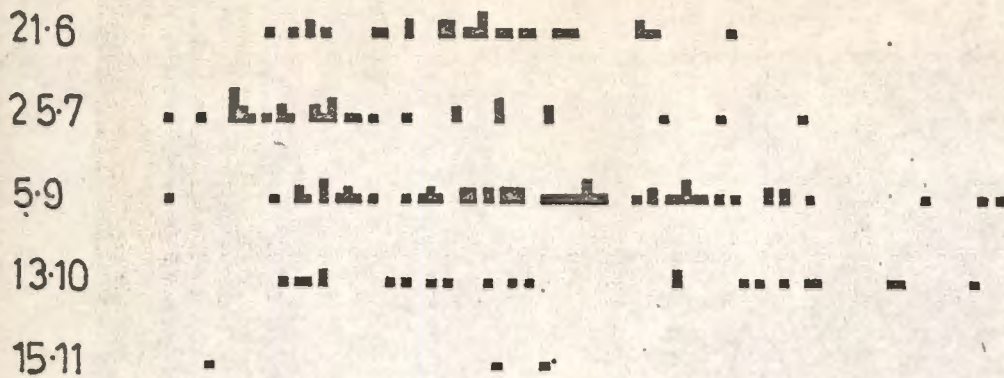
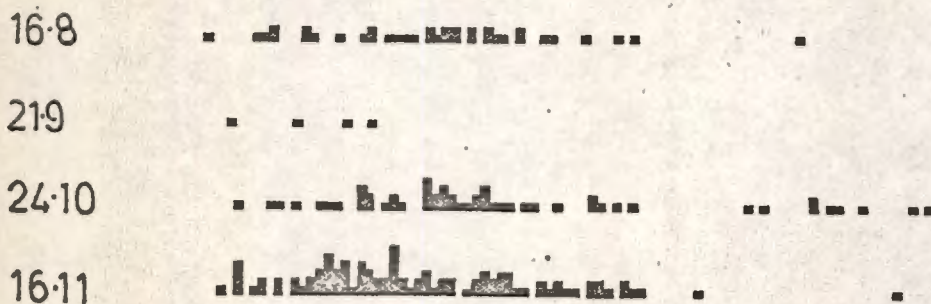
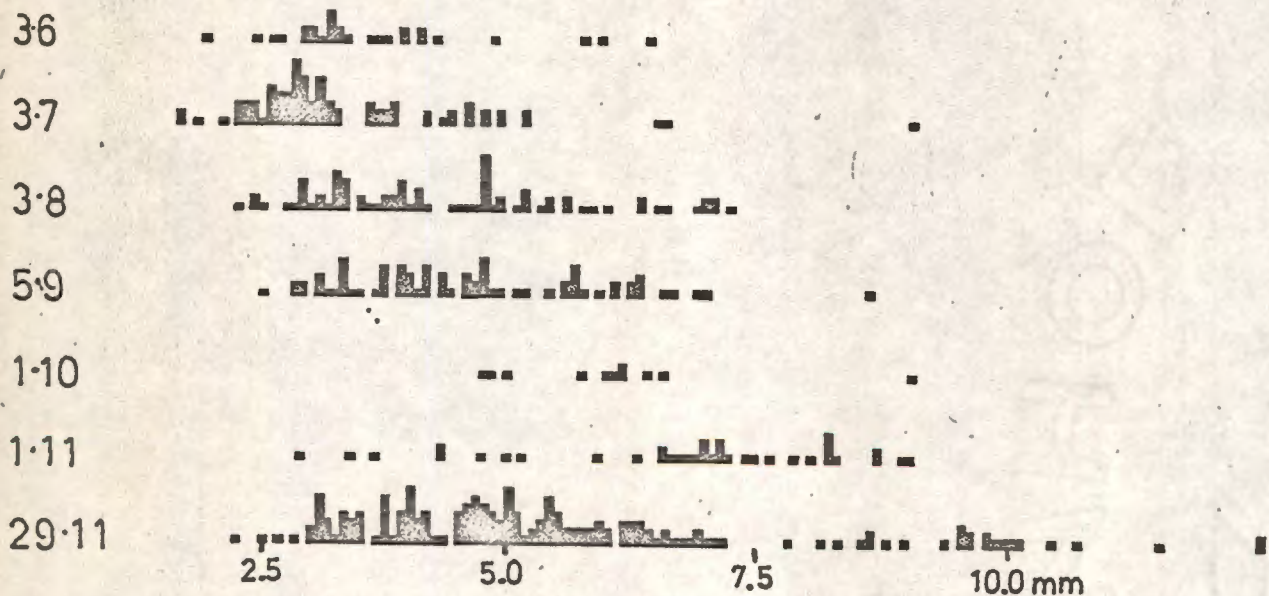
196119621963

Fig.83. Size frequency distribution of *B. tropicus* in Habitat 9 for certain months during the years 1961 - 1963.

October/November peak. Habitat 9. Fig. 83 shows that the snails of habitat 9 were generally rather small, in comparison with those of habitats 21b and 21c. This is not a genetically controlled character as the population was, on rare occasions as on 26.3 - 1962 found to consist of adults approaching 18mm shell length. It is clear in the representations of 1962 and 1963 populations, that during October there was a marked increase in the number of young snails. This was not apparent in 1961 and may perhaps be ascribed to fluctuations in the water level of the habitat. In September the dam was very low and brown, frothy algae were present on the surface. In October the water level seemed to have risen slightly but by November the dam was again very low and most of the swampy area had dried up.

Habitat 21b. Fig. 84. The few small snails found on 12.10.61 may indicate the onset of the breeding season but such an early increase was not visible in 21c or in habitat 9 and the sample is rather small to be conclusive. Although breeding may begin at the end of September the height of activity seems to be reached during October. The sample taken on 29.11.63 showed a decrease in the number of smallest snails, indicating that during November there was a decrease in the oviposition rate. This was also apparent, though to a lesser degree, in the sample taken on the same date from habitat 9.

Habitat 21c. Fig. 85. In contrast to the closely situated habitat 21b which was never found to be dry, habitat 21c was, on several occasions, found to ^{be} much reduced and twice, found to be completely dry. The collections made on 1.11.63 showed a marked increase in the number of juveniles in habitats 9 and 21b but, not in 21c where the new generation became apparent only in the following sample. This lag in the onset of breeding was most likely caused by the drying out of the habitat during October. When the sample 1.11.63 was taken there were only a few inches of water present, probably derived from the rains during the last third of October, and the intervening time had been too short to allow development of a new generation.

June peak. Of special interest is this breeding peak which occurred ~~during~~ the middle of winter.

Habitat 9 The May 1961 sample revealed the presence of many snails ranging from small to large and of the latter there were many individuals present. The June 1961 sample contains many small snails, the number of which had increased still further by July. It is notable that the June collection included only one large snail, showing that most of the large snails had died. The cause of this is unknown. In both Physa fontinalis (Duncan, 1959) and Physa gyrina (DeWitt, 1955) the death of the adult population after oviposition is reported and Comfort (1957) expresses the opinion that in monocarpic forms, especially certain nudibranchs, oviposition

TABLE

TOTAL AND MEAN MONTHLY RAINFALL IN mm. FOR 1961 - 1963

	Jan.	Feb.	Mar.	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Total	$\bar{X}$
1961	50.8	54.5	78.0	121.4	40.5	10.3	1.7	0.1	4.3	3.0	124.5	133.0	622.1	51
1962	61.1	112.8	65.2	90.1	3.8	0.0	0.0	2.3	2.5	24.0	155.3	51.2	568.3	47
1963	112.3	48.7	83.6	92.6	37.0	41.7	3.1	0.0	3.4	38.3	127.4	58.3	646.4	53
	74.7	72.0	75.6	101.4	27.1	17.3	1.6	0.8	3.4	21.8	135.7	80.8		

is normally followed by death. In view of the fact that I found B. tropicus to be a continuously spawning form it is not possible that the death of the B. tropicus was a kind of parental death. The 3.7.63 sample indicated a great increase in the number of young snails, so that in this case, the increase must have taken place in June. Although July and August are our driest months, June, as a rule is also fairly dry (table 5). In 1963, however, an unusually wet June was experienced, 41.7mm rain fell, compared to 0 and 10.3mm in the other two years. There is unfortunately no comparable sample for 1962 but the range for the August sample of that year is not inconsistent with a similar increase as that which took place in 1961 and 1963.

Hab. 21b. The 27.6.61 sample showed a large number of small snails and, for this habitat in which the snails attain large size, an absence of the largest snails. By 25.7.61 no small snails were found, indicating a cessation of breeding. August 1962 again indicated that breeding had occurred a short while before sampling, while the 3.6.63 sample showed an increase in the number of young snails so that in this case, breeding must have begun towards the end of May. Once more the July sample shows a shift to slightly larger snails, showing that breeding had ceased.

Hab. 21c. As in the previous instance there was, in June, a large number of small snails and in July a decrease in the:

number. The sample 3.7.63 showed that a burst of breeding activity had just taken place. This lag, when compared with 1961, or with the June peak of 21b suggests a delay in breeding owing to the drying up of the habitat.

In the distribution of the smallest snails during the winter breeding peak, habitat 9 differs from the other two habitats kept under observation. When the samples are compared it becomes apparent that there was a marked increase in the number of small snails during June with a subsequent decrease in the number during July. This indicates a breeding peak in late May or early June. In habitat 9 the two winter collections differed from those of the other habitats in having the greatest number of small snails present in July. The cause of this later breeding cannot be stated with certainty but the fact that this habitat is a marshy area, inclined to dry up at certain times, may be of importance. At the June 1963 collection it was noted that there was a little more water than during May, and by July the water level had risen till there was a fair amount of water present. It may therefore be that this slow reestablishment of the habitat after the winter drought is responsible for the lag in population growth when compared with the two other habitats.

The most important aspect of the winter peak is that, although the exact time of the peak cannot be pinpointed, and it may already begin in May, the very small snails in the 27.6.61 (21b) sample suggest that breeding had occurred in June (mid-

winter) and this seems to be the case also in habitat 9, (1961, 1963) and in habitat 21c, (1963) even when allowance is made for slower growth which occurs during the winter months (DeWitt, 1955, Gaud, 1956 and Duncan, 1959).

Records for the months between June and December are fairly complete and no bursts of oviposition are shown in the population histograms for this period. Unfortunately almost nothing is known of the population growth during the first half of the year. For habitats 21b, 21c a few fragments of information are available. At the end of January 1962 both habitats possessed fair numbers of small, but not very small, snails indicating breeding some time prior to this, probably in December. About a month later there had been a marked shift to larger sizes. In January 1963 habitat 21c had dried up but the sample taken at 21b showed no sign of small snails. From the February 1963 collection it is clear that there was, in that year no increase in the young snails, comparable to that of the beginning of 1962. A factor which might have played some part in stimulating oviposition in December 1961 and January 1962 is the exceptional amount of rain which fell during December 1961 (Table 5). This should probably not be regarded as a separate peak but rather as an extension of the October/November peak. Normally, it appears, there is a decrease in the rate of oviposition during January and probably February because the two samples taken at the beginning of March 1962 show no sign of an increase

in the number of juveniles. From a study of the breeding cycles of other freshwater snails, and taking into consideration the fact that there is a breeding peak in May, it seems unlikely that there is a third peak in April or March but

this hypothesis must be confirmed by better sampling methods. Although the population curves vary from year to year and from habitat to habitat, an overall consideration of many samples suggests that the population density is at a maximum towards the end of the year, and that the biggest snails occur around April. The pattern of the breeding cycle of B. tropicus thus seems to follow that which is basic to a number of freshwater snails, namely two breeding peaks in the course of the year; though in this instance there is the remarkable fact that one breeding peak apparently occurs in winter.

Breeding cycles of other Basommathophora. It has been found that Physa fontinalis in Britain (Duncan, 1959) and Physa gyrina in North America (DeWitt, 1955) oviposit in early Spring (April). A second, and smaller, breeding season may follow in autumn but the pattern depends on the latitude, a difference of 250-300 miles being capable of influencing the pattern of both these species (Duncan, 1959). It would be most interesting to compare the breeding cycle of Physa mosambiquensis of the Republic with that of the northern relatives.

There have been numerous studies made on the breeding cycles of B. truncatus in North Africa and Iraq, and of

special interest are the observations on breeding at low temperatures. Both Carosse (1930) and Pouhin (1939) (both quoted by Gaud, 1956) report Bulinus egg masses in January, which Gaud, (1956) states are contrary to his findings. Watson (1956) also rejects claims that B. truncatus may breed during the winter in water of 10°C and states that the snails were probably misidentified.

Egg masses of B. tropicus were recovered at Potchefstroom during all the winter months and a glance at the air temperature graphs shows that the water temperature in the shallow snail habitats must have dropped to below 10°C . There are also a number of other authors who report the breeding of freshwater snails, throughout the year/viz. Gordon et al., 1934, Cridland and Webbe (both quoted by Teesdale, 1962) and also Deschiens (1956) who quotes several authors who note similar findings. However it must be remembered that the findings of Gordon et al., (1934) and the authors quoted by Deschiens (1956), apply to laboratory conditions and may thus differ totally from occurrences in the field. Another point to bear in mind, is that the regions mentioned lie nearer to the equator, and this might be the explanation for year-round breeding, which is more remarkable in the highveld area.

The general pattern of breeding in the Basommatophora of importance in connection with bilharziasis seems to be then, two peaks, one of which may generally be distinguished as the major. In Africa generally, the major peak occurs during

April/May and the minor peak November/December. Though the two-peak pattern occurs in many of these snails, this is not always the case, for instance in Kenya the pattern of the breeding cycles in the various snails is so complicated and variable that it is wellnigh impossible to draw general conclusions from the graphs of Teesdale (1962). That author states that there is a distinct increase in the number of young snails after the early rains and a lesser increase after the November/December rains, thus fitting in with the general picture of population cycles.

10.3

Causes of oviposition

There is as yet, no agreement among the various authors as to whether or not oviposition is dependent on some internal regulating mechanism. It is obvious though, from the seasonal variations in oviposition that, if such an innate (regulating) mechanism is present in the Basommatophora, it is greatly subject to external influence. Gaud (1956) and Webbe and Msangi (1958) think it unlikely that such a regulating mechanism is present in the Basommatophora (chiefly Planorbidae) studied by them while Duncan (1959) on the other hand is of the opinion that in P. fontinalis such an intrinsic mechanism may be present. The last mentioned author has approached the question from the behavioural point of view which is a novel approach in planorbid studies and seems well worth exploiting.

The most important external factor involved in oviposition is apparently temperature. This was found to be the most important factor in the regulation of the breeding cycle in P. fontinalis (Duncan, 1959) and P. gyrina (DeWitt, 1955), it being found that oviposition commenced when the water temperature had risen from 7-11°C and from 10-12°C respectively. A rise in temperature has also been shown experimentally to induce spawning in L. stagnalis (experiments of Niewenhoven and Lever in Raven, 1948 quoted by Duncan, 1959).

Workers in North Africa and Iraq have found temperature ^{to} play an important role in the control of breeding activities (Watson, 1956, Gaud, 1956). An illustration of this is the fact that B. truncatus produces two or more generations per year in warmer regions, but only a single generation in cooler regions (WHO, 1956).

In warmer areas the midsummer temperature may rise so much as to be inimical to the snails, eg. in Iraq a spate of oviposition takes place when the hottest months are over (Watson, 1956) and heat-inhibition of breeding in Tanganyika is described by Webbe and Msangi (1958).

Although the summer maximum may be a limiting factor in breeding in hot regions, it may not be high enough in the cooler regions. In certain parts of Britain it has been postulated that, in the case of Patella intermedia, and other marine invertebrates, the distribution in the more northerly

and eastern parts of Britain is limited, not by the winter minimum but by the low summer maximum.

10.4 Rainfall

In the abovementioned instances of breeding of Physa, temperature is apparently the most important regulator of that process. Although temperature may still play a large part in the determination of the breeding cycle of snails in countries nearer the equator, the widespread occurrence of a distinct, often sharply delimited rainy season, or seasons, brings into the foreground rainfall, or floods resulting from this, as a factor influencing oviposition, eg. the floods are said to stimulate oviposition in Oncomelania (WHO, 1956).

Potchefstroom lies in the summer rainfall area and the seasons are clearly defined (table 5). The rainy season extends from October to March with the maximum in January (Baier, 1963) while during the months June to August it may so happen that no rain falls. During the three years of this study the mean annual rainfall was 612.3mm. From table 5 it appears that November is the wettest month and April the second wettest but this must not be associated with the April and November rains which occur in other parts of Africa; it is coincidental.

Although the amount of rain falling at any one time is occasionally large, eg. 58 or even 77.5mm. have been registered, this is not the rule. During the period 1961-1963 rain fell

on 239 days and of those precipitations, 149 measured 5mm or less (fig. 86). Such showers are much more beneficial to the habitat especially in certain cases, notably small streams, than gross precipitations. Besides bringing about a sharp decrease in the salt concentration in drying pools the flooding of vleis and pools apparently has little ill effect on the snails whereas a deluge, resulting in a flushing of small streams often results in high turbidity, current velocity and consequent dislodging of the snails.

Rainfall which occurs in the form of several light showers is of great benefit both to the habitat and the snails. A light shower is not accompanied by great runoff, and the intermittent showers tend to reduce oscillations in pool size. This is of importance to the fauna of the pond, but also to a great extent to the marginal vegetation which is composed of hygrophilous elements.

One cannot but be impressed by the general, though sometimes inexact correlation between breeding peaks and the rainy seasons as described by so many investigators working in Africa. The effect of the rainfall on breeding is also mentioned by Pretorius (unpublished^{thesis}) though I do not accept his interpretation of the correlation between rainfall and breeding in L. natalensis. The supposition that differing rainfall patterns may be held responsible for differences in population fluctuations in Iraq and two Mediterranean countries most effectively explains the population changes in those countries (Gaud, 1956).

Rainfall affects populations not only through its influence on breeding but, as in certain rivers and streams, is responsible for the drastic reduction of population numbers through creating unfavourable ecological conditions and/or washing away large numbers of the snails.

Although no definite proof is furnished certain conclusions as to the effect of temperature and rainfall on oviposition in B. tropicus may be drawn from the following observations:-

In my field notes for 1961 the first record of very many egg masses is on 15th September, while in 1962 many and very many egg masses are recorded as early as 16th August. Rainfall was first examined as a possible cause for the earlier oviposition in 1962. In 1961 only 0.1mm fell on 28th August and the following rainfall occurred on 20th September, after the field observations had been made. In 1962 the first rains after the winter were registered on 28th August and no rain fell during June or July so again, in this case, the possibility of rain as stimulus is ruled out.

Another possible cause of the initiation of oviposition is temperature. A glance at the temperature charts for August (fig. 82) reveals the following: In 1961 the last pentad of July showed a sharp increase in the temperature but this was soon followed by a sharp drop in both the maximum and the minimum temperature. Initially there was a slight lag in the decrease of the minimum temperature but this soon fell so far

TABLE 6

MEAN MAX. AND MIN. AIR TEMP. (PENTAD VALUES) FOR JULY - SEPT. 1961 and 1962

	Year	July	Aug.	Sept.
Mean maximum	1961	19.2	19.0	27.6
air temperature	1962	19.7	22.8	28.7
Mean minimum	1961	1.7	1.0	8.4
air temperature	1962	- 0.6	2.5	9.3
Difference between the	maximum	0.5	3.8	1.1
values for the two years	minimum	2.3	1.5	0.9

as to be the lowest of the year. In 1962 August was marked by a sudden increase in the temperature (table 6) and although during the month a drop in temperature was also experienced, the maximum temperature for August 1962 was considerably higher than for August 1961. It is possible that this may be held at least partly responsible for the more marked oviposition during August 1962. The warmer climate in August 1962 is not so apparent when the mean maximum temperatures for the two years are compared and the difference is more readily appreciated by referring to fig. 82 . It is also apparent that in August 1962 there are two drops in temperature. A lowering of the temperature has also been found to stimulate oviposition (see below). That these decreases in temperature were not responsible for the oviposition is clear because the samples were taken on the 16th August (fourth pentad) while the first drop in the temperature occurred during the 5th pentad .

It may be of some significance that the greatest changes in the monthly mean maximum air temperatures are those between August and September and the drop between April and May. It is not to be overlooked that these temperature fluctuations may play some part in determining the population peaks which follow soon after.

Although increasing temperature seemed to stimulate oviposition during August and September it may not be assumed that temperature is the only controlling factor in the breeding of B.

tropicus because when considering the October/November peak the summer rains must also be taken into account. The precise effect of rainfall upon oviposition in B. tropicus is unclear but it is possible that the October breeding peak bears some relation to the onset of the summer rains in October. The supposition that this may be so, is strengthened by the fact that in habitats 9 and 21c there were exceptionally large numbers of young snails present in 1963, the year in which an unusually wet June was experienced. Nevertheless, in spite of the high rainfall during midsummer, there is no sign of the population increase during January or February and it is perhaps as found by other authors, the high temperatures of midsummer which exert some inhibiting or depressing effect on spawning.

It has also been suggested that the stimulating effect of rain is not to be ascribed to the rain as such, but to the accompanying drop in temperature. This was suggested in 1934 by Gordon et al. who found that, in the laboratory, oviposition was stimulated in P. pfeifferi and P. globosa when the snails were transferred to cooler water. It is difficult to distinguish between the effect of the rain as such, and the lowering of the temperature and this is aggravated by the fact that, as pointed out by Teesdale (1962) the temperature curves (here) roughly coincide with those of rainfall, making it almost impossible to distinguish between their separate effects.

Various other factors are said to play a part in regulating spawning. Several snails have been found to exhibit

increased oviposition upon changing of the water in the aquarium. This occurred in P. fontinalis (Duncan, 1959 and Lymnaea elodes Say (Linville, 1900, in Duncan, 1959) and certain unnamed workers have found the same to be true in the case of the clawed toad, Xenopus laevis (Bondesen, 1950) Duncan (1959) also mentions experiments described by Raven (1948) in which it was found that, under certain conditions, oxygen resulting from photosynthesis could induce egg laying in L. stagnalis. A good shower of rain may cause changes in the water of the habitat, notably a lowering of the salt concentration, especially in a drying pool and such an osmotic change may then provide the stimulus to oviposition. Also an activity of the snails viz. copulation, has been ^{mentioned} as possibly initiating egg-laying. Although self fertilization has been found to occur in so many of the Basommatophora; it occurs in all the families of Basommatophora and in some Stylommatophora, (Laramberge^u, 1939 quoted by Baker, 1945) both Bondesen (1950) and Duncan (1959) note that copulation is followed by oviposition. Both these authors regard copulation as being essential to oviposition but in Physa fontinalis copulation may act as the normal initiator of the process (Duncan, 1959).

Besides these factors which may directly cause spawning there are various factors which may influence the process.

A factor which may be of ^{importance} / in many countries with a low or sharply delimited rainfall, is desiccation. Besides, its influence on the life cycle, this factor is also supposed

to have an effect on oviposition. It has been stated that snails emerging from ~~anhydrobiosis~~ ^{anhydrobiosis} oviposit rapidly when the habitat is again filled (Barlow et al, 1945 - 1954 (quoted by Watson, 1956).

It was also established (Barlow, 1935) that snails which had suffered desiccation initially laid more eggs than snails which had not been subject to drought (see also under habitat 6).

The effect of parasitism on reproduction is discussed later. The condition of the animal is dependent largely on its diet and ^{it} has been found in various Planorbidae that starvation, or a poor diet, hinders egg laying and it is postulated that an unknown thermostable vitamin is the factor involved (Lagrange, 1957). Abdel Malek (1952) found a reduction in the number of eggs in poorly fed snails and performed experiments in this connection.

Another factor which was sometimes thought to influence oviposition is the light and this factor is discussed under flora. It is here sufficient to say that though light was found to have an effect on the breeding size of Ancylus fluviatilis this was only an indirect influence through the effect of light on the algae constituting the food of the snails (Hunter ^{quoted by} 1953 (Duncan, 1959) and it has been found that the oviposition rate in Bulinus is greatly influenced by temperature but unaffected by light (Gaud, 1956). Oviposition occurs mainly at night (Bondesen, 1950, Duncan, 1959) and this is also seemingly unaffected by light and may be due to an intrinsic rhythm

(Duncan, 1959 and certain worker mentioned by Bondesen, 1950).

In B. tropicus oviposition in the laboratory occurred also during the day though the frequency relative to the nocturnal oviposition was not established.

10.5 Oviposition sites of B. tropicus

Unlike L. natalensis which can oviposit on damp mud, (Shiff, 1960) B. tropicus is, under normal conditions, strictly aquatic and therefore the egg masses are deposited under water. One of the most obvious situations for oviposition is the aquatic plants and in many instances the leaves (Duncan, 1959), and especially the under surfaces of the leaves are mentioned as oviposition sites (Abdel-Malek, 1952), (Webbe and Msangi, 1958). Bulinids are also said to oviposit on the under surfaces of small stones (Pouhin, 1939 quoted by Gaud, 1956), but the local B. tropicus are not fastidious about oviposition sites. Though in the present study, the under surface of leaves, eg. waterlily or Marsilia was a favourite site for spawning, many egg masses were found also on the vertical surfaces or on the bottom (polythene material) of the aquarium. Gordon et al. (1934) also note that P. globosa often oviposits on the upper surfaces of leaves. The five families of Basommatophora are said to prefer a flat site for oviposition (Bondesen, 1950), but this is not apparent in the case of B. tropicus. Egg masses were not infrequently found attached to the corners and angles of perspex strips in the

aquarium even though plenty of flat surfaces were available. Egg masses were frequently found on plant stems, even the thin stems of grass. This was very noticeable in habitat 11b where although the bottom of the pool was covered by a thick carpet of grass, offering many leaves as suitable flat surfaces for oviposition, a very large number of the egg masses were found on the thin grass stems, or on the curved shells of snails, up to three, or even four, egg masses occurring on a single shell. Deposition of egg masses on the shells of other Bulinus has been noted by Pouhin (1939 quoted by Gaud, 1956) and this phenomenon is said to be very conspicuous in P. globosa under natural conditions (Gordon et al. 1934). These are further indications that to several bulinids, a flat surface is of little importance in oviposition.

The shells of the snails are a very suitable oviposition site as by the snails' ability to move away from noxious influences, the eggs may be maintained in a favourable milieu, and by the snails' movement in the water they are well oxygenated. The explanation for the popularity of shells as a spawning site is probably to be sought in the texture of the surface which, by growth lines or costulations, or both, is uneven enough to allow adhesion of the egg mass.

Unattached egg masses, generally found on the bottom of the aquarium but on one occasion found floating, were rare in B. tropicus. Egg masses found loose on the bottom of the aquarium were also discovered in the case of Physa fontinalis

(Duncan, 1959). Bondesen (1950) views such an occurrence of a loose egg clutch on the floor of the aquarium as being the result of an oviposition on a rough surface. Although a too rough surface may be unsuitable it seems very likely that a too smooth, slimy surface such as develops on aquarium walls is also unsuitable. My records of oviposition in the laboratory showed that loose egg masses were encountered only after one month. Bondesen (1950) also notes that Ancylus fluviatilis Müller was on several occasions observed to ~~graze at~~ a certain spot until all "coatings" (Pl35), probably mainly algal, had been removed, before depositing its egg mass on the exposed surface. Also the observation of Gordon et al. (1934), that oviposition only occasionally occurred on dead or rapidly decaying plant material, may perhaps be connected with the development of a slimy layer on such vegetal matter.

Frequency of oviposition.

Laboratory records of B. tropicus show that during March and April, oviposition occurred once a day, once every second day or irregularly at longer intervals. The snails under observation were kept isolated in finger bowls and these cannot be considered optimal conditions so that possibly, under favourable conditions, the snails would produce more than one egg mass per day. This was found to be so by Gaud (1956), who noted that during July and August Bulinus snails produce more than one egg mass per day. Also Ritchie et al. (1961)

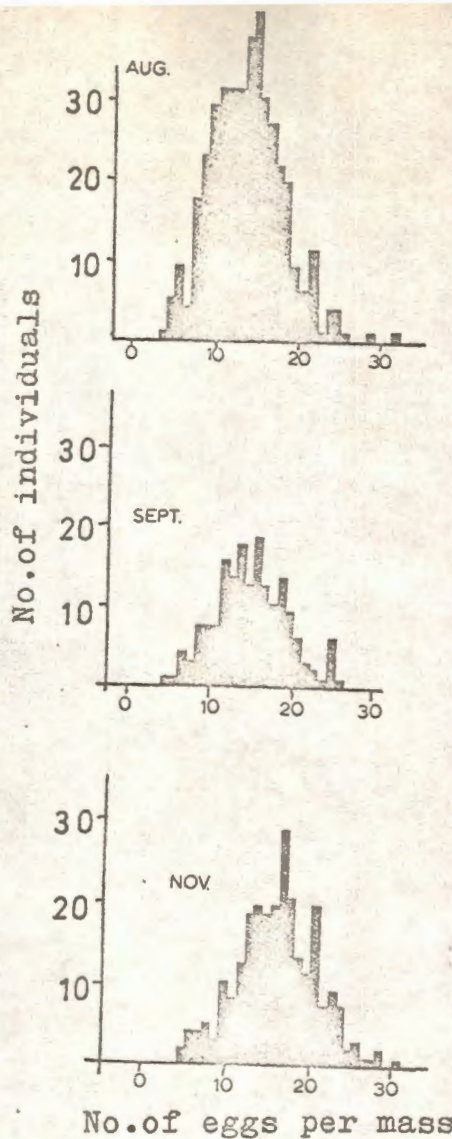


Fig.87. Frequency distribution diagram of the no. of eggs per mass, in Habitat 11b during the months August, September and November 1962.

TABLE 7 NO. OF EGG MASSES PER 10 SAMPLES AND MEAN NO. OF OVA PER MASS.

Sample number	Date	No. of collections	Actual No. of egg masses	Presumed value	$\bar{x}$ no. eggs per mass.
136	16.8.1962	8	168	210	15.2
149	17.9.1962	9	250	277.8	16.6
162	16.11.1962	10	428	428	13.8

found that A. glabratus are capable of producing two egg masses per snail daily for a period of as much as 6 weeks. On the other hand Physa fontinalis was found to oviposit only every two to four days (Duncan, 1959).

In 1962 large numbers of egg masses were recovered from habitat 11b for the purpose of counting the number of egg masses, and also performing egg counts. This was done in the months August to November, excluding October, when the habitat was dry. From the histograms (fig. 87) it can be seen that the number of egg masses increased considerably during the three months. These histograms represent the actual numbers of egg masses taken but, as in August only 8 and in September 9, samples were taken instead of 10, the theoretical values for ten collections were calculated and are reflected in table 7 . These presumed values too, show a sharp increase in the number of eggs per mass laid during the last months of the year.

The number of eggs per mass varies from 4-32, and the mean number of eggs per mass varies from sample to sample. In rearing B. tropicus Swart and Reinecke (1962) found the number of eggs per mass to average 17. This is slightly more than both the mean or the mode of the present collections (table 7). In the August sample the range is from 5-26. By September the maximum number of eggs had increased to 31 and there had been an increase in both the mode and the mean number of eggs per capsule. It may be concluded that this was caused by an increase in the size of the snails. In August the greater

part of the population had been between 5.6-8.1mm in length but by September this had been increased to 6.4-8.9mm. In November the increase in capsule number was associated with a decrease in both the mode and the mean number of eggs per capsule. There are fewer capsules containing more than 20 ova and there is a corresponding increase in the number of capsules containing less than 10 ova. This is apparently to be attributed to i) a decrease in the number of large snails and/or a decline in their reproductive capacity, and ii) the initiation of breeding in snails of the younger generation.

Because of the morphological similarity between the tropicus and truncatus groups, it was thought desirable to determine whether there is any significant difference in the mean number of eggs per capsule.

Gaud (1956) found an average of 7-8 eggs per mass in B. truncatus though the average for that species for the month of June reached a value of 11.4. Bondesen (1950), quoting other authors, cites the following values: 3-9, 6-9, 1-11 with a maximum of 19 for Bulinus contortus. Barlow (1935) gives a value of 11-18 eggs while Perry (1922, quoted by Pilsbry and Bequaert, 1927) found B. contortus in Egypt to produce 20-30 eggs. Though B. truncatus under certain conditions produce capsules containing fewer eggs than seems normal for B. tropicus, it appears that the ranges of egg number in the two species overlap to such an extent that this feature cannot help be used to distinguish between the two

A factor which is generally ignored but is very important in this connection is the degree of parasitism of the snails. For example a specimen of B. tropicus infected with a trematode was maintained in the laboratory for $2\frac{1}{2}$ months. During this time it produced only one small gelatinous mass in which no ova could be distinguished, and a capsule containing one egg. During this period a non-infected specimen of roughly the same size, and maintained under similar conditions, produced 33 capsules containing on the average 14.5 eggs but several masses contained 21 ova, and even the last capsule recorded contained as many as 19.

Conclusions

Although the importance of temperature as a basic factor in the regulation of breeding activities is widely accepted, it is becoming generally recognised that many factors are involved. Gaud (1956) stated clearly that it was not alone temperature, but also possibly floods and the drying up of bodies of water that control population dynamics in the vicinity of the Mediterranean. It should be fully realized, however, that, as pointed out in the case of Tanganyika (Webbe and Msangi, 1958), the population curves are not dependent solely on the climatic factors such as rain and temperature but also on the ecological changes wrought by these factors.

The complexity of the mechanism controlling breeding in molluscs is thus great, and the operation of the controls obscure. It is for example, not known what regulates A. glabratus!

breeding cycles of which there may be several annually in any one habitat (Harry and Aldrich, 1958). The same is true for many other Basommatophora.

It has not been possible to single out any one factor as THE factor responsible for causing oviposition in B. tropicus and the same applies in the case of Physa fontinalis (Duncan, 1959). That author postulates an increase of action specific ^{the} energy prior to the onset of breeding season and when the energy value has reached a certain level, one of several stimuli, (environmental changes) may act as releaser. Such releasers mentioned in various instances are: either a rise or drop in temperature, change of water; a change wrought in the water, as in the Hydrocharis experiments; copulation; and even, in the case of Patella vulgata L, mechanical stimulation (Orton et al. 1956, quoted by Duncan, 1959).

STILL AND RUNNING WATER

From a perusal of the literature it seems that, excluding the ancyliids, still water is a more favourable habitat for freshwater snails, than running water. It has for example been found that in streams which do not differ chemically, snails are absent from those having a swift current (de Meillon et al. 1958). Pallary (1933 quoted by Gaud, 1956) also states that Bulinus does not like running water but Abdel-Malek (1956) is of the opinion that this genus can better endure the effects of a current than either Physopsis or Biomphalaria. In the

present investigation only one well established colony was found in flowing water, but the occurrence of this colony was discontinuous. Whether this scarcity of B. tropicus in streams should be attributed to the direct effect of the current is not known; it may be that the absence of snails from these habitats was caused by factors unconnected, or only indirectly connected with, the current.

Various localities, either streams or the Mooi River itself were examined but although numerous specimens of Burnupia occurred on the rocks, no B. tropicus were discovered in a current of any velocity. At the outlet of Boskop dam a few B. tropicus were usually to be found but in such small numbers that they could hardly be regarded as constituting a colony. The stream in this region is about 3m broad and along the banks grow willow trees among the roots of which B. tropicus and Lymnaea and Burnupia were to be found. Sometimes B. tropicus were also to be seen clinging to the floating leaves and other debris.

A second locus at which B. tropicus was found was the outlet below the municipal dam. This was about 70cm broad and only a few cm deep. Generally a few small snails were to be found among the plants along the margin, where there was hardly any current. A large number of snails was, on certain occasions, found on the bottom, and in the centre of, the stream. This was not regarded as their normal position and they were thought to have been washed into this situation by

the strong current resulting from the increased flow at the outlet.

The only known well established colony in a stream was that occurring in habitat 1. A thriving colony existed here for some time and then declined. The water was at certain times found to be oily as a result of mosquito control measures and this factor may have contributed to the decline of the colony but it is not possible to assess the effect of the oil upon the snails as oil occurred also on the surface of habitats 8a and 21b. Observations at/8a were cut short by the drying of the habitat, but flourishing colonies continued to exist in habitat /21b throughout the years of this study and also in 1964.

The current affects not only the establishment of colonies of snails, but probably also the very size, texture, aperture shape and general form of the shell. It has been observed that the larger A. glabratus inhabit marshes and slow flowing streams while adults of smaller size occur in streams having a current velocity approaching the maximum tolerated by the snails (Harry and Aldrich, 1958).

According to Boettger, (1944) shells from agitated waters are often much more sturdy than those from placid waters. A difference in both the sculpture and the shape of specimens of the pelecypod Io fluviatilis from running and still waters has also been noted (Adams, 1900 ^{quoted by} Pilsbry and Bequaert, 1927). The stillwater forms of this species are spiny and rough while the currentforms are free of such ornamentation. Another



form of ornamentation of freshwater snails viz. costulation, is regarded (Boettger, 1944) not as a character which develops in still water, but as a consequence of rapid growth under very favourable conditions. During such growth the periostracum bends outward before it is supported by the deposition of calcium salts. In B. tropicus costulation, pronounced (fig. 87a) periostracal ridges/is clearly distinguishable from the normally present fine "growth lines". The occurrence of costulation was studied in habitats 9, 21b, 21c for the year 1963 and although the impression is gained that a certain ovoid form was more frequently costulated than the elongated form, the factor is very variable and cannot be said definitely to coincide with any particular shape of shell. Neither could any correlation with a certain habitat or month of the year be detected. It is assumed that costulation is an ecologically induced factor, successive samples of the same population exhibiting varying degrees of costulation. Frequently in any one population, small snails are costulated while older snails exhibit this character only in the last part of the shell, suggesting that at a certain time, environmental conditions inducing this character came into operation. This supposition is strengthened by the observation that often the costulated portion of the adult shell is formed after a thickening representing a period of interrupted growth. The above observations do not completely exclude the possibility that costu-

lation may have some genetic basis, forms possibly existing which are incapable of developing costulation under any circumstances.

The shape of the aperture too has been found to vary in accordance with the type of habitat, becoming larger and more rounded in moving water until in stream forms, it may become practically circular (Boettger, 1944). This broadening of the aperture Boettger (1944) explains thus: in order to obtain a firmer grip on the substratum the foot broadens, pushing the enveloping mantle edge into the same shape, and this is then the shape of the shell secreted by the mantle. Baker (1928) also notes an increase in the aperture size in the Planorbidae which are exposed to wave action in larger lakes, and this phenomenon is noted in contrast to the usual river or stream form. In B. tropicus a rounded aperture cannot be correlated with occurrence in flowing water but is often encountered in the shells of snails inhabiting pools (Stiglingh et al. 1962).

With regard to the influence of running water on shell form there are conflicting opinions. Baker (1928) found the creek form of Amnicola limosa (Say) to be elongate and to possess a narrow umbilicus and shallow sutures. The lake form on the other hand was globose, had a wide umbilicus and deep sutures. In contrast to this Simroth and Grimpe (1918) Boettger (1944) and Wright (1957) are all of the opinion that forms from moving water have relatively shorter spires, the elongated forms being found in still water. In this connection it is interesting

to note that B. forskali, is said to prefer fast flowing streams to placid waters (Adams, 1934 quoted by Watson, 1956).

After studying the shapes assumed by B. tropicus from various localities in South Africa it was postulated (Stiglingh et al., 1962) that in this species the reaction to water movement was the same as that described for Amnicola; elongated forms occurring in streams and squat forms in still waters. It has since become apparent that water movement is not the only factor concerned in the determination of elongated or squat forms.

The local B. tropicus were not found in streams of sufficient current velocity to enable me to come to any definite decision on the effect of current on this species. There are however two facts which seem to counterindicate acceptance of the "moving water - long spire" theory as being valid in this instance. The fact that ovoid forms are found in both pools 21b, 21c rule out the possibility of their being formed in response to current action. A further important detail is that in several instances both longspired and depressed forms have been found in a single population eg. sample 170, (21b) (fig. 88); 205, (21b); and 207 (9) fig. 89 and these are probably no exception.

As pointed out by Simroth and Grimpe (1918) such variation in shell form may occur in a lotic habitat because some of the individuals may exist in microhabitats in which the current effect is negligible. In this case however, both forms occur

in lentic habitats. It is here suggested that though current action can, and does influence shell shape, population differences sometimes ascribed to the effect of water movement have possibly resulted from other ecological factors. In B. tropicus the occurrence of long and short spired forms in a single pool indicates that the causative factor should be sought, in this case, among such factors as varying growth rate at different seasons, more or less favourable environmental conditions, or internal parasites or possibly even genetic determination.

It seems to me that if the shell is considered to be composed of a cone wound about a central axis, shell form may be said to depend on four factors:

the length of the cone constituting the shell;

the rate at which the cone increases in size (diameter);

the tightness of coiling about the central axis, and lastly

the extent to which the succeeding whorls overlap one another.

With regard to these characters, four types of shell may result,

- a) greatly overlapping but loosely coiled whorls - no example in my series.
- b) greatly overlapping but tightly coiled whorls (fig. 90)
- c) slightly overlapping loosely coiled whorls (fig. 91)
- d) slightly overlapping tightly coiled whorls (fig. 92).

For practical purposes the shell forms a and d may be said not to occur in B. tropicus, the figured specimen of type d

88



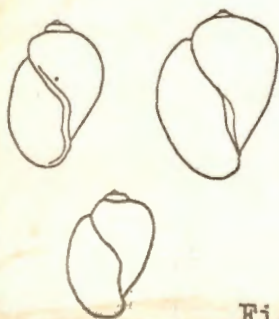
89



Fig.88. Long-spired and depressed forms in a sample taken from Habitat 21b.

Fig.89. Long-spired and depressed forms in a sample taken from Habitat 9.

90



91



92



20 mm

Figs.90 - 92. Shells representing types b, c and d respectively.

being the only one encountered. Types b and c represent the basic forms of this species. Type b corresponds to the type previously designated "juvenile type" (Stiglingh et al., 1962) because it is the common form of young snails. Type c represents the "adult type" referred to in the same paper.

There is no sharp demarcation between these types, a series of intermediate forms connecting them. Both types may occur in a single population (fig. 88 and 89) but generally however, one predominates greatly in frequency over the other.

Failure to recognize which factors are responsible for variations in shell form result in conflicting statements. Thus Pretorius (unpublished thesis) states that in L. natalensis there is no difference in the ratio length/breadth (L/B) in younger and older snails, but that significant differences exist in the slenderness of shells in the two groups. This is contradictory because the ratio L/B may be taken as the simplest expression of the degree of slenderness of the shell.

Discussion of various shell ratios employed to reflect shell form

The ratio shell length/aperture length (L/AL) depends on both the rate of increase in cone diameter and the degree of overlap. In B. tropicus the ratio L/AL exhibits a strong correlation with increasing shell length and increases with age. This was earlier stated (Stiglingh et al., 1962) to be the case in B. tropicus and was confirmed in the present study.

An increase in this ratio during growth has been found to

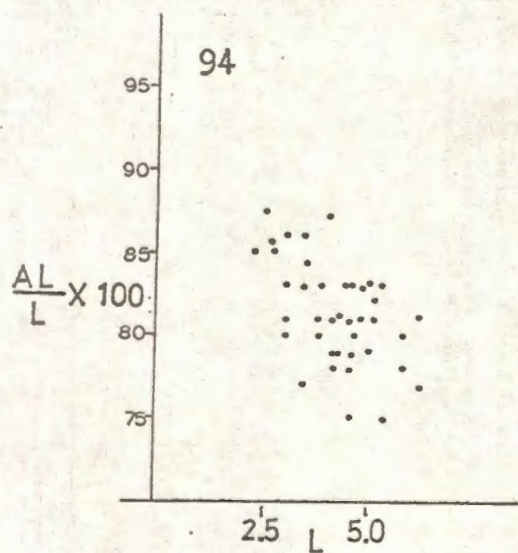
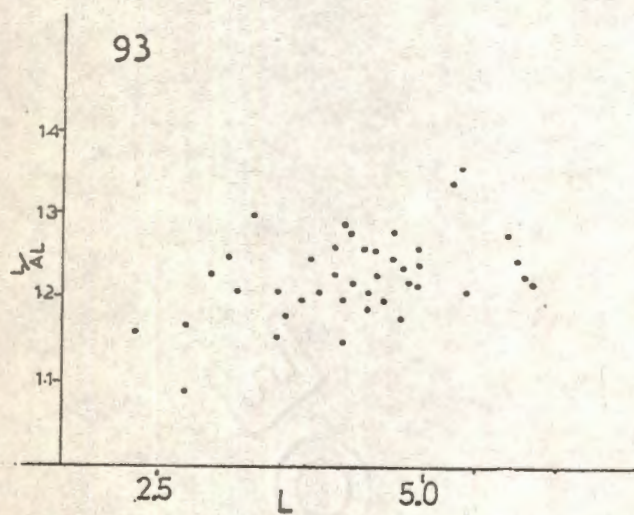
occur also in the case of L. palustris (Peters, 1938), B. jousseaumei and B. globosus (the last two species described by Wright, 1957). Contrary to these findings Hubendick (1951) detected no meaningful changes, correlated with growth, in the ratio $AL/L \times 100$ in L. peregra. Also Pretorius (unpublished thesis) concluded that L/AL does not change as L. natalensis increases in length. As this ratio is in a large measure dependent on the number of whorls and therefore generally speaking, on the age of the snail, it is difficult to imagine a spiral shell in which the ratio L/AL would not increase with increasing shell length. The only instances in which the ratio would probably not increase is in certain depressed forms such as B. depressus Haas, or certain forms which consist of very rapidly increasing cones eg. the ampla form of L. peregra.

In B. tropicus the $L/AL : L$ correlation was strong, values of 1.2 - 1.7 being obtained for shells ranging from about 5.5mm to 11.3mm in length (Stiglingh et al., 1962). In the present instance values ranging from 1.08-1.62 were obtained for snails of 1.4 to 13.8mm in size. In these few samples values less than 1.3 may be said, broadly speaking, to represent the juvenile form, while higher values occur in typical adult forms. The various Lymnaea species are generally composed of cones which increase in diameter more rapidly than the cone of B. tropicus. Consequently the value L/AL will be smaller than in B. tropicus and a weaker correlation is to be expected eg. in L. natalensis of 11.3mm and over, the ratio varies from

1.3-1.4, exceptionally 1.45 (Pretorius, unpublished thesis). A closer study of the graphs of Pretorius (unpublished thesis) and Hubendick (1951) nevertheless show that a correlation, even if slight, between these shell features, might also occur in the forms studied by them.

The lack of correlation exhibited in the graph (fig.45) for L. natalensis may not represent a real lack of correlation in the population. The ratio L/AL was studied only in shells ranging from 10.3-11.8mm and it is possible that this range, merely 1.5mm, is too narrow to reflect any correlation. Also, as is evident (from Wright, 1957 fig. 3, 28 etc.) the correlation between L/AL and L is most clearly apparent in that part of the graph representing the younger snails. In the older snails a more or less constant ratio seems to be attained. Another feature of these graphs is the greater scatter of the points representing older snails, demonstrating that, in this respect, ratios of older snails are liable to greater variation than those of younger snails. This finding is in agreement with that of Schutte and van Eeden (1959) who declared that in B. pfeifferi, older snails exhibit greater variation in form than the younger snails.

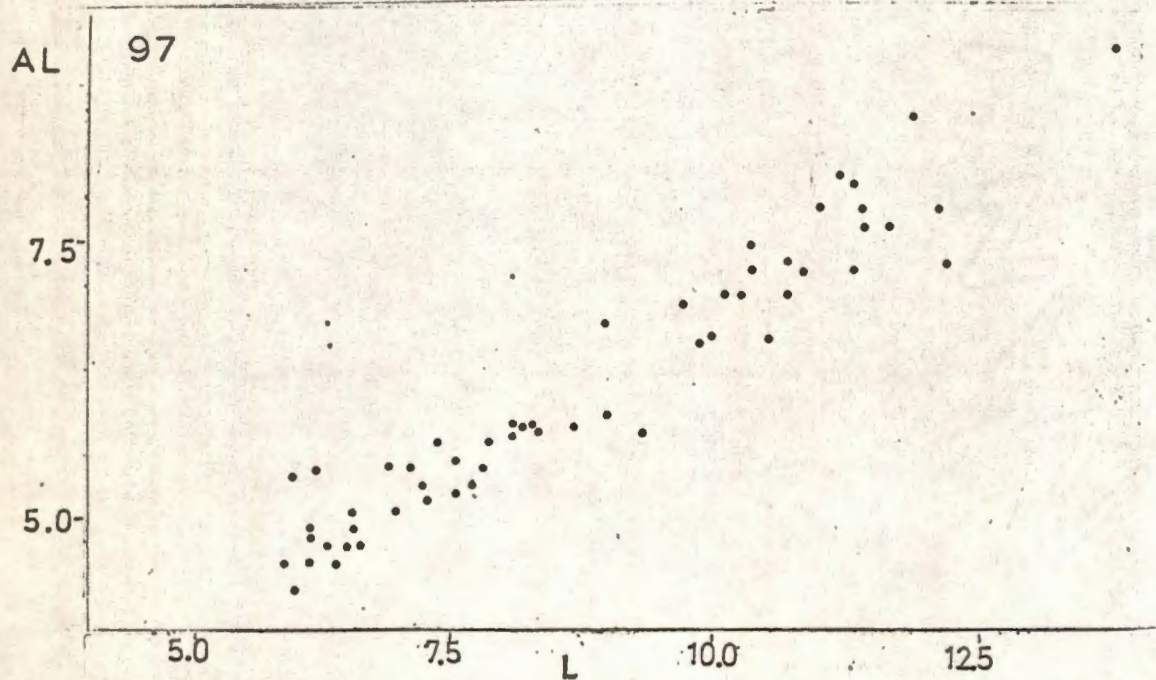
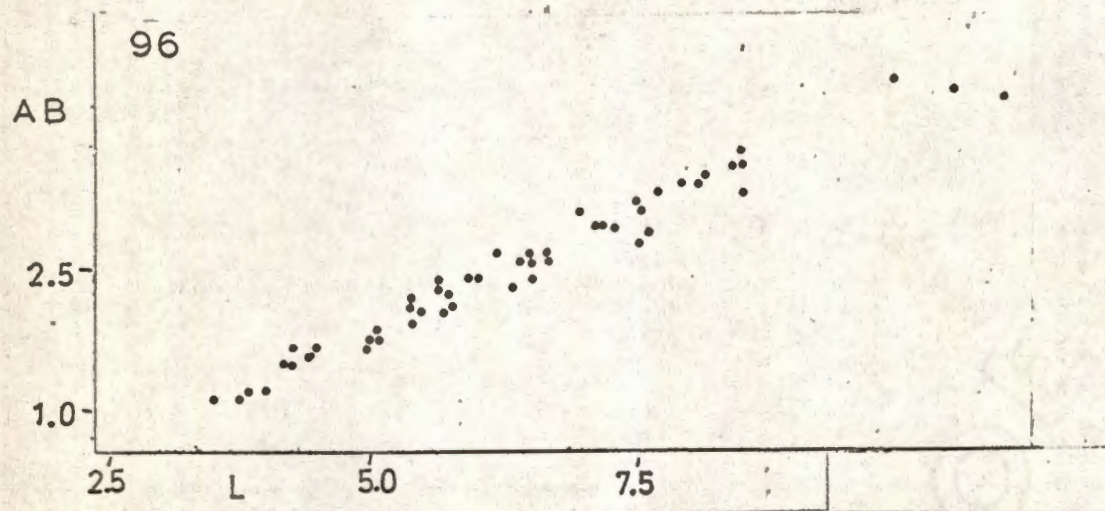
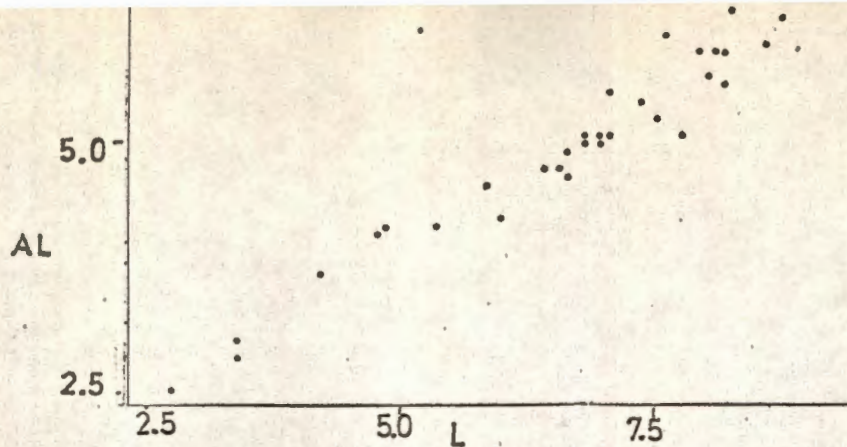
Hubendick (1951) drew various graphs reflecting the relation between the aperture length and shell length, and to this he employed the ratio $AL/L \times 100$. That no correlation was visible between this ratio and shell length in that author's figure 5, is possibly to be ascribed to the fact that the



Figs.93,94. Two means of plotting the same set of data.

points in his graph do not represent individuals of a single population, but only an abstraction. Each point represents a different population, being the mean value of the ratio $AL/L \times 100$ plotted against the mean length of that population. Furthermore, a clear correlation is not to be expected in this instance because of the highly divergent population forms being represented in the graph. The typical peregra form ratio, as employed by Hubendick, would decrease with age, while in the ampla form it would probably increase with age. That the ratio does, in some lymnaeids, behave as in B. tropicus, B. jousseaumei etc. is in fact indicated by populations 13 and 48 in Hubendick's (1951) fig. 4. These populations are of the peregra and ovata type, and I would expect the same result from a representation of this ratio in a lagotis population.

It is puzzling that Hubendick (1951) found no correlation between the ratio $AL/L \times 100$ and shell length in the sample from Karesuando (fig. 3). The shells of this population incline slightly to the peregra form and the various sizes are well represented in the sample. The lack of correlation is thus difficult to explain. That the findings cannot be ascribed to a less sensitive method of representing the data is shown in figs. 93 and 94 in which I have, using the same data, plotted L/AL , and Hubendick's inverse ratio $AL/L \times 100$ against shell length. Hubendick (1951 p.9) found an "uncommonly great heterogeneity" in the Karesuando sample, and that feature seems to me, to be a possible explanation for his finding no corre-

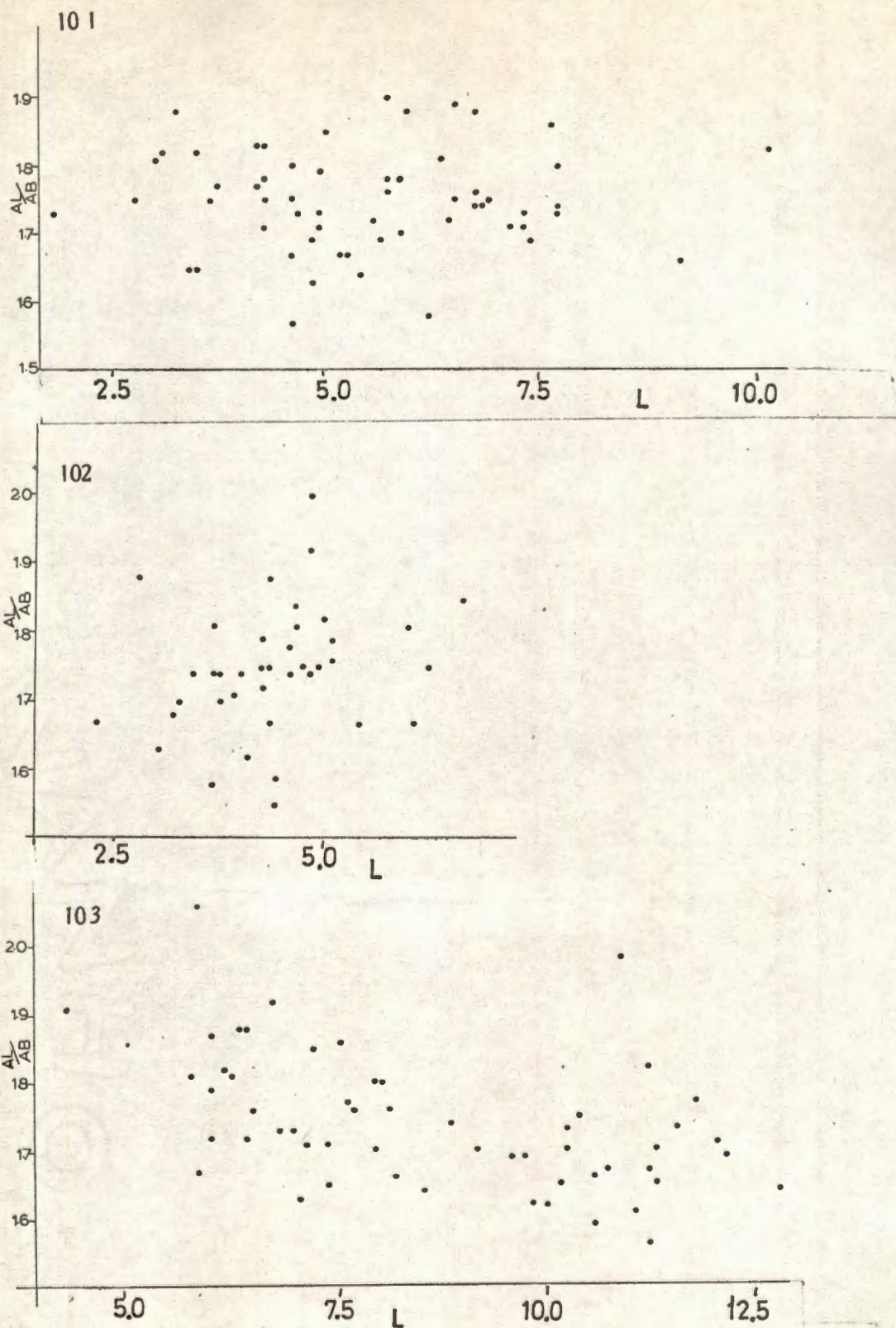


Figs.95 - 100. Aperture length (AL) and aperture breadth (AB) plotted against shell length (L) in mm., for samples 60, 194 and 204.

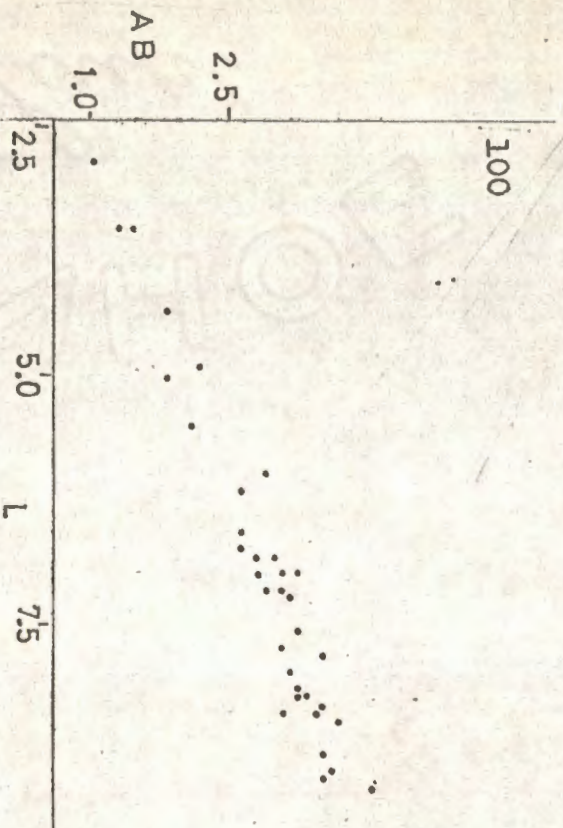
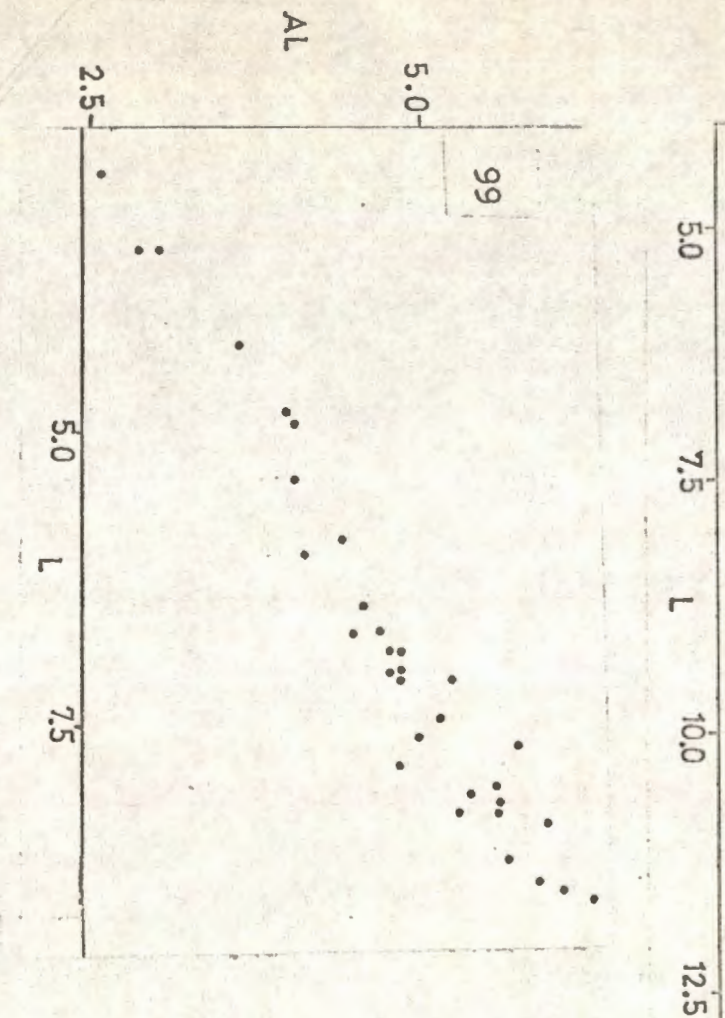
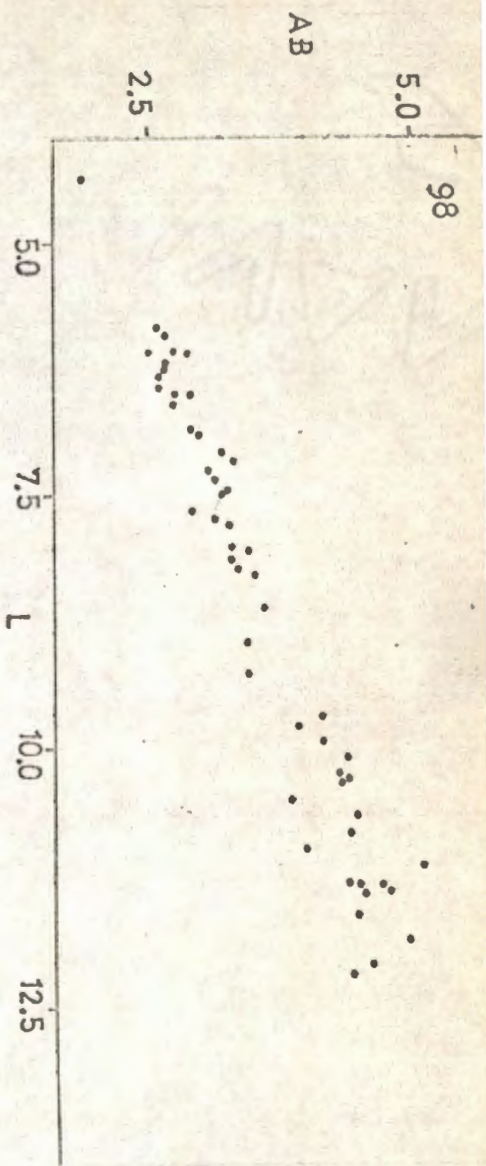
lation between $AL/L \times 100$ and L .

The ratio shell length/aperture breadth (L/AB) is employed by Wright (1957) to indicate an increase in the diameter of the body whorl in relation to the increase in shell length, but he does not state whether he considers the ratio L/AL unsuited to this purpose. He uses the ratio L/AL to reflect the degree of exertion of the spire and graphs of this ratio in B. jousseaumei and B. globosus reveal an interesting feature. The first part of the graph runs almost horizontal and then, at a certain point, between the 6-8mm shell length mark, begins to slope upward. This sudden inclination is interpreted by Wright (1957) as being the result of the attainment of sexual maturity, the shell changing shape to accommodate the enlarged accessory glands.

Although, in fact there is, during most of the period of growth, an increase in the absolute value of both AL and AB , this is not necessarily always apparent in graphs incorporating these values. Even a rather marked increase in cone size, as represented by AL or AB , may go unnoticed. An increase in the ratio may be marked by a great scatter of the values eg. Wright's (1957) fig. 2; or the relatively slight increase in cone size, tending to reduce the ratio L/AL , might be overshadowed by the effect of a lesser degree of overlap in the younger parts of the shell, having the opposite effect on the ratio.

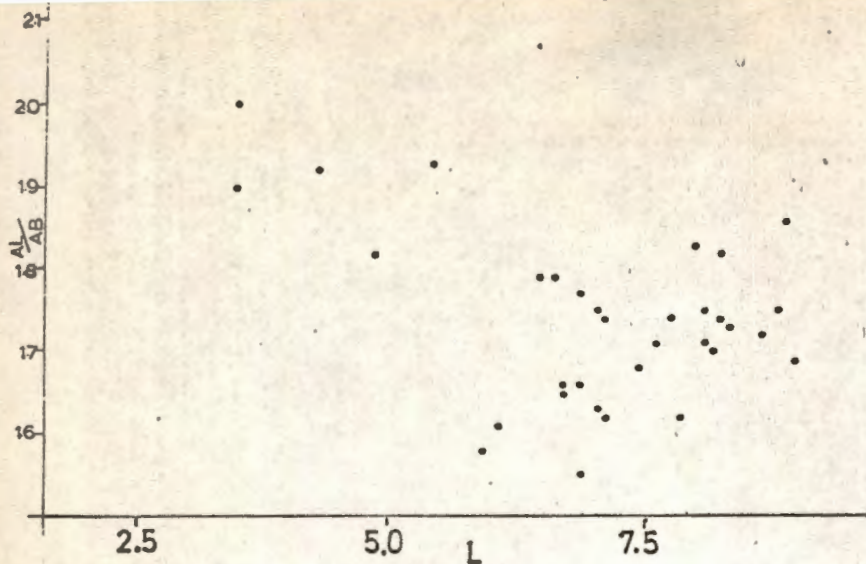


Figs. 101 - 103. The ratio aperture length/aperture breadth (AL/AB) plotted against shell length (L) in mm for samples from Habitat 9.



In B. tropicus no sudden increase in cone size as represented by AL or AB could be detected. Both ratios were plotted against L for samples 60, 194, and 204 (from habitats 9, 21b and 9 respectively) (figs. 95 - 100) and all exhibited a straight line correlation showing that any increase in shell volume is gradual or masked in the populations studied.

An attempt was also made to find out whether any change in aperture shape could be indicated by the ratio AL/AB. None of the graphs drawn shows a clear correlation between the ratio AL/AB and L, and all of them exhibit a large degree of scatter (figs. 101-104). In a previous paper (Stiglingh et al., 1962) it was stated that this ratio does not change with age. This was subsequently also confirmed in the case of L. natalensis (Pretorius, unpublished thesis) ^{figs. 101 and 102} (both samples from habitat 9), demonstrate the scatter of these values, and are drawn from samples with low spires and large apertures (fig. 100). In figs. 103 and 104 (both samples from habitat 9) of more typical B. tropicus with well developed spires (fig. 106) there is some indication, more clearly to be seen in fig. 104 (habitat 9) that there is indeed a difference in this ratio when young and old snails are compared. This should have been anticipated as there is generally a clear difference between juvenile and adult forms in this type of shell. The juveniles have greatly overlapping whorls and a relatively long, narrow aperture, hence the high values for AL/AB in young snails. The depressed type of shell (figs. 101 and 102)



107

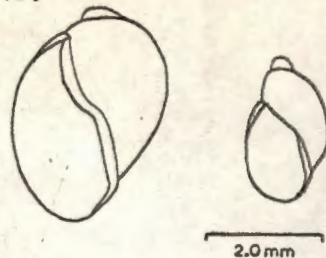
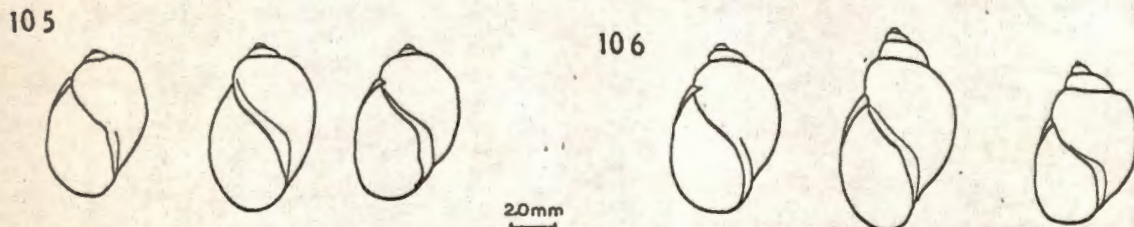


Fig.107. A typical, and a long-spined juvenile.

Fig.104. The ratio aperture length/aperture breadth (AL/AB) plotted against shell length (L) in mm, for sample 104 from Habitat 9.



Figs.105, 106. Depressed and long-spined forms of B. tropicus.

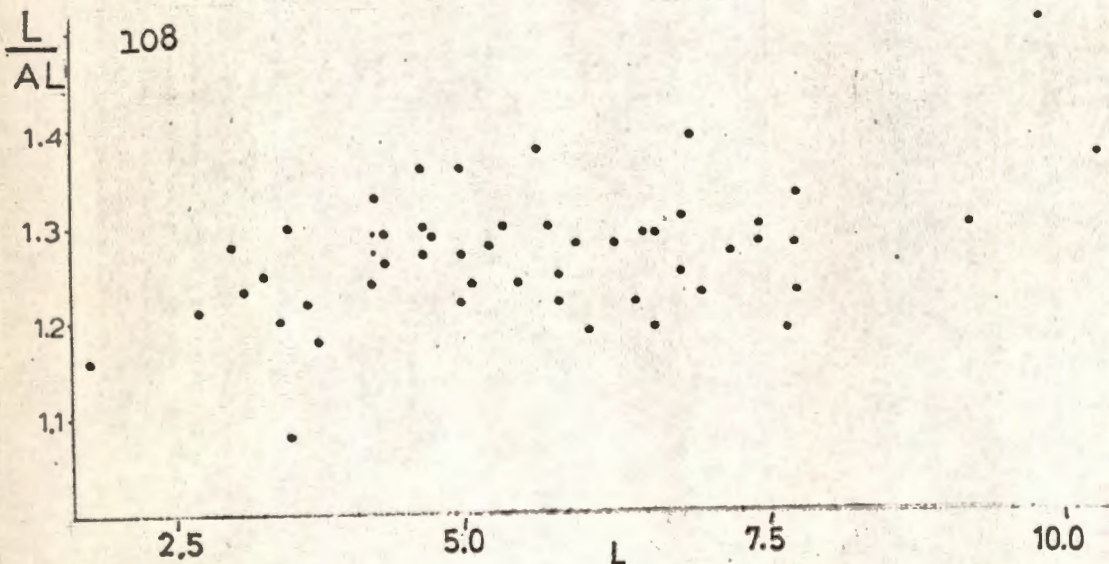
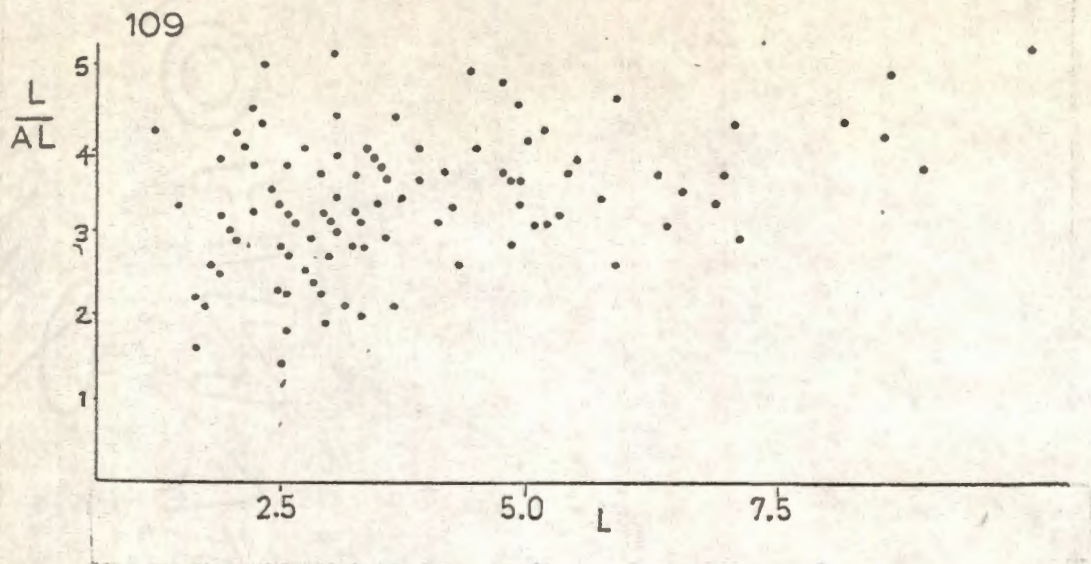


Fig.108. The ratio shell length/aperture length (L/AL) plotted against shell length (L) in mm for a sample from Habitat 9.

taken from habitat 9) presents a rather juvenile appearance because of the great overlap and large aperture and there is consequently no clear-cut difference between the form of such adults and typical juveniles. An interesting aside, here, is the fact that juveniles may also assume a typical adult form (fig.107) as in sample 173 (habitat 9). This type of juvenile apparently gives rise to the longspired adults.

Although there is clearly a correlation between L/AL and L (figs.108-111) the precise nature of this correlation is variable. Normally the graphs of this ratio in B. tropicus differ markedly from those plotted by Wright (1957) for B. jousseaumei and B. globosus. The graph for B. tropicus is initially steeply inclined and there are possible indications that the last part of the scatter diagram is sometimes less steeply inclined. (figs.108, 110 and 111) from habitats 9, 21b and 9₁). Fig. 109₁ from habitat 9 does not clearly exhibit this tendency. In these graphs the value L/AL increases from a minimum of 1.08 in the smaller snails, to 1.62 in the large snails. Fig.109 (habitat 21c) deserves special mention because it demonstrates the great variation sometimes occurring in a single sample. This is the more remarkable for occurring in such small snails. Though some of these juveniles possess the normal juvenile value for the ratio L/AL , many fall into the adult range, two having ratios of 1.5 or more.



Figs.109, The ratio shell length/aperture length (L/AL) plotted against shell length (L) in mm. for a sample from Habitat 9.

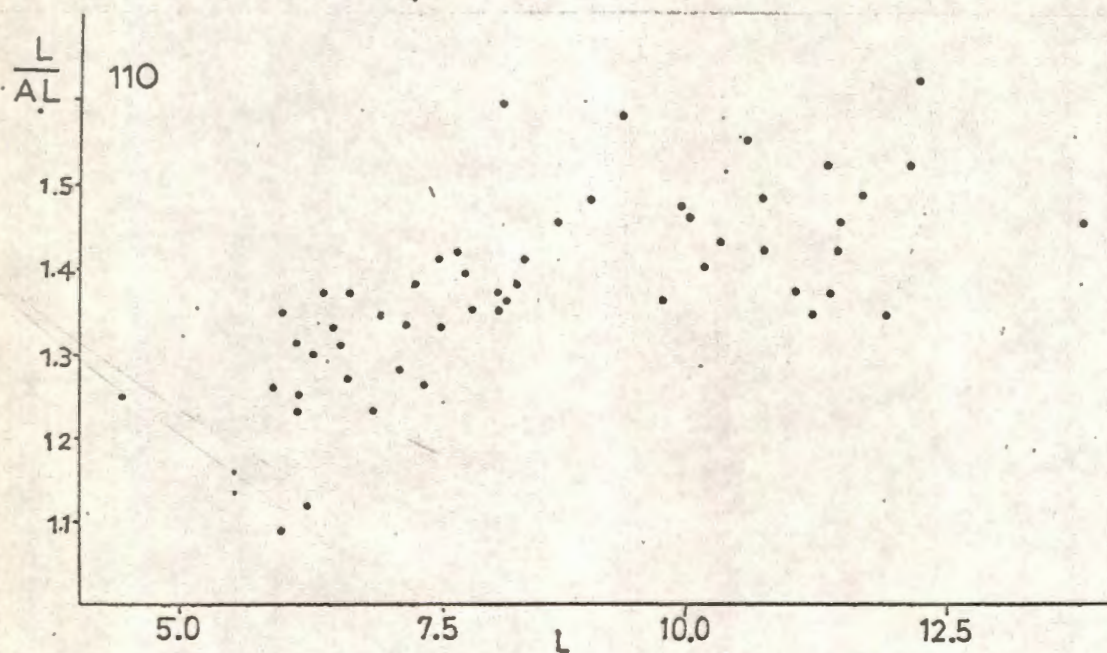


Fig.110. The ratio shell length/aperture length (L/AL) plotted against shell length (L) in mm for a sample from Habitat 21b.

The most important point emerging from these graphs is, however, the great differences which occur in this ratio, and therefore in shell shape, in various samples taken from the same habitat but at different times. Sample 60 (fig.105) taken from habitat 9 on 15.9.1961 differs greatly from sample 204 (fig.106) taken on 1.11.1963. The former is characterized by the shells generally having a large aperture, depressed spire and flattish whorls and traces of costulation; the latter has shells with a relatively smaller aperture, well developed, though not prominent, spire, obese whorls, and is costulated. Some shells of the kind occurring typically in sample 204, are to be found in sample 60. This is clearly seen in the high value for the second largest shell. When the graphs of L/AL in the two samples are compared they are seen to differ not only in the inclination of the line but in the value of the ratio for snails of comparable sizes. Thus for snails of between 6.4 and 8.1mm in sample 60 (fig.108) the value for L/AL is mostly between 1.2 and 1.3 while in sample 204 (fig.111) the values are mostly between 1.35 and 1.4, owing to the relatively smaller aperture.

A similar difference in shell form in samples taken from the same habitat at different times, has also been found to occur in habitat 21b. One sample (117) taken on 2.3.1962 (fig. 112) is considerably more depressed than the sample 205 (fig. 113) taken on 29.11.1963. The latter is also characterized by a high degree of heterogeneity while the former is not.

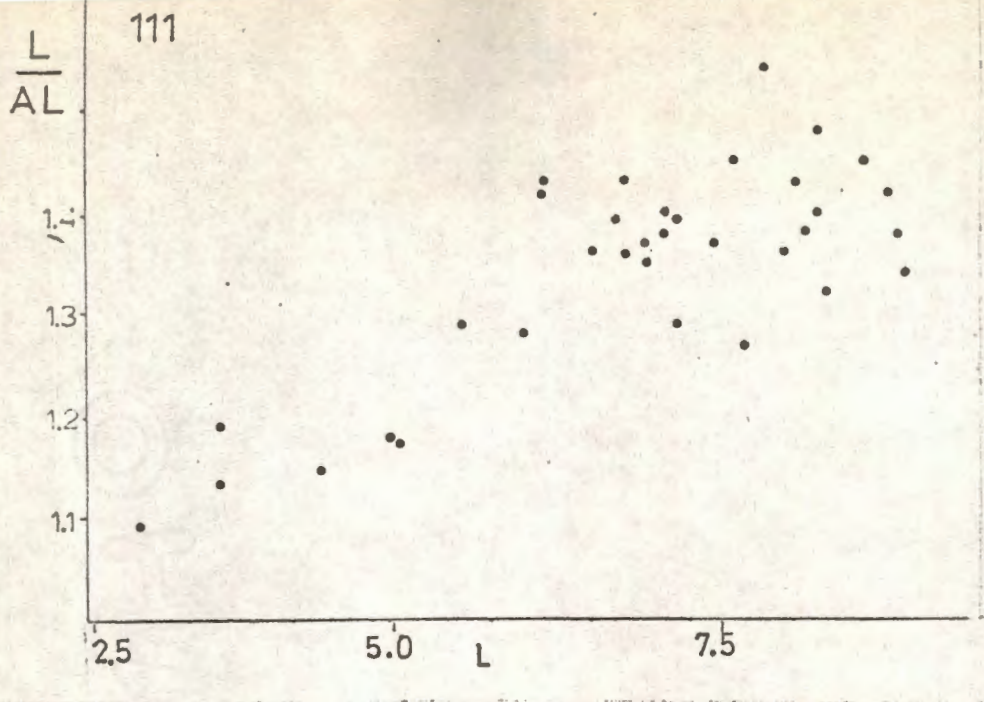
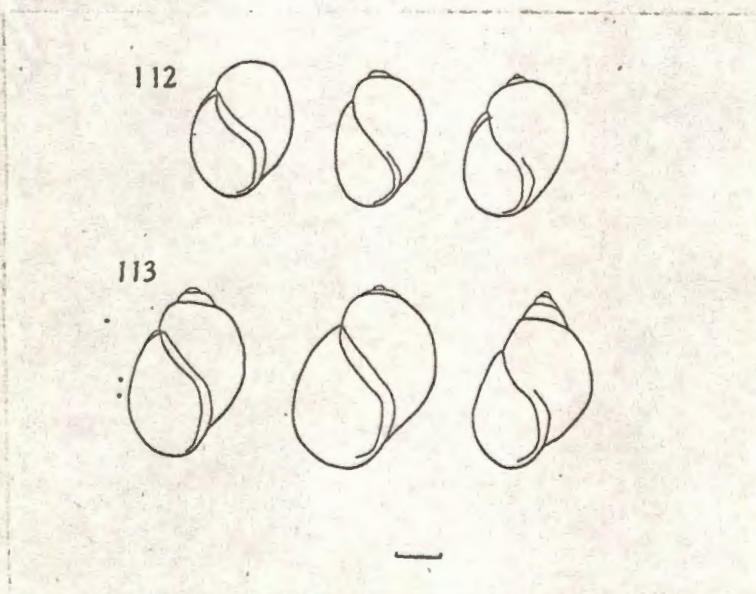


Fig.111. The ratio shell length/aperture length (L/AL) plotted against shell length (L) in mm for a sample taken from Habitat 9.



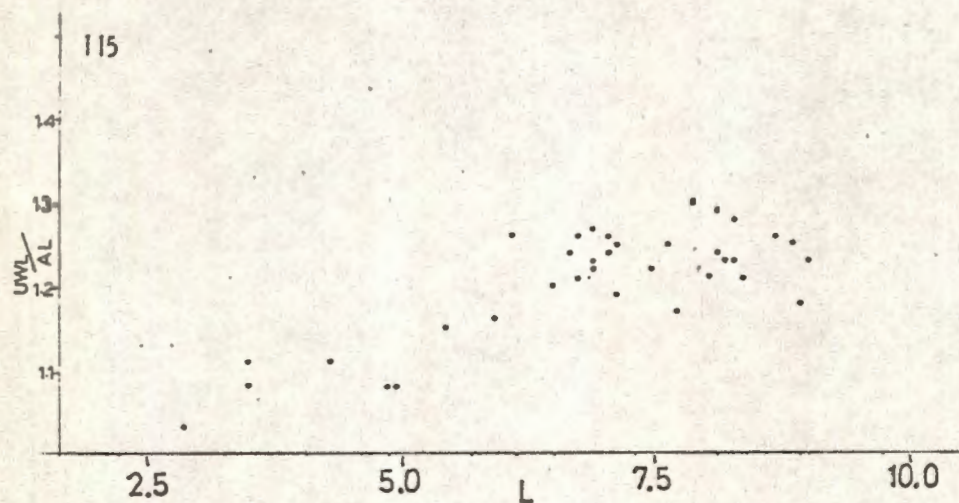
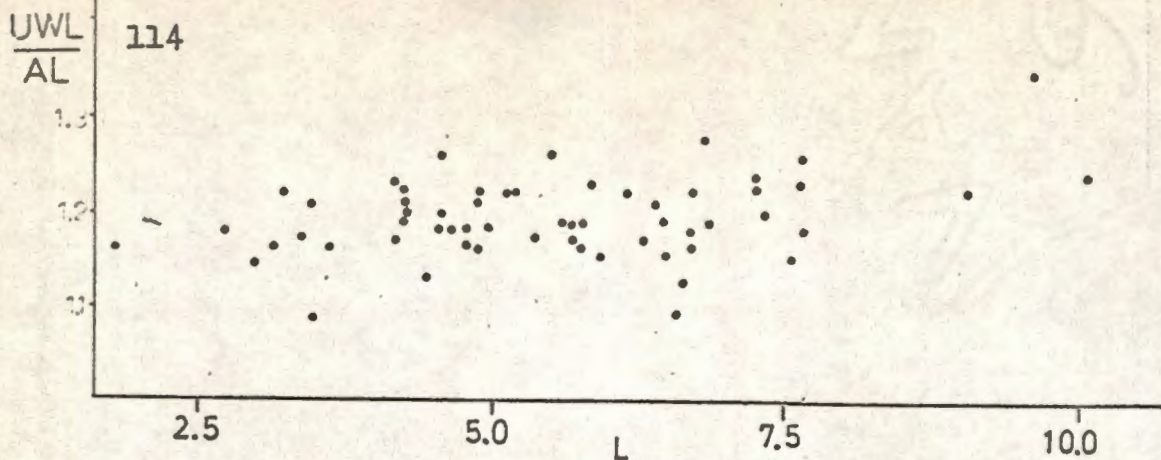
Figs.112, 113. Specimens from samples taken on 2.3.1962 and 29.11.1963 from Habitat 21b.

In the present paper the degree of overlap as expressed by the ratio ultimate whorl length/aperture length (UWL/AL) was investigated in the same two Loxton samples which were compared with regard to L/AL and L, viz. samples 60 and 204. As in the previous instance, the ratios of older and younger snails differed clearly (figs. 114, 115). In fig. 114 the graph runs more or less horizontally but from the beginning and end of the graph it can be seen that the values for younger snails are indeed smaller than those for older snails. In fig. 115 this trend is expressed beyond all doubt, proving that there is considerably less overlap in the more recently formed parts of the shell, than in the older.

122 ASSOCIATED FLORA

Because so many of the Basommatophora occur in shallow water they are to be found on, or amongst the marginal plants. This association should not be taken to imply any direct dependence on the plants even though, in most habitats, the snails are found in the presence of lower and/or higher plants.

One of the most important elements is the algae, generally regarded as indispensable to the snails' existence for it is on the algae, especially in the case of the young snails, that they are largely dependent for food (see feeding). In the aquarium the extent to which B. tropicus feed on the algal bloom which develops on the aquarium walls is apparent in the feeding trails and other bare patches where they have rasped



Figs. 114, 115. The ratio ultimate whorl length/aperture length (UWL/AL) plotted against shell length (L) in mm for two samples taken from Habitat 9.

off the algal coat. The algae are, however, not always beneficial to the snails. The Cyanophyceae which may be harmful to birds and mammals drinking water rich in these forms, (several authors quoted by Lewin, 1962) can also be noxious to the snails. Young snails are said to be averse to Cyanophyceae and to die if that is the only available food. Cyanophyceae were present in habitat 21b and I have a record of their being present in great numbers in July. These algae may exert a noxious influence in the habitat but - this cannot be of the most extreme kind, because there is always an abundance of other food available to the snails.

Even plants which do not contain toxic substances may be harmful to the snails eg. when the pool is surrounded by a dense growth of trees and shrubs and their leaves fall into the water and cause an accumulation of organic matter (Boycott, 1936). The decomposing plant material leads to the formation of excess humic acids. The effects of the accumulation of decomposing plant material is considered by Pilsbry and Bequaert (1927) to be of such importance that they regard it as a possible cause of the small number of molluscan species in African swamps. None of the selected habitats was surrounded by much overhanging vegetation; where an abundance of plants was present it was in the form of aquatic vegetation.

Another important chemical factor which is largely dependent on the plants is the oxygen evolved, not only from the algae, but also from the higher plants, and released during

photosynthesis. This oxygen is usually beneficial though the effects of an excess is uncertain and there are indications (Abdel-Malek, 1956) that it may be harmful.

If too many plants are present their unfavourable influence may be extended beyond the effect of photosynthetic oxygen, for at night the carbon-dioxide factor is correspondingly increased. The acid resulting from this gas is held partly responsible for the pitted appearance of certain shells (Pilsbry and Bequaert, 1927). Locally several habitats, 21b, 8a, and 11b have a very heavy plant growth yet the Potchefstroom snails do not normally become pitted during active life in the field, but only during ~~an~~^{an}hydrobiosis. The explanation of this may lie in the high calcium-content of the water which neutralizes the excess acid.

An excess of filamentous algae is also in some instances thought to be inimical (Boycott, 1936, van Someren, 1946). The filaments may hinder or prevent surfacing and, as mentioned by Boycott (1936), and observed by myself, algae tend to become attached to the apices of the shells and so aggravate the condition. Filamentous algae both of the branched (*Cladophora*,) and unbranched type have been found on the apices of B. tropicus. Smallish tufts also become anchored to other parts of the shell, including the ultimate whorl, but have not, in these positions been found to form the dense "beards" which develop on the apices.

It is probably easier for snails to surface in a pool overgrown by higher plants, than in one overgrown with algae which form an enmeshing net. In plants with leaves the snails can crawl through the spaces between leaves and may even, as I have observed in the case of local B. tropicus, crawl about on the upper surfaces of Marsilia leaves. The same behaviour has been observed in other pulmonates in a foul pond thickly overgrown with Lemna (Boycott, 1936).

Ponds which are heavily shaded, and in which the shading is not caused by aquatic vegetation, are generally found to be poor snail habitats (Boycott, 1936). Besides the possibility of accumulation of excess organic material in the water, the beneficial effect of sunlight in maintaining the pool in a healthy condition, is probably a major factor. In such pools vegetation is generally suppressed and the shaded nature of pool 21a seems to be the reason for the small number of snails which are found there in comparison with the densely populated, and adjacent, pools 21b and 21c which contain abundant aquatic vegetation and stand in full sunlight.

Although the snails are not directly dependent on higher plants for survival, B. tropicus seem to thrive in habitats rich in plants. When snails were found in habitats possessing only a few plants, the number of snails was small, eg. in habitat 21a. This pool lies a few hundred metres from pool 21b which has already been described. Habitat 21a is a small pool

of still water which was cut off from the Mooi River. There were willow trees overhanging portions of the pool and it was perhaps partly due to their shade that there was very little plant growth in the pool which was shallow and clear but contained mainly only sparse sedges. Occasionally snails were found here but only in small numbers, and they could hardly be considered to constitute a colony. In the unshaded habitat 21b which had an abundance of plants, a flourishing colony of B. tropicus existed. Another example illustrating this point is pool 13, a small pool more or less 3 by 3m only a short distance from habitat 2. As in the previous example (habitats 21b, 21c) habitats 2 and 13 have the same type of bottom. Pool 13 was usually turbid and contained only very sparse sedges. Habitat 2 was a disused quarry which was partly used as a rubbish tip. Periodically there were large amounts of Potamogeton present and snails could be found in fairly large numbers along the edges. These snails had fragile shells and really large specimens were never encountered.

The vegetation of any pool varies cyclically with the seasons and just as Mozley (1944) distinguishes juvenile, intermediate and senile stages in the evolution of a habitat, the seasonal fluctuations result in a rather analogous condition. Winter is characterized by scantiness of vegetation, summer-height of productivity and autumn - a dying down of the vegetation sometimes accompanied by pollution of the habitat and blooming of the algal flora. In spite of this

oscillation, the basic elements remain constant. The habitats are characterized by being permanent or fairly permanent, sunny pools containing clear and fairly shallow water, often only a few cm. deep. A characteristic is the abundance of plants. The nature of the flora differs from one locus to another but seems most dependent on the amount of water present. In shallow pools the essential feature is the short grass which covers the bottom of the pool, and sometimes may emerge above the surface of the water. Grass may be virtually the only vegetation present or may be interspersed with Marsilia. These two plants occur in frequent association. Snails and egg masses are frequently found on the under surface of the Marsilia leaves and Porter (1938) found B. tropicus in the same situation at Brakpan. Other plants of note in this connection, are clumps of sedges, long grass, Lagarosiphon and a temporary growth of filamentous algae and Lemna. In deeper habitats the grass and Marsilia grow along the margins while in the deeper water some Marsilia may be found, but Potamogeton forms the bulk of the vegetation. Snails were also commonly found on the sedges and though they do not seem to be present there in large numbers, this may, in fact, be due to the difficulty of sampling amongst sedges.

13. ASSOCIATED FAUNA

It is known that aquatic molluscs, except for some Pelecypoda, (Boycott, 1936), are not at all dependent on other

animals and this apparent independence is confirmed by other authors (Deschiens, 1956 and de Meillon et al., 1958). Even though the omnivorous feeding habits of the Basommatophora rule out competition with other types of animal, for food (Boettger, 1944), Boycott as long ago as 1936 concluded that, although no definite evidence was available, interspecific competition between snails seems to exist. This idea seems not have been followed up for a long time, but a few years ago it came under the spotlight after the findings of Oliver-González et al., (1956) on Marisa cornuarietis. It has been known for years (Fischer, 1950, in Deschiens, 1956) that Bulinus and Physa eat the eggs of other gastropod species. To these two species Deschiens (1956) adds Lymnaea as another guilty species and thinks that the egg-eating is the limit of competition between snails. The devastating effect that this may have in nature is illustrated by the effect of M. cornuarietis on a population of A. glabratus. By eating the egg masses and the young snails while it devours the plants, it may virtually exterminate a once flourishing population (Radke et al., 1961). Such a drastic effect of one snail species on another seems to be exceptional and numerous authors are of the opinion that the various enemies of snails cannot succeed in eradicating an established population (Boycott, 1936, Abdel-Malek, 1956 and Harry and Aldrich, 1958), while Teesdale (1962) goes so far as to say that he found no instance of the numerous predators in nature having any discernable effect on the num-

bers of bilharzia vectors. Nevertheless it seems logical to assume that where predators or parasites are present in considerable numbers, even if only seasonally, they must play some role in reducing the population. This viewpoint is supported by the opinion of Baker (1928) who states that planorbid and other freshwater snails constitute an important source of food for animals such as fish and birds.

13.1 Protozoa

In the present investigation it was noticed that unidentified ciliates of the Vorticella type occur sometimes on the shells of B. tropicus. They are also to be found in dense growths on the non-viable egg masses of the snails. Whether they are the cause of the death of the developing embryos, ^{or} whether they only become established on the egg masses when they are beginning to decompose, was not established. That the former is possibly true is suggested by the findings of the WHO (1956) who state that ciliates destroy the egg masses.

13.2 Trematoda

Parasites possibly harboured by B. tropicus. Of all the animals associated with snails it is the trematodes which are by far the most important. Various trematodes employ B. tropicus as intermediate host, though generally only one species occurs as active parasite at any specific time (Porter 1938).

Table 8

List of trematodes for which B.tropicus is
intermediate host.

(Compiled from Porter, 1938)

AMPHISTOME CERCARIAE

Paramphistome group : C.paramphistomi cervi; C.paramphistomi
calicophorum; C.stelliae
Convoluta group : C.convolutoides
Diplocotylea group : C.vaalensis

DISTOME CERCARIAE

Cymnocephalans group : F.hepatica

ECHINOSTOME CERCARIAE

Cucumeriformis group : C.cucumeriformis
Echinata group : Echinostomum fulicae

CYSTOPHOROUS CERCARIAE

Appendiculata group : C.kimberleyana; C.pedicellata

XIPHIDIOCERCARIAE

Cercariae microcotylae
Paracellulosa group : C. sansoucia; C.parapaucadena
Cercariae armatae : C.cawstoni; C.glena; C.barbertoni

FURCOCERCOUS CERCARIAE

Apharyngeal brevifurcous monostome : C.sewelli
Apharyngeal brevifurcous distome : S.haemotobium
S.mansoni
S.spindale
Apharyngeal longifurcous distome : C.brakpanensis

Precisely which are the larval trematodes harboured by B. tropicus remains an unsolved problem. In table 8 an impressive list of cercariae issueing from B. tropicus has been compiled from Porter (1938) who claims to have identified 22 different types of cercaria from that snail.

The first group of cercariae is the amphistome group, to which B. tropicus is particularly susceptible, five out of the six amphistomes listed, occurring in this snail. Trematode taxonomy is unfortunately a thorny question, aggravated by the occurrence of larval forms which have not yet all been linked to a specific adult form. In a previous paper (Stiglingh et al., 1962) it was stated that B. tropicus served as host to both Paramphistomum and Cotylophoron. According to Dawes (1946) these two genera are considered to be synonymous and Paramphistomum the correct designation. Swart, however (pers comm.) considers these two as separate genera. In a recent paper (Swart and Reinecke, 1962) B. tropicus is said to serve as host to both Paramphistomum microbothrium ^{Fischöeder} and Calicophoron calicophorum ^{Fischöeder}. Both Neveu-Lemaire (1936), and Dawes (1946) consider P. microbothrium to be synonymous with P. cervi (Schrank) a designation employed also by Porter (1938). B. tropicus was also said to harbour P. explanatum ^(Creplin) (Neveu-Lemaire, 1936) but this is considered (Dawes, 1936) to be probably synonymous with P. cervi. B. tropicus is also thought by some investigators to act as host to Fasciola hepatica (L.)

(Neveu-Lemaire, 1936 and Porter 1938). In the Echinostome group Porter (1938) lists Echinostomum fulicae for which the red knobbed coot Fulica cristata Gmelin serves as the definitive host. This may then be of considerable importance locally as the redknobbed coot is of common occurrence here in a number of the habitats (see later).

Although B. tropicus is not numbered amongst the generally accepted vectors of schistosomiasis, this has been claimed by certain investigators, and in any case the snails are capable of harbouring schistosomes. Trematodes of this group said to be parasites of B. tropicus are S. haematobium (Bilharz)^e S. mansoni ^{Samson} (both claimed by Porter, 1938), S. spindale ^{Montgomery} (Neveu-Lemaire, 1936 and Porter, 1938) and also another animal schistosome, probably S. bovis ^(Sonsino) (Teesdale, 1962). Whether B. tropicus is in fact capable of transmitting human schistosomiasis seems not definitely established. Teesdale (1962) has never found B. tropicus alluaudi (Dautzenberg) to be infected with human schistosomes in the field in Kenya and neither could it be experimentally infected with S. haematobium. Both Neveu-Lemaire (1936) and Porter (1938) regard B. tropicus as being the vector of S. haematobium and S. mansoni and Porter (1938) lays claim to having infected laboratory reared B. tropicus with S. haematobium and to have used the cercariae issueing from these snails to infect a puppy with the same parasite. As far as I know there are no later records confirming this and it is futile to debate the question till the

relationship between B. tropicus and B. truncatus has been clarified. The need for this was pointed out as early as 1939 (Connolly) and it may be that some of the so-called tropicus which occur in South Africa are in reality members of the truncatus group. This is suggested by a study of the radulae of a sample from Grahamstown (Stigling et al., 1962) and this aspect has been further investigated by Schutte (in press).

In the present study no attempt was made to identify the trematodes concerned but it was obvious that at least three different cercarial types occurred. The most commonly occurring type contains no pigment. In snails from certain habitats it was noticed that larger parthenitae are present. These seem to be rediae as feet seem to be present in the thicker (posterior?) part of the larva and in these forms a black rod was noticed. In certain infections this black rod was discrete and compact while in others the black material was scattered more or less throughout the length of the larva. It is not known whether this last mentioned form is a younger stage of the discrete rod type, or whether these two types represent two different species.

Host-parasite relation. In the literature much is made of the balance between parasite and host. The parasite is dependent on the host for its very life and so destruction of the host is destruction of its life source. For the pre-

servation of this balance there are diverse mechanisms and it is these that cause the predators and parasites in an anti-snail campaign only to limit the numbers of, but not eradicate, the snails (Abdel-Malek, 1956). This is in agreement with the point of view of Boycott (1936) who stated that he knew of no example, in nature, of extermination of an established colony of snails by a predator. It is for this reason too, that Olivier (1956) sees no hope in the use of worm parasites to control snails in the field. Even a heavy infection can be withstood for a lengthy period because of the well developed regenerative powers of the tissues of all molluscs (Fretter and Graham, 1962).

It is true that in some cases infection, especially a light infection, may have little or apparently no effect upon the host. Such an example is the case of the encysted stages of Echinostomum ^(Froelich) revolutum in Physa ^{Tryon} occidentalis (Hurst, 1927). This is understandable because the parasite is in an inactive phase and in reality not a parasite, yet even such inactive stages appear sometimes to be harmful.

In a previous paper (Stiglingh et al., 1962) the presence of round white objects occurring most frequently in the pericardial cavity was noted and it was stated that these probably represent the eggs of some unidentified organism. After reading the papers of Johnson (1920) and Hurst (1927) it became clear that these were metacercariae. Contrary to the findings of Hurst (1927) these encysted forms cannot be

regarded as inconsequential. At certain times of the year I found the pericardial cavity of almost every individual in a population to contain metacercariae and snails as small as 2.6mm possessed 5 metacercariae in the tiny cavity. Of the larger snails, many were found to have the pericardial cavity greatly enlarged and crammed full of metacercariae so that circulation and heart function must surely be hampered. The organ which is next in importance in metacercarial infection is apparently the kidney. Numbers ranging from a few to many, metacercariae were found in many instances embedded in the tissue of the saccular part of the kidney. In one instance clusters of metacercariae were found in the stomach region but this was unusual, although scattered metacercariae sometimes occurred in the haemocoel or embedded in superficial tissues such as the collar, or even the gill.

Active stages of the larvae may also be relatively harmless. If, for example the infection is localized, occurring only in the digestive gland, the larvae may mature, and eventually abandon the host which is then free to recover, (Baker 1928). Even the reproductive activities of the snails are not necessarily interfered with. Marcuzzi (1950) could detect no changes of any importance in the reproductive system of infected snails, and Coelho (1954) found even heavily infected snails to possess active sex cells, though these were present in smaller numbers.

One should nevertheless guard against reducing the effect of parasitism to a sort of benign commensalism. The deleterious effect of the parasite on the host is very real. Under laboratory conditions A. glabratus is often killed about 5 months after infection with S. mansoni (Barbosa and Olivier, 1958), and the widespread and sometimes exceedingly heavy infections found in B. tropicus do great harm to the snail. Baker (1928) also found that in many instances the liver, reproductive organs and the stomach were largely destroyed by trematode infection.

Not all trematodes have an equally harmful effect on the host, and the heaviest destruction is said to be caused generally by those trematodes in which a redia stage occurs because the rediae cause much damage when feeding on the tissues (Abdel-Malek, 1956). S. spindale and S. bovis are then probably less harmful than most of the other trematodes infecting B. tropicus as those two lack a redia stage. The worm E. fulicae also lacks a redia though most of the echinostomes pass through such a stage (Porter, 1938). The most common infection of B. tropicus is almost certainly amphistome infection and the amphistomes possess a voracious redia. Porter (1938) found that, in the case of C. calicophorum infection the digestive gland of the snails was, almost without exception, to a large extent replaced by hordes of rediae which in many cases penetrated into, and sometimes destroyed, the gonad. Heavy infections of local B. tropicus are so bad that the haemocoel

is sometimes filled with a compact mass of pathenitæ which sometimes also lie in the blood vessels of the mantle and gill where they no doubt impede the circulation of the blood.

That it is not only the presence of a voracious redia which must be taken into account in trying to assess the damage done by a parasite is indicated by an observation by Boycott (1936). He thought it doubtful that much harm was caused to L. truncatula even by large numbers of liver flukes (these contain a redia stage), while in Portugal and India Planorbis was harmed and even killed by bilharzia parasites (no redia stage). It is therefore possible that the various snail species possess a differing inherent ability to cope with parasites.

Pathology. Infestation of the snail by parasites is able to effect profound changes in the snail. Sometimes, there are no external signs, morphological or behavioural, which betray the presence of parasitism. In other instances it is apparently possible to detect parasitism by external means. Porter (1938) states that infected snails move sluggishly and feed poorly, while Boettger (1944) states that the snails, in order to compensate for the loss of body substances through the parasitism, feed excessively. It may be, therefore that the appetite of the snail varies according to the stage, or degree of parasitism.

Besides behavioural differences, external morphological variations have also been noted such as changes in the tenta-

cles, foot and the colour of the animal (Porter, 1938, Boettger 1944). In the later stages of parasitism the host is said to be pale (Boettger, 1944) but it is not known whether this paleness is the result of a decrease in the pigmentation as found in parasitized P. occidentalis (Hurst, 1927), or whether the paleness is perhaps the result of lower Hb content. In my material of B. tropicus distinct variations in the haemolymph colour, ranging from yellowish to dark red, were noted but the relation between haemolymph colour and the degree of parasitism was not established.

One of the external changes is that wrought in the shell, and as this is one of the most obvious it will be discussed first. Abdel-Malek, (1952) who studied parasitism in H. Corpulenta Say found the infected specimens to be slightly lighter than the non-infected. He detected no sign of gigantism which has been found to be a feature of several kinds of snail harbouring a trematode infection. Further it has been found (Rothschild A. and M., 1939, Rothschild, 1941a) that besides being ultimately larger, trematode infected Littorina neritoides (L.) also grow faster than uninfected individuals. Such gigantism has been reported in parasitized individuals by Rothschild (1936, A. and M. Rothschild, 1939, quoted by Rothschild, 1941a), Boettger, (1953b) while Harry and Aldrich (1958 report that A. glabratus specimens infected naturally with S. mansoni are usually among the largest specimens. The difference in size is, in certain circumstances very great. Thus Rothschild

(1936) found in Peringia ulvae, in which specimens of more than 5mm are rare, numerous specimens 8-9mm long. 90% of this population was found to be infected and no uninfected snails larger than 5 mm were found. In B. tropicus it has been noticed that populations of exceptionally large snails, are invariably heavily parasitized, often practically bursting with parthenitae.

There is no agreement as to the factors responsible for gigantism and various explanations have been suggested by Wesenberg-Lund (1934), Rothschild (1936), Rothschild (1941a) and Boettger (1953b).

Not only the absolute size but also the proportions of the shell are affected and in some cases, eg. the stylomma-
(Müller)
tophoran Zebrina detrita, infected with Dicrocoelium dendriti-
(Rudolphi)
cum, even though gigantism does not occur, the shells are relatively more slender, there is a smaller aperture and a longer spire (Boettger, 1953b). Zebrina detrita specimens
Stiles and Hassall
infected with Dicrocoelium lanceatum (according to Dawes (1946) synonymous with D. dendriticum) are also said (Neuhaus 1939) to be slimmer than uninfected. This slimmess is also reported for Peringia ulvae,
(Pennant)
but has not been observed in B. tropicus. Other shell features said to be the result of parasitism are brittleness of the shell, which has been noted in L. natalensis, and Planorbis pfeifferi, also irregularities or corrosion of the shell (Rothschild 1936, Boettger, 1944).

I found no corrosion of the shell of parasitized B. tropicus, no matter what the degree of parasitism, and corrosion of the shell in this species (locally) seems to be limited to periods of anhydrobiosis.

The changes apparent in the shell are, however, merely an expression of changes wrought in the soft parts of the animal. As pointed out by Abdel-Malek (1956) the parasites cause both mechanical, (feeding and migration) and physiological (feeding and excretion) damage to their hosts.

The gravity of the morphological damage depends in part on the location of the parasites, those in the headfoot obviously being less harmful than those occurring in a vital organ such as the liver. Echinostoma was found encysted in any kind of tissue in Physa (Johnson, 1920, Hurst, 1927). It has also been noted that the various trematodes parasitic in Heliosoma corpulentum affect many organs, the prostate, hermaphrodite gland, heart, kidney and connective tissue in general.

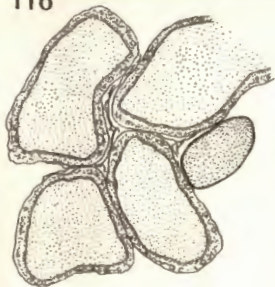
Baker (1928) also found infection to be of a general nature, though sometimes localized in the liver, genitalia and stomach.

No special study of the development of an infestation in live snails was made in the present instance but from dissections the impression was gained that during the maturation of an infection the larvae follow an "up and down" path in the snail. In the lightest infection observed there were a fair number of very small rodshaped parthenitae in the sinus of the stomach region. In light infections there

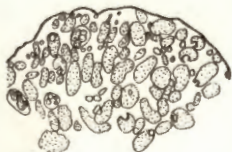
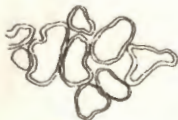
are generally also a fair number of parthenitae in the haemocoel dorsal to the foot. It seems that, from the haemocoel in the lower part of the snail the parthenitae soon find their way to the gonad. Even in snails which are apparently lightly infected the gonad was sometimes found to be totally destroyed. They reach the gonad apparently mainly via the intestinal sinus because numerous parthenitae are usually to be seen lying in this blood space. The smallest cercariae are often found in the gonad region, indicating that they perhaps develop there and later migrate into the liver. In very heavy infections the whole surface of the liver may be totally obscured ^{by} ~~with~~ parthenitae and cercariae. The largest cercariae are found abapically, which may be taken to indicate a downward migration before leaving the snail. This impression of a simple up and down path is not always confirmed. In certain instances larval forms may be found in many other parts of the snail, notably the large sinus around the rectum. Occasionally in very heavy infections both parthenitae and cercariae may be found in the blood vessels of the mantle, the gill, and the collar where they cause a very uneven surface and apparently, great congestion.

The two organs which are generally most gravely affected by the parasitism are the digestive gland and the hermaphrodite gland. The liver tubules of Helisoma corpulentum may become virtually a mass of rediae in heavy infections (Abdel-Malek, 1952) but the acini are not ruptured because of the

110



117



— 0.1mm

FIG. 116. LIVER OF PARASITIZED SNAIL, AND MASS OF PARASITE WHICH HAVE PARTLY REPLACED THE LIVER.

FIG. 117. LIVER OF HEALTHY SNAIL.

relatively massive epithelial cells constituting the walls. In the case of B. tropicus the higher degree of infection of the liver which is usually infected, is mentioned by Porter (1938) who states that masses of cercariae and parthenitae may fill it. This was found to be the case in many of the locally infected snails. In many instances the whole of the liver surface was covered by a thick layer of cercariae and/or parthenitae, and parts of the organ replaced by a mass of larva (fig. 116).

In B. tropicus there is a distinct histological difference in the livers of parasitized and healthy snails (figs. 116 and 117). In the parasitized forms the liver tubules become hypertrophied; the cells of the epithelial lining are much shorter and the enclosed lumen relatively much larger.

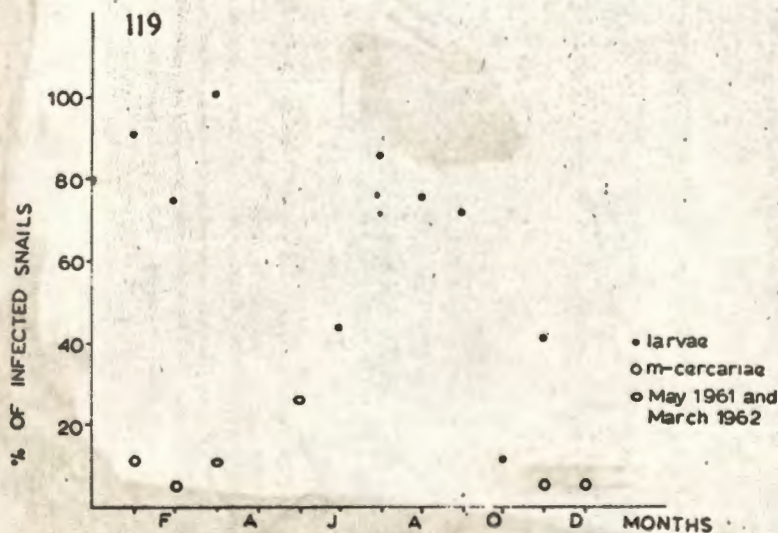
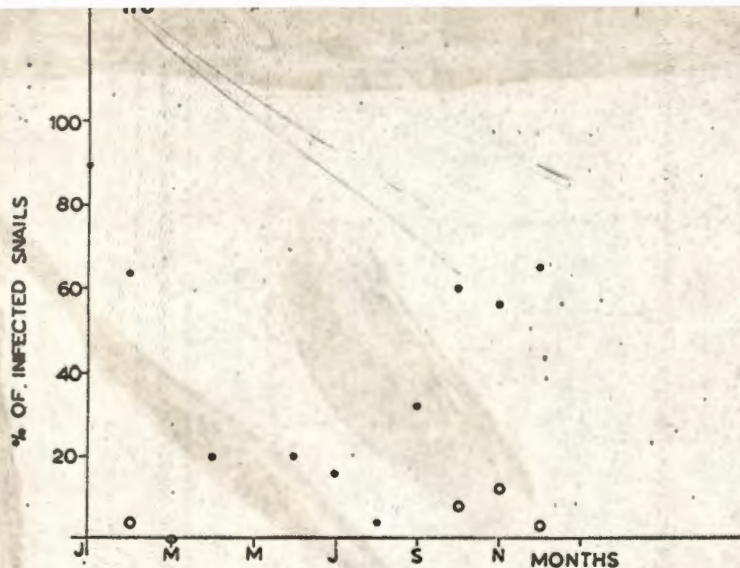
Three snails were selected, a large parasitized specimen, a large parasite free, and a small parasite-free specimen. These snails were 16.0, 13.4 and 4.7mm in size respectively. The height of five epithelial cells in the liver of each snail was measured, and the procedure repeated for the two larger snails. The mean values obtained in the case of the large parasitized specimen were 12.2 and 12.7 micra; for the large parasite-free snail 19.5 and 17.3 micra, and for the small snail 16.5 micra. This illustrates the extent to which parasites may cause a reduction in the height of liver epithelium, for the cells of the parasitized snail are con-

siderably shorter than even the tiny snail, which was healthy.

In a large, heavily parasitized specimen examined the lumina of the tubules were filled with a bluestaining secretion which is present only in small amounts in the tubules of healthy snails. This gives the liver the appearance of stagnation, a feature enhanced by the apparent reduction in both the number of faecal vacuoles and the number of secretory droplets.

Besides the copious secretion in the liver tubules the parasitized snail also had accumulations of a dense substance in certain tubules. Such aggregations were not observed in the other slides and it is possible that they are the result of the parasites.

Infection rate. The infection rate of the snails does not remain constant throughout the year but undergoes cyclic changes. In the present instance these changes have been found to occur not only with regard to the number of snails infected with active larval forms, but also the encysted stage. These oscillations are climatically induced, (Porter, 1938) and Teesdale, 1962) and may also be partially governed by the migration of certain birds which act as host to a number of the flukes (Porter, 1938).



igs.118, 119. Graphs demonstrating the percentage of parasitized snails in Habitats 21b and Q respectively.

Webbe and Msangi (1958) mention that in certain habitats a high infection rate was associated with the presence of numerous large snails in the population, and this appears to be so also in the case of B. tropicus. For example infection rates in the total population and in only the large snails, were found to differ greatly, 5%-27%; 11%-55%. In one sample, 26.3.62 in which only large snails were collected, 78% were found to be infected while a total population sample 2.3.62, from the same habitat, had an infection rate of only 11%. Older B. tropicus may therefore be much more prone to infection by their trematode parasites, than are the young snails.

The infection rate in two habitats was studied, habitat 21b, as a stable habitat, and habitat 9 as a "temporary" habitat.

21b. fig. 118. In this habitat samples of roughly 25 snails per month were examined. The pattern of metacercarial infection seems simple. It is very high at the beginning of the year. In January the pericardial cavity and the kidney were often found to be crammed with metacercariae and snails as small as 2.6mm were found to be infected. In February there was a tremendous decrease in the number per snail, sometimes only one or two metacercariae being found. This low infection rate was continued throughout the winter until September when, although the number of snails infected had risen sharply, the gravity of the infection per snail was

about the same as during the winter months. From October till December the individual rate of infection increased but did not approach the value attained in January.

The rate of infection with active larval forms could not be traced with the same degree of certainty, owing to the small number of infected snails in the samples. In January no infected snails were found and in February there was an indication of a possible slight rise in the infection rate. After February no infected snails were found again till the last three months of the year when a peak in infection rate is indicated.

In habitat 9 (fig. 119) larger numbers of snails were used and in comparison with habitat 21b, habitat 9 shows a March to May increase and then a smaller peak beginning in November (12.2%) and continuing into the beginning of the year, with a sharp drop in infection rate in December.

These findings are in rough agreement with those of Porter (1938) who found that in the Transvaal the beginning (October/November) and the end (March/April) of the summer rains appear to be the periods when most snails are infected, and that during the very hot and very cold weather there is a decrease in the infection rate. The low December and January values are therefore probably real. In habitat 9 the rising infection rate in March is in closer agreement with Porter's (1938) findings than is the condition in 21b. It is possible that, as in the case of differences

in population dynamics, differences in infection rate in these two habitats are to be attributed to the ephemeral quality of habitat 9.

The annual infection pattern in local B. tropicus and also in B. tropicus described by Porter (1938), coincides roughly with that of B. pfeifferi infected with S. mansoni in Kenya (Teesdale, 1962), viz. high infection rates during the summer and low rates during the winter. Whereas Porter (1938) describes a drop in the infection rate in midsummer, Teesdale (1962) after a three year study, mentions that there is, in March, a decrease for which he can find no explanation.

It has also been determined that in Littorina saxatilis (Olivi) parasitized by a larval trematode, the infection rate is high in summer and low in winter, (Berry, 1962) and apparently such a pattern is basic to many infections by larval trematodes.

This pattern of a summer maximum is however, the inverse pattern of that found to occur in B.(P.) nasutus in Tanganyika (Webbe and Msangi, 1958). In that species there seems to be an absolute peak in infection rate during August/September, with the lowest point in midsummer. I can think of no explanation for the great difference between the infection rate of this species and that of B. tropicus.

Great variation has been found in the degree of parasitism in successive years.

A high infection rate cannot always be assumed to occur in large snails, even in infected habitats and during months when infection is to be expected. In March 1963 no really small snails were found in habitat 21b, but none of the population was infected. Also in habitat 11b, during the months of October, November and December 1961, out of a total of 105 snails, including many large individuals, not a single infected specimen containing active larvae or metacercariae, was found.

It was at first thought that the absence of infection in this habitat might be caused by the fact that this habitat, being dependent on the irrigation canal, intermittently dries up. In many instances drought has been shown to have an unfavourable effect on parasite infections and this factor might then be responsible for a parasite free population. The population samples for the last few months of 1962 were examined and parasitism was discovered as early as September; 27/47 snails examined had metacercarial infection and one light immature infection was discovered. In October the habitat was dry but in November some very heavy infections were found, and one of these was mature. This pool does therefore, in certain years, harbour infected populations and no reason for the absence of infection during 1961 could be found.

13.3 ANNELIDA

The best known example of oligochaete association with aquatic snails is that of Chaetogaster. This is a small commensal found in the mantle cavity and around the neck region of B. tropicus. Chaetogaster limnaei Baer occurs on several Basommatophora (Boettger, 1944) and a species of Chaetogaster is also reported to occur on L. caillaudi from East Africa (van Someren, 1946) suspected Chaetogaster are abundant on A. glabratus in Puerto Rico (Harry and Aldrich, 1958) and a number of Chaetogaster species from living B. pfeifferi have been noted in Kenya (Teesdale, 1962). The widespread occurrence of this genus is emphasized by the fact that it occurs also on freshwater prosobranchs and accompanies the animals on which it is commensal, even into the slightly salty Ostsee (Boettger, 1944). In Potchefstroom Chaetogaster species were observed also on Lymnaea and Physa.

Leeches are generally recognised as snail parasites and (Macen and Worthington, 1951) molluscs are a favourite food of these annelids. The most frequently mentioned genera are Helobdella and Glossosiphonia. References to either or both of these genera in connection with snails are contained in Boettger, (1944), Boycott (1936), Olivier (1956) and Abdel-Malek (1956).

Small unidentified leeches occurred in habitat 1 and 21b and in habitat 6 a larger type of leech was encountered,

Olivier (1956) is of the opinion that leeches may possibly play a part in controlling vectors in the field, and from my own observations I gained the impression that in a certain instance this occurred in connection with B. tropicus. Habitat 6, after a drought of at least 6 months, was partially filled by rains in October 1963. On the 1st November a fair number of large snails ^{was} ~~were~~ found, and soon after the rains a number of small snails were present. On 29th November another visit was paid to this habitat and only two large snails could be found. On the previous occasion no leeches had been recorded but on 29th leeches were present in very large numbers, crawling on the rusty drums on which snails were usually to be found. From this I concluded that the sudden disappearance of the snail population was possibly correlated with the sudden appearance of the great number of leeches.

13.4 ARTHROPODS

Of the arthropods which have an influence on snails, crabs and crayfish are frequently mentioned as predators, and of the insects, adult Dytiscidae, and the larvae of flies, dytiscids and Odonata are cited. Examples and references to other authors are given by Pilsbry and Bequaert (1927), Boettger (1944), Abdel-Malek (1956) WHO (1956) and Olivier (1956). Fresh-water crabs of the genus Potamon occur in several of my habitats but the influence of these forms on

on the snail population, in the fields could not be established. Of the insects, I only observed larval Odonata to prey on B. tropicus. These larvae were present in very great numbers in several of my habitats during the summer and may have been a fairly important factor in reducing the snail population, for these larvae, when brought to the laboratory, were observed to feed readily on the snails. A species of Nepa, usually occurring with L. caillaudi, was never seen, by van Someren (1946) to prey on Lymnaea. Nepa occurred locally in habitats 2, 8a, 9 and probably other habitats as well, but, as in the previous instance, was not observed to feed on B. tropicus.

13.5 MOLLUSCA

The interaction of molluscan species has been briefly discussed and to that I can add but little. It is noteworthy that Lymnaea was seen to eat the eggs of B. tropicus in the laboratory (Combrinck pers. comm.) but B. tropicus has also, on several occasions, been observed eating its own eggs in the laboratory in absence of suitable food. It is doubtful whether this occurs to any great extent in nature. Deshciens (1956) is of the opinion that in certain circumstances, when the population density is high, egg eating, especially by Lymnaea and Physa, may reduce a Bulinus population. In pool 21b these three genera have coexisted in fluctuating numbers for years, Physa being the least successful.

13.6 FISH

Boycott (1956) stated that fish are very fond of snails which form a large part of their diet (Pilsbry and Bequaert (1927)). According to Baker (1928) and Baettger (1944) they are the main predators of snails. Fish have even been thought of in connection with the control of bilharzia vectors in small dams (Olivier, 1956). An investigation in Kenya (Teesdale, 1962) revealed, however, that of a total number of 271 Tilapia (nigra and mossambiqua) none contained snails in their stomachs. A Tilapia sp. did occur in the local pools and shallow waters of the dams where B. tropicus were collected although they were generally rather small. It is doubtful whether they could have had any influence on the snail population since, moreover, they occurred in small numbers. Abdel-Malek (1956) offers a list of fish which eat molluscs. It is of interest to note that the guppy, Lebistes reticulatus eats the egg masses of A. glabratus (Oliver-González, 1946, quoted by Abdel-Malek, 1956). It might well be that Tilapia or other small fish too small to be efficient predators of B. tropicus, also have this habit.

13.7 AMPHIBIANS

References to amphibians which are predators of snails, have been given by Abdel-Malek (1956). Xenopus species are mentioned by WHO (1956) as eating snails and Teesdale (1962) was also informed that Xenopus fed on Biomphalaria

pfeifferi. Xenopus locally occurs in fair numbers and has been observed to eat snails in the laboratory, and may therefore play some part in reducing snail populations. Their presence could, however, not be correlated with low snails numbers.

13.8 BIRDS

The birds observed in, or near, snail habitats are listed in table 9 .

Birds are potentially important to snails in three ways. They may serve as the final host for parasites whose larval forms infest the snails; they may be agents in the dispersal of snails and finally, they may be inimical to the snails by feeding upon them, or their egg masses.

The red-knobbed coot (Fulica cristata) is a very common aquatic species and, on the authority of Porter (1938), is believed to harbour the intestinal parasite Echinostomum fulicae for which B. tropicus acts as vector. This is the only locally occurring bird known to act as host to a parasite for which B. tropicus is the intermediate host.

The opinion has been expressed (Pilsbry and Bequaert 1927) that aquatic birds are probably the worst enemies of freshwater snails and mussels. Whenever the question of birds feeding on molluscs arises, the first bird to be discussed is usually the duck, probably partly because this type of bird would be most important in the control of bilharziasis if it could be demonstrated that ducks were able to help in

Table 9

List of birds observed in the vicinity of the habitats.

Anas undulata	96	Yellow-bill. duck.
Anthus richardi	692	Richard's pipit.
Anhinga . rufa	52	Darter.
Ardea purpurea	57	Purple heron.
Ardea cinerea	54	(Common) Grey heron.
Ardea melanocephala	55	Black-headed heron.
Anas erythrorhyncha	97	Red-bill teal.
Amadina erythrocephala	820	Red headed finch.
Ardea purpurea	57	Purple heron.
Apus caffer	383	White-rumped swift.
Actitis hypoleucos	258	Common sandpiper.
Apus affinis	385	Little swift.
Bubulcus ibis	61	Cattle egret.
Corythornis cristata	397	Malachite kingfisher.
Circus ranivorus	167	African marsh harrier.
Cisticola tinniens	646	Le Vaillant's cisticola.
Corvus albus	522	Pied crow.
Creatophora cinerea	735	Wattled starting.
Columba guinea	511	Rock pigeon.
Charadrius tricollaris	238	Three banded sand plover.
Calamocichla gracilirostris	604	Cape reed warbler.
Certhilauda albafasciata	474	Spike-heeled lark.
Capella nigripennis	250	Ethiopian snipe.
Dendrocygna viduata	100	White-faced duck.
Diatropura progne	818	Long-tailed widow-bird.
Euplectes orix	808	Red Bishop bird.
Gyps coprotheres	106	Cape vulture.
Hoplopterus armatus	245	Blacksmith plover.
Hirundo albigularis	495	White throated swallow.
Himantopus himantopus	270	Black-winged stilt.
Hirundo rustica	493	European swallow.
Lanius collaris	707	Fiscal strike.
Macronyx capensis colletti	703	Cape longclaw.
Motacilla capensis	686	Cape wagtail.
Oena capensis	318	Nemaqua dove
Ortygospiza atricollis	844	Quail finch.
Phalacrocorax africanus	50	Reed cormorant.
Petrochelidan spilodera	505	Cliff swallow.
Passer domesticus	784	House sparrow.
Passer melanurus	786	Cape sparrow.
Ploceus velatus arundinorias	803	Masked weaver.
Plocepasser mahali	780	White browed, sparrow weaver.
Plectiopterus gambensis	88	Spurwing goose (wilde Makou).
Porphyrio porphyrio	208	Purple gallinule koningriethaan.
Platalea alba	85	Spoonbill.
Podiceps ruficollis	6	Cape dabchick.
Quelea quelea	805	Red-billed quelea.

Table 9 (Contd.)

<i>Riparia paludicola</i>	509	African sand martin.
<i>Streptopelia capicola</i>	316	Cape turtle dove.
<i>Stigmatopelia senegalensis</i>	317	Laughing dove.
<i>Saxicola torquata</i>	576	Stone chat.
<i>Stephanibyx coronatus</i>	242	Crowned plover.
<i>Spatula capensis</i>	94	Cape shoveller.
<i>Steganura paradisaea</i>	852	Paradise whydah.
<i>Scopus umbretta</i>	72	Hamerkop.
<i>Sprea bicolor</i>	746	Pied starling.
<i>Threskiornis aethiopicus</i>	81	Sacred ibis.
<i>Telephorus zeylonus</i>	722	Bokmakierie.
<i>Tricholaema leucomelas</i>	432	Pied barbet.
<i>Tringa nebularia</i>	263	Greenshank.
<i>Upupa africana</i>	418	African hoopoe.

controlling the vectors. Opinions are divided as to the effect of these birds on a snail population. Olivier (1956) states that there is no evidence that birds are important in diminishing the number of snails; that only poorly fed ducks in an isolated body of water might be of help in this respect. On the other hand Abdel-Malek (1956), though quoting another author's doubts as to the usefulness of ducks because of the occurrence of many molluscs in habitats frequently visited by certain duck, nevertheless, still thinks that ducks might be the cause of a decrease in the snail population in certain localities in the Sudan. Pilsbry and Bequaert (1927) and van Someren (1946) have a high estimation of domestic duck as snail eaters and Boycott (1936) goes so far as to say that domestic and domesticated ducks are the worst enemies of fresh-water molluscs. Ducks are said to be capable of eradicating snails from a pool in a short while (Pilsbry and Bequaert, 1927). Once more the necessity of stipulating which species of duck is involved, becomes apparent. In the following discussion all the birds mentioned feed upon molluscs and all notes on their distribution and habits are, unless otherwise indicated, taken from Roberts (1957).

Numerous duck are thought to feed exclusively on vegetable matter. Some of these are the spurwing goose (Plectropterus gambensis Linnaeus) the Egyptian goose (Alopochen aegyptiacus Linnaeus), yellowbill (Anas undulata Dubois) all

of which occur locally. Others have a more catholic taste and may, under certain circumstances, be important enemies of snails. Of the locally occurring ducks only the white-faced duck (Dendrocygna viduata (Linnaeus)) and the South African pochard (Netta erythrophthalma (Wied)) are listed as eating snails, but it is possible that there are several other species of duck which feed on snails, even if only in the absence of their preferred food.

The possible importance of wild duck in reducing snail numbers should not be overlooked. One of these is the Lesser Scaup (Nyroca affinis) which is mentioned by Baker (1928). There is also a record (Chapin, in Pilsbry and Bequaert, 1927) though not very impressive, of Hartlaubs Teal (Pteronetta hartlaubi) having eaten small pelecypods. It is further known that molluscs are devoured by Cape Teal (Anas capensis (Gmelin)) and the Moccoa duck (Oxyura maccoa (Eyton)) and what is more, of these the former occurs throughout, and the latter virtually throughout, the Republic. Besides these facts to be considered, there are also several species concerning the feeding habits of which we remain ignorant eg. the locally occurring Cape Shoveller (Spatula capensis (Eyton)), or the White-backed duck (Thalassornis leuconotus (Eyton)) which I did not observe here, but which is distributed over the whole of South Africa.

Besides duck, there are several other types of bird that feed on molluscs; the Great Crested Grebe (Podiceps

cristatus (Linnaeus)) which is found locally; the cattle egret (Bubulcus ibis (Linnaeus)) which is very common in this district and was found frequently at habitats 6, 9, 21b and 21c and also occurs throughout the Republic; Little Egret (Egretta garzetta (Linnaeus)), Green-backed Heron (Butorides striatus (Linnaeus)), Little bittern (Ixobrychus minutus (Linnaeus)), Sacred ibis (Threskiornis aethiopicus (Latham)) which occur here in large numbers at several habitats, especially 9, 11b and in the vicinity of habitats 21b and 21c. The Purple gallinule (Porphyrio porphyrio (Linnaeus)) has been observed among the rushes at habitat 9 and although Roberts (1957) does not list molluscs as part of the diet of this bird, Macworth Praed (1962) makes mention of Mollusca forming part of the gallinule diet.

In the present connection the bird groups to which the greatest number of molluscs eaters belong are the plovers and the waders. Locally occurring plovers, all said to eat molluscs, are Kittlitz's sandplover (Charadrius pecuarius Temminck) and the three-banded sandplover (Charadrius tricollaris Vieillot) and the blacksmith plover (Holopterus armatus (Burchell)). The Crowned plover (Stephanibyx coronatus (Beddaert)) which is also common and occurs throughout South Africa, is often to be seen near water amongst the Blacksmith plovers. Its diet, however seems not to include snails probably as a result of its generally preferring habitats which are drier than those favoured by the Black-

smith plover. The Ringed plover (Charadrius hiaticula Linnaeus) occurs throughout the Republic, though chiefly along the coast and also devours molluscs.

The Ethiopian snipe (Capella nigripennis (Bonaparte)) was recorded at the Loxton Dam (habitat 9) which offers the kind of habitat preferred by Ethiopian snipe, viz. large marshy area with flooded, short grass.

Numerous waders are present in this district. Generally speaking their food consists mostly of insects (including larval forms), small crustaceans and molluscs. All the waders found locally, except the Little stint (Calidris minuta (Leister)) are said to include molluscs in their diet. The Little stint is said to feed on Daphnia, copepods, and mosquitoes

and chironomid larvae. The waders here observed are Ruff (Philomachus pugnax (Linnaeus)) Common sandpiper (Actitis hypoleucos (Linnaeus)), Marsh sandpiper (Tringa stagnatilis (Bechstein)), Greenshank (Tringa nebularia Gunnerus) and the Wood sandpiper (Tringa glareola Linnaeus).

The Black-winged stilt (Himantopus himantopus (Linnaeus)) in the Sudan (Abdel-Malek, 1956) and the White-headed stilt in Australia (Himantopus leucocephalus Gould) (Cleland, 1918, in Pilsbry and Bequaert, 1927) have been found to feed on three species of Planorbidae and on Bulinus species respectively. The Black-winged stilt occurs locally, in fact throughout South Africa, as does the closely related avocet (Recurvirostra avosetta Linnaeus).

On the whole it seems quite possible that the influence of birds on snail populations is underestimated, especially when one considers the large flocks which congregate on certain waters. Prominent among these are the huge flocks of flamingoes. In the greater flamingo (Phoenicopterus ruber Linnaeus) flocks may number up to 2000 birds and it is this species which must be taken into account as its diet is mainly animal; insect larvae, shrimps and small molluscs. The lesser flamingo (Phoeniconaias minor (Geoffroy) may be disregarded as it is mainly vegetarian in its habits, feeding particularly on unicellular algae.

Other birds not observed here, but having records of mollusc eating are amongst others the Open bill stork (Anastomus lamelligerus Temminck) according to Chapin (in Pilsbry and Bequaert, 1927) the only specialized mollusc eater in Africa, and various crakes. The African crane (Crax egregia (Peters)) and the Black crane (Limnocorax flavirostra (Swainson)) are specified snail eaters as is also the African jacana (Actophilornis africanus (Gmelin)).

Another snail eater which I did not observe here is Peters' finfoot (Podica senegalensis Vieillot). This species occurs also in most of the Transvaal and in the Natal coastal belt and as far south as Port Elizabeth. In the present context the diet of the finfoot is interesting as both in the Congo recorded, and Roberts (1957), the molluscs

eaten are specified snails, though the species is not mentioned.

Besides the birds known to feed upon snails, one must bear in mind that there are many others of whose diet we know nothing. Furthermore, stomach content examinations which are negative for snails do not indicate that particular species never feeds on snails. For example, the "hamerkop" (Scopus umbretta Gmelin) and reed cormorant (Phalacrocorax africanus (Gmelin)) have a varied diet which is not said to include molluscs. Porter (1938) however reports seeing several hamerkop feed on Physopsis africana and small Unio caffer by dropping them on the ground and so cracking them and obtaining the soft internal parts. Such a method of getting at the body of Anodonta is also reported to be employed by two species of crow (unnamed author in Pilsbry and Bequaert, 1927) and is now well known in bird behaviour, further examples being described by Tinbergen (1958).

Neither is the reed cormorant (Phalacrocorax africanus) stated by the textbooks to feed on molluscs, but Chapin (quoting another author (in Schmitt 1926)) states that ~~this~~ bird also eats Mollusca.

Some of the records of mollusc eating are rather unusual. Thompson (1841 in Boycott, 1936) mentions a grey wagtail full of Ancylus fluviatilis, and Pilsbry and Bequaert (1927) observe that wagtails (Motacilla) prey to a great extent on

certain molluscs in Europe. Students in this Zoology department have, on various occasions, seen the rock pigeons (Columba guineae Linnaeus), said to feed largely on grain, fish snails from a small artificial stream on the roof of the laboratory.

Local birds which are not listed as eaters of molluscs, but which seem worth investigating in this respect are the Cape dabchick (Podiceps ruficollis (Pallas)) and various herons which are fairly well represented in this area. In particular the Purple heron (Ardea purpurea Linnaeus) which usually feeds along the waters edge, and the black-headed heron (Ardea melanocephala Vigors and Children) which has a varied diet and devours insects (Roberts and Macworth Praed, 1962) are of possible importance. Another aquatic bird and possible mollusc eater is the spoonbill (Platalea alba Scopoli) and the red-bill teal (Anas erythrorhyncha Gmelin). Also worthy of mention is the pied crow (Corvus albus Müller). Crows occur in large numbers at habitat 8a and are said to eat "almost anything in the shape of an animal diet" (Roberts, 1957 p. 277). It may be that their habits do not bring them often enough in contact with water yet, according to Pilsbry and Bequaert (1927). two species of crow feed on mussels.

It is unfortunate that in the description of stomach contents the term molluscs, without specification as to what kind of mollusc, even snail or mussel, so frequently appears.

It is however not the task of the ornithologist to classify in detail the organisms eaten by birds, and in this lies a rewarding field of study for the malacologist.

13.9 MAMMALS

Ironically it is the visitors, birds and mammals, that are most affected by, and exert the greatest influence on, the snails. Cattle, horses, donkeys, sheep and humans are visitors to most of the habitats studied.

The ungulates may increase the organic content of the water by their faeces and urine but when organic matter is present above a certain level it is harmful. The animals also stir up the mud and, especially in soft-bottomed pools, cause varying degrees of turbidity. In consequence of this snails are hardly ever found at watering places of cattle, the urine, dung and turbidity being unfavourable to them (van Someren, 1946). Habitats 6, 8a, 9, 21b and 21c were used by animals as watering places and these water bodies consequently contained urine and dung, but in most of the habitats many snails were found. Some distance from my sampling site (habitat 9) at Loxton dam, was a much frequented watering place. In contrast to the other habitats mentioned this locus was denuded of plants by the trampling of the animals, pollution occurred, and no snails were found.

The main importance of the animals however is their acting as hosts to certain trematode parasites, the larval stages of which employ snails as intermediate host. B. tropicus serves as vector for the larval forms of trematodes which are finally parasitic in a variety of mammals. If it were possible to exclude the final host from the habitat, the snails would also be free of the trematode parasite. The animals are also responsible for cropping the emergent vegetation and the reduction in shade might lead to slightly higher temperature in some cases. By wandering from pool to pool they may act as agents in the dispersal of snails or their egg masses.

The humans do not seem to have any direct effect on the snails except possibly when the habitat is used as a washing place. This was the case in Pool 6 but in that instance there was probably no influence as the washing seemed to affect only a small part of the pool. Porter (1938) concluded that South African molluscs are impervious to a large amount of soap, even when exposed to its action for a long period: this is particularly true for hard water, such as occurs in this district.

14 FEEDING

14.1 FEEDING RATE

It has been established by a number of authors that several factors operate in regulating the feeding rate

TABLE 10

NUMBER OF RASPS PER MINUTE

No. of rasps per Minute					No. of rasps per Minute				
SNAIL Size in mm.	TRIAL 1	TRIAL 2	TRIAL 3	$\bar{x}$	Size in mm.	TRIAL 1	TRIAL 2	TRIAL 3	$\bar{x}$
4.43	40	42	48	44	3.8	42	48	52	48
7.1	42	46	44	44	8.1	38	38	36	38
10.9	34	42	38	38	10.6	36	36	24	36

of snails. Besides temperature which is a controlling factor in all physiological processes, the size of the animal, its physiological condition, the duration of the feeding time, and probably also the nature of the food influence the tempo of feeding.

To determine the effect of size on the feeding tempo the number of rasps performed by the snails of differing sizes was counted. Two snails each of small, medium and large size, were selected and their rasps counted for a period of half a minute. The values thus obtained were then doubled to facilitate comparison with the data supplied by other authors. The values relate to uninterrupted feeding periods; when the snail stopped feeding the trial was disregarded. Table 10 shows that the two largest snails performed, on the average, 37 rasps per minute while the two smallest snails averaged 46 per minute. Hubendick (1957) found that in Lymnaea snails of 10mm length performed as many as 50-60 rasps per minute at 15°C, while B. tropicus of this size average more or less 40 per minute, at 23.5°C. Further, the large Lymnaea (45mm) gave only 24 rasps per minute. The wide range of rasping speeds in Lymnaea, when compared to B. tropicus, is to be correlated with the far greater mass attained by the former species. Both Hubendick (1957) and Komiya^{et al.} (1958) investigated the effect of temperature on the rate of feeding but came to conflicting conclusions. Hubendick (1957) found the feeding rate to decrease simultaneously with temperature

in Lymnaea (no figures given) and Komiya (1958) found the rate to increase with decreasing temperature in Oncomelania. This does not imply that the physiological processes differ because the temperatures chosen by the authors vary greatly. This results in the phenomenon that in one instance a decrease of temperature could be a movement away from the optimum while in another, (at a high temperature) a decrease would be a movement towards the optimum, and apparently conflicting conclusions may be drawn.

Feeding movements are performed not only during actual feeding but also at other times when the snail is apparently not ingesting food. This has been noted also by other authors including Boettger (1944) and Komiya^{et al.} (1958). The latter author terms these movements "food groping activity". In B. tropicus the rasps during such groping movements do not follow one another as quickly as during genuine feeding. In two sets of 5 observations each, on snails moving over food the number of rasps per minute was 33, + 21 as opposed to 26 + 17 during movement over a bare surface. Such a discrepancy is functionally economical; occasional rasps during progress over the substratum, being sufficient to inform the animal as to the suitability of the substratum as a feeding ground. The slower rasping rate thus conserves energy which may be used during actual feeding, or other activities of the snail.

When food is encountered the rasping is accelerated and the food groping activity then becomes food taking activity. When feeding upon a massive piece of food the snail is more or less stationary and makes irregular excavations into the food. When browsing upon algae or other periphyton however, feeding takes place during locomotion, as is also the case in other browsers, enabling the snail, to cover a large area to obtain sufficient nourishment from the small amount of food ingested at each rasp.

14.2 FEEDING TRAILS

The walls of aquaria in time develop an algal layer. In an aquarium containing snails, tiny clear areas, mostly arranged to form some sort of pattern, are generally to be seen. These clear areas are spots in which the algal coat has been rasped from the glass, and the pattern which they form is known as a feeding trail.

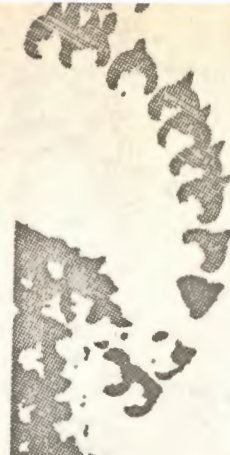
The nature of the feeding trail is dependent on the rate of locomotion, and the animal's inclination to feed. The nature of the track is subject to great variation, trails showing variations in the shape of the track, the number and proximity of consecutive rasps, and the shape of individual rasps.

The shape of the trail.

An animal disinclined to feed may pass over a suitable substratum without performing, or performing only occasional, feeding movements. A trail left by such a snail consists



132



133



134



135

Figs.132 - 135. Various rasps and trails made by B.tropicus.

126



127



128



129



130



131



Figs.126 - 131. Various rasps and feeding trails made by B.tropicus.



120

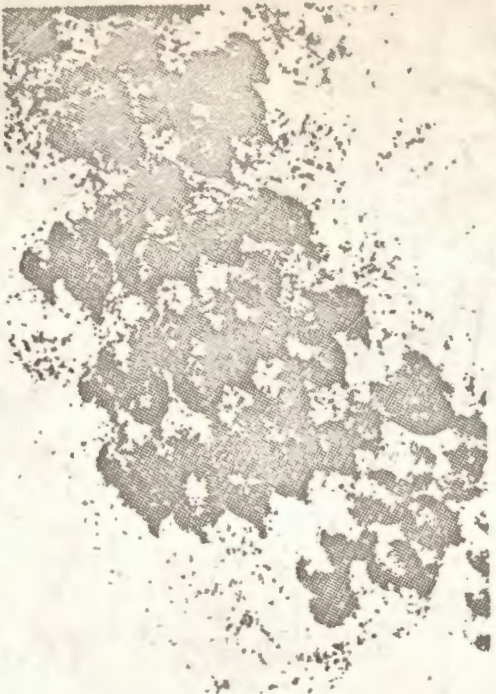
12



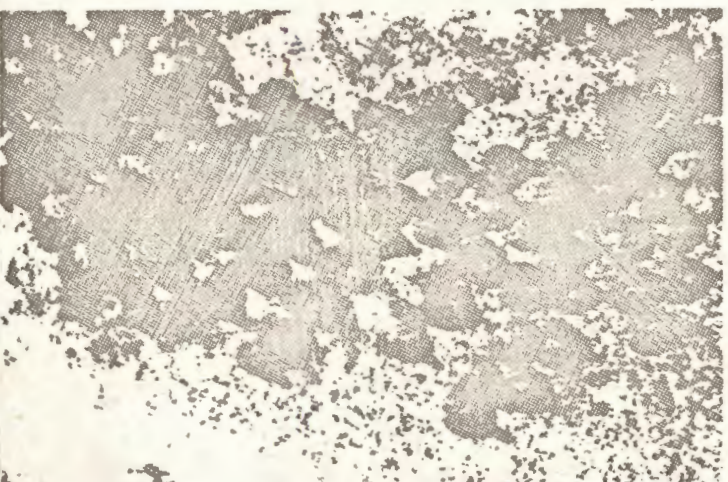
122



123



124



125

of a series of rasps which are random or only loosely organized into a pattern. Such rasps often occur at the beginning of a feeding trail. This disorganised beginning of a feeding trail has also been noted by Boettger (1944), and is well demonstrated in the feeding trail of B. tropicus, shown in fig. 120. During swift locomotion as often occurs in Physa, the rasps may be arranged to form a single line (Boettger, 1944). In the representation of the feeding track of L. stagnalis (Hubendick, 1957) the rasps are arranged alternately left and right, after the manner of human foot prints.

In slowly moving animals the swinging of the head from side to side, a feature also of vertebrate browsers, becomes more exaggerated. In this manner several rasps are juxtaposed ~~sitioned~~ during the sideways sweep of the head and a curved line is etched into the algal coat with every sweep. During the progression of the snail consecutive curves are added to the preceding, forming a continuous zig-zag line (fig. 121).

Ankel (1929) found that Helcion pellucidum (L.) leaves a trail similar to that just described and he goes on to claim that Lymnaea leaves a similar trail. It is likely therefore, that the trail made by Lymnaea, as described by Hubendick (1957) was made during fast locomotion.

Judging from the figure of a feeding track of Omalogyra (Philippi) atomus (Fretter and Graham, 1962) the component parts of the track are not necessarily curved. A possible explanation for this discrepancy in the shape of the trail of O. atomus and

B. tropicus, is that the former possesses a planoid shell, balanced vertically on the body of the animal, and any great flexure in the nuchal region would tend to throw the animal off balance.

The angle between the successive curves of the trail is determined by the rate of locomotion and Boettger (1944) states that feeding trails usually originate as single bites or curves which are separated from each other by wide angles, the curves later following one another so closely that they overlap, this being ^{the} result of a decrease in the speed of locomotion. This was found to be true also in B. tropicus but there are a number of instances of the beginning of a feeding trail being composed of curves so closely consecutive that they overlap (figs. 122 and). Neither are wide-angled curves restricted to the beginning of feeding trails; this type of feeding may continue over long distances (fig. 121) Individual variation in trail pattern is great, because of the interplay of several factors, and no simple relation between temperature and trail angles was found.

The number and proximity of rasps per curve. Feeding trails vary also in respect of the number and proximity of consecutive rasps found in each curve. As is to be expected, the curves of a feeding trail rasped by a large snail are generally broader than the curves of a small snail of the same species. It may not be deduced from this that the number of rasps per curve is necessarily greater in large snails than

ones.

in small ^ In B. tropicus the number of rasps per curve in a trail made by a small snail was often found to be greater than the number of rasps left by a large snail at the same temperature. There is some relation between the number and proximity of the rasps per curve, and the angle between consecutive curves. As a rule wide angled curves possess well-spaced rasps (fig.123), while in trails in which the consecutive curves are closely placed, or overlap, the rasps are more numerous and tend to be contiguous or overlap (fig.124), sometimes producing a snakeskin effect (fig.125). In instances of extreme overlap this results in areas being scraped completely free of algae (fig.126).

Form of individual rasps. A glance at the feeding trails left by B. tropicus shows that the appearance of the rasps made by any particular snail is not constant.

In some trails it is noticeable that the ultimate rasp in a curve is sometimes asymmetrical, the last rasp to the right side of the animal being represented only by the right half of the rasp and vice versa (fig.127). This seems to be caused by a slight inclination of the odontophore, in the direction in which the snail is twisting. This inclination does not occur when the snail is only slightly bent from the axis representing the line of progression, but comes into effect when the snail is greatly extended in a lateral position.

When the radula scrapes the substratum, it is the area anterior, and lateral, to the collostylar hood that is brought into contact with the substratum. This region is a softly rounded, roughly horse-shoe shaped area on the dorsal part of the odontophore. During feeding, the right and left halves of the odontophore (and so also the horse-shoe) are brought closer together at each rasp. This results in the central part of the horse-shoe (anterior margin of the collostylar hood) being pinched to form a narrow scoop. This part of the radula will be referred to below as the tip of the radula, as it is functionally, though not morphologically, so.

From feeding traces it is apparent that occasionally only the central or only the lateral parts of the radula are used in feeding. As a rule, however, both these areas are employed and it is the balance between the importance of the central and lateral parts which is responsible for the variety of rasp traces.

When feeding upon a bulky piece of food, eg. a piece of banana, it is the tip of the radula which is used to make a small excavation in the banana and to "spoon" the substance into the buccal cavity. For this purpose the large cones of the central teeth, being both firm and sharp, are well suited. As pointed out by Heidermans (1924) these teeth are also used to cut the leaves of plants.

When grazing upon algae it is the lateral parts of the transverse fold, clothed with the marginal teeth, which are

of prime importance. Heidermans¹ (1924) drew attention to the fact that the fine denticles of these teeth are used as brushes in removing the algae during grazing.

While grazing on algae or starch covered slides, rasps made by the central part of the fold are visible as small irregular spots¹ (fig.128). They may occur more or less randomly in a zig-zag pattern. During this type of feeding the radula is protruded and small pecks given at the substratum. This is basically only a simplified version of the same type of feeding behaviour that takes place while a leaf is being eaten and it occurs only rarely during grazing, possibly only on a thick carpet of algae. This is deduced from the fact that such pecks were noted only in those parts of the slide where the starch layer was exceptionally thick. Rasps in which the radula tip is responsible for virtually all the scraping, are portrayed in fig. 129 . In typical grazing the lateral parts of the fold clothed with the marginal teeth are used, resulting in two short more or less parallel lines (fig.130). The radula is but slightly protruded and is tilted well forward so that the lateral parts of the transverse fold lightly touch the substratum. During this kind of feeding the tip of the radula is at no time in contact with the substratum. Such rasps are ineffective in feeding, as witnessed by the fact that only a minimal amount of the algal layer is removed and the rasps tend to be arranged in a more or less straight line, or as footprints. As a rule they are not situa-

ted close to one another and are often found leading into, or trailing from the end of, a feeding track.

Normally during grazing, both the central and the lateral parts of the fold are involved in the scraping, the lateral teeth by virtue of their number and fine denticles playing the more important part. Examples of varying importance of the tip and lateral parts of the radula are given in figs. 131, 132 and 133, , the last-mentioned merging into the normal type of rasp, which may be described as roughly leaf-shaped. In most rasp traces it may be observed that the central area is not completely scraped free of algae. This is the result of mainly the lateral parts of the radula being used in the initial stages of the rasp (fig. 134). If both the central and lateral areas are initially used, the rasp is more effective, practically all the algae being removed from that area (as indicated in fig. 135).

Examination of these highly effective rasps thus suggests that Heiderman'sⁿ (1924) distinction between the functions of the marginal and lateral teeth is not as clear cut as it initially appears. It is true that the marginal teeth serve to remove the algae from the substratum and probably they are too delicate to be of any use in the cutting of plant tissues. However, it is also apparent that the lateral, by their shape classed primarily as cutting teeth, play an important role not only in feeding on thick algal layers, but also in normal grazing over a surface but sparsely coated with algae. That

they are not as effective in removing algae as the marginals is suggested by the fact that the central part of the rasp almost always contains more algae than the lateral parts. It must be taken into account though, that the lateral parts of the rasp are formed by oblique rows of teeth, so that the scraping in this region is more effective than in the central region. By analogy, the effect of the central and lateral portions of the transverse fold, is the same as that of a comb being pushed forward at right angles to a line in the direction of motion, or pushed forward while held at an angle; the latter resulting in a narrower but more thoroughly scraped area.

In a single rasp, the marks made by individual teeth are often distinguishable, and in some cases it is possible to distinguish between the small scratches etched by the central and lateral teeth of the radula. The marks of the central tooth are joined to form a line which is thinner than the lines on either side of it. It has symmetrical widenings at intervals representing the two cusps of the central tooth. The marks made by the first lateral on either side are broader because of the size of the tooth, and the thickenings are also larger. It is interesting to note that these lines are asymmetrical, showing that in grazing, it is the endo- and mesocone which are of greater importance.

14.3

THE RELATION BETWEEN SWIMMING AND FEEDING

The normal feeding of B. tropicus takes place whilst crawling over plants and other submerged objects which offer suitable surfaces for the development of the periphyton which serves as food for the snails. It has however been noticed that B. tropicus not infrequently floats inverted at the water surface, and this phenomenon might be related to feeding.

In browsing through the literature one comes across several references to aquatic snails swimming inverted at the water surface. In B. tropicus no series of waves passing from the posterior to the anterior part of the foot of inverted snails could be detected. Instead, large depressions may sometimes be seen moving slowly in a posterior direction. These depressions occur only in the anterior half of the foot and it is thought that they are correlated with contractions of the columellar muscle righting the shell which offers resistance to the water through which the snail is moving. Another factor of possible importance in this connection, is the asymmetrical shape of the shell which tends to swivel on the neck. Locomotion at the water surface is in B. tropicus apparently brought about by the action of the cilia clothing the sole of the foot.

The possibility of a relation between this swimming and feeding has been brought to light through the work of

Goldacre (1949). He examined naturally occurring bodies of water in England and discovered on almost every one, a thin surface film. He expressed the opinion that this monomolecular layer results from the decomposition of plant material and, in most cases, probably consists of a protein or lipo-protein substance. Such a film may then be assumed to occur also on many waterbodies in South Africa especially those habitats where lush vegetation and high temperature would seem to favour the formation of such a film. In the aquarium thick surface films have been noted to occur and have been associated with the decaying of organic matter in the aquarium.

Snails found swimming at the surface were found to be depending from this layer which Goldacre (1949) considers to be important in feeding. Although the film is only a monomolecular layer, its potential importance is not to be underestimated as it has been calculated (Goldacre, 1949) that a tadpole feeding on this layer, could eat its own dry weight in protein in a single day. He further considers this layer to be important not only for the protein it contains, but also on account of the many micro-organisms which are ensnared in the layer, and thus incidentally ingested.

A good example of a snail feeding on this protein layer, is furnished by Cheesman (1956) who found that Pomacea canaliculata curves the anterior part of its foot at the water surface to form a funnel into which the protein layer is drawn. At intervals the snail bends over and ingests the

collected protein. This type of behaviour was not observed in B. tropicus. Sometimes the surface film may be seen to collect on the sole of the foot while the animal is swimming but as was found in Pomacea canaliculata, this incidentally collected film was not eaten.

The finding that B. tropicus apparently has no specialised surface feeding mechanism does not preclude the possibility that the snail feeds on this surface layer. The snail often performs feeding movements while swimming at the surface, or while attached by the posterior part of the foot, to the aquarium wall. In the latter instance the anterior part of the body is reflected so that it is in the same position as during swimming. Feeding movements are performed and the head is swung from side to side as during grazing. These feeding movements suggest that the animal is probably ingesting a certain amount of the surface film and the entangled micro-organisms which it contains.

Surface swimming and surface feeding are nevertheless not necessarily associated in B. tropicus, the snails fairly often being observed to swim at the surface without performing feeding movements. It is here suggested that it is sometimes a lack of oxygen which may induce surface swimming. In the laboratory the number of snails floating at the surface generally increases when fresh water is let into the aquarium, and the low oxygen tension of the tap water is possibly responsible for the upward movement of the snails.

A number of observations seems to support this supposition.

Large snails have a relatively small surface area in comparison with their mass, and they therefore have a less favourable gas diffusion rate than small snails. In B. tropicus it is conspicuous that large snails are found at the surface more frequently than small snails. In a dish containing 20 large and 20 small snails, the number of large snails found at the surface was four times as great as the number of small snails.

In contrast to this Cheesman (1956) found the number of small snails at the surface to be much greater than the large snails. This finding is perhaps related to the size of the snails, for whereas adult B. tropicus are relatively small and float comfortably at the surface, large Pomacea canaliculata may weigh~~t~~ as much as 52gm. Another fact, possibly supporting the supposition that swimming is related to the oxygen-content of the water, is connected with the chemical law that at high water temperatures, there is a decrease in the amount of dissolved gases. Hot weather would therefore, in pools with scant vegetation, cause a reduction in the amount of dissolved oxygen, and Boycott (1936) noted that, especially during such weather, pulmonates are to be found crawling at the surface.

After all these "pro's" in favour of the oxygen theory, there is one important "con", viz. the fact that the pseudo-branches of the snails found at the surface are not markedly

distended (in the manner described on p. 58) as one would expect in snails suffering from lack of oxygen.

Through its necessity for normal photosynthesis, light also plays an important role in the determination of the oxygen content of a volume of water; during the night the amount of this gas may be considerably lower than during the day. Gordon et al. (1934) found that large numbers of P. globosa swim at the surface of the water shortly after sunrise, but once the sun is up, they sink to the bottom. Deschiens (1956) also noted that numbers of snails are to be found near the surface at twilight, and I have observed the same with regard to B. tropicus in the laboratory. The authors mentioned do not connect this phenomenon with oxygen tension, and it may prove upon examination that it is the light factor which is directly responsible for this twilight swimming.

Besides the above-mentioned possible causes of surface swimming, there is yet another viz. that swimming is simply one of the methods of locomotion open to the snails and that they therefore employ it.

FOOD PREFERENCES OF B. TROPICUS

The omnivorous feeding habits of freshwater snails, have been noted by a number of workers. B. tropicus must also be classed as an omnivore though not all food is taken equally readily.

The normal food of these snails appears to be periphyton,

consisting largely of unicellular algae and diatoms. Because of the trituration resulting from the sand grains in the alimentary canal, identification of a large part of the stomach contents was impossible. Filamentous algae, Spirogyra and other species, have been identified in the gut of B. tropicus but it is not thought that they constitute an important item in the diet. Even in the laboratory when snails were not fed lettuce leaves, and they spent a large part of their time crawling among the algal filaments, pieces of spiral chloroplast were only rarely found in the stomach. The bulk of the stomach contents consisted of unicellular green algae, including such forms as diatoms, and Oophila and Scenedesmus types.

Deschiens (1956) states that the bilharziasis vectors have microphagous habits but considers them only supplementary, to the preferred food consisting of aquatic or land phanerogams. Whether or not aquatic snails normally feed on the higher plants is as yet a bone of contention eg. Boettger (1944) and Morton (1960) both express the opinion that Lymnaea eats green plants, while Boycott (1936) and van Someren (1946) consider this exceptional.

The feeding habits of the snails should be considered in conjunction with the structure of the radula. Boettger (1944) (quoting Heidermanns) is of the opinion that lymnaeids are the best adapted of all Basommatophora to eat the living

136



 0.1mm

Fig.136. Physid rasp.

tissues of plants, with which they not infrequently gorge themselves. The structure of the lymnaeid radula with its sturdy yet sharp denticles seems to be in accordance with such a habit, but this should be experimentally established. Morton (1960) states that Physa crops green plants but in the light of the structure of the physid tooth which serves to brush algae off the surfaces to which they are attached, this seems highly unlikely. Observation of living Physa mosambiquensis serves further to support the opinion of Heidermanns. The odontophore is but slightly protruded; there is no long, forward rasp and the main movement is one of lateral radular compression. The radula is fairly broad and when it is brought into contact with the substratum the two lateral halves are moved mediad, scraping a roundish or oval area clear of algae (fig. ¹³⁶~~126~~). This movement may more accurately be described as a mopping movement than a rasping movement.

The radula of B. tropicus suggests that it can feed upon periphyton. In practice it does not seem as though the higher plants normally form an important part of the diet. B. tropicus has not been observed to eat living plants in the field, and when provided with lettuce in the laboratory, these leaves are much more readily taken if they are first boiled. The rather tough epidermis is apparently a hindrance to the snail in finding a starting point for its feeding activities. When leaves are left in the aquarium for any

length of time it is frequently found that the bulk of the leaf has been eaten but that the tough veins, and the epidermis are left untouched, the softer tissue being scraped from the inner surface of the epidermis. Spinach was found to be more acceptable to the snails than the lettuce; possibly because the epidermis is apparently less tough, but it may be that the snails are more strongly attracted to the spinach leaves. Pieces of banana proved to be a favourite item of food. Nymphaea leaves which Azvedo^{+ Medeiros} (1955) found to be readily eaten by some snails, were usually not eaten by B. tropicus, and these thus proved useful as floats upon which egg masses were deposited and from which they were later easily removed for examination. The leaves were found to be slow in decaying, and only when decay had set in, were they attacked by the snails. It is probably true that B. tropicus accepts decaying vegetation more easily than living tissues.

It is certain from a study of the stomach contents, that B. tropicus subsists on vegetal matter but it will also, on occasion take animal matter. Moths that fall into the aquarium are attacked by large numbers of old and young snails, and dead snails are also eaten, though often only partially.

CHROMATOGRAPHY15. A PRELIMINARY REPORT ON THE FREE AMINO ACIDS IN
THE HAEMOLYMPH OF B.TROPICUS15.1 INTRODUCTION

As an aid to taxonomy the comparison of chromatographic patterns of substances taken from the bodies of different individuals, are compared, but the substances responsible for the pattern are not identified.

This method was first employed using the foot, ground into chromatographic paper, but the method was later abandoned for two reasons.

Firstly, the possibility of chromatographic patterns becoming taxonomic tools, which seemed so promising from the results of some early experiments, does not seem to have materialized eg. pattern differences which are attributed to sex (Micks, 1954), or even differences of diet, have been detected.

Secondly it seems wiser to attack the problem from the other end; first to determine the range of chromatographic pattern variations in a single species, before comparing it with others. It has for instance been found that the chromatograms may reflect many types of metabolic difference. Besides the factors mentioned above, there are others eg. differences which may possibly be attributed to the seasons (Wright, 1959), to whether

the specimens are wild or lab-bred, or even whether they are parasitized or not.

In the present instance therefore, an analysis of the free amino acids in the haemolymph of B. tropicus was undertaken.

15.2 MATERIALS AND METHODS

15.21 Obtaining and preparation of sample

Haemolymph extraction presented some difficulties. The technique used by Friedl (1961) could not be used as the shell of B. tropicus seems to be less transparent than that of Lymnaea stagnalis jugularis Say which was used by Friedl, and besides this, B. tropicus are considerably smaller so that large numbers of snails had to be used. The snails used by Friedl were at least 25mmⁱⁿ length while those of B. tropicus were sometimes in the region of 18mm, but often such large snails were unobtainable and snails of 12mm or less had to be used.

This difficulty was aggravated by the low concentration of amino acids in freshwater forms, eg. Wright (1959) mentions a freshwater pelecypod having a concentration of free amino acids less than 1/12 of that of a marine pelecypod.

Another difficulty was the high degree of parasitism obtaining in many of the sacrificed snails. Because of the small number of parasite free individuals in the

densest population, both parasitized and non-parasitized snails were used. As soon as possible however, chromatograms should be run to ascertain any possible discrepancies in the amino acids of these two kinds of snail.

Initially the snails were narcotized and haemolymph extracted by means of a syringe inserted into the cephalic sinus. Later a method based on that of Targett (1962) was employed. The snails were dried on filter paper and held spire-down, but instead of inserting a syringe into the apex, the tip of the apex was removed and the exuding haemolymph collected by means of a Pasteur pipette. Pricking the sole of the foot caused further retraction of the snail into the shell and consequently greater exudation of haemolymph at the spire tip.

The collected haemolymph was diluted with absolute ethanol, to five times the original volume and then centrifuged to remove the cells and precipitated protein. The clear supernatant was then evaporated till the volume was equal to about half of the original blood volume. It was not found necessary to desalt. Samples were used as soon as possible and, when necessary, stored at 4° C.

15.22

Spotting

Originally the spots were used, later strea-

king between rhomboidal windows (cut into the paper by means of a stencil) was used. The streaking greatly improved separation and relatively narrow bands could be obtained by this method. Aliquots of 50 - 200 μ l were applied.

Because of the difficulties involved in obtaining an adequate amount of haemolymph, a fairly large number of trials was made using standard solutions of amino acids, and these standards were also used later to test the suitability of various solvents. Standard solutions of twenty amino acids were made, the concentration of these solutions being 25mg/2ml. These were applied in 2 μ l aliquots more or less 25 γ per spot.

15.23 Paper chromatography

Various types of paper were used S and S 2043, Whatmans No. 3 and Whatmans No. 1, last mentioned finally being selected and used throughout.

Basic chromatographic pattern

No results of any importance were obtained and therefore these techniques will be only briefly mentioned. Spots were made by using slime scraped from the body of the snail, or by squashing pieces of the foot directly onto the paper. Both the circular and the ascending methods of development were used,

the solvents employed being the well-known butanol/glacial acetic acid/water (4:1:5) and the same mixture in the proportions 4:1:1 (Merck). The latter solvent was subsequently employed as it is a one-phase solvent. The papers were then treated with ninhydrin. No differences could be detected in the pattern obtained from small or large snails, and the work was abandoned in favour of investigating the free amino acids present in the haemolymph.

Identification of amino acids

Descending

The descending method proved the most satisfactory in the present instance and chromatograms could be run almost the length of the paper without unduly diffuse spots resulting. In the early stages it was not possible to regulate the temperature but later the work was done at 21° C.

The apparatus was a large glass tank to hold four papers. The lid was furnished with holes for replenishing the solvent and for filling after acclimatization.

Whole sheets of Whatmans paper were suspended from the two glass troughs in which they were held by fairly heavy glass rods. It was found that the quality of the spots could be improved greatly by cutting rhomboidal windows in the paper and then applying aliquots, either in the form of a dot or a line, between the obtuse

angles of two adjacent windows. This caused the amino acids to separate not as a series of rather diffuse spots, but as a series of discrete narrow bands.

15.24 Solvents

The most frequently used solvent was the butanol/glacial acetic acid/water (4:1:1) but several others were tried, including phenol/water 75:25g + 20mg NaCN. It was found that pyridine/isoamyl alcohol/water (in various proportions, 35:30:30 Merck, or 35:35:30 Edman, 1945) gave beautiful narrow bands (3mm) but the front was uneven and the distance run small, eg. in 26 hours the distance run was less than 30cm. The most promising of the two-dimensional techniques seems to be butanol/glacial acetic acid/water (4:1:1) and butanol/methyl-ethyl-ketone/water (2:2:1). This is a variation of the technique of Mizell (1961) who used a butanol/glacial acetic acid/water mixture in the proportions 25:6:25 for the first dimension.

15.25 Silica gel chromatography

The gel used was Kiesel gel G Merck. A variety of solvents including those suggested by Brenner and Niederwieser (1960), Honegger (1961) and Mutschler and Rochelmeyer (1959) were tried, as also two dimensional techniques of several authors. Certain of these two dimensional techniques seem worth pursuing further, especially in view of the smaller aliquots

needed in comparison with paper chromatography.

15.26 Spray

Numerous types of spray were tested, including some to which agents, inducing the spots to assume different colours, had been added. The best of these was found to be the solution suggested by Merck, 0.3gm ninhydrin in 95ml acetone + 5ml collidine.

15.3 RESULTS

In some instances the colour assumed after collidine treatment was of much assistance in distinguishing between closely situated or overlapping spots, eg. there are slight colour differences between cystine and cysteine, and clear colour differences between lysine and histidine; and also between glutamine and threonine which to some extent overlap. Tryptophane is a poorly defined and faint spot and it seems possible that this will not be visible unless it is present in a high concentration in the haemolymph.

The spots were found to differ in two respects from the reports of other authors. It has been stated that the basic amino acids in an acid solvent, yield diffuse bands (Williams and Kirby, 1948) and that histidine must be present in a great quantity before it is detected by ninhydrin (Wright, 1959). In the present instance the bands of the basic amino acids were among the most discrete, in spite of the glacial acetic acid in the sol-

vent; and histidine was found to be one of the most conspicuous amino acids.

The results of 7 chromatograms of snail haemolymph seem to indicate that the following free amino acids are present in the blood of B. tropicus: cystine (and/or cysteine,) lysine, histidine, arginine (and/or aspartic acid), glycine, glutamine, threonine, alanine, valine and leucine. In two instances phenylalanine seemed to be present, and in the same two chromatograms there are also possible traces of tyrosine.

15.4 CONCLUSIONS

These results are interesting in that Targett (1962 and 1962b) found that in several species of bilharziasis vector investigated by him, arginine and histidine are present in the tissues but absent from the blood.

At the moment it cannot be stated with certainty that these two amino acids are present in the blood of B. tropicus, as a small amount of these amino acids may have contaminated the sample either by exuding from damaged tissues, or from the parasites which were often crammed together in the apex of the shell.

The fact that histidine and arginine may be present in the blood of B. tropicus makes it worth while investigating. In doing this there are two important points to bear in mind; laboratory-bred material should be used

to be assured of parasite-free snails, and the possibilities of silica gel chromatography should be further explored as the necessity for large aliquots for paper chromatography greatly hampers the work.

16. SUMMARY

1. The morphology and histology of the headfoot of B. tropicus were investigated and the conclusion drawn that the translucent granules and white flecking, visible in the tissues of the snail, are probably caused by calcium carbonate compounds and calcium glands respectively.
2. An attempt was made to distinguish between the various types of gland and vesicular cell, occurring in the snail, and also to relate form and function in the pulmonary cavity.
3. The alimentary system was studied, with special reference to the buccal mass, the histology of the digestive gland and the lining of the rest of the alimentary canal.
4. There is a short digression on the nature of the various types of muscular tissue in B. tropicus, and also the reason for the asymmetrical passage of the buccal retractors through the ganglionic ring.
5. The reproductive system was described, also with special reference to its lining. There is some discussion on the phenomenon of double nucleoli and nature of the supporting cells found in the walls of the reproductive tract.

6. The geology of the Potchefstroom district was briefly sketched and an attempt made to determine the influence of several ecological factors on the snails.
7. Observations on desiccation in B. tropicus and related forms, were made, and there follows some discussion on the terms employed in describing the types of diapause in snails.
8. From population samples taken over three years it appears that there are two breeding peaks per year in local B. tropicus; one in October/November, and the other in June.
9. The influence of some biotic factors on the snails is discussed, with special reference to the Trematoda which are apparently one of the most important factors. Broadly speaking trematode infections of the snail studied seem to be highest in summer and low in winter, but they vary from one habitat to another.
10. Some aspects of the interaction of birds and snails are described and attention drawn to the fact that we are ignorant of the feeding habits of many birds which are potential predators of snails.
11. Certain aspects of feeding in B. tropicus are examined, eg. the feeding rate and the manner in which the radula is employed during rasping. This was found

to differ markedly from the rasping method of Physa but to resemble that of Lymnaea. There is also some speculation as to the possible causes of surface swimming observed in this species.

12. Various chromatographic methods, both paper and silica gel, were employed in an attempt to determine the free amino acids in the haemolymph of this snail.

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List of Abbreviations.

A	=	amoebocyte
AC.W.	=	acinus wall
ALV.C.	=	alveolar cell
BA.C.	=	basal cell
BL.S.	=	blood space
BL.V.	=	blood vessel
B.M.	=	basement membrane
BU.M.	=	buccal mass
C	=	cilia
CA.G.	=	calcium gland
CA.D.	=	calcium deposits
CL.C.	=	club shaped cell
CON	=	concretion
CU	=	cuticle
D.	=	debris
D.G.	=	dorsal glands
DIG.C.	=	digestive cells
DIG.GL.	=	digestive gland
DIL	=	dilatation of duct
D.SU.GL.	=	duct of subepithelial
DU.GL.	=	duct of gland
E	=	epithelium
E.GL.	=	empty gland cell
EP	=	epiphallus
FB	=	fibroblast
FIB	=	fibrous connective tissue
F.M.	=	follicular membrane
FO	=	foot
F.V.	=	faecal vacuole
GA.C.	=	ganglionic cell
GLO	=	secretory globules
GR	=	granule
H	=	haemocoel
H.V.	=	hepatic vestibule
I	=	intestinum
I.C.	=	intestinal contents
I.M.R.	=	intermediate mantle ridge
I.P.L.	=	inner pallial lobe
K.W.	=	kidney wall
K.L.	=	kidney lamella

List of Abbreviations continue

L.C.	=	Leydig's cell
L.GL.	=	lighter gland
LI.C.	=	lightly staining cells
LI.ME.C.	=	lime cell
L.J.	=	lateral jaw
L.R.R.	=	lateral rectal ridge
M	=	muscle
MA	=	matrix
MAN	=	mantle (general surface)
M.GL.	=	mucous gland
M.P.L.	=	middle pallial lobe
MU	=	mucus
N	=	nucleus
N.C.	=	nerve cell
NU.C.	=	nurse cell
O	=	ovum
O.P.L.	=	outer pallial lobe
OR.G.	=	orange globule
OR.L	=	oral lobe
P.B.	=	pallial band
PIG	=	pigment cell
P.L.	=	pallial lobe
P.P.C.	=	PAS-positive cells
P.S.	=	penis sheath
P.S.C.	=	purple staining cell
R.T.	=	radula teeth
S	=	sensilla
SCR	=	secretory droplets
SH	=	shell
SU.C.	=	supporting cell
T.L.	=	trematode larvae
VAC	=	vacuole
VE.C.	=	vesicular cells
V.G.	=	ventral glands
V.M.	=	vitelline membrane