A CONTRIBUTION TO THE RESPIRATORY PHYSIOLOGY

OF

THREE SOUTH AFRICAN FRESHWATER PULMONATE SNAILS,

BULINUS (PHYSOPSIS) AFRICANUS (KRAUSS),

BULINUS (BULINUS) TROPICUS (KRAUSS),

AND

LYMNAEA NATALENSIS KRAUSS

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BY

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"No, no!" said the Queen. "Sentence first -

verdict afterwards."

Lewis Carroll.

C O N T E N T S

I N T R O D U C T I O N

The use of chemical molluscicides in the control of schistosome transmitting snails has been largely based on empirical studies of toxicity, rather than on a clear understanding of basic physiology and biochemistry of snail hosts. It is therefore possible that purely physiological studies may in themselves provide information which could be of value in the development of more effective mollucicides.

In this investigation an attempt has been made to understand some aspects of the respiratory physiology of three species of fresh-water pulmonate snails, all of which are of medical and veterinary importance in the Rupublic of South Africa. Bulinus (Physopsis) africanus (Krauss) is the intermediate host of Schistosoma haematobium, the causative organism of human schistosomiasis (Bilharziasis). Bulinus (Bulinus) tropicus (Krauss) is the intermediate host of the conical flukes Calicophoron calicophorum and Paramphistomum microbothrium and of the liver fluke Fasciola hepatica, while Lymnaea natalensis Krauss is the intermediate host of the liver fluke Fasciola gigantica.

Apart from the obvious economic and Public Health importance of these snails, the interest in this investigation was also stimulated by the complicated respiratory mechanisms that these snails possess. All three species possess a functional lung (mantle cavity) and atmospheric air is admitted into the mantle cavity through the pneumostome which is an outgrowth of the mantle. Bulinus africanus and Bulinus tropicus possess haemoglobin and Lymnaea natalensis possibly possesses haemocyanin. In addition, Bulinus africanus and Bulinus tropicus have an accessory gill, the pseudobranch, which is pallial in origin and situated outside the mantle cavity. This structure is absent from Lymnaea natalensis.

Previous work on various aspects of respiratory physiology of aquatic snails has shown varied, unpredictable and often

contradictory results with regard to the extent to which the snails utilize their respiratory machinery and their respiratory behaviour under different conditions.

Some species are essentially air breathing e.g. the British Lymnaeids Lymnaea truncatula and Lymnaea palustris. According to Hunter (1952) lengthy immersion in water will drown these species. On the other hand the Ancylids are purely aquatic and can live submerged throughout their lives. Many observations have also shown that some pulmonate species can live continuously submerged. Carter (1931) and Cheatum (1934) quotes various authors who have found Lymnaeid and Planorbid species living continuously submerged with water filled mantle cavities at depths varying from 25 - 250 m, making surfacing most unlikely. More recently Noland and Reichel (1943) and Noland and Cariker (1946) have succeeded in breeding several generations of Lymnaea stagnalis in the laboratory without access to the atmosphere. Most of the pulmonates however, occupy and intermediary position, and although aquatic in habit also indulge in periodic surfacing to take atmospheric air into the mantle cavity. The primary function of the air bubble in the mantle cavity is not known for sure. Although, it is generally agreed that the bubble has a respiratory function, some authors are of the opinion that pulmonary respiration is auxiliary to cutaneous respiration and that the bubble functions mainly in buoyancy regulation.

As far as pulmonary respiration is concerned, the suggestion has been put forward that the air bubble may function as a physical gill as in some aquatic insects, but the evidence for this view is contradictory. The general impression, with regard to the function of the pseudobranch, is that it supplements cutaneous respiration. (Baker 1945). According to Hunter (1957), cutaneous respiration under water replaces surface breathing most completely in the two Basommatophoran families in which secondary gills have

developed, namely the Ancylidae and Planorbidae.

The oxygen uptake of aquatic snails in relation to starvation, temperature, live weight and oxygen content of the water, has been studied by various workers. Although their experimental results showed similar tendencies in most instances, the respiratory behaviour of the animals with regard to oxygen content of the water did differ in that some species maintained a steady respiratory rate in the face of a decreasing oxygen supply, whereas other species decreased their respiratory rate as the oxygen supply diminished.

It has also been shown that aquatic snails have a certain tolerance to survive in anaerobic conditions. As far as the pulmonates are concerned it appears that the Planorbids are more resistant to anoxia than the Lymnaeids and Physids.

It is clear from these brief introductory observations that the freshwater pulmonates show wide variation in the use to which the respiratory apparatus may be put. As a result it seemed relevant to provide a detailed review of previous work and to discover in how far the South African species fitted into the rather broad spectrum of respiratory activity. In so doing particular attention has been paid to (1) the relative importance of pulmonary and cutaneous respiration, (2) individual survivalship in anoxic conditions, (3) cutaneous respiration in relation to starvation, temperature, live weight and oxygen content of the water, (4) the anatomy, histology and function of their respiratory organs,and (5) the possible role of the air in the mantle cavity as a physical gill.

M A T E R I A L.

The shells of the three species studied are given in Plate 1. Bulinus (Physopsis) africanus were obtained from the Bilharzia Research Unit, South African Institute for Medical Research, Johannesburg. These snails were collected in the Klein Jukskei river on the outskirts of Johannesburg, and particulars regarding this locality are given in a paper by De Meillon et al (1958). Bulinus (Bulinus) tropicus were obtained from a pond near the Grahamstown aerodrome, and from a farm dam near Kenkelbosch, approximately halfway between Grahamstown and Port Elizabeth. The depth of the water from these sources varied from 0.15 to 0.3 m and the snails were usually found on vegetation at the margins. Lymnaea natalensis were collected from a concrete sump, below ground level, in an irrigation canal, on the farm Mosslands, twelve miles from Grahamstown on the main road to Port Elizabeth. The water in this sump was stagnant and varied in depth from 0.3 -0.6 m. The snails were usually found, floating on the surface, or attached to the walls immediately below the surface.

All the animals were kept in the laboratory, in water varying in depth from 6 - 10 cm., in large glass dishes and in wooden baths lined with plastic sheeting. Grahamstown municipal water, continously aerated and enriched with plants was used to supply the aquaria. The snails were fed on dehydrated lettuce leaves, and the dishes and baths in which the animals were kept, were cleaned out regularly.

During the course of the investigation, the temperature of the water in the laboratory varied from 12°C - 17°C in winter and from 18°C - 22°C in summer. The pH of the water varied from 6.5 - 8.0 with a mean of 7.3.

All three species bred quite prolifically, except during the winter months when the temperature was obviously affecting fecundity. Shiff (1964) found that temperature exerted a strong influnce on the rate of increase of Bulinus (Physopsis) globosus, the optimal temperature for this species was 25°C.

Plate 1: Representative specimens of the shells of the three species.

Upper row: Bulinus (Physopsis) africanus
Middle row: Bulinus (Bulinus) tropicus
Lower row: Lymnaea natalensis

Unfortunately with the facilities available it was not possible to follow the careful culturing routine used by Shiff (1964) for Bulinus (Physopsis) globosus, so that fresh adult material was obtained when needed.

APPARATUS AND TECHNIQUES

As a result of the varied requirement of physiologists in studies on respiratory physiology, numerous methods are available for the determination of dissolved oxygen, the analysis of respiratory gases, and the measurement of oxygen consumption.

The method used for the determination of dissolved oxygen in this study is based on the Winkler method (Welsh and Smith, 1963), and modified as follows.

By allowing the entire reaction to take place in a 10 ml. all glass syringe, it was possible to estimate the dissolved oxygen in approximately 5 ml. water. For this procedure, the Winkler reagents were diluted 1 in 20, and orthophosphoric acid (Fox et al 1937) replaced concentrated sulphuric acid for liberating the iodine. The free iodine was partioned in chloroform (Johnson and Whitney, 1939), resulting in a deep purple to mauve coloured iodine - chloroform complex, which was found stable enough to allow the intensity to be measured colorimetrically.

The standard markings and divisions on the barrels of the syringes were unsuitable for accurate measurement of volumes, and to overcome this difficulty, hairlines were etched on the barrel corresponding roughly to 5, 6, 7 and 8 ml., when coincident with a hairline on the plunger. Reproducible masses of water taken up were obtained in this way. Table 1, in the appendix, reports the results of this standardization. For all practical purposes the respective volumes in ml. were as follows:-

Etched marks	1	2	3	4	needle
Syringe No. 1	5.03	6.04	6.95	7.95	0.21
Syringe No. 2	5.07	5.97	6.87	7.80	0.22

The volume of water used for the estimation was 5.24 ml in syringe No. 1 and 5.29 ml in syringe No. 2, and approximately 1 ml of the reagents were used in each case. The oxygen

concentration in a water sample was determined as follows. Water was drawn into the syringe up to mark 1, followed by manganese sulphate up to mark 2, and alkaline iodide up to mark 3. The syringe was rotated in an horizontal plane at intervals for 3 minutes to distribute the precipitate evenly, and laid flat for 2 minutes to allow the precipitate to settle. Orthophosphoric acid was then drawn in up to mark 4, and the syringe rotated gently until the precipitate had dissolved and all the iodine liberated and evenly distributed.

The colorimetric estimation of dissolved oxygen was made by taking a volume of the resulting iodine solution, from the syringe, containing 4 ml water and shaking with 6 ml chemically pure chloroform until partition was complete. The iodine-chloroform complex was separated from the clear supernatant layer directly into 8 x 1.5 cm. cylindrical cuvettes, which were then dipped in warm water (40 - 50°C), for a few seconds to expel any bubbles, and read in an EEL colorimeter using a green filter (500 - 570 mμ). A calibration curve was constructed, using water samples, covering a range of oxygen concentrations. These concentrations were obtained by bubbling nitrogen through originally well aerated water for increasing lengths of time. It was possible by this simple technique to obtain water almost free of dissolved oxygen. The oxygen content of each water sample was determined in duplicate by the direct Winkler titration method, and expressed in mgm/l. These results were compared with those obtained colorimetrically from chloroform treated iodine solutions taken from the Winkler bottle and syringe. Figure 1 shows graphically the comparisons which were made. Statistically there was no significant difference between the Winkler and syringe calibration curves, ($t_{0.05} = 0.08$, d.f. = 42) and for all determinations by the syringe method, the Winkler calibration curve was used.

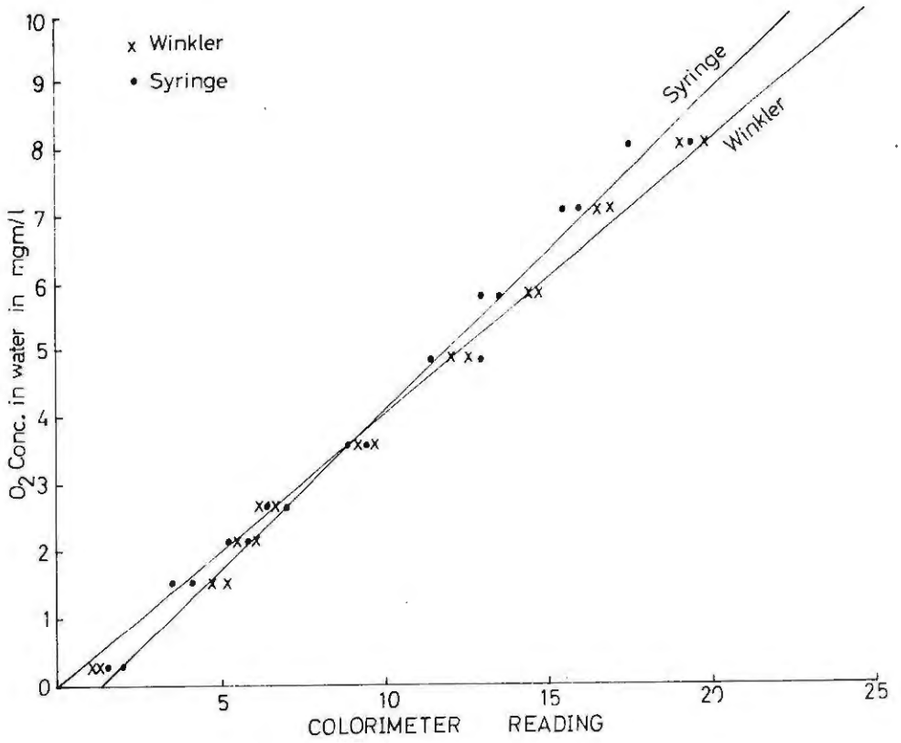

Figure 1: Calibration curve for determining the dissolved oxygen concentration in water, showing the two regression lines which were obtained for the Winkler and syringe methods respectively.

Measurement of oxygen uptake by the snails.

The oxygen consumption of the animals, under standardized conditions, was measured in respirometers constructed from 10 ml all glass syringes. The snails were placed in approximately 10 ml water, and the actual volumes were determined by weighing, as previously described. The results of this standardization are shown in the appendix, Table 2. For all practical purposes, the total volume of water in the respirometers A and B, in which the snails were placed, were 10.26 and 9.67 ml respectively. The displacement of the snails were measured as accurately as possible in a 5 ml measuring cylinder, and these volumes were subtracted from the volume of water in the respirometers. By securing syringe needles in reverse in the respirometers, it was possible to connect a Winkler syringe directly to the respirometer, and to withdraw a sample of water for the determination of the oxygen concentration, without contact with air. (See Plate 2).

The oxygen uptake of the snails was taken as the difference in the oxygen concentration of the water in the respirometer, before the snails were introduced, and after the animals had been in the respirometer for a given length of time. It is appreciated that this "static" approach to respirometry is not necessarily good technique and could be misleading in view of the concomitant effects of increased carbon dioxide and waste products upon animal respiration. While this argument is certainly valid for current dwelling animals, I have taken the liberty of considering it of less importance to animals such as these which are not normally found in flowing water.

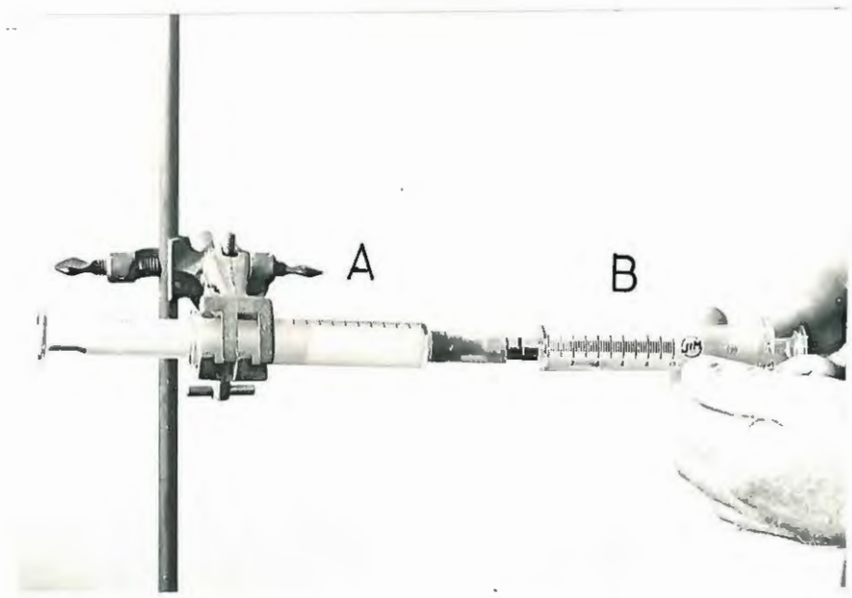

Plate 2: Procedure for withdrawing a water sample from the respirometer (A) directly into the estimating syringe (B).

Analysis of gas from mantle cavity

Air bubbles from the mantle cavities of the snails were analysed according to the method described by Welsh and Smith (1963). The apparatus consisted of a tonometer, constructed from a 0.1 ml serological pipette, graduated in 0.001 ml divisions. (Plate 3).

All gas samples for analysis were collected into a 5 ml syringe filled with a slightly acidified saturated sodium chloride solution. This solution has very low gas dissolving capacity. The gas bubbles were allowed to equilibrate at the same temperature as the syringe for 3 minutes before being transferred to the tonometer for analysis. For the absorption of carbon dioxide and oxygen, 20% KOH and 5% KOH -Pyrogallol were used respectively. All volumes were converted to volumes of dry gas according to the calculations by Campbell and Taylor (1935). Although this apparatus had the advantages of ease of construction and use and was suitable for the analysis of gas samples of less than 0.1 ml., it was, however, not sensitive enough to measure the carbon dioxide concentration in atmospheric air.

Collection of gas bubbles.

The method described by Hunter (1953 b), whereby air from the mantle cavities of snails was obtained, by piercing the cavity with a syringe fitted with a blunt needle, and withdrawing the air bubble into the syringe filled with 80% glycerol, was found to be impractical as far as these particular species were concerned.

An alternative procedure using gas forcibly expelled from the mantle cavity was developed, and found satisfactory The animals expelled their mantle gas bubble if the foot or pneumostome region was prodded with a blunt seeker. The gas bubbles were collected under water, into an inverted water filled funnel, connected by a length of tygon tubing to a 5 ml syringe, as shown in Plate 4. The syringe and tygon

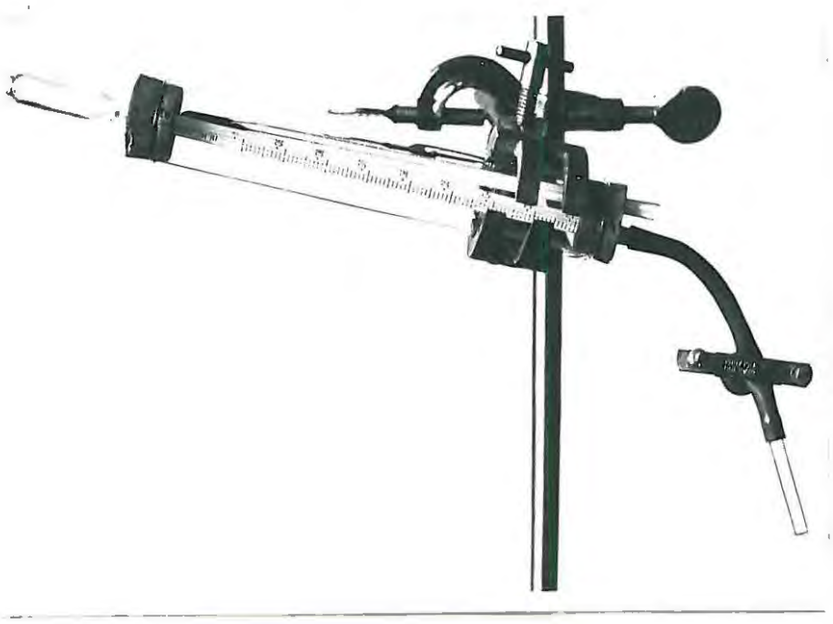

Plate 3: Tonometer for the analysis of gas bubbles.

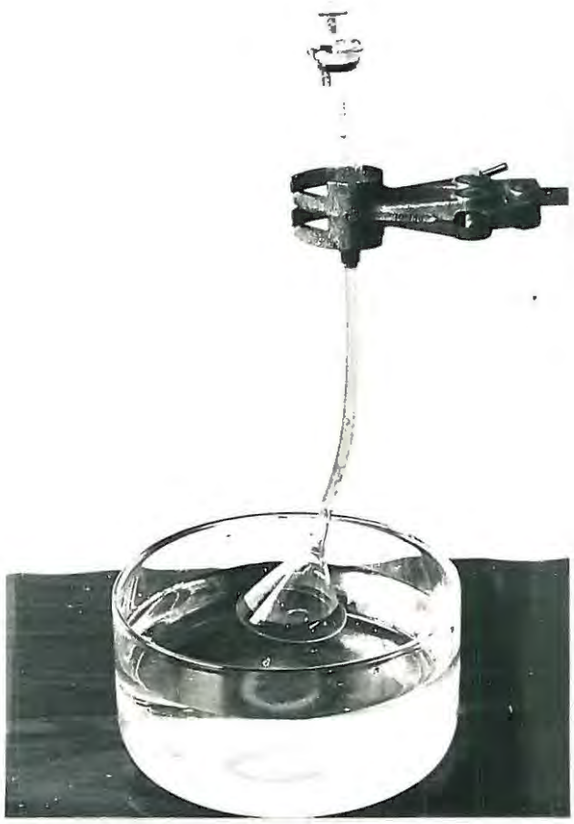

Plate 4: Apparatus for collecting the gas bubbles expelled from the mantle cavities of the snails.

tubing are filled with saturated sodium chloride. The bubble was then carefully, but rapidly, withdrawn into the syringe.

In order to ascertain if any significant gas exchange occurs between the bubble and the surrounding water during its passage up to the neck of the funnel and into the syringe, (a period of between 5 to 10 seconds), atmospheric air and nitrogen bubbles of 0.05 and 0.10 ml volume were introduced under the funnel and left for varying time periods, before being withdrawn into the syringe and transferred to the tonometer for analysis. The results are shown in Table 3.

In the case of atmospheric air there is no significant exchange of gases between the bubble and the surrounding water as it can be assumed that the gases in the water are very nearly in equilibrium with atmospheric air. The nitrogen bubbles show clearly an increase in oxygen concentration after 30 seconds, as is to be expected. As the time limit of 30 seconds far exceeded the time of 5 - 10 seconds usually taken for withdrawing the bubbles into the syringe, this particular procedure of collecting gas bubbles from the snails was considered to be satisfactory, and sufficiently accurate.

A further check on this method of collecting gas bubbles was made by comparing the composition of atmospheric air and nitrogen, when collected under the funnel, and when taken directly into a syringe filled with saturated sodium chloride. The results are reported in Tables 4 and 5, and show that there is obviously no significant difference between the two methods.

TABLE 3.

The composition of atmospheric air and nitrogen bubbles, when exposed to water containing 5.6 ml O_2/l for increasing lengths of time.

	Time	Temp. (°C)	Bar. (mm Hg.)	Volume of bubble	% CO_2	% O_2	% N_2
					(expressed in volumes of dry gas)		
Atmospheric Air.	10 seconds	19	720	0.05 ml	0.0	21.7	78.3
	10 seconds	19	720	0.10 ml	0.0	20.2	79.8
	1 minute	19	720	0.05 ml	0.0	21.4	78.6
	1 minute	19	720	0.10 ml	0.0	21.7	78.3
	5 minutes	19	720	0.05 ml	0.0	20.4	79.6
	5 minutes	19	720	0.10 ml	0.0	20.7	79.3
	15 minutes	19	720	0.05 ml	0.0	20.4	79.6
	15 minutes	19	720	0.10 ml	0.0	20.0	80.0
			---oooooo---				
Nitrogen	10 seconds	20	718.5	0.05 ml	0.0	0.0	100.0
	10 seconds	20	718.5	0.10 ml	0.0	0.0	100.0
	30 seconds	20	718.5	0.05 ml	0.0	0.98	99.02
	30 seconds	20	718.5	0.10 ml	0.0	1.05	98.95
	1 minute	20	718.5	0.05 ml	0.0	2.0	98.0
	1 minute	20	718.5	0.10 ml	0.0	2.0	98.0
	5 minutes	20	718.5	0.05 ml	0.0	4.7	95.3
	5 minutes	20	718.5	0.10 ml	0.0	4.4	95.6
	15 minutes	20	718.5	0.05 ml	0.0	7.0	93.0
	15 minutes	20	718.5	0.10 ml	0.0	6.8	93.2
	30 minutes	20	718.5	0.05 ml	0.0	10.6	89.4
	30 minutes	20	718.5	0.10 ml	0.0	10.3	89.7
	60 minutes	20	718.5	0.05 ml	0.0	15.5	84.5
	60 minutes	20	718.5	0.10 ml	0.0	15.0	85.0
	75 minutes	20	718.5	0.10 ml	0.0	18.0	82.0

TABLE 4

Analysis of atmospheric air. (all volumes expressed as dry gas.)

Funnel method of collection.					Direct method of collection.				
Temp. (°C)	Bar. (mm)	% CO_2	% O_2	% N_2	Temp. (°C)	Bar. (mm)	% CO_2	% O_2	% N_2
22.0	714.5	0.0	20.6	79.4	21.0	712.0	0.0	19.9	80.1
22.0	714.5	0.0	21.9	78.1	19.0	713.0	0.0	20.7	79.3
22.0	714.5	0.0	21.2	78.8	21.0	719.0	0.0	21.6	78.4
21.5	714.0	0.0	21.5	78.5	19.0	719.0	0.0	21.6	78.4
22.0	717.0	0.0	21.9	78.1	19.0	719.0	0 0	21.2	78.8
22.0	717.0	0.0	20.1	79.9	19.0	718.0	0.0	22.1	77.9
22.0	717.0	0.0	21.9	78.1	18.0	712.0	0.0	21.0	79.0
22.0	717.0	0.0	20.9	79.1	19.0	712.0	0.0	20.0	80.0
22.0	717.0	0.0	22.1	77.9	19.0	719.0	0.0	21.4	78.6
22.0	717.0	0.0	20.7	79.3	19.0	719.0	0.0	21.2	79.8

Mean of % O_2	= 21.28	Mean of % O_2	= 21.07
S.D.	= 0.65	S.D.	= 0.66
Standard error of the mean	= 0.2	Standard error of the mean	= 0.2

TABLE 5.

Analysis of Nitrogen (all volumes expressed as dry gas).

Funnel method of collection.					Direct method of collection.				
Temp. (°C)	Bar. (mm)	% CO_2	% O_2	% N_2	Temp. (°C)	Bar. (mm)	% CO_2	% O_2	% N_2
18.5	709.0	0.0	2.9	97.1	18.5	711.5	0.0	2.2	97.8
18.0	709.0	0.0	2.9	97.1	18.5	711.5	0.0	2.3	97.7
18.5	708.5	0.0	2.7	97.3	18.5	711.0	0.0	2.7	97.3
18.5	708.5	0.0	2.8	97.2	18.5	711.0	0.0	2.7	97.3
18.5	712.0	0.0	2.6	97.4	18.5	711.0	0.0	2.8	97.2
19.0	712.0	0.0	2.7	97.3	17.0	711.0	0.0	2.6	97.4
20.0	713.0	0.0	2.5	97.5	16.0	709.0	0.0	2.7	97.3
21.0	715.0	0.0	2.4	97.6	16.0	710.0	0.0	2.6	97.4
20.0	714.0	0.0	2.8	97.2	16.0	710.0	0.0	2.8	97.2
21.0	716.0	0.0	2.7	97.3	15.0	711.0	0.0	2.9	97.1

Mean of % O_2	=	2.7	Mean of % O_2	=	2.3
S.D.	=	0.49	S.D.	=	0.66
Standard error of the mean	=	0.15	Standard error of the mean	=	0.2

INVESTIGATION AND RESULTS

This investigation has been divided into three sections. The first is concerned with general observations on the respiratory behaviour of the snails as observed in the laboratory, in the presence of a water-air interface, in the absence of a water-air interface and in anoxic conditions respectively.

The second has as its purpose the cutaneous oxygen consumption of the animals in relation to starvation, temperature, live weight and oxygen content of the water.

The third deals with pulmonary respiration. Particular emphasis is placed on the functional efficiency of the mantle cavity as a lung by relating the oxygen uptake from the air bubble in the mantle cavity with the anatomical and histological structure of the respiratory organs.

1. General observations on respiratory behaviour

(a) Respiratory behaviour as observed in the laboratory.

All three species visited the surface periodically to replenish the air in the mantle cavity. The snails, in general, followed the pattern as described by Hunter (1953 b). The animals ascend to the surface by crawling up the vertical sides of the dishes, until the tentacles reach the surface. The edge of the shell is raised and the whole shell is rotated - clockwise in Bulinus africanus and Bulinus tropicus, and anticlockwise in Lymnaea natalensis. This action exposes the pneumostome which then protrudes in a siphon-like extension, just breaking through the surface film. The pneumostome opens, usually with an audible click, and remains open for periods varying from 2 - 6 seconds, before closing. After closing the lung, the animal normally dives and only returns to the surface for the next lung-filling.

Various factors appear to determine the surfacing behaviour in aquatic snails, for example (i) the amount of air inspired, (ii) oxygen tension in the water, (iii) temperature of the water, (iv) size of animals and (v) buoyancy of the shell and visceral mass.

(i) Amount of air inspired

Cheatum (1934), was of the opinion that the amount of air inspired may be a factor that determines the intervals between breathing periods. By taking in a large volume of air at the surface, the snails provide themselves with a greater volume of oxygen and thus maintain a rate of respiration for a longer time than by taking in a smaller volume of air.

(ii) Tension of oxygen in the water.

The tension of oxygen in the water and in the mantle cavities of aquatic snails seems to have some effect on surfacing. Cheatum (1934) found that snails remain submerged about three times as long when the water contained 6.4 ml O_2/l than when the oxygen content of the water reached a value of 1.7 ml/l. Precht (1939) showed that Lymnaea stagnalis came to the surface when the oxygen concentration of the water reached a value of 2 - 3 ml/l and when the percentage oxygen in the mantle cavity decreased to 1 - 4%. This finding was confirmed in Planorbis corneus* by Füsser and Kruger (1951), who found that an oxygen tension of 1 - 3% in the mantle cavity forced the snails to the surface.

(iii) Temperature of the water

As far back as 1877, Clessin (vide Cheatum 1934), advanced the theory that Lymnaeids normally

* Planorbis corneus = Planorbarius corneus (Jones 1964 a)

depend on cutaneous respiration and come to the surface only when compelled to do so by high temperatures. Cheatum (1934), showed in his experiments on various Lymnaeid, Planorbid and Physid species that there was a substantial increase in the intervals between breathing at 11°C, than at 21°C. Precht (1939), found that Lymnaea stagnalis resorted entirely to cutaneous respiration at tempertures below 5°C. Wessenberg-Lund (1939), reported that at temperatures below 5°C, Planorbis corneus buried itself in the mud and remained in a state of hibernation. According to Hunter (1953 a), Lymnaea peregra could obtain sufficient oxygen by cutaneous respiration and exchange of gases through the air bubble in the mantle cavity at water temperatures below 12°C, whereas in summer when the temperature exceeded 12°C, the snails came to the surface to breathe.

(iv) Size

The size of aquatic snails show an inverse relationship to the surface area available for cutaneous respiration. Precht (1939) and Hunter (1953 b) found that small individuals of Planorbis corneus and Lymnaea peregra and Physa fontinalis respectively, remained submerged longer than the larger individuals.

(v) Buoyancy of the shell and visceral mass.

It is to be expected that the presence of gas in the mantle cavity of aquatic snails will have a buoyancy effect. Cheatum (1934), noted that aquatic snails can float up to the surface or alternatively sink down to the bottom. Henderson (1963) found that Physa fontinalis uses the mantle gas as a means of flotation to reach the water surface and quotes various authors

who have observed this phenomenon in a variety of pulmonate snails. Precht (1939), Jones (1961), Henderson (1963) and Jones (1964 b) have shown that the buoyancy properties of the air bubble could be important in influencing the surfacing behaviour of aquatic snails. These authors found that an increase in atmospheric pressure (thus decreasing buoyancy) applied soon after the snails had surfaced and dived, forced the animals to return to the surface. In view of these findings, Jones (1961 and 1964 b) and Henderson (1963) advanced the hypothesis that the surfacing behaviour in the freshwater pulmonates could be initiated in part by the stimulus of reduced buoyancy as pulmonary oxygen is consumed. A proprioceptor mechanism would suffice to mediate the response. It can also be argued that the decrease in the partial pressure of the pulmonary oxygen is as likely a stimulus to release surfacing behaviour in aquatic snails. However, the convincing experimental evidence of Jones (1961) appears to favour the former viewpoint. Jones found that the analysis of pulmonary gas composition at the end of a dive in Planorbis corneus and Lymnaea stagnalis showed considerable variation which did not indicate a critical pulmonary oxygen partial pressure. Henderson (1963) working on the same species of snails came to the conclusion that the buoyancy of the shell-visceral mass could be detected by the animals and used to determine the quantity of gas inhaled at the surface.

The aim of the following experiment was to ascertain if temperature, oxygen tension of the water and size of the animal have any effect on the duration of the dive in these three species. Snails of different sizes were placed in water at depths of 7.5 cm. and 30 cm. and observed constantly for six hours. Prior to the experiments, the animals were forced to expel as much of their mantle gas as was possible by stimulation of the foot or pneumostome region with a blunt seeker. The criterion for the complete or almost complete expulsion of the mantle gas was that the animals sank to the bottom of the containers due to loss of buoyancy. All observations were made at ambient temperature in daylight. The duration of the dive was taken as the intervals between definite lung fillings at the surface. These results are shown in Tables 6 and 7, which report the mean of the intervals between breathing periods and the number of breathing periods respectively.

The considerable individual variation in these results indicate that there was no clear pattern in the surfacing behaviour of these snail species. While most of the animals dived after surfacing, a number of snails from each species remained just below the surface making no effort to dive. Furthermore, some individuals of Bulinus africanus and Bulinus tropicus remained submerged and never surfaced during the observation periods. As regards the intervals between brething periods (Table 6) the data are not sufficient to indicate any systematic trend. Bulinus tropicus appeared to be the most constant in this respect and showed the least variation at the different temperature levels.

TABLE 6

Mean of intervals in minutes between breathing periods

Species	Temp. °C	O_2 conc. ml/l	Depth of water: 7.5 cm.			Depth of water: 30 cm.		
			Size of snails (length of shell in mm.)			Size of snails (length of shell in mm.)		
			15-20	10-15	5-10	15-20	10-15	5-10
Bulinus africanus	17-19	6.0		161(2)	123(2)		53(5)	98(2)
Bulinus tropicus	17-19	6.0		25(4)			70(3)	38(5)
Lymnaea natalensis	17-19	6.0	33(1)	50(3)		29(2)	66(5)	88(2)
Bulinus africanus	20-22	5.6		180(3)	28(4)			
Bulinus tropicus	20-22	5.6		25(2)	25(2)			
Lymnaea natalensis	20-22	5.6		72(4)	60(1)			
Bulinus africanus	24-25	5.2		58(2)	94(2)			
Bulinus tropicus	24-25	5.2		24(3)				
Lymnaea natalensis	24-25	5.2	90(1)	76(2)				

The figures in brackets refer to the number of snails observed.

TABLE 7

Number of breathing periods in six hours

Species	Temp. °C	O_2 conc. ml/l	Depth of water: 7.5 cm.				Depth of water: 30 cm.			
			Size of snails (length of shell in mm.)				Size of snails (length of shell in mm.)			
			15-20	10-15	5-10		15-20	10-15	5-10	
Bulinus africanus	17-19	6.0		2,3	2,0	(1.8)		4,2,7,6,0	3,2	(3.4)
Bulinus tropicus	17-19	6.0		3,14,6,12		(8.8)		4,4,2	3,3,4,0,4	(3.0)
Lymnaea natalensis	17-19	6.0	6	5,3,8		(5.5)	8,3	2,2,3,2,3	3,2	(3.1)
Bulinus africanus	20-22	5.6		2,0,0	2,0,11,2	(2.4)				
Bulinus tropicus	20-22	5.6		2,0	10,10	(5,5)				
Lymnaea natalensis	20-22	5.6		4,4,5,5	2	(4.0)				
Bulinus africanus	24-25	5.2		7,0	4,0	(2.7)				
Bulinus tropicus	24-25	5.2		10,10,16		(12)				
Lymnaea natalensis	24-25	5.2	5	2,7		(4.7)				

The figures in brackets refer to the mean of the number of breathing periods. In those instances where more than one size is shown, the mean has been taken irrespective of size.

The results shown in Table 7 indicate that Bulinus africanus surfaced less frequently than either Bulinus tropicus or Lymnaea natalensis. Taken overall it would seem that Bulinus tropicus is the most frequent breather.

Under the conditions of the experiment, there was no indication that temperature, depth of the water, and the size of the animals had any marked effect on the surfacing behaviour of these species. The small decrease in the oxygen concentration of the water from 6.0 ml/l at 17 - 19°C to 5.2 ml/l at 24 - 25°C was surely not sufficient to influence the surfacing behaviour of the animals. It is appreciated that these factors cannot be lightly dismissed and that the observation period of six hours was clearly not sufficient to warrant any definite conclusions. However, the highly irregular pattern in the surfacing behaviour of these snails should be examined in the light of the following findings. Henderson (1963) found that the mantle gas volume is very easily upset by disturbing or handling the snails. In view of this it is possible that the initial handling of the snails to expel the mantle gas may so have disturbed the animals, which could explain in part the wide variations in the freqency of surfacing.

Furthermore, the suggestion put forward by Henderson (1963) that the buoyancy of the shell-visceral mass determines the volume of gas uptake at the surface may also account for the irregular breathing periods as observed. This implies that the volume of air taken into the mantle cavity is variable which suggests that pulmonary respiration as such may not be all that important. In view of this, the next logical step was to ascertain how essential is pulmonary respiration in these species,

(b) The importance of pulmonary respiration

Firstly, it was necessary to determine if and when newly hatched snails indulge in pulmonary respiration. Egg masses of all three species were placed in separate beakers in water at depths of 5 and 10 cm. The beakers were placed in an oven at 26°C, and the water changed regularly to avoid excessive bacterial growth. The oxygen concentration of the water was determined daily. During the incubation period the egg masses were examined at regular intervals of two hours under a binocular microscope. After hatching a similar procedure was adopted to determine the time when pulmonary respiration first occurred. These results are shown in Table 8.

In all three species pulmonary respiration occurred at an average of 2 - 3 days after hatching, when the young snails were seen at the water surface with air bubbles clearly visible in their mantle cavities.

In the case of the adult snails, experiments were carried out whereby the animals were prevented from surfacing by means of plastic gauze at two temperature levels.

The first experiment was carried out during the winter months when the ambient temperature was 15° - 16°C. Some snails were acclimated from 15° to 25°C over a period of ten days. During this period the animals were fed, but not during the experiment. Prior to the experiment all the snails were forced to expel as much of their mantle gas as was possible. Lots of 5 snails of each species chosen so as to be of equal size were placed in separate beakers in 1 litre of water, which was continually aerated. Similar groups

TABLE 8

Depth of water in cm.	Temperature of water (°C)	Mean of O_2 concentration in ml/L	Time (days) from hatching of eggs to first appearance of pulmonary respiration		
			BULINUS AFRICANUS	BULINUS TROPICUS	LYMNAEA NATALENSIS
5	26	5.0	3	3	3
5	26	5.0	2	3	2
5	26	5.1	3	1	1
5	26	4.9	3	2	3
10	26	5.2	3	3	2
10	26	4.8	3	3	2
10	26	5.0	2	4	3
10	26	5.0	2	3	3

were placed in water which was not aerated. In both treatments the snails were prevented by a cone of plastic gauze from surfacing. Controls were set up in non-aerated water in which the snails had free access to the atmosphere. The beakers were placed in water baths at 15° and 25°C. These results are summarized in Table 9(a).

A similar experiment was conducted during the summer months. Snails were acclimated from an ambient temperature of 20°C to 15° and 25°C over a period of five days. In this instance lots of 15 snails of each species were used, the subsequent procedure being similar to that of the above experiment. A summary of these results are shown in Table 9(b).

The survival of the three snails species in both these experiments in aerated water at 15°C and 25°C and in non-aerated water at 15°C did not differ markedly from the controls in which the snails had free access to the atmosphere. Very marked mortality was found in those lots in non-aerated water at 25°C. Within a few hours some of the individuals of all three species were lying motionless and fully extended on the bottom of the beakers. This condition increased progressively among the snails and the experiments were stopped when all the snails were lying motionless at the end of the time periods given in Tables 9(a) and 9(b). None of these snails from either experiment recovered when placed in fresh water. This behaviour resembles a condition noted by Von Brand et al (1950) when snails were exposed to anoxic conditions and defined as the distress syndrome by Harry and Senturia (1964) (vide page 35). An important factor which should be considered as contributing towards the high mortality of the snails is the possibility of carbon dioxide accumulation. Harry and Senturia (1964) showed that distress could be evoked in

TABLE 9(a)

Experiment	Number of snails used of each species	Temp °C	BULINUS AFRICANUS			BULINUS TROPICUS			LYMNAEA NATALENSIS		
			Duration of experiment in hours	Decrease in O_2 conc. in ml/L	Number of snails alive	Duration of experiment in hours	Decrease in O_2 conc. in ml/L	Number of snails alive	Duration of experiment in hours	Decrease in O_2 conc. in ml/L	Number of snails alive
Snails prevented from surfacing. Water aerated continuously.	5	15-16	96	6.8-6.8	5	96	6.8-6.8	5	96	6.8-6.8	5
	5	15-16	96	6.9-6.8	5	96	6.9-6.9	5	96	6.9-6.9	5
	5	25	96	5.5-5.3	5	96	5.5-5.4	5	96	5.5-5.3	5
	5	25	96	5.2-5.0	4	96	5.2-5.1	4	96	5.2-5.0	3
Snails prevented from surfacing. Water not aerated.	5	15-16	96	6.8-1.5	4	96	6.8-3.2	5	96	6.8-1.7	4
	5	15-16	96	6.5-1.9	5	96	6.5-3.0	5	96	6.5-1.3	5
	5	25	20	5.5-0.9	0	27	5.5-1.2	0	18	5.5-1.0	0
	5	25	27	5.8-1.5	0	34	5.8-1.0	0	30	5.8-1.0	0
Control: Snails with free access to the atmosphere.	5	15-16	96	6.8-4.8	5	96	6.8-4.8	5	96	6.65-4.4	4
	5	15-16	96	6.7-4.3	5	96	6.7-4.9	4	96	6.75-4.5	5
	5	25	96	5.2-2.6	5	96	5.3-2.3	5	96	5.2-2.0	5
	5	25	96	5.3-2.3	4	96	5.3-2.4	5	96	5.3-2.0	4

TABLE 9(b)

Experiment	Number of snails used of each species	Temp. °C	BULINUS AFRICANUS			BULINUS TROPICUS			LYMNAEA NATALENSIS		
			Duration of experiment in hours	Decrease in O_2 conc. in ml/L	Number of snails alive	Duration of experiment in hours	Decrease in O_2 conc. in ml/L	Number of snails alive	Duration of experiment in hours	Decrease in O_2 conc. in ml/L	Number of snails alive
Snails prevented from surfacing. Water aerated continuously.	15	15	25	6.5-6.0	0	96	6.5-6.4	14	96	6.5-6.4	14
	15	15	96	6.15-6.0	15						
	15	25	96	5.3-5.1	15	96	5.3-5.1	14	96	5.3-5.1	11
	15	25							96	5.4-5.2	15
Snails prevented from surfacing. Water not aerated.	15	15	96	6.5-0.95	2	96	6.5-1.4	15	96	6.5-0.95	13
	15	15	96	6.15-2.7	15						
	15	25	25	5.3-0.5	0	48	5.3-0.49	0	48	5.3-0.3	0
Control: Snails with free access to the atmosphere.	15	15	96	6.5-4.3	15	96	6.5-4.6	15	96	6.5-4.3	15
	15	25	96	5.3-1.6	10	96	5.3-2.5	15	96	5.3-2.2	15
	15	25	96	5.2-2.7	15						

Taphuis glabratus (Australorbis glabratus) by exposing the snails to carbon dioxide. (vide page 35). In order to test this, lots of 15 snails of each species from the same summer stocks as used in the second experiment were placed under gauze in non-aerated water at 25°C. The carbon dioxide concentration in the water was determined at intervals, by equilibrating an air bubble with 10 ml. of water in a syringe. Part of the bubble was then transferred to a tonometer for analysis. The dissolved oxygen concentration of the water was also checked simultaneously. These results are shown in Table 9(c). The expected high mortality rate with accompanying distress in these lots of snails confirmed the experimental finding as found previously at 25°C. A notable exception, however, was one lot of Lymnaea natalensis which showed no no distress and a high survival over a period of 96 hours. Replication of this treatment with snails from the same stocks resulted in the expected high mortality. No free carbon dioxide could be detected in the water by the tonometric method used, and this gas was therefore not considered to be a direct cause seriously affecting the survival of the snails under the conditions of the experiment.

Table 9(b) also reports some results which seem to be at variance with the overall picture of survival and mortality. Distress, with marked mortality was evident in the case of Bulinus africanus in both aerated and non-aerated water at 15°C. Also four individuals of Lymnaea natalensis in aerated water at 25°C showed the distress syndrome. In replicate experiments on snails from the same stocks, survival was in keeping with the expected results which indicated that the initial findings were very likely abnormal for some reason or another, or due to some unknown

TABLE 9 (c)

Survival of Snails when prevented by gauze from reaching the surface.
Water not aerated.

Species	Number of snails used	Time in hours	Temp. ^{o}C	O_2 conc. ml/L	CO_2 %	Remarks	Final Result Alive	Final Result Dead
Bulinus africanus		zero	25	5.3	0.0			
		24	25	2.0	0.0	10 Snails distressed		
	15	30	25	1.3	0.0	All Snails distressed	-	15
Bulinus tropicus		zero	25	5.3	0.0			
		24	25	1.15	0.0	8 Snails distressed		
	15	32	25	0.8	0.0	All Snails distressed	-	15
Lymnaea natalensis		zero	25	5.2	0.0	No distress		
		22	25	1.6	0.0	No distress		
		42	25	1.0	0.0	No distress		
		69	25	0.9	0.0	No distress		
	15	96	25	0.3	0.0	No distress	14	1
Lymnaea natalensis		zero	25	5.3	0.0			
		24	25	1.15	0.0	12 Snails distressed		
	15	30	25	0.8	0.0	All Snails distressed	-	15

factor or factors. In this connection it should be noted that overcrowding could be a contributing factor as 15 snails of each species were used in the second experiment whereas 5 snails of each species were used in the first experiment. A further aspect to be taken into account is that the snails used in these experiments were from different stocks.

Notwithstanding this variation in the respiratory behaviour of the Bulinus africanus and Lymnaea natalensis, the results from these experiments suggest that all three species can survive for 96 hours and probably for longer periods, without access to the water-air interface at 15°C, despite the decreasing oxygen tension in the water, and at 25°C in well oxygenated water.

Presumably cutaneous respiration is adequate to cater for the oxygen requirements of the animals under these experimental conditions. However, survival is sericusly affected if access to the atmosphere is denied in water at 25°C in which the oxygen tension is being progressively decreased by the animals to levels which do not differ markedly from those at 15°C in non-aerated water. In such conditions the cutaneous oxygen uptake does not seem adequate to supply the oxygen requirements of the snails. This implies that pulmonary respiration in these species becomes increasingly important in conditions in which the cutaneous respiration is not sufficient to cater for the metabolic needs of the animals.

In this connection it is interesting to note that Jones (1961) found that a fall in the dissolved oxygen concentration with a consequent decrease in cutaneous uptake resulted in a compensatory increase in pulmonary oxygen uptake in the case of Planorbis corneus and Lymnaea stagnalis.

The behaviour of the animals when denied access to the atmosphere was in no way abnormal, except in those lots of snails in which extensive mortality occurred at 25°C in non-aerated water. The general impression was that these animals were in respiratory stress after a few hours and they probably died from asphyxiation. Taken overall, pulmonary respiration does not appear to be essential, provided the cutaneous respiration can supply the oxygen requirements of the animals.

(c) Survival of the snails in anoxic conditions.

Aquatic snails are known to possess a certain tolerance towards anoxic conditions. Von Brand et al (1950), in studies on various species of freshwater pulmonates found a considerable variation in the resistance towards total anoxia, in that the Lymnaeids and Physids tolerated total anoxia for only 6 hours whereas the Planorbids were more resistant by surviving in such conditions for 16 - 24 hours, with the exception of Helisoma trivolvis which survived for 64 hours. All these species consumed carbohydrate during exposure to anaerobic conditions and produced carbon dioxide and lactic acid. Subsequent studies by Mehlman and Von Brand (1951) indicated that the species not resistant to anaerobic conditions were killed primarily by the accumulation of lactic acid, whereas the more resistant species were more tolerant to the lack of oxygen due to the fact that they accumulated in their tissues less toxic fatty acids such as propionic and acetic acid. Berg (1952), showed that the limpet Ancylus fluviatilis living in well aerated streams was less tolerant to anoxia than Acroloxus lacustris, a limpet living in stagnant water. Biomphalaria sudanica, the intermediate host of Schistosoma mansoni in East

Africa has been found by Jones (1964 b) to live in swamp water in which the dissolved oxygen concentration seldom exceeds 5% of air saturation and often the water is completely devoid of oxygen. In these environments a high concentration of carbon dioxide usually occurs and Jones recorded values of 2.9 - 3.0%.

Von Brand et al (1950), noted that all the pulmonate species studied showed a characteristic behaviour under anaerobic conditions, in that within a few hours after exposure to total oxygen lack the snails lay motionless and fully extended. Provided the animals remained extended they recovered completely in aerobic conditions, but if the anaerobic period lasted too long, haemorrhage occurred and the snails retracted into their shells, a state from which they did not recover when placed in aerobic conditions. This behaviour was defined as distress by Harry and Senturia (1964). These authors subjected Taphius glabratus (Australorbis glabratus) to anaerobic conditions, but in contrast to the experimental findings of Von Brand et al (1950), no distress could be evoked in this species. This could be ascribed to the experimental designs of the various workers. Von Brand et al (1950) employed a closed system of small volume, whereas Harry and Senturia used a constant flow system of large volume which lessened the possible accumulation of toxic waste products from the snails. However, Harry and Senturia found distress symptoms when the snails were exposed to carbon dioxide. In the absence of oxygen, 5% carbon dioxide produced mild distress from which the snails recovered, 20% carbon dioxide caused severe distress from which the animals did not recover, whereas 100% carbon dioxide resulted in immediate retraction

of the snails into their shells and death. Small amounts of oxygen considerably lessened the toxic effects of carbon dioxide, for example 5% oxygen and 20% carbon dioxide caused no well defined distress symptoms.

The aim of the following experiments was to obtain a measure of the tolerance towards anoxia in these South African pulmonate species. The experiments were carried out at 15° and 25°C on two different stocks of snails.

The first experiment was carried out during the winter months when the ambient temperature was 15 - 16°C. After prior acclimation and expulsion of the air bubbles from the mantle cavities of the animals, lots of 5 snails of each species were placed in litre aspirators in 900 ml water through which nitrogen had been bubbled. The air in the space above the water was displaced by nitrogen. The aspirators were closed with rubber bungs provided with manometers and immersed in water baths at 15°C. Similar groups of snails were placed in aspirators which were immersed in water baths at 25°C.

The pressures in the aspirators were adjusted to atmospheric pressure. Controls were set up in aspirators in well aerated water with an atmospheric air interface. The results are summarized in Table 10(a).

The second experiment was conducted during the summer months when the ambient temperature varied from 20 - 22°C. The procedure was similar to the first experiment, except that lots of 15 snails of each species were used. These results are summarized in Table 10 (b). Despite the low oxygen concentration of the water which varied from 0.93 to 1.25 ml O_2/l in the first experiment

TABLE 10 (a)

Experiment	Number of snails used of each species	Temp. °C	BULINUS AFRICANUS			BULINUS TROPICUS			LYMNAEA NATALENSIS		
			Duration of experiment in hours	Decrease in O_2 conc. in ml/l	Number of snails alive	Duration of experiment in hours	Decrease in O_2 conc. in ml/l	Number of snails alive	Duration of experiment in hours	Decrease in O_2 conc. in ml/l	Number of snails alive
Snails exposed to water-N_2 interface in an aspirator	5	15	96	0.93-0.45	4	96	0.93-0.45	5	96	0.93-0.45	5
	5	25	96	1.25-1.0	5	96	1.25-1.0	5	96	1.25-1.0	5
	5	25	96	1.0-0.7	5	96	1.0-0.7	5	96	1.0-0.7	5
Control:											
Snails exposed to water-air interface in an aspirator	5	15	96	6.2-2.7	5	96	6.2-2.7	5	96	6.2-2.7	5
	5	25	96	5.8-2.7	5	96	5.8-2.7	5	96	5.8-2.7	5
	5	25	96	5.9-2.4	5	96	5.9-2.4	5	96	5.9-2.4	5

TABLE 10 (b)

Experiment	Number of snails used of each species.	Temp. °C	BULINUS AFRICANUS			BULINUS TROPICUS			LYMNAEA NATALENSIS		
			Duration of experiment in hours	Decrease in O_2 conc. in ml/L	Number of snails alive	Duration of experiment in hours	Decrease in O_2 conc. in ml/L	Number of snails alive	Duration of experiment in hours	Decrease in O_2 conc. in ml/L	Number of snails alive
Snails exposed to water-N_2 interface in an aspirator.	15	15	96	0.8-0.3	12	96	0.95-0.4	14	96	0.95-0.3	14
	15	15				96	0.8-0.4	15	96	0.8-0.4	15
	15	25	96	0.8-0.3	14	96	0.95-0.2	14	96	0.95-0.2	7
	15	25				96	0.7-0.3	13	96	0.7-0.2	13
Control:											
Snails exposed to water-air interface in aspirator.	15	15	96	5.2-4.4	15	64	6.15-4.9	1	96	6.15-3.2	14
	15	15				96	6.2-1.3	15			
	15	25	96	5.3-1.4	15	96	5.2-1.9	0	96	5.2-1.6	13
	15	25				96	5.3-1.3	14			

and from 0.7 - 0.95 ml O_2/l in the second experiment, the survival of the snails over a period of 96 hours at both 15° and 25°C did not differ markedly from the controls in which the snails had access to atmospheric air. These results indicate that these species can exist in very nearly anoxic conditions and do not appear to be sensitive to an oxygen lack.

Two aspects merit further consideration in connection with this high survivalship of these species in these anoxic conditions.

(i) Lymnaea natalensis, which does not possess haemoglobin did not appear to be at any disadvantage and showed as high a survivalship as Bulinus africanus and Bulinus tropicus, both possessing haemoglobin. Although it is granted that haemoglobin in the Planorbids (at least in Planorbis corneus) has a high affinity for oxygen and may be of use in the transport of oxygen at low oxygen tensions (Leitch 1916, Borden 1931, Fox 1945, and Zaaizer and Wolwekamp 1958), the possible role that this pigment may have had in the high survivalship of the snails under the conditions of this experiment is considered as insignificant.

(ii) Von Brand et al (1950) and Berg (1952) considered the anaerobic consumption of carbohydrate by snails during exposure to anoxia as an important factor for survival in such adverse conditions. Although this aspect was not investigated in this study, the high survivalship of these species may possibly be ascribed to the incurring of an oxygen debt and the anaerobic metabolism of carbohydrate stores.

During the course of the experiments the snails of all three species which were exposed to low oxygen tensions tended to remain at the junction of the water-nitrogen interface. It is assumed that the animals took Nitrogen into their lungs as they were buoyant. Occasionally a few animals were seen to move to the bottom of the aspirators, only to return to the surface. No distress was seen in the first experiment but in the second experiment distress was evident only in one lot of Lymnaea natalensis at 25°C where eight individuals collapsed and in Bulinus tropicus at 25° where two individuals collapsed. A replicate experiment on Lymnaea natalensis showed no distress. A most confusing and contradictory aspect however, was the distress accompanied by a high mortality rate in Bulinus tropicus in the controls at 15° and 25°C. On repeating this experiment with snails from the same stocks, no distress was seen. No explanation can be given for these confusing results, except that not sufficient replicates were done.

The high survivalship of the snails in anoxic conditions in this series of experiments is in complete contradiction to the results of the preceding experiments. In one instance, if the animals are denied access to a water-gas interface at 25°C, in water which is progressively being depleted of oxygen, extensive mortality results, caused by what seems to be a dire respiratory need. In the other instance, survival was high in the almost total absence of oxygen. The only difference in the experimental design was, that in this case the snails had access to a water gas interface, even though this gas was nitrogen. Unfortunately it was not possible to provide the additional control where snails are exposed to anoxic conditions in the absence of a water-gas interface. In this respect,

the preceding experiments in which the snails were denied access to a water-air interface in non-aerated water at 15° and 25°C, and in which the oxygen tension fell to low levels may be considered as controls.

Provided this is granted, it would seem as if access to a water-gas interface is important in these species at high temperatures in anoxic conditions. Based on the assumption that the animals in this series of experiments took Nitrogen into their mantle cavities as they were buoyant, the presence of this inert gas in the lung would have no respiratory value which suggests that the buoyancy effect due to Nitrogen in the mantle cavities of the animals was important for their survival.

2. Cutaneous respiration under standardized conditions.

The oxygen uptake of aquatic snails in relation to starvation, temperature, weight, and oxygen tension of the water has been investigated by various workers.

Studies by Von Brand et al (1948) on Australorbis glabratus, Helisoma dyryi, Physa gyrina and Physa sp. showed that starvation resulted in a pronounced decrease in the oxygen consumption during the first few days of starvation, this decrease becoming more gradual later on. Berg and Ockelmann (1959), found that starvation during a period of 1 - 24 hours after collecting the animals resulted in a distinct decrease in oxygen consumption in Lymnaea palustris whereas Lymnaea pereger and Physa fontinalis showed only a small decrease.

All the species studied by Von Brand et al (1948) and Berg and Ockelmann (1959) showed an increase in respiratory rate with a gradual increase in temperature. Von Brand found that a temperature of 41°C was lethal to Australorbis glabratus.

Von Brand et al (1948) found that oxygen consumption decreased with increasing size of the snails if referred to unit weight, but remained approximately constant if referred to relative surface area. It should be pointed out that the data of these authors were based on the total weight of the snails (soft parts plus shell), and not on the actively metabolizing soft parts. Berg and Ockelmann (1959) found an increase in oxygen consumption with an increase in live weight (soft parts only) in the species they studied. This increase, however, was subject to seasonal variation which was ascribed to reproductive activity. In previous work Berg et al (1958) showed that the oxygen consumption of Ancylus fluviatililis is greater during the period of reproduction than during other seasons.

The respiratory behaviour of freshwater snails, in relation to the oxygen content of the water, showed

considerable variation in that some species maintained their oxygen consumption at a reasonably steady rate in the face of a declining oxygen supply, whereas other species decreased their oxygen consumption as the oxygen supply diminishes. Von Brand et al (1950), found that Australorbis glabratus was able to maintain an approximately steady rate of oxygen consumption over a range of a partial pressure of oxygen varying from 760 - 13 mm. Hg. Below a tension of 13 mm. Hg. the oxygen consumption of the snails declined rapidly. Zanijer and Wolwekamp (1958), found that Planorbis corneus and Lymnaea stagnalis showed a decreased respiratory rate with a decreasing oxygen tension. Berg and Ockelmann (1959) also found that some pulmonate species for example, Lymnaea auricularia and Physa fontinalis are able to maintain their oxygen consumption with a decreasing oxygen tension to a critical point of oxygen supply, whereas other species such as Lymnaea palustris show a decrease in oxygen consumption in response to a declining oxygen supply.

The aim of the following experiments was to determine the oxygen consumption of these South African pulmonate species in relation to starvation, temperature, live weight and oxygen content of the water. All experiments were conducted in closed respirometers. Prior to an experiment the snails were forced to expel as much of the mantle gas as was possible by stimulation of the foot or pneumostome region. Where experiments were conducted at temperatures other than that of the ambient, the snails were acclimated from the ambient to the experimental temperature by 1°C per day. During this period the animals were fed. In order to minimise any possible toxic effects due to accumulation of waste products from the animals in the respirometers, all experiments were of 30 minute duration. The procedure of Berg and Ockelmann (1959) was adopted whereby the oxygen uptake of the animals is expressed in terms of the actively metabolizing soft

parts (live weight) of the animals. After completion of an experiment, the snails were dried thoroughly on filter paper, weighed and killed in boiling water. The soft parts were extracted and the shells dried and weighed. The difference between the two weights gives the live weights of the animals.

(a) Oxygen consumption in relation to starvation.

One snail of each species were kept in separate beakers in waterbaths at 15° and 25°C. The animals were given no food and their oxygen consumption was measured at 24 hour intervals over a period of six days. The snails were weighed before and after completion of the experiment. The loss of weight during the six day starvation period varied from 1 - 2% in all three species. Controls were set up in which the animals were fed.

The results are shown in Figure 2. All three species showed a gradual decrease in oxygen consumption during the starvation period of six days. This decrease was not very marked but was more pronounced at 25°C than at 15°C. The results of this experiment are in agreement with the experimental findings for other pulmonate species as found by Von Brand et al (1948) and Berg and Ockelmann (1959).

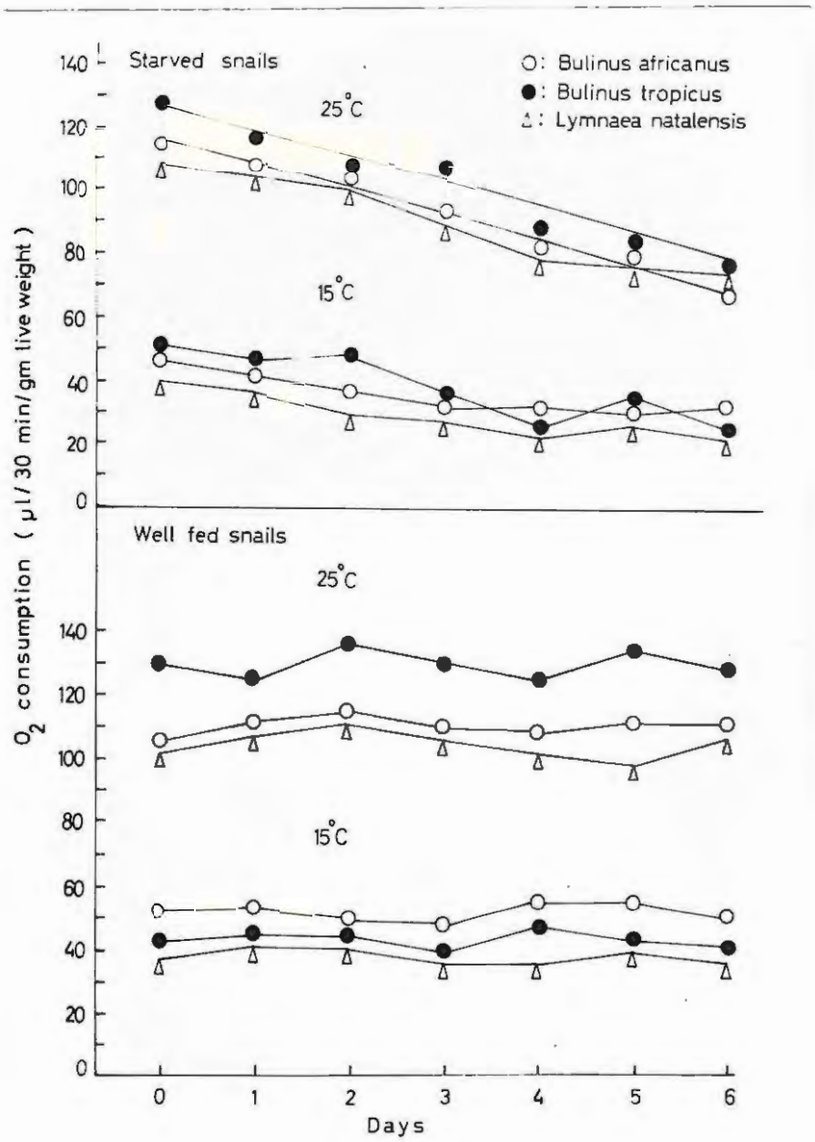

Figure 2: Oxygen consumption of starved snails as compared to oxygen consumption of well fed snails.

N.B. The symbols used to designate the different snail species will be used in all the following figures.

(b) Oxygen consumption in relation to temperature.

The influence of temperature on the respiratory rate of the snails was investigated over the range of 15° - 35°C. Two individual snails from each species were used in this experiment, These results are shown in Figure 3.

All three species showed a marked increase in oxygen consumption with an increase in temperature. This increase was very pronounced in the case of Bulinus tropicus and Bulinus africanus. The temperature coefficients (Q_{10}) over the range of 15° - 25°C of the increase in metabolic rate for Bulinus africanus, Bulinus tropicus and Lymnaea natalensis are 3.0, 2.4 and 2.9 respectively, which are within the normal limits. The maximum temperature which these snails were able to tolerate ~~were~~ was 30°C for Lymnaea natalensis and 37.5° for Bulinus africanus and Bulinus tropicus. Above these respective temperatures the animals appeared to be adversely affected as shown by the decrease in their oxygen uptake. The temperature relationships of these species are thus in agreement with the experimental findings of other pulmonate species studied by Von Brand et al (1948) and Berg and Ockelmann (1959).

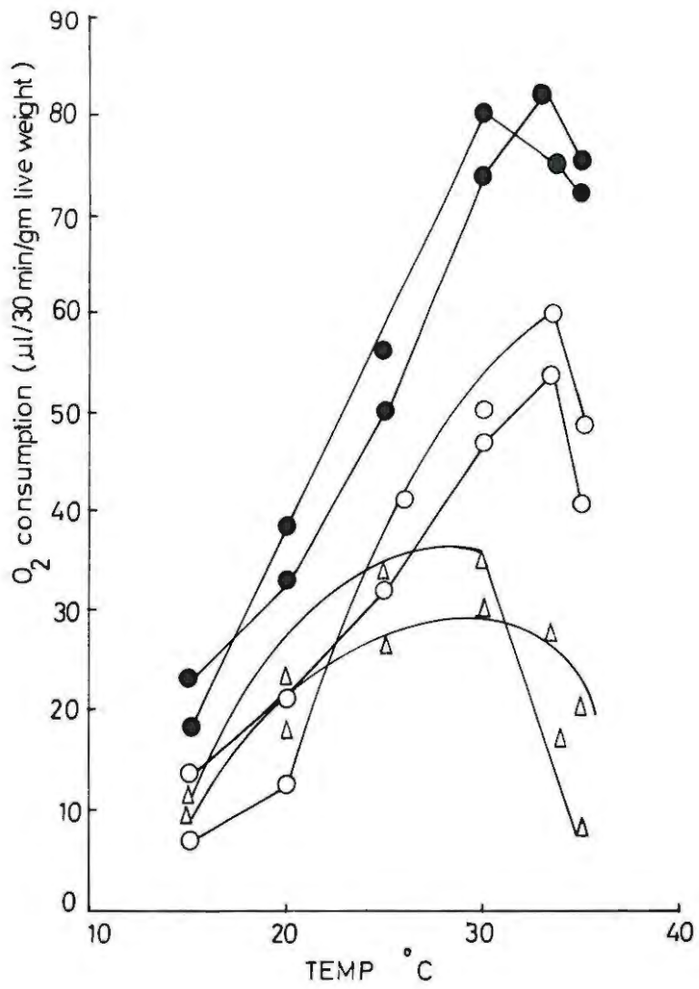

Figure 3: Oxygen consumption in relation to temperature.

(c) Oxygen consumption in relation to live weight.

Snails of different sizes were used in this experiment and their oxygen consumption was measured at 15° and 25°C. The results are shown in Figure 4.

All three species showed an increase in oxygen consumption with an increase in live weight at both temperature levels. No attempt was made to correlate the oxygen consumption of the animals with their surface area, as Perlowagora-Szumlewicz and Von Brand (1958) found that the formula $W\frac{2}{3}$ (W = weight of snail) for determining relative surface is not sufficiently accurate. These authors point out that the weight of the metabolically inert shells of snails of different sizes could vary significantly, and thus result in erroneous calculations. This point is illustrated in Figure 5, which shows graphically the variation in the relationship between the total weight of the snails and the weight of the shells of the animals used in this experiment.

The results of this experiment confirm those found by Berg and Ockelmann (1959) for the relationship between oxygen consumption and live weight.

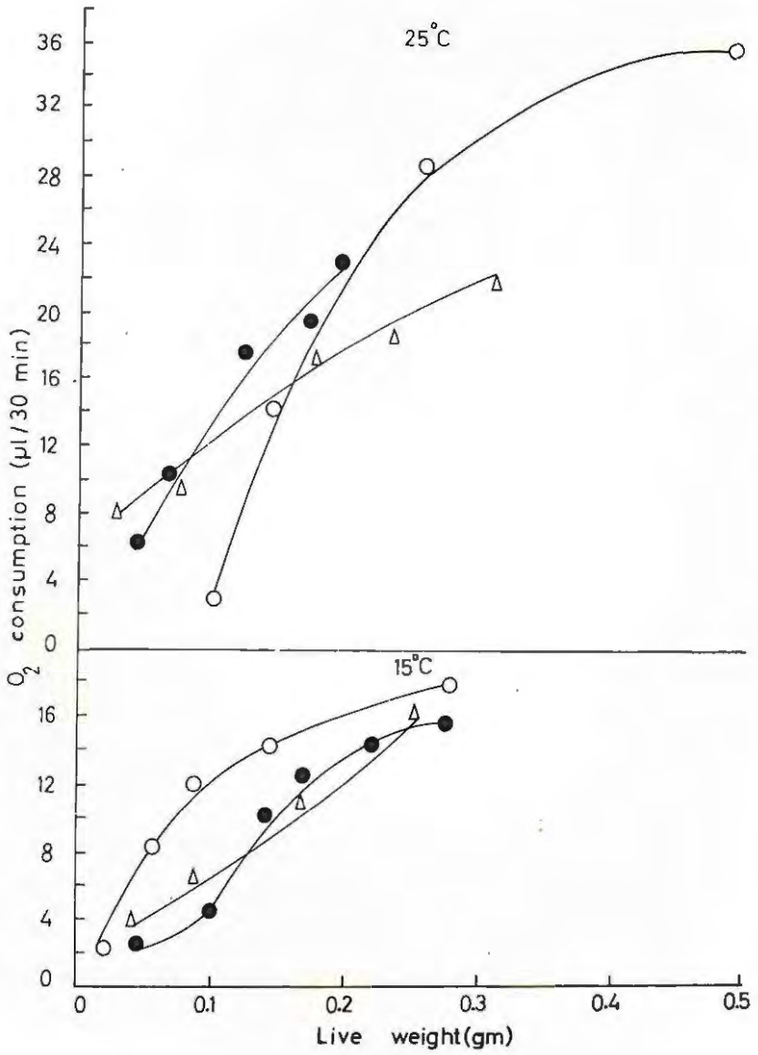

Figure 4: Oxygen consumption in relation to live weight.

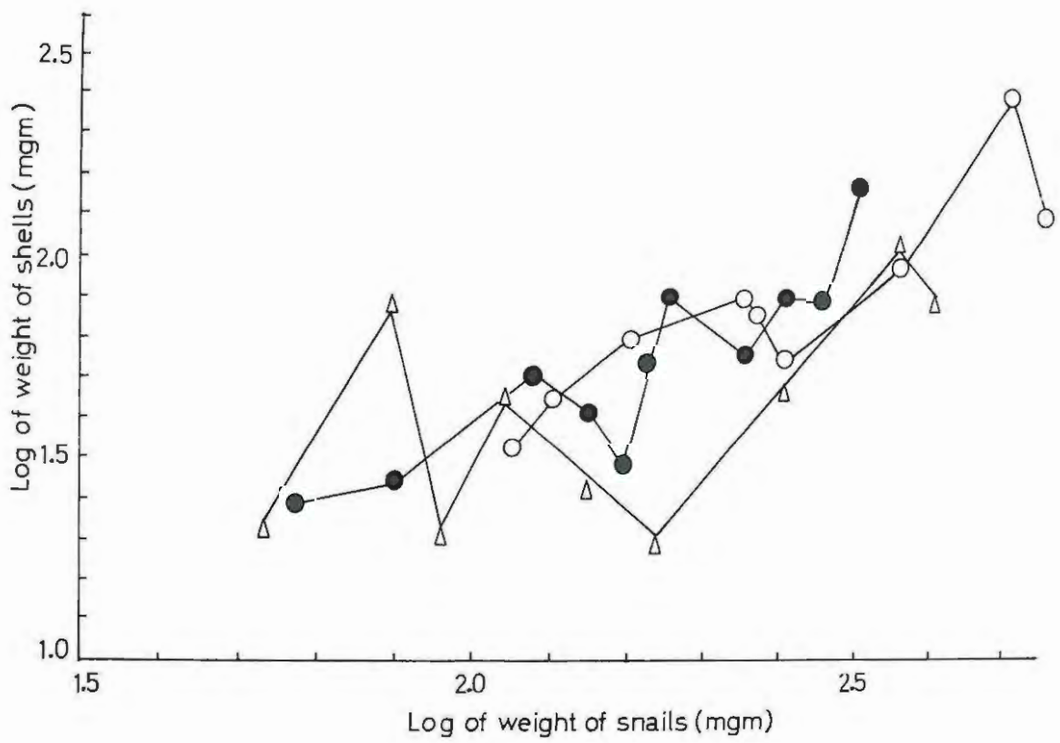

Figure 5: Relationship between the total weight of snails and the weight of the shells.

(d) Oxygen consumption in relation to oxygen content of the water.

Experiments were carried out at temperatures of 15°, 20° and 25°C. One snail of each species was used for the experiments at 15° and 20°C and a separate lot of individual snails was used for the experiment at 25°C. Water containing different oxygen saturation levels were prepared by bubbling nitrogen through for various lengths of time. For the purposes of this experiment the oxygen concentration in well aerated water at the different temperatures was taken as 100% saturation. The results are shown in Figure 6 which show that these species decrease their oxygen consumption as the oxygen supply diminishes. At 15° and 20°C the decrease was gradual, whereas, at 25° it was much steeper. Bulinus africanus, Bulinus tropicus and Lymnaea natalensis are thus oxygen dependent, as they do not maintain a steady rate of respiration as the oxygen content of the water diminishes. There is clearly a considerable variation in this aspect of respiratory physiology as far as freshwater pulmonates are concerned, and these results are in agreement with those of other workers in this field.

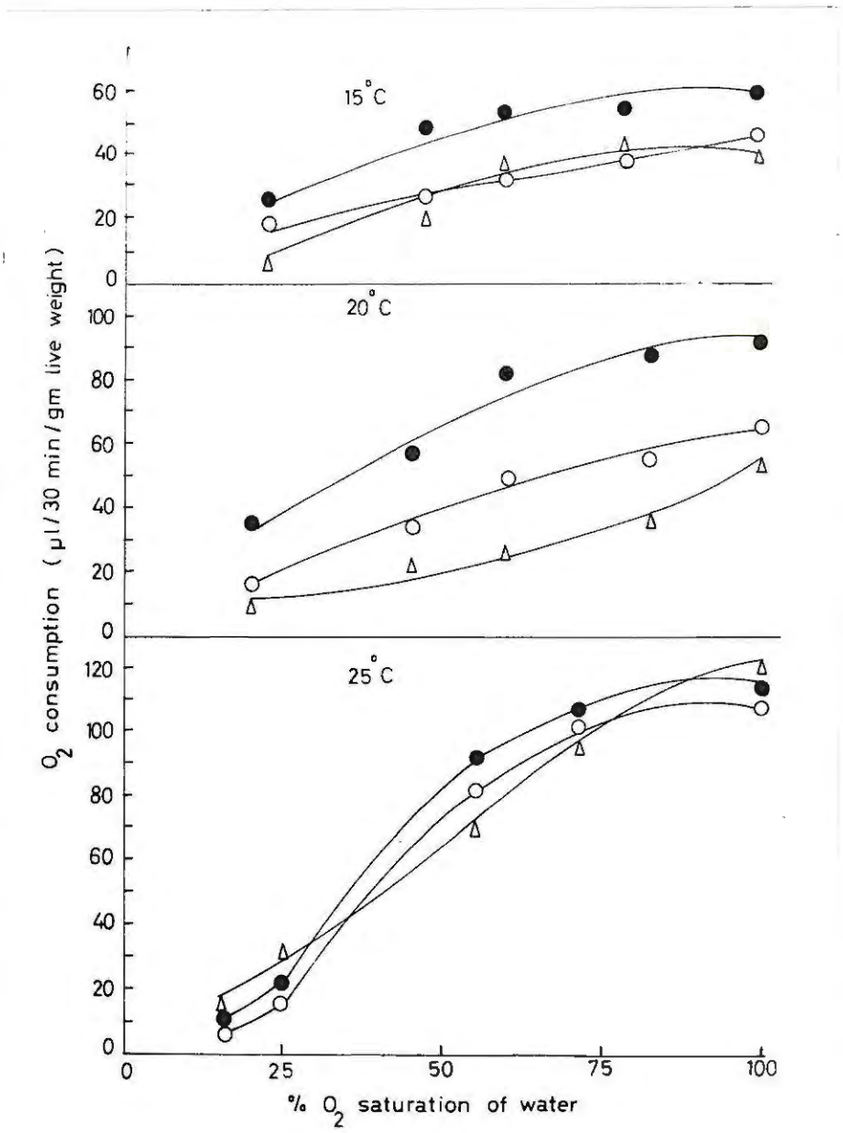

Figure 6: Oxygen consumption in relation to oxygen content of the water at 15°, 20° and 25°C.

3. PULMONARY RESPIRATION

(a) Anatomy and histology of the respiratory organs:

A knowledge of the anatomy and histology of the respiratory organs is essential in order to assess the physiological significance of the mantle cavity in all three species and of the pseudobranch in the Planorbids. Fresh material was used for the dissections. The snails were anaesthetized by a mixture of menthol and chloral hydrate (Personal communication from Professor J. A. van Eeden, Zoology Department, Potchefstroom University). For the histology of the mantle and pseudobranch, the material was fixed in Bouin (Pantin, 1960). All sections were cut 6μ in thickness and stained with Mallory's triple stain (Pantin 1960).

The Planorbid species Bulinus africanus and Bulinus tropicus differ from Lymnaea natalensis in having certain organs on the left side (sinistral) these organs in Lymnaea natalensis being on the right side (dextral). The breathing organ is the lung or mantle cavity. An extension of the mantle, the pneumostome provides a passage for atmospheric air to reach the mantle cavity by forming a short temporary siphon which is protruded through the surface film of the water. In addition, Bulinus africanus and Bulinus tropicus possess an accessory gill, the pseudobranch. This structure is absent from Lymnaea natalensis. The arrangement and relationships of the pneumostome region of the Planorbids and Lymnaeids in general are depicted diagrammatically in Figure 7(a) and (b) respectively. (For the purposes of these diagrams the shells of the animals were removed).

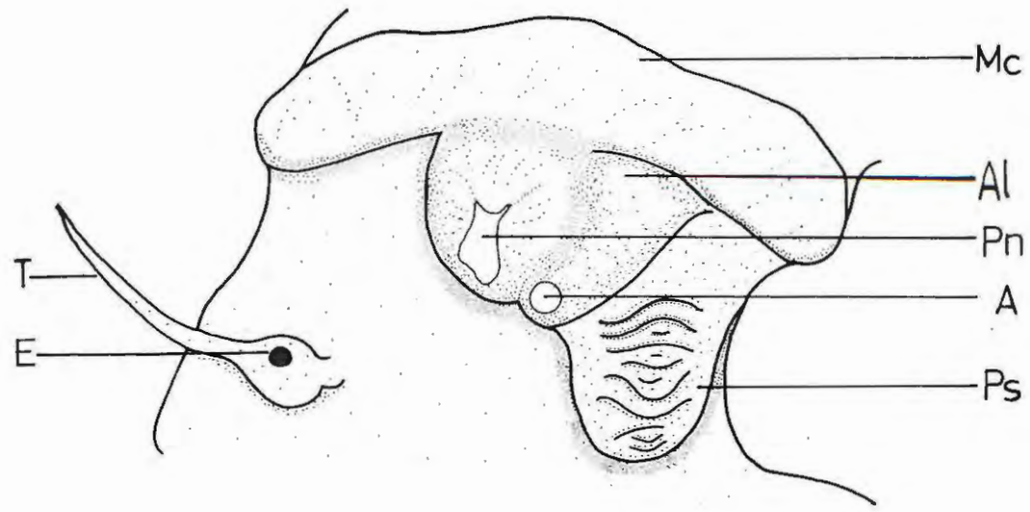

Figure 7(a): Diagram showing dorsal view of the pneumostome region of Bulinus africanus.

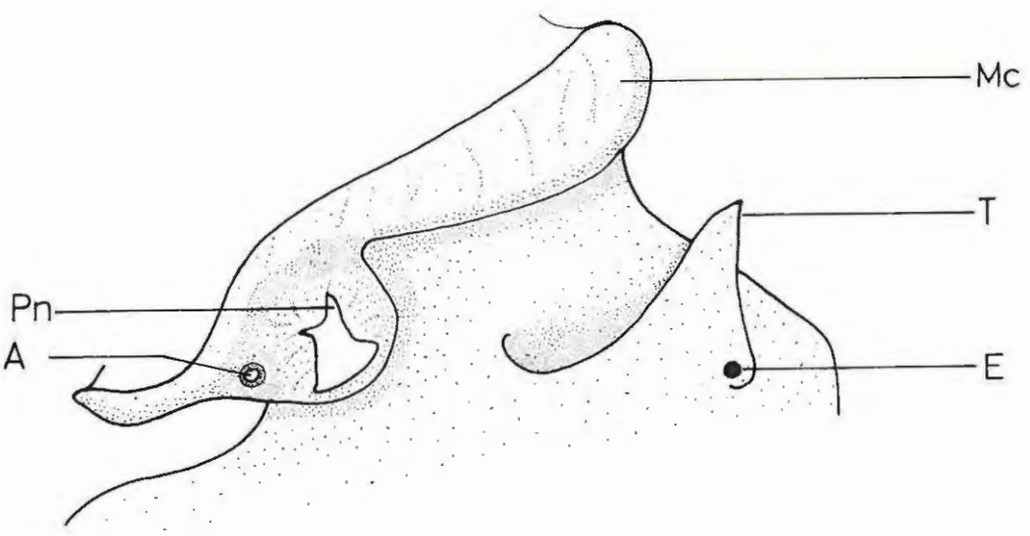

Figure 7(b): Diagram showing dorsal view of the pneumostome region of Lymnaea natalensis

Legend for Figures 7(a) and (b)

A : anus
Al: anal lobe
E : eye
Mc: mantle collar
Pn: pneumostome
Ps: pseudobranch
T : tentacle.

The Mantle

As the morphology of the mantle of Bulinus africanus and Bulinus tropicus is essentially similar, only the mantle of Bulinus africanus and that of Lymnaea natalensis will be described.

The mantle is a thin membranous structure, deeply pigmented in patches in all three species. By the injection of Methyl blue into the pedal sinuses of the snails, the bloodvessels of the mantle could be clearly distinguished and the direction of the bloodflow followed. Figures 8 and 9 show the ventral surface of the mantle with attendant structures of Bulinus africanus and Lymnaea natalensis respectively.

The kidney is a conspicuous organ bisecting the mantle roof and is well developed in these species.

The use of the above mentioned injection technique revealed a distinct difference in the vascular arrangement of the mantle between the Planorbids and Lymnaeids.

In Bulinus africanus six pronounced blood-vessels are present, namely, a lateral, and a medial rectal bloodvessel running along the intestine, a bloodvessel which follows the intermediate mantle ridge, a vessel running within the mantle collar, a renal vein, and, a pulmonary vein, both lying alongside the kidney, the latter flowing directly into the atrium of the heart. No bloodvessels were evident in the mantle roof anterior to the kidney. On the other hand, in Lymnaea natalensis only one bloodvessel running along the mantle roof was seen. Histological sections of the mantle of this species did not reveal any additional bloodvessels.

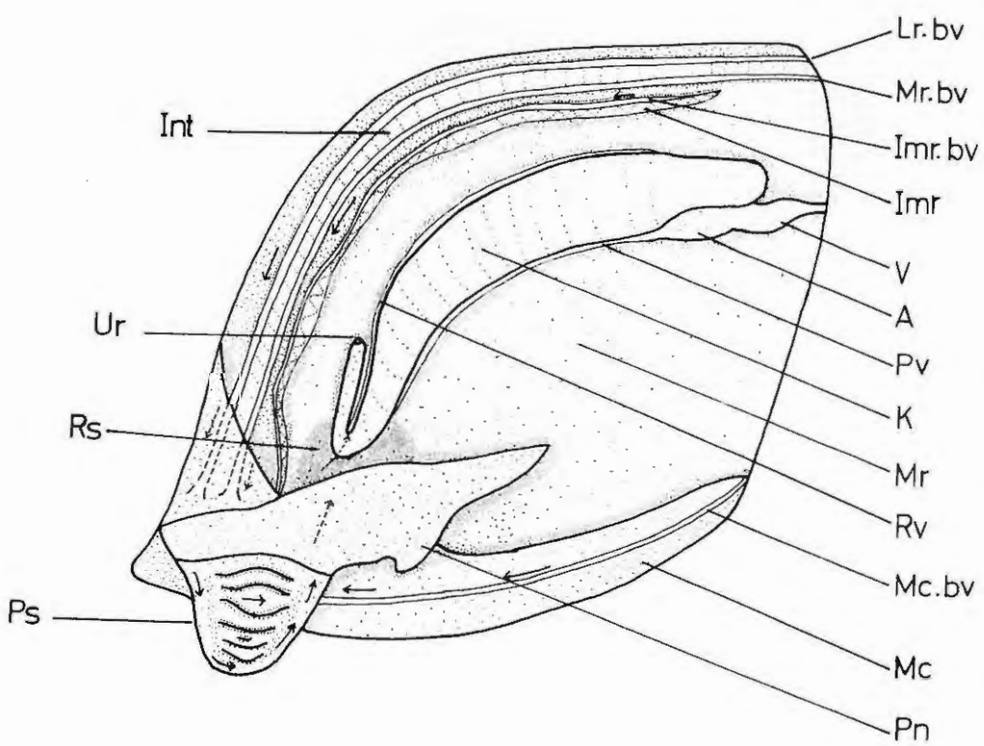

Figure 8: Diagram depicting the ventral surface of the mantle complex of Bulinus africanus

A: Atrium of heart
Int: Intestine
Imr: Intermediate mantle ridge
Imr-bv: Intermediate mantle ridge bloodvessel
K: Kidney
Lr.bv: Lateral rectal bloodvessel
Mc: Mantle collar
Mc.bv: Mantle collar bloodvessel
Mr: Mantle roof
Mr.bv: Medial rectal bloodvessel
Pn: Pneumostome
Ps: Pseudobranch
Pv: Pulmonary vein
Rs: Renal sinus
Rv: Renal vein
Ur: Ureter
V: Ventricle of heart.

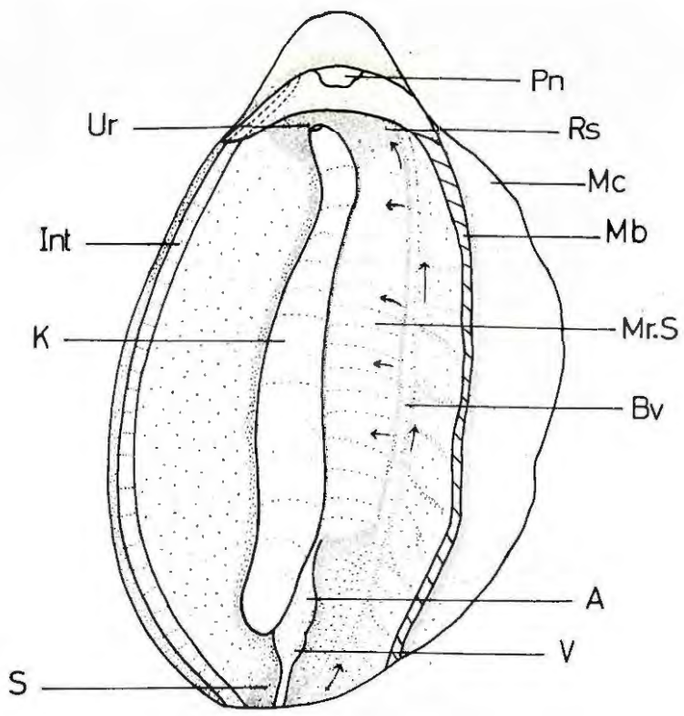

Figure 9: Diagram depicting the ventral surface of the mantle complex of Lymnaea natalensis

A: Atrium of heart
Bv: Bloodvessel in mantle roof
Int: Intestine
K: Kidney
Mb: Mantle border (cut)
Mc: Mantle collar
Mr.S: Mantle roof sinus
Pn: Pneumostome
Rs: Renal sinus
S: Sinus
Ur: Ureter
V: Ventricle of heart.

It appears that the pseudobranch in the Planorbids is supplied by blood from the visceral mass flowing along the lateral and medial rectal bloodvessels and by blood, possibly from the mantle itself, flowing within the mantle collar and intermediate mantle ridge vessels. Unfortunately, the exact course followed by the blood entering the afferent pseudobranch vessel could not be determined. The efferent drainage of the pseudobranch appears to be related to a vascular area of the mantle which is closely associated with the distal region of the kidney or renal sinus, and from where blood is carried to the renal vein. From this vessel blood flows through the kidney into the pulmonary vein and thence to the heart. This confirms the conclusion of Stiglingh et al (1962) for the efferent drainage of the pseudobranch in Bulinus tropicus

In Lymnaea natalensis the flow of blood is different. A renal sinus is present as is also a sinus in the region of the heart, (S - figure 9), which appears to be closely associated with the visceral mass. The mantle roof anterior to the kidney consists of a brownish coloured vascular area, termed the mantle roof sinus (Mr.S - figure 9). It seems as if blood flows from the visceral mass through the mantle roof vessel into the mantle roof sinus and also into the renal sinus. The entire kidney seems to be an efferent drainage system for taking blood back to the heart and it also appeared that blood may flow more directly from the mantle roof vessel via the mantle roof sinus into the heart. The area of the mantle posterior to the kidney appears to be devoid of any bloodvessels or vascular spaces.

Histologically the mantle of both the Planorbids and Lymnaeids consist of epithelial cells, smooth muscle and connective tissue. The natural relations of the various tissue elements are most readily understood by referring to Plates 5 and 6 which represent typical transverse sections through the mantle roof anterior to the kidney, of Bulinus africanus and Lymnaea natalensis respectively. The outer surface of the mantle consists of a layer of pigment cells. Immediately beneath this is a layer of smooth muscle followed by connective tissue. In Bulinus africanus the smooth muscle layer is not as well defined as in Lymnaea natalensis. The inner surface is an extremely thin single layer of epithelial cells. It is clear that there is a distinct difference between the mantle roofs of these species. Whereas, in Bulinus africanus the connective tissue elements are densely packed, with relatively few sinuses, or vascular spaces, Lymnaea natalensis shows a highly vascular area with numerous large sinuses, which is equated with the mantle roof sinus. This implies that in Bulinus africanus the blood flow through the mantle is not as extensive as that in Lymnaea natalensis.

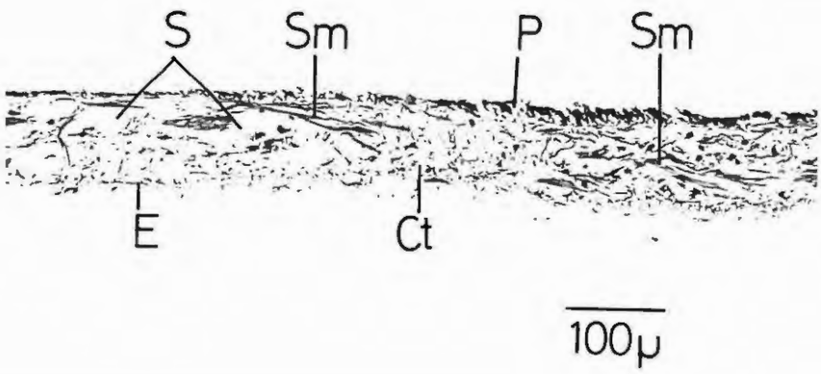

Plate 5: Transverse section through mantleroof (Mr.) of Bulinus africanus

Ct: Connective tissue
E: Epithelium
P: Layer of pigment cells
S: Sinus
Sm: Smooth muscle

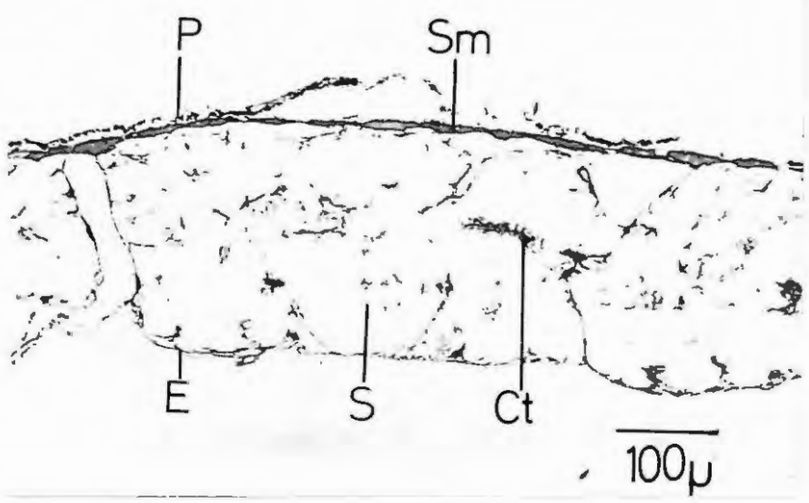

Plate 6: Transverse section through mantle roof sinus (Mr.S) of Lymnaea natalensis.

Ct: Connective tissue
E: Epithelium
P: Layer of pigment cells
S: Sinus
Sm: Smooth muscle

The Pseudobranch

The pseudobranch of Bulinus africanus and Bulinus tropicus is a U-shaped structure (Figure 10). It consists of a number of transverse primary folds protruding on both the ventral and dorsal surfaces. Each primary fold is thrown into smaller transverse secondary folds, an arrangement which consequently greatly increases the total surface area. Histologically the pseudobranch consists of connective tissue forming large sinuses or vascular spaces which are bounded on the outer surface by a single layer of cuboidal epithelium cells. Plate 7 (a) and (b) show typical transverse and longitudinal sections through the pseudobranch of Bulinus africanus. The histological appearance of the pseudobranch of Bulinus tropicus is essentially similar.

The pseudobranch itself has a separate afferent and efferent bloodsupply. From the afferent bloodvessel, the blood flows through the large sinuses and thence into the efferent bloodvessel from where it goes to the heart as described previously.

Examination of newly hatched and adult Bulinus africanus and Bulinus tropicus showed that the external surface of the pseudobranch is ciliated. By means of carmine particles it was found that in the living animal the actively beating cilia create currents of flow around the pseudobranch and in the pseudobranchial chamber. Although these currents were not very well organised, inhalent and exhalent currents could be distinguished which appeared to run counter to the flow of blood through the pseudobranch. Even this rather ill defined counter-current mechanism would greatly increase the efficiency of the pseudobranch in obtaining

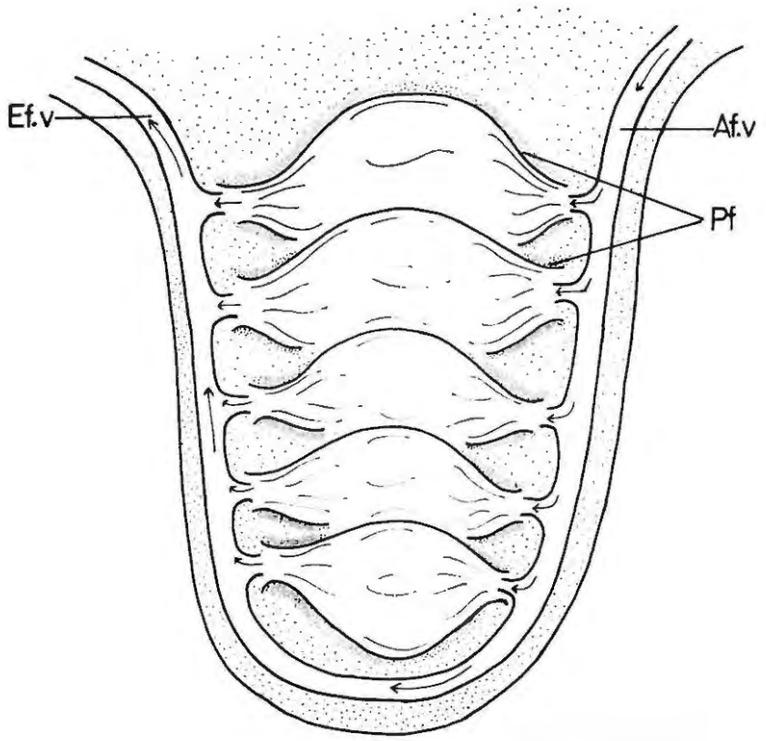

Figure 10: Diagrammatic representation of the dorsal aspect of the pseudobranch of Bulinus africanus

Af.v: Afferent pseudobranch bloodvessel
Ef.v: Efferent pseudobranch bloodvessel
Pf: Primary folds.

The direction of blood flow is indicated by arrows.

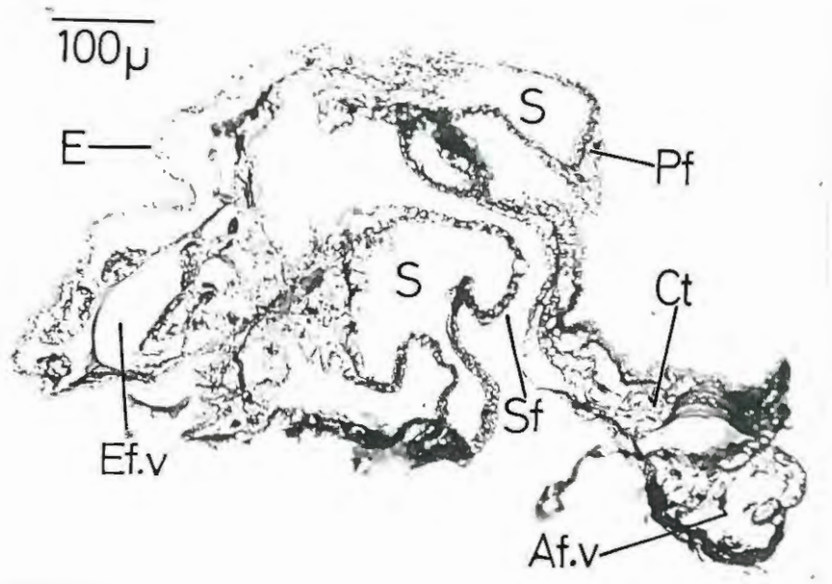

Plate 7(a): Transverse section through pseudo-branch of Bulinus africanus.

Af.v: Afferent bloodvessel
Ct: Connective tissue
E: Epithelium
Ef.v: Efferent bloodvessel
Pf: Primary fold
S: Sinus
Sf: Secondary fold

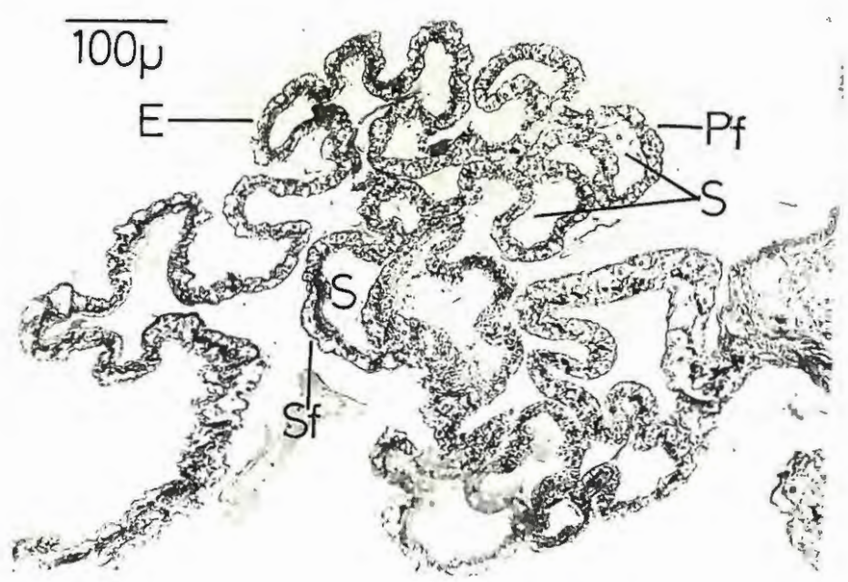

Plate 7(b): Longitudinal section through pseudo-branch of Bulinus africanus

E: Epithelium
Pf: Primary fold
S: Sinus
Sf: Secondary fold

oxygen from the surrounding water.

It should be pointed out that although the pseudobranch is ciliated, no cilia were seen in the histological sections. This could be due to the preparation methods.

(b) Oxygen uptake from the air bubble in the mantle cavity.

The presence of air in the mantle cavity of freshwater pulmonates has prompted the suggestion that the bubble may function as a physical gill as in some aquatic insects. Ege (1918) showed that some species of aquatic insects (Dytiscidae, Corixidae, Hydrophilidae and Notonectidae) carry air stores in the form of bubbles on their body surfaces by means of which they are able to obtain some of their oxygen requirements when submerged, by diffusion across the air water interface of the exposed bubble surface. These bubbles, however, decrease progressively in size due to the outward diffusion of nitrogen and eventually the exposed surface becomes inadequate for the efficient diffusion of oxygen into the bubble. The insect is consequently forced to return to the surface to renew its air supply. In view of this, Crisp and Thorpe (1948) and Thorpe (1950) point out that the functional efficiency of these bubbles as physical gills is limited and are more accurately described as air stores of diminishing volume.

Very elegant work by Thorpe and Crisp (1947) has shown that some aquatic Hemiptera and Coleoptera species have overcome this difficulty of a progressive decrease in the size of their air supplies by holding an extremely thin layer of air of negligible volume but large surface area, on their body surfaces by means of densely packed hydrofuge hairs. The physical forces involved can maintain this air film

at a constant volume. This type of air supply is known as a plastron and as its volume is negligible but constant, it need not be renewed at intervals, and functions solely as a physical gill whereby a constant flow of oxygen from the surrounding water to the respiratory system of the animal can be maintained. Provided the water is well aerated, insects possessing a plastron can remain submerged indefinitely and thus become completely independent of atmospheric air.

The evidence that the air bubble in the mantle cavities of freshwater pulmonates functions as a physical gill is contradictory.

Hunter (1953 a and b) stated that the air bubble may or may not be used as a physical gill. Hunter (1953 a) noted that Lymnaea peregra were able to live submerged for long periods at water temperatures below 12°C, obtaining their oxygen requirements by cutaneous respiration and by gas exchange via the air bubble in the mantle cavity. To quote Hunter (cf. page 87) "it is sufficient to state here that the bubble in the mantle cavity is used as a physical gill by those individuals of Lymnaea peregra which live in these populations on the Loch shore". In subsequent studies on Lymnaea peregra and Physa fontinalis, Hunter (1953 b) observed that these species were living continuously submerged in the shallows of Loch Lomond, with either a gas bubble, or water in the mantle cavity, whereas other members of the same population made regular visits to the surface to breathe. Analysis of the gas bubbles from the snails which were living continuously submerged varied, as some contained a high percentage of carbon dioxide (1.6% - 2.3%), which led him to conclude that it was most unlikely that such bubbles could function efficiently as a physical

gill while retaining such a high concentration of carbon dioxide.

Hunter's inferences, with regard to physical gill function are open to criticism and not convincing. It seems that he relied mainly on observations in the field with no carefully controlled experimental evidence to substantiate his viewpoints.

Henderson (1963), approached this aspect of physical gill funtion from a purely experimental point of view by determining the change in the volume of the mantle gas in Lymnaea stagnalis and Planorbarius (Planorbis) corneus as reflected by the gradual increase in the underwater weight of the snails after the animals had surfaced and dived. This implied that the volume of gas was decreasing. Henderson concluded from his experimental findings that the gas in the mantle cavity of these two pulmonate species was functioning as an air store of constant pressure but steadily decreasing volume and not as a physical gill in the sense defined by Crisp and Thorpe (1948).

In order to determine if the air bubble in the mantle cavities of Bulinus africanus, Bulinus tropicus and Lymnaea natalensis function as a physical gill or as an air store, a more direct approach was adopted by analysing the mantle gas tonometrically.

The air in the mantle cavities of the snails was expelled, and the animals were allowed to surface into atmospheric air. After they had breathed and dived into water at 20°C containing 5.6 - 6.2 ml O_2/l they were trapped under gauze, and the gas bubbles expelled at regular intervals of time for analysis.

Except in two instances (Figure 11) attempts to determine the oxygen concentration in the gas bubbles from individual snails at intervals during a time period were unsuccessful, most probably because the mantle gas volume is easily upset by repetitive handling of the snails as reported by Henderson (1963), and therefore the results reported here give an overall picture of gas bubble composition from different snails at different times.

As it was not possible to carry out these analyses involving atmospheric air all at the same time, at every sampling of a gas bubble from the mantle cavity the air was sampled as well. The variation of the oxygen content in the atmosphere at varying temperature and barometric pressure is shown above the utilization curves in Figures 11 and 12, which show the results from two different stocks of snails.

In all three species the oxygen concentration in the bubbles decreased with time, implying that the snails were using the oxygen for respiratory purposes and/or that oxygen was diffusing from the bubbles into the surrounding water. In view of the fact that the partial pressure of dissolved oxygen in the water was approximately 150 mm. Hg. the loss of oxygen from the bubble to the surrounding water by diffusion was considered insignificant. Henderson (1963) found that the greatest increase in the underwater weight was during the first two hours after surfacing, which implied that the gas volume in the mantle cavity was decreasing due to the utilization of the oxygen in the bubble for respiratory purposes. From Figures 11 and 12 the greatest reduction in gaseous oxygen occurred during the first 1 - 2 hours which appears to be the reciprocal

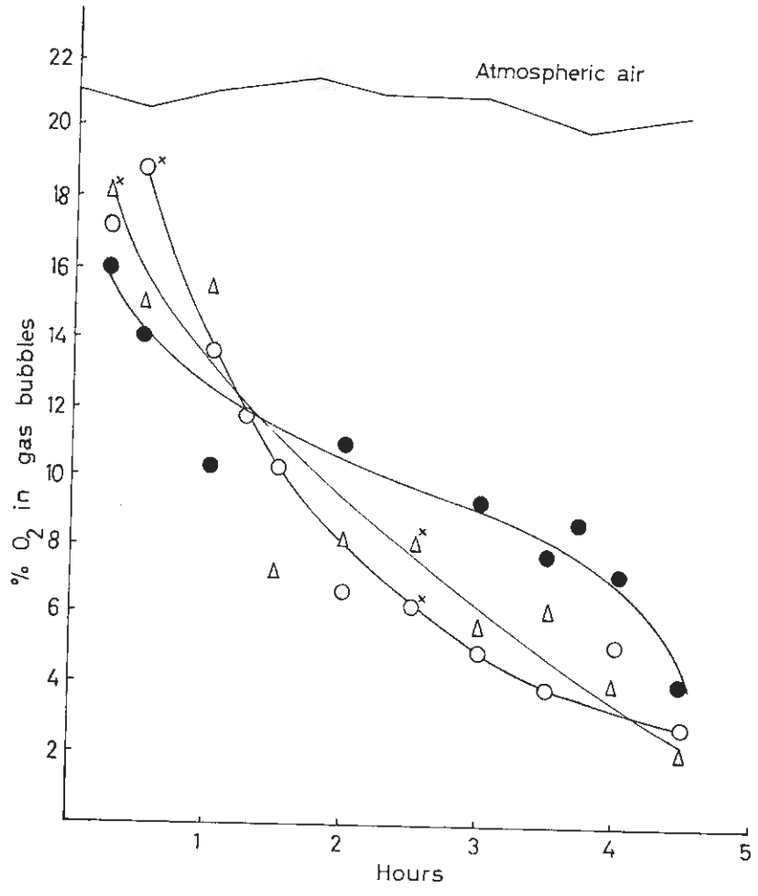

Figure 11: Effect of time on the oxygen concentration of the gas bubbles expelled from the mantle cavities of the snails.

(x - denotes the same snail of Bulinus africanus and of Lymnaea natalensis)

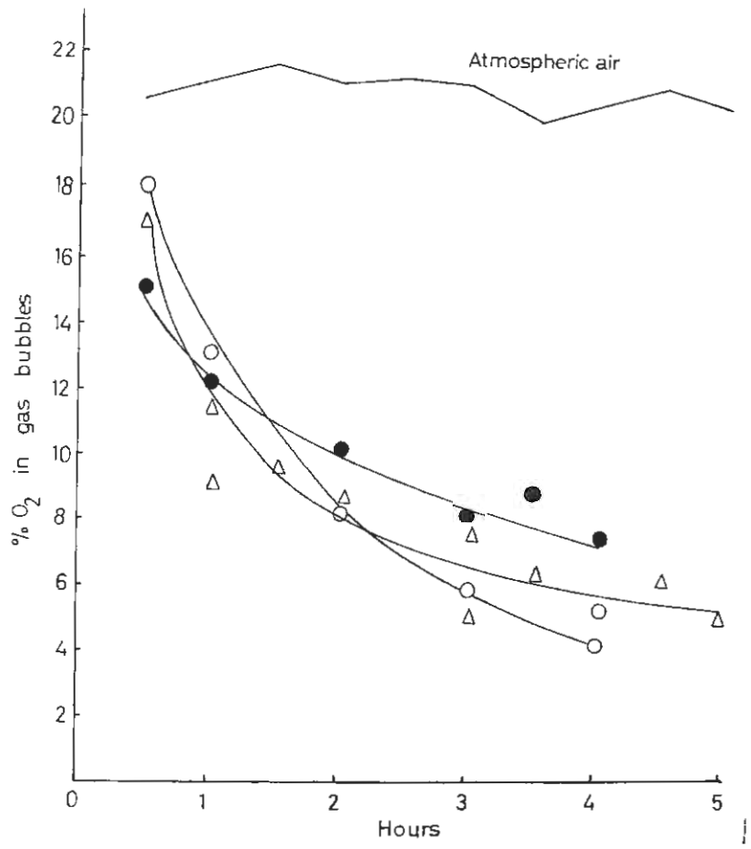

Figure 12: Effect of time on the oxygen concentration of the gas bubbles expelled from the mantle cavities of the snails.

of the increase in underwater weight of snails as shown by Henderson (1963). This reciprocal relationship is illustrated in Figure 13, which compares the decrease in oxygen tension of the bubbles from the mantle cavities of these three snail species (vide figure 12) with the increase in underwater weight in Planorbis corneus and Lymnaea stagnalis (Henderson 1963) over a period of four hours.

The progressive decrease, in the oxygen concentration in the mantle gas from all three species clearly implies that the bubbles were decreasing in size. These results suggest that the bubbles are functioning as air stores of diminishing volume and not as physical gills in the strict sense of the word.

In order to provide more conclusive evidence, the snails were allowed to surface into nitrogen, before diving into water at 20°C containing 5.6 - 5.9 ml O_2/l. The nitrogen bubbles were expelled at intervals to determine their composition. Although most of the snails behaved as if they were surfacing into atmospheric air, some individuals from all three species were very reluctant to open their lungs in nitrogen. A control was provided by introducing nitrogen bubbles of 0.2 ml. under a funnel under water and these were analysed at various intervals to show the increase in oxygen. The chosen volume of 0.2 ml is based on an approximate estimate that the snails take in 0.2 ml of gas into their lungs when surfacing (vide page 74). It is appreciated that this type of control is not necessarily good technique, but under the conditions of the experiment I have taken the liberty of adopting this procedure in order to obtain a reasonable approximation to an exposed physical gill.

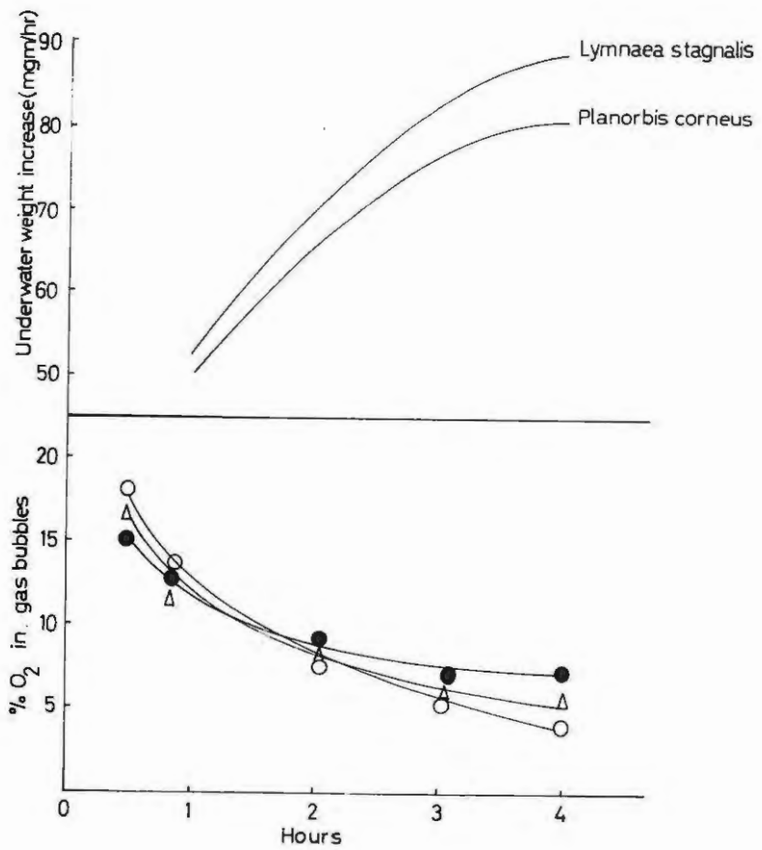

Figure 13: Showing reciprocal relationship between the decrease in oxygen tension of the mantle gas of Bulinus africanus, Bulinus tropicus and Lymnaea natalensis and the increase in underwater weight of Planorbis corneus and Lymnaea stagnalis, the latter information taken from Henderson (1963).

Figure 14 shows these results. The oxygen concentration in the nitrogen bubbles exposed to the surrounding water under the funnel increased rapidly as is to be expected, whereas the rate of increase in the oxygen concentration in the nitrogen bubbles expelled from the snails was considerably slower. The rate of diffusion of the respiratory gases would be directly proportional to the area of interface. As the pneumosome, when visible was usually closed except on a few occasions when it appeared as a fine slit, the interface at the pneumostome was very small. This fact is borne out by the difference in the rate of increase in oxygen between the nitrogen bubbles in the mantle cavities of the snails and the nitrogen bubbles exposed to the surrounding water under the funnel.

This slow rate of diffusion of oxygen into the nitrogen bubbles in the mantle cavities of the snails is certainly not indicative of a physical gill, as Thorpe and Crisp (1947) and Crisp and Thorpe (1948) have pointed out that a prerequisite for efficient physical gill function is an air bubble or film of constant volume with a large surface area exposed to the surrounding water. Nevertheless, oxygen from the surrounding water did diffuse into the nitrogen bubbles in the mantle cavities of the animals. This increase was considered to be mainly due to diffusion of oxygen through the cutaneous tissues as the interface at the pneumostome was very small. The subsequent decrease in the oxygen concentration in the bubbles suggest that the animals were using the oxygen for respiratory purposes.

In view of the results of this and the previous experiment the conclusion is reached that the air bubble in the mantle cavities of Bulinus africanus, Bulinus tropicus and Lymnaea natalensis

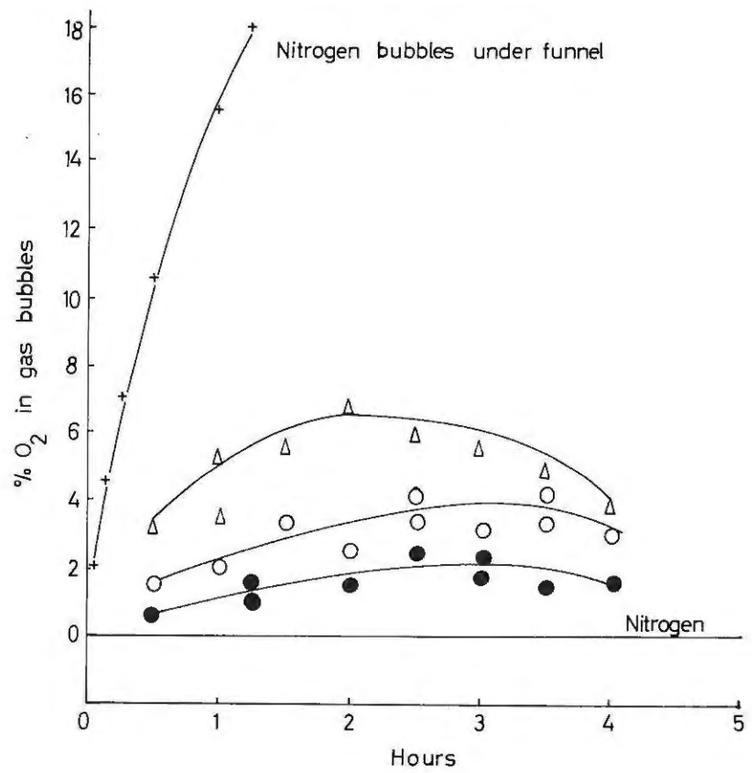

Figure 14: Comparison between the increase in oxygen concentration in nitrogen bubbles from the mantle cavities of the snails and in nitrogen bubbles under water.

functions as an air store of diminishing volume and not as a physical gill.

Although the exact volumes of air taken into the mantle cavities of these species could not be accurately determined, tentative attempts were made to obtain a reasonable assessment of these volumes, by measuring the volume of air expelled by snails immediately after surfacing. These volumes varied from 0.05 - 0.25 ml. Assuming that the volume of air taken into the mantle cavity is of the order of 0.2 ml, the volume of oxygen in the air bubble immediately after surfacing into atmospheric air is 0.042 ml. (42µl). From figures 11 and 12 the percentage oxygen in the air bubbles decreased from 21% to values varying between 8% and 2% over a period of 4 to 4½ hours. If the value of 4% is taken as the final figure, then based on the above assumption the snails consumed 7.5µl oxygen/hour from the air bubble.

From figure 3 the cutaneous oxygen uptake of these species varies from 40 - 60µl /hour/gm live weight at 20°C. Assuming that the pulmonary oxygen consumption is 7.5 µl/hour at 20°C, it is estimated that pulmonary oxygen uptake constitutes approximately 12 - 20% of the cutaneous oxygen consumption at 20°C per hour per unit weight.

(c) Relation between structure and function of the mantle and pseudobranch.

Although the preceding experiments have shown that the mantle gas in all three species has a respiratory function its contribution to possible requirements is low. This appears to be borne out histologically in Bulinus africanus and Bulinus tropicus where the structure of the mantle roof (Mr - Plate 5) does not suggest an effective absorptive surface for oxygen. By contrast the vascular

mantle roof sinus (Mr.S - Plate 6) of Lymnaea natalensis suggests that in this species the diffusion of oxygen from the mantle gas is relatively more efficient. In view of this it is reasonable to assume that the bloodflow through the mantle roof of Bulinus africanus and Bulinus tropicus per unit time is less than that in Lymnaea natalensis, and consequently the volume of oxygen which can diffuse from the mantle gas into the blood is correspondingly less in the two Planorbid species than in Lymnaea natalensis. With a more efficient system for the diffusion of oxygen from the mantle gas, Lymnaea natalensis may compensate for the absence of an accessory gill and possibly a respiratory pigment.

Based on the histological structure of the mantle in these species, it seems as if the function of the mantle cavity as a lung may be more important in the Lymnaeids than in the Planorbids. This statement substantiates the conclusions reached by Jones (1961) that the lung is relatively more important in Lymnaea stagnalis than in Planorbis corneus. In very elegant experiments in which the pulmonary and cutaneous oxygen uptake of these snails were determined separately but simultaneously, Jones found that pulmonary uptake exceeded cutaneous uptake at dissolved oxygen partial pressures below 140 mm in Lymnaea stagnalis, but only below 40 mm in Planorbis corneus. On the other hand, cutaneous uptake exceeded pulmonary uptake above partial pressures of dissolved oxygen of 150 mm in Planorbis corneus but only above 190 mm in Lymnaea stagnalis. Furthermore, Jones found that the values for the percentage of total oxygen uptake which is pulmonary were higher for Lymnaea stagnalis than for Planorbis corneus at all values of dissolved oxygen tensions. These results clearly indicate that Lymnaea stagnalis relies on oxygen uptake from the mantle cavity to a much greater extent than does Planorbis corneus.

A most striking aspect of the mantle complex

of Bulinus africanus, Bulinus tropicus and Lymnaea natalensis is the very large kidney in relation to the size of the mantle surface (vide Figures 8 and 9). This is understandable as these species live in freshwater and a well developed kidney would be of obvious significance for maintaining and regulating their ion and water balance. However, such a large kidney mass would necessarily diminish the total functional surface area of the mantle available for the absorption of oxygen from the mantle gas.

Taken overall, the conclusion is reached that the functional significance of the mantle cavity as a lung in these three pulmonate species is not of great importance. Provided cutaneous respiration is adequate to supply the oxygen requirements of the snails, pulmonary respiration does not appear to be essential, and the primary function of the mantle gas may be in buoyancy regulation. The following observations can be used as evidence to substantiate this point of view. In the laboratory snails from all three species were observed to use the mantle gas as a means of flotation to reach the water surface. Alternatively they were seen to sink to the bottom from the surface. (This behaviour has also been observed in other pulmonate species by Cheatum (1934) and Henderson (1963)). Furthermore, newly hatched snails from all three species took up surface breathing within 2 - 3 days after hatching at a temperature of 26°C. It should be pointed out that the young snails may have been forced to the surface by this high temperature. However, young snails were also observed to indulge in surface breathing at a much lower temperature, but the exact time after hatching at which this occurred is not known.

Hunter (1953 b) and Jones (1961) also report that the young of Lymnaea peregra and Planorbis corneus respectively, indulge in surface breathing, immediately after hatching in the case of Lymnaea peregra and in Planorbis corneus before the shell height reaches 2 mm. The respiratory value of the mantle gas bubble is presumably much less in newly hatched or young snails than in mature animals. These observations on the behaviour of both adult and juvenile snails indicate that buoyancy may play an important part in the normal surfacing behaviour of the freshwater pulmonates in general.

The presence of smooth muscle in the mantle of Bulinus africanus, Bulinus tropicus and Lymnaea natalensis suggest that the volume of the mantle cavity is subject to alteration by the contraction or relaxation of the mantle itself. Any decrease in the pulmonary gas volume is probably accommodated by partial apposition or contraction of the cavity walls. It is possible that buoyancy adjustment in pulmonates may occur by compression of the mantle gas, although Henderson (1963) found no evidence of this in Planorbis corneus and Lymnaea stagnalis.

As is to be expected, the histological structure of the pseudobranch of Bulinus africanus and Bulinus tropicus suggest that this organ could be most efficient for the diffusion of oxygen from the surrounding water into the blood. With its large surface area, thin epithelium, and extensive blood supply, it is clear that the pseudobranch may allow for the maximum oxygenation of the blood flowing through the large blood sinuses. Furthermore, the currents of flow in the immediate vicinity of the pseudobranch set up by the actively beating cilia on the external surface of the pseudobranch suggest that a counter-current system is operating, which no doubt would further enhance the diffusion of oxygen into the blood.

D I S C U S S I O N

1. Results.

As far as the surfacing behaviour in these pulmonate species is concerned, the termination of the dive appears to be determined by the rate at which the pulmonary gas volume decreases. This in turn depends on the volume of gas uptake at each successive surfacing and the respiratory needs of the snails in relation to the temperature and oxygen tension of the water. Two other factors, however, may regulate surfacing behaviour. The animals may respond to a decrease in the pulmonary oxygen partial pressure, and/or a reduction in pulmonary gas volume with a consequent loss of buoyancy or increase in specific gravity (Jones 1961, Henderson 1963). The highly irregular surfacing pattern in these three pulmonate species suggests that the buoyancy of the shell-visceral mass was probably the most likely factor responsible for the irregularity in the diving periods. This statement is substantiated by the following findings.

(i) These snails can exist solely by cutaneous respiration in favourable conditions when denied access to the atmosphere, implying that pulmonary respiration may not be all that important. It is therefore difficult to imagine that the irregular dives as observed in these animals could be initiated in response to a decrease in the oxygen tension of the mantle gas.

(ii) Henderson (1963), found that surfacing in Planorbis corneus and Lymnaea stagnalis normally occurs during the time when the snails are least buoyant as reflected by the greatest change in the under-water weights of the animals, namely the first two hours of submersion. This present study (vide Figure 13) has shown that a very close reciprocal relationship exists between the increase in

underwater weight in Planorbis corneus and Lymnaea stagnalis (Henderson 1963) and the decrease in the oxygen tension of the mantle gas in Bulinus africanus, Bulinus tropicus and Lymnaea natalensis. This implies that as pulmonary oxygen is consumed the consequent loss of buoyancy due to the diminishing mantle gas volume may be the stimulus driving the snails to the surface. It can be argued that the decrease in pulmonary oxygen partial pressure is as likely a stimulus to terminate the dive, but in view of the fact that pulmonary respiration does not appear to be essential in these three species worked upon, it seems much more likely that the loss of buoyancy is the factor releasing surfacing behaviour.

(iii) The considerable variation in the diving periods also suggest that the volume of gas uptake at the surface is variable. This was confirmed in a subsequent experiment (vide page 74) which showed that the volume of the mantle gas immediately after surfacing could vary from 0.05 - 0.25 ml. These observations would seem to be in agreement with the suggestion put forward by Henderson (1963) that the buoyancy of the shell-visceral mass determines the volume of gas uptake at the surface.

(iv) As pointed out by Jones (1961), a simple proprioceptor mechanism sensitive to loss of buoyancy of the shell complex would be sufficient to mediate the surfacing response. Such a sensitive mechanism may have an additional advantage. In view of the unwieldy nature of an unbuoyant shell a loss of buoyancy is likely to provide the stimulus driving the snails to the surface to replenish the air in the mantle cavity in order to become buoyant and thus more manoeuvreable. This point was illustrated in the experiments in which the

snails were forced to expel their mantle gas and subsequently prevented from surfacing by means of plastic gauze. Although the animals did not appear to be unduly inconvenienced by the loss of buoyancy, their movements were sluggish and they gave the impression of experiencing some difficulty in supporting the burden of the shell. This was particularly noticeable when the snails were crawling up the vertical sides of the glass beakers.

Notwithstanding the fact that surfacing in the freshwater pulmonates in general seems to occur at intervals of short duration, prolonged diving periods are possible. This is borne out in the series of experiments in this study in which the snails were denied access to the atmosphere for 96 hours, even in the absence of gas bubbles in their mantle cavities. Cheatum (1934), Hunter (1953 b) and Henderson (1963) also found that other pulmonate species are able to remain submerged for long periods. It is clear that in these instances cutaneous respiration was responsible for the major part of the respiratory exchange.

The air bubble in the mantle cavities of Bulinus africanus, Bulinus tropicus and Lymnaea natalensis clearly has a respiratory function as an oxygen store and the importance of this supply of oxygen varies with the respiratory needs of the animals. The gaseous composition of the air bubbles from the mantle cavities of all three species suggests that even under very nearly natural conditions this source of oxygen is not very substantial. Although based on an approximate estimate, the oxygen in the air bubbles constitute 12 - 20% of the cutaneous oxygen requirements

of the snails per unit weight per hour at 20°C. By far the greatest utilization is via the cutaneous route.

In view of the fact that survival was always high in both aerated and non-aerated water at 15°C and in aerated water at 25°C, access to a water-gas interface would seem to be unnecessary, even in the absence of the greater part of the gas bubble in the mantle cavity. Presumably cutaneous respiration was sufficient to cater for the oxygen requirements of the snails. In non-aerated water at 25°C denial of access to a water-gas interface coupled with a falling oxygen tension causes extensive mortality. In this instance the respiratory function of the mantle gas would seem to be essential, as the cutaneous uptake of oxygen was probably not adequate to cater for the increased respiratory requirements of the snails at 25°C. (vide Figure 3). It would seem thus that pulmonary respiration in these species is auxiliary to cutaneous respiration, but that pulmonary respiration becomes increasingly important in conditions in which the cutaneous uptake is not adequate to supply the metabolic needs of the snails.

On the other hand, survival was high in all instances when the snails of all three species were exposed to almost completely anoxic conditions in the presence of a nitrogen water interface at both 15° and 25°C. It is well appreciated that presenting the snails with an anoxic environment may cause them to switch over to anaerobic respiration, possibly by incurring an oxygen debt and the anaerobic breakdown of carbohydrate stores, as the long exposure time of 96 hours would seem to indicate. As far as the concentration of dissolved oxygen is concerned there was no marked difference

between this series of experiments and the previous experiments in which the oxygen concentration fell to low levels in the absence of aeration. The only difference in the experimental design lay in the availability of a gas-water interface, even though this gas was nitrogen. Is, therefore, the high survival in anoxic conditions, particularly so at 25°C, coupled in some way with the availability of this interface? The following explanation is offered to account for this state of affairs.

The development of anoxic conditions may effectively release surfacing behaviour to relieve the stress condition which is developing. By so doing the animal is brought normally into water of increased oxygen content at the surface. Failure to relieve this stress condition resulted in the extensive mortality of the snails as found in these experiments if the snails are denied access to the atmosphere at 25°C. The high survivalship of the snails in conditions of virtually no oxygen as provided in the second series of experiments, suggests that surfacing albeit into nitrogen, satisfied what in effect seems to be a first component in stress relief, namely surfacing and filling the mantle cavity with gas. The continued lack of oxygen then invokes anaerobic respiration which ensures survival. It would seem as if access to a water-gas interface is important in these species in anoxic conditions at high temperatures. In view of the fact that the respiratory significance of Nitrogen is nil, these experimental results suggest that the buoyancy effect of this gas in the mantle cavities of the snails was in part responsible for their high survivalship. Although it is appreciated that the design of these experiments was artificial in so far as subjecting the snails to a Nitrogen environment is concerned, the results indicated that buoyancy is important in these pulmonate species. The results of these experiments can also be considered as further evidence that the buoyancy properties of the mantle gas is of more importance than the respiratory function.

The oxygen consumption of these species in relation to starvation, temperature, live weight and oxygen content of the water, confirm in general the findings of Von Brand et al (1948), Zaaijer and Wolwekamp (1958) and Berg and Ockelmann (1959) for other aquatic snail species.

Starvation for a period of six days appeared to have little effect on the oxygen uptake of the snails.

As regards the oxygen uptake in relation to temperature, the animals showed a marked increase in metabolic rate with increasing temperatures. The maximum temperatures which was tolerated by the snails under the conditions of the experiment was 30°C for Lymnaea natalensis and 33.5°C for Bulinus africanus and Bulinus tropicus. Above these respective temperatures the oxygen uptake of the animals decreased suggesting that some damage had occured. In the case of Bulinus africanus and Bulinus tropicus the maximum temperatures tolerated are in agreement with those reported by Frank (1964). This author found that the highest temperature recorded in the natural habitats of Bulinus africanus and Bulinus pfeifferi in Eastern Transvaal was 35.5°C, which did not appear to harm the snails. However, temperatures only a few degrees higher than 35.5°C were rapidly fatal under laboratory conditions.

All three species showed an increased oxygen consumption with increasing live weight at both 15° and 25°C, thus confirming the experimental findings of Berg and Ockelmann (1959) for other aquatic snail species.

These species decrease their oxygen consumption as the oxygen tension in the water diminishes. They are thus oxygen dependent. It is interesting to speculate on the essential physiological difference between oxygen dependent and oxygen independent species. According to Berg and Ockelmann (1959), oxygen dependent species are at some disadvantage as compared to independent species, as it indicates that one or more physiological functions of the organism must have been depressed or inhibited. These

authors point out that this physiological difference need not be so decisive that oxygen dependent species cannot exist in environments with a poor oxygen supply, but that such species probably do not thrive as well as independent species. While this point of view is granted, a more important aspect seems to be that oxygen dependent species may be at an advantage in terms of survivalship in bad respiratory conditions. Berg (1952) found that the limpet Ancylus fluviatilis, an oxygen independent species living in well aerated streams has not the same ability to survive in anaerobic conditions as another limpet species Acroloxus lacustris which is oxygen dependent and lives in stagnant water. The three pulmonate species worked upon in this study are oxygen dependent and showed a remarkable ability to survive in almost completely anoxic conditions for 96 hours. In view of this the suggestion is put forward that the ability of aquatic snails to regulate their metabolic rate according to the oxygen tension of the water may be an important factor for their survivalship in conditions of low oxygen tension. As the natural habitats of freshwater snails may be subject to wide fluctuations in dissolved oxygen tensions due to the prevailing plant growth this suggestion is not unjustified. Jones (1961) reports the conditions in a ditch 15 cm. deep near Leiden, the natural habitat of Planorbis corneus and Lymnaea stagnalis, both of which are oxygen dependent. (Zaaijer and Wolwekamp 1958). On hot sunny days in June when the ditch was choked with plants, the surface oxygen tension of the water covered the remarkable range of 0.4 ml/l at 3.30 am. at a temperature of 13° to 16.9 ml/l at 1.30 pm. at a temperature of 30°C. The corresponding oxygen tensions at the bottom of the ditch varied from 0.3 ml/l to 4.3 ml/l. Photosynthetic processes no doubt accounted for the supersaturated conditions, as in May when the plant growth was less abundant, the oxygen tension of the water was correspondingly less. It is therefore possible that oxygen dependent species may be at an advantage in habitats in which violent diurnal and seasonal fluctuations in the dissolved oxygen tension are likely to occur.

The histological structure of the mantle in Bulinus africanus and Bulinus tropicus do not suggest that the mantle cavity of these species functions as an efficient organ for respiratory exchange. On the other hand the vascular mantle roof sinus of Lymnaea natalensis indicates that the mantle cavity of this species may be a relatively more efficient organ for respiratory exchange, which implies that the functional significance of the mantle cavity as a lung could be of more importance in the Lymnaeids than in the Planorbids. Although the mantle gas in these snail species clearly has a respiratory function, they are able to exist solely by cutaneous respiration for long periods, in the absence of pulmonary oxygen and without access to the atmosphere. However, pulmonary respiration seems to become increasingly important in conditions in which cutaneous respiration is not sufficient for the oxygen requirements of the animals. Taken overall, however, pulmonary respiration in all three species appears to be auxiliary to cutaneous respiration, and it would seem as if the primary function of the mantle gas is in buoyancy regulation. The observations that adult snails from all three species use the mantle gas as a means of flotation to enable them to reach the surface and that the young snails take gas bubbles into their mantle cavities within a few days after hatching, indicate that the buoyancy properties of the mantle gas is important in their normal surfacing behaviour.

The histological structure of the pseudobranch of Bulinus africanus and Bulinus tropicus leaves no doubt that it is a very efficient organ for respiratory exchange, and probably contributes a considerable portion of the total cutaneous oxygen uptake in these two Planorbid species.

2. General

Although the aspects of respiratory physiology investigated in this study did not indicate any marked differences between the respiratory behaviour of Lymnaea natalensis and that of the two Planorbid species, it is to be expected that the presence of an accessory gill and haemoglobin in the Planorbids would be of some respiratory significance. From a physiological point of view the Planorbids must be better adapted for an aquatic life and less dependent on aerial respiration than other species.

The selective pressures necessitating the development of a pseudobranch and the occurence of haemoglobin are difficult to assess. The chemical and physical properties of haemoglobin in Planorbis corneus indicate that this pigment may be of use in conditions of low oxygen tension. (Leitch 1916, Borden 1931, and Zaaijer and Wolwekamp 1958). As the natural habitats of freshwater snails may be subject to violent fluctuations in the dissolved oxygen tension, the possession of a pigment with the characteristics of haemoglobin would be an advantage. This histological structure of the mantle of Bulinus africanus and Bulinus tropicus indicate that the mantle cavity in these species is not an efficient lung. Furthermore, as the total surface area of the mantle is considerably decreased by the presence of a large kidney mass, it is not unreasonable to suggest that the development of a pseudobranch in these Planorbid species may compensate for the diminished functional efficiency of the mantle cavity as a respiratory organ. The same argument can be applied to Lymnaea natalensis. Notwithstanding the large kidney in this species, the histological structure of the mantle indicates that the mantle cavity could be a relatively efficient lung by virtue of the vascular mantle roof sinus. In this way Lymnaea natalensis may possibly compensate for the lack of an accessory gill and haemoglobin.

However, this study did not indicate that Lymnaea natalensis is at any particular disadvantage in lacking a pseudobranch and haemoglobin, two characteristics which would suggest a respiratory advantage. In view of this it seems likely that the anatomical and physiological differences between the two Planorbid species and Lymnaea natalensis could be of ecological significance. In this respect, the hypothesis put forward by Jones (1961 and 1964 a) with regard to Lymnaea stagnalis and Planorbis corneus merits further consideration. This author found, in observations on these snails in their natural habitat that there was an important difference in the range of the two species. Lymnaea stagnalis tended to remain within easy reach of the surface whereas Planorbis corneus spends a considerable time on the bottom of the ditch, and consequently came to the surface less frequently. Furthermore, in experiments on gas exchange Jones (1961) found that the lung in Lymnaea stagnalis was relatively more important than in Planorbis corneus. On the basis of these observations and experimental findings Jones advanced the hypothesis that these two species occupy separate regions of the habitat. Planorbis corneus by virtue of the possession of haemoglobin with a high affinity for oxygen is able to exploit the pulmonary oxygen to a greater extent than Lymnaea stagnalis. The former species is thus able to indulge in longer dives and journeys further away from the surface, while Lymnaea stagnalis, being more dependent on the lung is limited to regions within easy reach of the surface.

In how far this proposed hypothesis of Jones applies to the South African species worked upon in this study can only be determined by field and laboratory studies. Two factors can be mentioned however.

Firstly, Lymnaea natalensis was always found in its local habitat either floating on the surface or immediately below the surface, whereas Bulinus tropicus was usually found in its local habitat at depths varying from 0.2 - 0.3 m.

Occasionally these snails were seen at the surface.

Secondly, the histological structure of the mantle of Lymnaea natalensis as compared with that of Bulinus africanus and Bulinus tropicus suggests that the mantle cavity of Lymnaea natalensis is functionally a more efficient lung than that of the two Planorbids. Consequently Lymnaea natalensis would seem to be more dependent on its lung and thus restricted to regions in the immediate vicinity of the surface.

In conclusion it can be stated that the aspects of respiration of these South African pulmonate species investigated in this study fit into the rather broad range of respiratory physiology for other pulmonate species worked upon. The following generalizations are accordingly warranted.

(i) Although these snails use the mantle gas for respiratory purposes, pulmonary respiration is auxiliary to cutaneous respiration, which is responsible for the major part of respiratory exchange. The primary function of the mantle gas appears to be in buoyancy regulation.

(ii) On an anatomical basis the mantle cavity of the Lymnaeids is functionally a more efficient lung than that of the Planorbids.

(iii) The oxygen consumption of the snails in relation to starvation, temperature, live weight and oxygen content of the water are in agreement with that of other pulmonate species.

(iv) The snails are able to survive in almost completely anoxic conditions for long periods.

In the light of the experimental findings of this investigation, the following hypothesis concerning the surfacing behaviour of the freshwater pulmonates in general is advanced.

Surfacing behaviour is initially released by the loss of buoyancy consequent upon the reduction of the mantle gas due to utilization of the oxygen for respiratory purposes and/or to the diffusion of gases into the surrounding water. The frequency of surfacing will thus be directly influenced by the rate at which the volume of the pulmonary gas diminishes. As far as respiration is concerned this will depend on the prevailing respiratory needs of the snails in relation to the temperature and oxygen content of the water and the size of the animals. When the metabolic rate of the snails is reduced, for example at low temperature, cutaneous respiration is adequate to cater for their oxygen requirements and consequently the oxygen uptake from the mantle gas will be reduced. On the other hand, should the cutaneous oxygen uptake not be sufficient, as for example in conditions of low oxygen tension or in the face of an increased metabolic rate at high temperatures, pulmonary respiration becomes important to supply additional oxygen, with a consequent increase in the rate of oxygen uptake from the mantle gas.

The decrease in buoyancy or increase in specific gravity resulting from a diminishing mantle gas volume then releases surfacing behaviour which drives the snails to the surface to replenish the air in the mantle cavity. Such a sensitive mechanism will have an additional advantage in that it will aid and expedite the return of the snail to the surface while the shell complex is still partly buoyant.

Finally, this study has once more illustrated the inter and intra specific variation in respiratory behaviour of the limnic gastropods when faced with a variety of environmental conditions, and as Hunter (1961) has pointed out the evolutionary significance of this plasticity in respiratory and other aspects of freshwater mollusc physiology, suggests that it is of fundamental selective value evoked by the peculiarities of freshwater environments.

S U M M A R Y.

1. Various aspects of the respiratory physiology of two Planorbid species, Bulinus (Physopsis) africanus and Bulinus (Bulinus) tropicus, and one Lymnaeid species, Lymnaea natalensis were investigated.
2. The need for the understanding of basic physiological mechanisms in schistosome snail hosts was pointed out.
3. A colorimetric method for the determination of dissolved oxygen in water is described.
4. A simple procedure for collecting gas bubbles expelled from the mantle cavities of the snails is described.
5. The gaseous composition of the gas bubbles were analysed in a tonometer constructed from a 0.1 ml pipette.
6. Under laboratory conditions, the three species showed a highly irregular pattern in diving behaviour.
7. The importance of the mantle gas in respiration depends on the respiratory needs of the snails in relation to the temperature and oxygen content of the water. When the animals were denied access to the atmospheric air, a high survivalship was evident at 15°C in both aerated and non-aerated water and also at 25°C in aerated water. Extensive mortality was found in non-aerated water at 25°C, in which the oxygen tension fell to low levels. The conclusion reached was that pulmonary respiration becomes increasingly important in conditions in which the cutaneous oxygen uptake is not sufficient to cater for the metabolic needs of the snails, as for example at high temperatures coupled with a decreasing oxygen tension in the water.
8. All three species showed a high survivalship over a period of 96 hours in almost completely anoxic conditions. These results were completely contradictory to the results mentioned above. The only difference in the experimental design was that in this instance the snails had access to a water-gas interface, the gas being pure nitrogen. A hypothesis to account for these contradictory and confusing results is advanced.

9. The cutaneous oxygen uptake of the three species in relation to starvation, temperature, live weight and oxygen content of the water showed similar tendencies namely, a slight decrease with starvation, a marked increase with a rise temperature, an increase with increasing live weight, and a decrease as the oxygen concentration of the water diminishes.

10. The general outlay of the anatomy and histology of the respiratory organs is given. From a morphological point of view the mantle cavity of Lymnaea natalensis appears to be a more efficient lung than that of the two Planorbid species.

11. Tonometric analysis of the mantle gas of all three species indicated that the gas bubbles function as an air store of diminishing volume and not as a physical gill.

12. It was concluded that pulmonary respiration is auxiliary to cutaneous respiration and that the primary function of the air bubble in the mantle cavity is in buoyancy regulation.

13. The hypothesis is advanced that surfacing behaviour of the freshwater pulmonates in general is initially released by a loss of buoyancy consequent upon the reduction of the mantle gas volume due to the utilization of the oxygen for respiratory purposes and/or the diffusion of gases into the surrounding water.

14. This study did not reveal any marked differences in the aspects of respiratory physiology which were investigated between the two Planorbid and one Lymnaeid species. The conclusion reached was that Lymnaea natalensis did not appear to be at any disadvantage in lacking an accessory gill and possibly a respiratory pigment, when compared with Bulinus africanus and Bulinus tropicus. The possibility that the anatomical and physiological differences could be of ecological significance is briefly discussed.

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A P P E N D I X

TABLE 1

Mass of water in gm. in needle and up to respective marks.

Etched marks:	1	2	3	4	needle
Syringe No. 1	mass in gm.	mass in gm.	mass in gm.	mass in gm.	mass in gm.
	5.0250	6.0366	6.9705	7.9579	0.2036
	5.0296	6.0304	6.9815	7.9556	0.2106
	5.0261	6.0316	6.9718	7.9722	0.2096
	5.0386	6.0446	6.9776	7.9639	0.2100
	5.0357	6.0450	6.972	7.9711	0.2088
	5.0414	6.0510	6.9836	7.9689	0.2124
	5.0287	6.0353	6.9214	7.9347	0.2246
	5.0320	6.0321	6.9016	7.9289	0.2124
	5.0277	6.0336	6.9326	7.9463	0.2214
	5.0293	6.0395	6.9215	7.9557	0.2234
mean:	5.031	6.038	6.953	7.955	0.214
SD =:	0.046				0.001
Syringe No. 2	5.0693	5.9342	6.8449	7.7873	0.2104
	5.0748	5.9276	6.8492	7.7786	0.2163
	5.0642	5.9241	6.8249	7.7891	0.2132
	5.0679	5.9269	6.8273	7.7760	0.2286
	5.0735	5.9321	6.8207	7.7997	0.2294
	5.0724	5.9478	6.8328	7.7824	0.2287
	5.0638	5.9336	6.8265	7.7794	0.2198
	5.0720	5.9228	6.8214	7.7827	0.2276
	5.0607	5.9367	6.8426	7.7958	0.2237
	5.0684	5.9389	6.8318	7.7877	0.2248
mean :	5.070	5.932	6.832	7.786	0.222
SD = :	0.035				0.002

TABLE 2.

	Weight of water in gm. up to 10 ml. division on barrel.	Weight of water in gm. in needle.
Respirometer A	10.0452	0.2305
	10.0468	0.2103
	10.0445	0.2006
	10.0463	0.2128
	10.0426	0.2237
	10.0457	0.2196
	10.0445	0.2286
	10.0397	0.2282
	10.0472	0.2197
	10.0481	0.2210
mean :	10.045	0.219
SD :	0.002	0.002
Respirometer B	9.4456	0.2251
	.4667	0.2620
	9.4392	0.2346
	9.4373	0.2535
	9.4392	0.2248
	9.4426	0.2262
	9.4375	0.2247
	9.4489	0.2402
	9.4524	0.2276
	9.4326	0.2207
mean:	9.444	0.234
SD :	0.001	0.015