ASPECTS OF THE BIOLOGY OF AESTIVATION IN *BULINUS (PHYSOPSIS) AFRICANUS* (KRAUSS) (GASTROPODA, PULMONATA)

by

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2. ABSTRACT

Aestivation, a period of dormancy during hot, dry conditions, is known to occur in both the Prosobranchia and the Pulmonata among freshwater Gastropoda. While there have been numerous accounts, mainly by field ecologists concerned with bilharziasis control, of the survival value of aestivation in the Gastropoda, little is known of its underlying physiological mechanisms. Results of investigations into the physiological basis of aestivation in the freshwater Prosobranchia, confined to the family Ampulariidae, have been conflicting, fundamental differences having been shown to exist between different species of the genus *Pila*. Only a single comprehensive study, that by von Brand and his co-workers on the planorbid snail *Australorbis glabratus**, forms the basis of our knowledge of the aestivation process in the aquatic Pulmonata. In view of the conflicting results obtained in investigations on freshwater Prosobranchia, the general applicability of the findings for *Australorbis glabratus* to other freshwater Pulmonata was open to question.

The present investigation on the planorbid snail *Bulinus (Physopsis) africanus*, a species known to be a successful aestivator, was prompted by the obvious need for a further comprehensive study on an aquatic pulmonate species. The results presented here show that aestivation in this species is a definite physiological state, characterised by a depression of the metabolic rate, which not only aids in husbanding the snails' metabolic resources during the enforced starvation which must necessarily accompany aestivation, but also constitutes a form of resistance adaptation to the transient high temperatures which aestivating snails are bound to encounter. Loss of body water is shown to be important in initiating aestivation, but it is also the factor most likely to prove

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*Cridland (1967) reports that *Australorbis glabratus* has been renamed *Biomphalaria glabrata* but quotes no authority. Since the old name, *Australorbis glabratus* is used in all relevant literature, it has, in the interests of clarity, been retained in this account.

lethal during the course of aestivation. The results confirm and extend the earlier findings for *Australorbis glabratus*.

The results are discussed in the context of our present knowledge of aestivation in other Gastropoda, both aquatic and terrestrial, and also in relation to other forms of resistance adaptation. As expected, the investigation raises more questions than it answers, thus directions for further research, arising out of the present findings, are suggested.

3. INTRODUCTION

A striking feature of the biology of land and freshwater molluscs is their ability to withstand adverse physical conditions in their environment. Early investigations into this ability were confined to terrestrial pulmonates (von Brand, 1931; Howes & Wells, 1934 a & b; Wells, 1944). *Helix pomatia L* has two physiological states which contribute to the survival of environmental extremes. During winter this species *hibernates*; this is a seasonal process, preceded by a period of considerably increased storage of liver polysaccharide, and the closure of the shell aperture by means of an epiphragm, a complex structure composed of mucus and calcium carbonate. Adverse summer conditions cause these snails to go into *aestivation*, a spontaneous inactivity with no abnormal storage of reserves, during which the shell aperture is closed by the so-called "mucus veil". Both states enhance the survival of the snails, and both involve a reduction in the rate of oxygen uptake.

Freshwater molluscs may be subjected to extremes similar to those which face their terrestrial counterparts. In cold temperate regions their habitat, particularly if it is shallow, is subject to freezing in winter; in tropical and sub-tropical areas both high water tempertures and the complete drying up of the habitat are not uncommon. Such drying up of water bodies is by no means confined to cooler seasons, and the animals therefore need to survive some of the extreme rigours of the terrestrial environment.

Aestivation is defined by Kenneth (1963) as "dormancy during heat and drought periods." Adaptation to a set of environmental conditions is the sum of the physiological, behavioural and morphological characteristics which enable a species to reproduce, and therefore maintain itself, under these conditions within the habitat in which it has evolved; it must be such as to ensure survival in the face of any extremes which may, from time to time, occur. Aestivation is such an adaptation, and its manifestation has been widely reported for both prosobranch and pulmonate gastropods which inhabit freshwater. Shiff (1960) and Cridland (1967) have conducted experimental studies into the survival of various

African intermediate host species of trematode parasites. These studies have shown the Planorbidae in particular to be very well able to survive prolonged periods of exposure after the drying up of their habitat. South American Planorbidae have been shown to share this ability (Olivier, 1956; Olivier & Barbosa, 1956). Mozley (1954), Olivier (1956), Shiff (1960) and Cridland (1967) have all discussed the implications of this ability to survive periods of drought in the context of bilharziasis control through intermediate host elimination.

While much is known of the occurrence and survival value of aestivation in gastropod molluscs, notable contributions to our understanding of the physiological basis of this phenomenon have been few. Meenakshi (1964) claims that aestivation in the Indian ampulariid snails *Pila virens* and *Pila globosa* (authorities not quoted) is an anaerobic process, with lactic acid accumulation during aestivation compatible with glycolysis. Coles (1968) by contrast shows the African *Pila ovata* (authority not quoted) to be aerobic during aestivation, but that the rate of oxygen consumption is depressed. This is in agreement with the results obtained for *Helix pomatia* by Wells (1944) and for *Australorbis glabratus* (Say) by von Brand, McMahon and Nolan (1957). Coles (1969b) reports a similar depression in the rate of oxygen uptake in aestivating *Bulinus (Physopsis) nasutus* (Martens).

The above results, while undoubtedly assisting in the elucidation of the aestivation process, are too varied to allow for any generalizaion. Only von Brand's studies on *A. glabratus* can in any way be regarded as comprehensive, and even this classic work leaves questions unanswered. *A. glabratus*, and many other freshwater snails show a depression in the rate of oxygen consumption when starved (von Brand, Nolan & Mann, 1948) and although the level does not reach that recorded for aestivating animals over the same period of time, it must contribute to the overall reduction in the rate. Furthermore, although aestivating *A. glabratus* survive much longer than do starving individuals, there appears to be little difference in the rate at which carbohydrate reserves are consumed by the two groups. There are indications that body water content

determines the onset of aestivation in *A. glabratus*, and this has also been suggested for *Pila virens* and *Pila globosa* (Meenakshi, 1964), but this has, to date, only been conclusively demonstrated by Little (1968) for *Pomacea lineata* (Spix).

A further comprehensive study of the physiology of a single species, which sets out to answer at least some of the questions raised by previous work is therefore desirable, and with this in view the present investigation sets out to gain as much information as possible on the aestivation phenomenon in *Bulinus (Physopsis) africanus* (Krauss). Such a study must perforce initially be laboratory orientated since, to be meaningful, various parameters need to be carefully controlled. The application of the findings presented here to snails under field conditions remains to be tested and the biochemical basis of the aestivation process remains virtually untouched. This investigation can, therefore, only be regarded as an intermediate step between the sphere of the field ecologist and the biochemist.

4. GENERAL DESCRIPTION OF METHODS

Established techniques were used in most of the analytical and quantitative determinations made in this investigation. Since many of these techniques were developed for material other than molluscan tissue, or involved selection from a number of alternatives, the following brief discussion on methods is included.

It is realised that the techniques described below do not in every instance represent the most accurate or sophisticated; certainly the analytical methods would not, for example, meet the rigorous requirements of the biochemist. However, in a comparative study such as this, it is likely that variability inherent in the material studied is such as to far exceed the inaccuracies of the methods employed. The choice becomes one of either gathering few very accurate data, which could be possibily offset by individual variability, or a sufficient body of information which could show up trends and allow for generalization in spite of a lack of absolute specificity of the technique.

WATER ANALYSIS

Analytical methods used in water analyses were selected from National Institute for Water Research (1966), Standard Methods for the Examination of Water and Wastewater, including Bottom Sediments and Sludge (1965) and Golterman (1969). Availability of apparatus and reagents, as well as the recommendations of authors, governed the choice of the following methods:

a. Hydrogen ion concentration: Measured directly using a calibrated pH meter. Field measurements were made using either a Beckman Model G or a "WTW" battery operated pH meter, while in the laboratory a Beckman Expandomatic was at times used as an alternative.

b. Conductivity: Field measurements were made with a "WTW" direct reading battery operated conductivity meter. In the laboratory a Phillips Conductivity Measuring Bridge (PR 9500) was used. Both instruments were calibrated against KCl

solutions of known conductivity (Golterman, 1969).

c. Total dissolved solids: Determined gravimetrically by evaporating a 100 ml sample, which had been filtered under suction through two layers of Whatman No. 1 filter paper in a Buchner funnel, to constant weight (National Institute for Water Research, 1966).

d. Total hardness: Determined by complexiметric titration with N/50 ethylenediaminetetra-acetic acid (di-sodium salt) (EDTA) using eriochrome black T as indicator (National Institute for Water Research, 1966; Golterman, 1969).

e. Calcium hardness: Determined by complexiметric titration with N/50 EDTA using solochrome dark blue (Calcon) as indicator (National Institute for Water Research, 1966; Golterman, 1969).

f. Alkalinity: Acidimetric titration with N/50 hydrochloric acid to a pH end-point determined by the conductivity of the sample (National Institute for Water Research, 1966).

g. Calcium and Magnesium: Calculated from total and calcium hardness.

h. Sodium and Potassium: Determined on an EEL flame photometer (Standard Methods, 1965).

i. Bicarbonate: Calculated from alkalinity (National Institute for Water Research, 1966).

j. Chloride: Volumetric titration with N/50 mercuric nitrate using diphenylcarbazone-bromphenol blue mixed indicator.

k. Sulphate: Turbidimetric, forming insoluble barium sulphate suspended in a glycerol/alchohol mixture (National Institute for Water Research, 1966).

DETERMINATION OF MAJOR TISSUE CONSTITUENTS

Techniques used in the determination of tissue carbohydrate, protein and lipid required testing for effectiveness and reproducibility on molluscan tissue, since they were developed principally for use with mammalian material. The full treatments of all tissues analysed are described below:

a. Tissue drying: All tissues were dried to constant weight

at 60°C, either in a wax-free oven (early experiments) or in an "Exitherm" thermostatically controlled vacuum desiccator. Tissue thus dried was stored, when necessary, in closed containers in a deep freeze.

b. Liquid extraction and estimation: Crushed, dried tissue was extracted with ether over a period of five hours in a Soxhlet reflux extractor, and the extract evaporated to constant weight. It is probable that the residue contained substances other than lipids, and it is, therefore, more correct to refer to it as the "ether extractible fraction". It will, however, include tri-glycerides, which form an important part of the animals' energy reserves (Lovern, 1955). The accuracy of this method was limited by the balance available; all weighings were done on a Mettler Model B5 analytical balance reading to 0,1 mg.

c. Carbohydrate extraction and determination: Quantitative estimation of the carbohydrates present were made on extracts of the fat free dried tissue using Dreywood's Anthrone Reagent as described by Morris (1948). More accurate methods depend on the determination of the concentrations of specific monosaccharides, and consequently on the hydrolysis and complete analysis of any di-, oligo- or polysaccharides present. Thus the less sophisticated anthrone method, which is still the standard technique for the quantitative determination of tissue glycogen (Stacey & Barker, 1962; Zwarenstein & van der Schyff, 1963), is the most versatile when dealing with unknown carbohydrates or mixtures. Disch (1963) points out the shortcomings of the technique. His most serious criticism is the different colour intensity obtained with different carbohydrates reported by Morris (1948). Glycogen and galactogen develop colour intensities of 111% and 54% relative to that developed by an equal weight of glucose (100%). In a comparative study involving individual organ systems absolute values are not essential, and would only be obtainable from detailed chemical analysis of the whole carbohydrate composition. The quantitative expression of all carbohydrates in terms of "equivalent mg glucose"

would adequately meet the requirements of a comparative study such as this, and could be readily converted to absolute values where the identity of a specific carbohydrate is known. Thus where glycogen is known, or can reasonably be assumed to be present, "equivalent mg glucose" x 0,9 would give a measure of absolute glycogen content whereas galactogen would require multiplication by a factor of 1,85.

Scott and Melvin (1953) have subjected the anthrone method to exhaustive and rigorous tests, showing, among other characteristics, an absolute adherence to Beer's law even at very low concentrations, and a minimal interference by extraneous substances and colours. These authors caution against negligence in cleaning glassware and thus leaving traces of carbohydrate, for so sensitive is the method that these might well affect results obtained, particularly where such traces contaminate reagent blanks. To this may be added that the colour developed depends on the formation of furfurals through the dehydrating action of sulphuric acid on the constituent hexose rings. These furfurals then condense with the anthrone to produce the blue-green colour. The test is, therefore, not affected by the rapid reduction in molecular weight of polysaccharides which follows extraction by methods at present in use (Stetten, Katzen & Stetten, 1956, 1958 quoted in Stacey & Barker, 1962). In this investigation the method was used as follows:

1. 5 ml anthrone reagent (0,2 g anthrone dissolved in 100 ml 25N sulphuric acid) was dispensed into each of the required number of 25 ml pyrex test tubes.
2. 1 ml of the sample solution to be tested was carefully layered on the anthrone reagent.
3. When all samples to be tested had been thus prepared, the reagent and sample were rapidly and thoroughly mixed in all the tubes.
4. All test tubes were placed simultaneously in a boiling water bath and heated for exactly ten minutes.
5. All test tubes were simultameously removed from the water bath, rapidly cooled to room temperature in running tap water, and the optical density of the contained solutions was read at 620 nm.

A glucose standard was included with each batch of determinations, and the colorimeter was set for zero absorbance on a reagent blank in which 1 ml of distilled water (or whichever solvent was used for the samples) was substituted for the sample solution. Two replicates were prepared for each sample; where these differed but slightly from each other in their optical density, the mean value for the two was taken, but where a large discrepancy occurred, the determination was repeated.

Sample volumes could be halved or doubled without loss of accuracy. Since the colour developed depends on the amount of carbohydrate present, and since the reagent was always well in excess, samples larger than 1 ml could be used where these were very dilute. Similar adjustments had, of course, to be made to standards and blanks in these instances. However, wherever it was possible, the technique was used as described above.

The range and accuracy of the method was assessed by doing a series of determinations on a glucose standard solution (Sigma 510-10) containing 0,1 mg glucose per ml, and dilutions of this solution containing 0,08, 0,05, 0,025, 0,01 and 0,005 mg glucose per ml. The effective range of the method as described above was from 0,08 mg glucose per ml down to 0,005mg glucose per ml. Within this range the determinations, using the 0,05 mg/ml sample as a standard, were all within 2% of the known concentrations, with the exception of the 0,005 sample. Using a 0,01 standard this sample also fell within the 2% range. The reproducibility of the method, when used on snail tissue extracts, where interference from other substances is possible, seldom showed differences greater than 2,6%.

Contoversy over the most effective method for the extraction of carbohydrate from mammalian tissue centres around the completeness of the extraction, and whether only carbohydrate is finally isolated. Carroll, Longley and Roe (1956), in a critical appraisal of the various methods used, have found that the more conventional method of Pflüger (1905, quoted in Stacey & Barker, 1962), which involves extraction in boiling 5N potassium hydroxide,

extracts a substance which is not glycogen, but is dialyzable, is anthrone sensitive, is precipitated by alchohol and reduces alkaline copper solutions. This fraction constitutes a source of error in glycogen determinations, but Carroll *et al.*, (1956) do not specifically rule out the possibility that it may be a polysaccharide. They contend that a series of five extractions with 5% trichloracetic acid (TCA) in a Servall Omnimixer at 14 000 rpm will extract all glycogen from rat liver but will exclude the unknown fraction described above.

In the present investigation the techniques of Pflüger and of Carroll *et al.* were compared. Individual tissues from three specimens of *B. (Physopsis) africanus* were approximately halved prior to drying; it was not possible to ensure that each portion had exactly the same proportion of individual organs, but this was attempted as far as possible. Each half was dried, and lipids were removed, after which each of the two halves of the same tissue was subjected to one of the above extraction methods, and the total carbohydrate content and total polysaccharide content estimated using Dreywood's anthrone reagent as described earlier. Details of the extractions used in the comparison between the two methods were as follows:

(i) Trichloacetic acid extraction:

1. Dry, fat-free tissue soaked in a small volume of 5% TCA for about one hour to prevent floating.
2. 2 ml TCA added and homogenised in an Ultra-Turrax homogeniser at 20 000 rpm for three minutes.
3. Centrifuged for three minutes at 3 000 rpm and decanted through Whatman No. 1 filter paper.
4. Residue subjected to four further one minute extractions in 1 ml TCA as described above; extract decanted through same filter paper; extracts pooled.
5. Homogeniser washed with six 1 ml changes of 5% TCA, which was pooled, centrifuged and decanted into the extract through the same filter paper.
6. Total extract made up to exactly 15 ml and thoroughly mixed.

7. Four samples of exactly 2 ml each pipetted into 15 ml centrifuge tubes, 10 ml of 95% ethyl alchohol added to each, and polysaccharides allowed to precipitate overnight.
8. Samples centrifuged at 3 000 rpm for five minutes.
9. Decanted and drained for ten minutes.
10. Precipitated polysaccharide redissolved in distilled water, samples pooled and made up to exactly 15 ml.
11. No consistent values for total carbohydrates could be obtained from the remainder of the extract in 6 above which at times gave no colour change at all with anthrone reagent; total polysaccharides were determined from the solution in 10 above.

In spite of the increased accuracy inherent in the above method (Carroll *et al.*, 1956) it has important disadvantages. Inconsistency of the total carbohydrate determinations, inevitable dilution and consequent increased manipulation of the determination technique, and the time consumed by each extraction all detract from the method. Also the use of a homogenizer, which was shown to require six washes with distilled water before all traces of carbohydrate were removed, does not appear to be the most suitable for the small tissue samples available.

(ii) Potassium hydroxide extraction:

1. Dried, fat-free tissue was boiled in 3 ml of 5N KOH until no further solids would dissolve (there was always a small insoluble fraction in muscle tissue).
2. Solution made up to exactly 5 ml and thoroughly mixed.
3. A single 2 ml sample precipitated with ethanol and treated as described for the TCA extraction method.
4. Precipitate dissolved and made up to exactly 5 ml with distilled water.
5. Remainder of extract could be used for the determination of total carbohydrates.

This extraction method, when compared with the TCA extraction method described above, has the advantage of being quickly executed, requiring few manipulations, and yielding more concentrated carbohydrate solutions. It could be used for

the estimation of the total carbohydrate content of the tissues, whereas the TCA extraction method could not. Its chief disadvantage is the yellow colour of the extract, which could interfere with colorimetric determinations, but this is considerably reduced on dilution to bring the solution within the range of the anthrone method, and is further minimised by dilution with the anthrone reagent, which is itself yellow prior to boiling with carbohydrate. It will, in any event, only affect determinations of total tissue carbohydrates, which cannot be satisfactorily done using TCA extraction. The unkown fraction reported by Carroll *et al.*, (1956) would presumably be a constant factor in the present comparative study. The results obtained for tissue polysaccharides by the two extraction methods are shown in Table 1. A paired comparison "t" test for this table, excluding the data obtained for the reproductive organs which were considered to be inadequate, showed no significant difference between the two methods (0,9>P>0,5). Further tests showed that sodium hydroxide was as effective as potassium hydroxide, and that 1N was as effective as more concentrated alkaline solutions.

On the strength of the above comparison, it was decided to use 1N sodium hydroxide in all further carbohydrate determinations. Results had, however, already been obtained from TCA extracted material; these were compared with the results obtained from tissues extracted with sodium hydroxide in order to determine whether the data could be pooled. Table 2 shows this comparison.

d. Protein determinations: Jacobs (1965) has reviewed the methods currently in use for the estimation of nitrogen in biological material. From this assessment the micro-Kjeldahl with direct nesslerisation, as described by Johnson (1941), was selected as the most suited to available equipment and materials. Initially the method was used only to standardise BDH Bovine Serum Albumen against snail tissue protein. The bovine albumen was then used as a standard for the determination of snail tissue protein content using Folin-Ciocalteau reagent as described by

Table 1 : Comparison between trichloracetic acid and potassium hydroxide extraction in the determination of tissue polysaccharides of *B. (Physopsis) africanus*.

		TCA extracted			KOH extracted		
		Tissue dry weight (mg)	mg Polysaccharide (≡ mg glucose)	% of dry weight	Tissue dry weight (mg)	mg Polysaccharide (≡ mg glucose)	% of dry weight
Muscle:	1	4,9	0,22	4,5	4,6	0,21	4,6
	2	2,7	0,11	4,1	3,9	0,18	4,6
	3	5,6	0,20	3,6	2,2	0,07	3,2
Gut:	1	2,8	0,16	5,7	4,5	0,26	5,8
	2	3,3	0,13	3,9	1,2	0,05	4,2
	3	1,6	0,11	6,9	3,9	0,27	6,9
Reproductive organs:	1	2,6	0,19	7,3	0,8	-	-
	2	3,7	0,30	8,1	2,0	0,15	7,5
	3	1,1	-	-	4,6	0,28	6,1

Table 2 : Comparison between polysaccharide contents of two batches of *B. (Physopsis) africanus* which had been extracted by the TCA and NaOH methods respectively.

	No. of snails	Mean polysaccharide content per snail (≡ mg glucose as % of dry tissue weight) Muscle	Gut	Reproductive organs
Fed snails				
TCA extracted	10	4,2 ± 0,257	6,1 ± 0,177	6,5 ± 0,567
NaOH extracted	10	4,0 ± 0,321	5,7 ± 0,236	6,7 ± 0,439
Difference		0,2	0,4	0,2
Probability		0,9>P>0,5	0,5>P>0,1	0,95>P>0,9
Starved snails				
TCA extracted	8	4,2 ± 0,274	2,2 ± 0,349	6,4 ± 0,346
NaOH extracted	7	4,4 ± 0,158	1,5 ± 0,296	6,4 ± 0,515
Difference		0,2	0,7	0,0
Probability		0,9>P>0,5	0,5>P>0,1	1,0>P>0,99

Lowry, Rosebrough, Farr and Randall (1951). The latter technique proved reliable for some time, but with a new batch of reagent discrepancies between replicates became frequent, and it was considered advisable to abandon it in favour of the micro-Kjeldahl for all further protein determinations. This introduced a further variable into the results; protein determinations using the Folin-Ciocalteau method of Lowry *et al.* had already been made on tissues from which carbohydrates had been extracted by both the methods discussed in 'c' above, and, with the introduction of the micro-Kjeldahl method of protein determination for use on the actual tissue protein extracts, three combinations of carbohydrate extraction/protein determination techniques had been used. These were:

1. TCA precipitated tissue, redissolved in 1N sodium hydroxide, the protein content of which was determined

using the Folin-Ciocalteau method;

2. Tissue dissolved in 1N sodium hydroxide, the protein content of which was determined using the Folin-Ciocalteau (F-C) method; and
3. Tissue dissolved in 1N sodium hydroxide, the protein content of which was determined using the micro-Kjeldahl method.

Table 3 shows the results obtained from each of these three combinations for snail muscle, which, of the three tissue complexes analysed, showed the greatest individual variation. An analysis of variance of the data in Table 3 showed no significant difference between the results obtained from the three combinations $F_{2/27} = 2,2$; $0,5 > P > 0,1$), and the results could therefore be pooled.

Table 3 : Comparison between the results obtained from the use of different combinations of protein extraction/protein determination methods on *B. (Physopsis) africanus* muscle. (For details see text).

Method	No. of snails	Mean tissue dry weight (mg/snail)	Mean protein content (mg/snail)	Mean protein content (% of dry weight)
TCA extracted/ F-C determined	10	9,15	5,76 ± 0,60	62,43 ± 1,0
NaOH extracted/ F-C determined	10	10,20	6,42 ± 0,71	62,34 ± 1,2
NaOH extracted/ Kjeldahl determined	10	9,49	6,18 ± 0,76	65,45 ± 1,4

A possible source of error in the sodium hydroxide extraction/ micro-Kjeldahl determination combination is the presence of non-protein nitrogen. Clinical analysts tend either to ignore this fraction, or to reduce the total protein nitrogen value by 0,15% (Varley, 1962), unless it is of specific importance. In the present comparative study it

was felt that it could safely be ignored as a constant, or at least a near constant, factor. It has been pointed out that the colour developed does not always follow Beer's Law throughout the full range (Varley, 1962); this was checked for the range of protein concentrations encountered, and the technique was found to be satisfactory provided that the protein content per sample tested was kept within the range 0,07 to 0,3 mg.

Both the micro-Kjeldahl and the Folin-Ciocalteau methods of protein determination were tested on protein solutions (BDH Bovine Serum Albumen) of known protein contents in the range 0,025 to 2,5 mg protein per ml. The micro-Kjeldahl method was also tested over a range of urea (BDH "Aristar") concentrations containing between 11,66 and 93,28 µg nitrogen per ml. The results of the range, accuracy and reproducibility tests are given in Table 4.

Table 4 : Range and reliability of the methods used in the determination of tissue protein content.

	Micro-Kjeldahl	Folin-Ciocalteau reagent
Range	0,07 - 0,3 mg protein per sample (bovine albumen) 11,16 - 46,64 µg nitrogen (urea)	0,005 - 0,1 mg protein per sample (bovine albumen)
Accuracy	All determinations within 2,1% of known protein content (bovine albumen) All determinations within 0,5% of known nitrogen content (urea)	All determinations within 1,0% of known protein content (bovine albumen)
Reproducibility	Within 2% (bovine and snail tissue) No discrepancy between replicates (urea)	Within 1% (initially) and 9% (new reagent) (bovine albumen and snail tissue)

e. Colorimetry: All colorimetric determinations were made using either a Bausch and Lomb "Spectronic 20" or a Hitachi Model 101 UV-VIS spectrophotometer. General colorimetric technique as described by Umbreit, Burris and Stauffer (1964) was followed.

MEASUREMENT OF OXYGEN CONSUMPTION

Determination of the rate of oxygen consumption needed to be made on snails both in and out of water. Neither the polarimetric methods used by Berg, Lumbye and Olckermann (1959) and by Harrison (1968) nor the titrimetric methods used by Berg and Ockelmann (1958) and by Zaaijer and Wolvekamp (1958, quoted in Ghiretti, 1966), meet this requirement, since they are suitable only for the measurement of cutaneous or physical gill respiration in water. The Warburg respirometer has been used for the measurement of oxygen consumption of pulmonate snails by von Brand, Nolan and Mann (1948); this instrument allows for the measurement of oxygen uptake both in air and in water, is extremely sensitive, is basically simple in its principle of operation and allows the carrying out of prolonged experiments without disturbing the experimental animals. The princple of operation of the Warburg respirometer depends on the animals utilizing the oxygen from an atmosphere isolated within a gas-tight flask; carbon dioxide is constantly being removed from the flask atmosphere by a solution of potassium hydroxide. Such a carbon dioxide free atmosphere, with a constantly diminishing oxygen content may be regarded as unnatural. This criticism is minor in a comparative study, and the rate of oxygen consumption of *Bulinus (Physopsis) africanus* is such as to deplete the oxygen content of the flask by no more than 1% per hour which seems hardly likely to affect the results materially.

The instrument used was a Braun Model V85 fitted with a Lauda flowthrough cooler. The system was set up for recirculation of the coolant through thermally insulated tubing, using a gravity feed through the cooler; the lift was provided by an ASTI 120 W washing machine pump. The range of water bath temperatures obtainable was continuous between 3°and 52°C.

Factory calibrated Dickens-Simer RQ flasks (Braun No. 32-315)

were used for active snails, since the slope leading to the gutter containing the carbon dioxide absorbant (10% potassium hydroxide) was such that the snails could not readily reach the reagent and thus injure themselves. Calibrated 20 ml conical reaction flasks (Braun 30-525) were satisfactory for dry, and thus immobile animals.

Calculation of flask constants (Umbreit *et al.*, 1964), required that the volume of each snail used in an experiment was known. Such volumes were derived from the live weight of the individual snail and the average density, determined by a modification of the method developed by Solomon (1969). Snails with air-filled mantle cavities were carefully dried with absorbant tissue paper and weighed. These were then placed, in batches of five, into specific gravity bottles filled with distilled water which had previously been weighed. The specific gravity bottles were placed in the vacuum chamber of a vacuum embedder which was evacuated until no further air bubbles emerged from the pneumostomes of the snails. Return to ambient atmospheric pressure replaced the air with water. A second evacuation was sufficient to remove the last traces of air. The specific gravity bottle was then re-stoppered, weighed again, and the specific gravity was calculated from the difference in weights. The density of *B. (Physopsis) africanus* was estimated by this method to be 1,13 which shows good agreement with Solomon's (1969) figure of 1,1 for *B. (Bulinus) tropicus*.

The respirometer was used as recommended by Umbreit *et al.* (1964). One hour was allowed for equilibration with experimental temperatures; periods between recordings varied depending on the rate of oxygen consumption and thus on the experimental temperature, always ensuring that a good, accurately measurable difference in pressure was evident before a reading was taken. Because of the intermittant opening and closing of the pneumostome, periods of less than one hour were never considered; four hours gave consistent results at all but the lowest temperatures.

5. MATERIAL

All specimens of *Bulinus (Physopsis) africanus* used in this investigation were collected from the following three localities:

Shongweni snails (prefix SH) : Collected from the cushion basin of the Vernon Hooper Dam on the Umlaas River at Shongweni.

Krantzkloof snails (prefix KK) : Collected from the large pool behind the weir on the Molweni River in the Krantzkloof Nature Reserve.

Nelspruit snails (prefix NPT) : Obtained from the Bilharziasis Research Unit, Nelspruit, and collected from a small dam near Mataffin.

Additional material was collected from the cushion basin of Nagle Dam on the Umgeni River, the lower reaches of the Palmiet River, and obtained from the South African Institute for Medical Research. These animals were only used in developing and testing techniques.

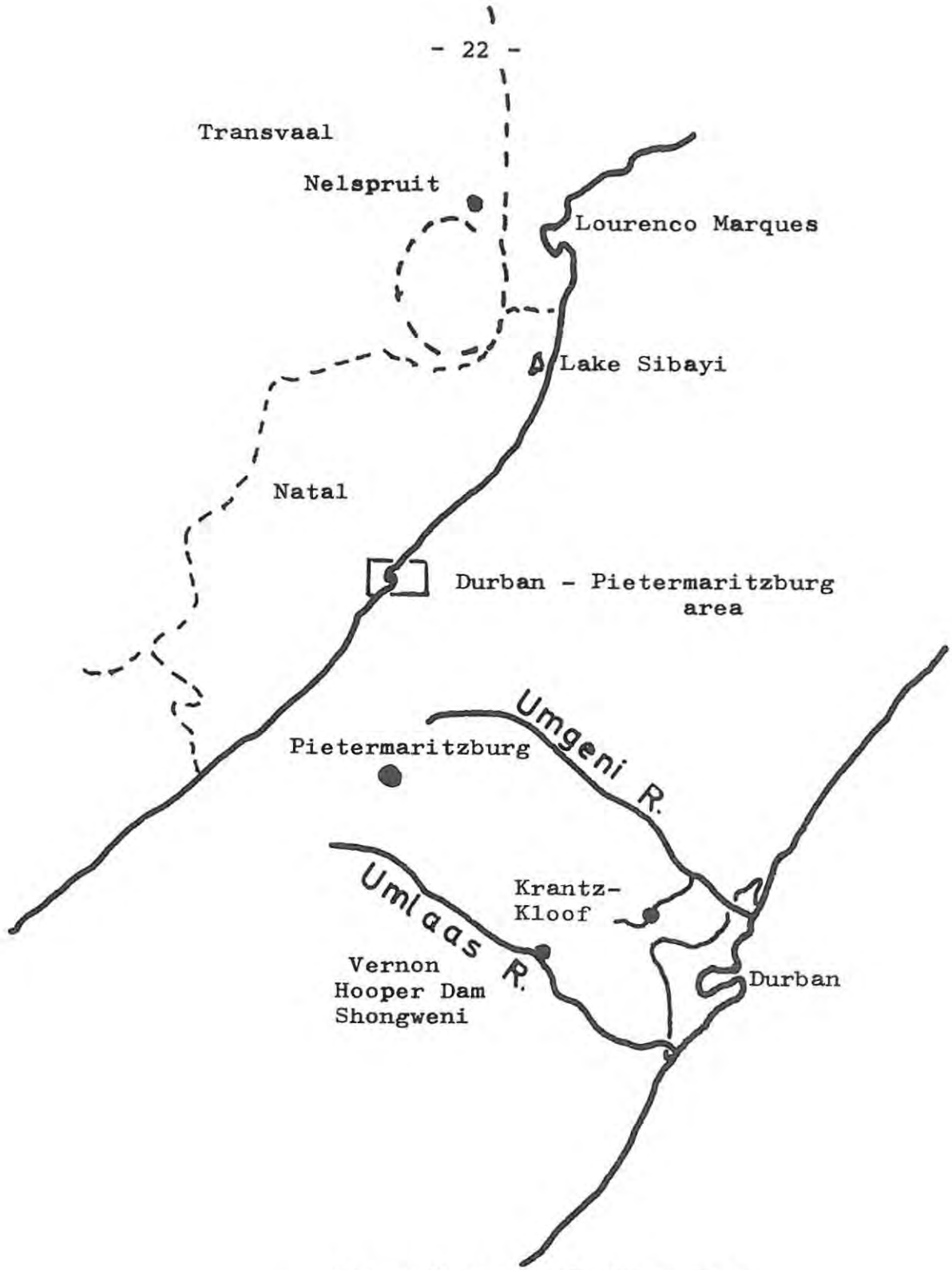

Figure 1 : Localities from which *B. (Physopsis) africanus* in the investigation were collected.

6. LABORATORY CULTURE METHODS

Harrison (1966) and Harrison and Schiff (1966) have shown that both temperature and chemical composition of water affect the intrinsic rate of natural increase, and hence the distribution and abundance of aquatic pulmonate snails. From these studies it appears that *Bulinus (Physopsis) globosus* (Morelet) while more sensitive to temperature changes than *Biomphalaria pfeifferi* (Krauss), is far more tolerant of a wide range of calcium and bicarbonate concentrations. The latter has been further elaborated by Williams (1970 a & b) who shows that in waters of "medium hardness" this species shows a low peak both in the relative numbers encountered in the field and in its intrinsic rate of natural increase under laboratory conditions. Harrison (1968) has also shown that calcium and bicarbonate concentrations can have a marked effect on the rate of oxygen consumption of *Biomphalaria pfeifferi*, which has a higher rate of oxygen uptake in a medium in which the bicarbonate concentration is optimal for its intrinsic rate of natural increase than in media with higher or lower bicarbonate contents.

The above findings all point to the desirability of keeping snails to be used in physiological studies under standard conditions. Field snails had to be adjusted to these standard conditions before use in experiments, and other factors, such as the possibility of infestation by the epizoötic oligochaete *Chaetogaster sp*, schistosome infection, and the possibility of physiological shock on transfer to standard laboratory conditions all had to be considered.

STANDARD CULTURE CONDITIONS

a. Aquaria and Culture Water: All experimental snails were kept in metal-free perspex aquaria, each containing 10 litres of standard aquarium water. Standard aquarium water was made up from matured pond water, collected from an artificial pond on the University of Natal campus. The water in the pond originated from the municipal water supply, but first passed through a filter bed planted with several indigenous marginal grasses. There was a slow but continuous flow

of water through the pond at all times. Appropriate volumes of 1M solutions of sodium bicarbonate (analytical grade) and calcium chloride (prepared from analytical grade calcium carbonate) were added to bring the calcium and bicarbonate concentrations up to 20 and 40 mg/l respectively.

Pond water was analysed for calcium, magnesium and bicarbonate concentration before each new batch of aquarium water was prepared, and a complete analysis was performed twice annually during 1968 - 1970, but this was discontinued when it became apparent that there were no significant fluctuations. The results of a complete analysis of both pond water and standard aquarium water are included in Table 6.

Each aquarium normally housed 25 snails, except when unusually large numbers were collected, in which case as many as 40 individuals would be kept in an aquarium for short periods. Such crowding had no apparent effect on the snails, but was nevertheless avoided whenever possible.

b. Temperature: While it was desirable to maintain the snails within a temperature range which would ensure maximal survival, acclimation to a single temperature, or to a narrow temperature range, had to be avoided, since the effect of environmental temperture was an aspect which was to be investigated. Seasonal differences, which could affect later comparisons, also had to eliminated as far as possible. The aim was, therefore, to subject the snails in culture to a standard, or near standard daily temperature range. Ideally the temperature fluctuations encountered during a typical day in the natural habitat should have been simulated in the laboratory. Attempts at obtaining temperature data from the collecting localities were frustrated by these being open to the public; three maximum-minimum thermometers concealed at Krantzkloof at various times were lost, after which further attempts were abandoned. Water temperature were recorded at all times when snails were collected, which was usually during the afternoon, and therefore near maximum for the day. These temperatures were all within the ranges 18°C -

26°C and 18° - 28°C for Krantzkloof and Shongweni respectively.

On the basis of these latter temperatures alone, it was decided to allow the water temperature in the cultures to fluctuate between 15°C (night) and 25°C(day). This had to be achieved by heating the culture room as a whole, since the stirring of the water necessary for direct heating of the aquaria tended to disturb the snails. Heating was effected by means of a convection heater controlled by a time switch. During summer the ambient temperature of the room was boosted between 1100 hours and 1400 hours to compensate for the lack of direct solar heating which would be experienced in bodies of water exposed to the sun. In winter longer heating periods were necessary during the day, and additional short periods of heating prevented too low a night temperature. The duration of these periods of heating had to be regulated from time to time according to season. The actual temperature ranges achieved were 15° - 27°C (summer) and 13° - 25°C (winter).

Bruton (personal communication) has made available to me temperature data collected for a shallow experimental pond at the Lake Sibayi Research Station. These data are summarised in Table 5.

Lake Sibayi has an Effective Temperature of 18°C, while the Durban area (in which both Krantzkloof and Shongweni are located) and Nelspruit fall between the 17 C and 18°C Effective Temperature isolines (Stuckenberg, 1969). This implies a lower mean annual temperature at the latter localities and, also, possibly lower maxima and minima in air temperature, since Effective Temperature is also dependent upon the difference between the means of the hottest and coldest months of the year. The laboratory culture temperature range falls well within that for Lake Sibayi experimental pond. It is, therefore, likely to fall within the compass of the ranges encountered at the collecting localities as well.

c. Illumination: Lighting was provided by a 2 meter double fluorescent lighting unit. All aquaria were approximately equidistant from the light source, and therefore received

Table 5 : Summary of temperature maxima and minima recorded in a shallow experimental pond at the Lake Sibayi Research Station, compiled from daily recordings.

	Maximum (°C)		Minimum (°C)	
	Mean	Range	Mean	Range
Summer (Dec.-Feb.)	30,4	23,4-35,0	24,0	20,3-28,9
Autumn (Mar.-May)	28,0	15,9-33,0	22,9	14,0-27,2
Winter (June-Aug.)	20,8	17,0-24,0	16,3	14,0-18,9
Spring (Sept.-Nov.)	25,9	17,2-30,9	20,5	12,2-25,1

approximately equal light intensity. The photoperiod to which the animals were subjected was ten hours full illumination per day, with dawn and dusk periods governed by day length.

d. Feeding: The snails were fed on dried boiled lettuce prepared according to Garnett (1964). Carrot as a supplement was given occasionally, but not regularly and never during pretreatment for an experiment. Lucerne dried in a plant drying oven at 60°C was also given as a supplement.

Food was given as and when necessary; uneaten food was not allowed to accumulate in the aquaria but the snails were never without food.

e. General maintenance: Aquarium water was not changed regularly. Daily inspection and the immediate removal of dead snails was sufficient to keep the cultures in good health. Faeces were removed from the aquaria when considered necessary, but their presence had no apparent effect on the snail population. Cloudy water was immediately changed, and periodic checks on the pH of the water helped to anticipate deterioration of the aquarium water. All aquaria were covered with sheets of glass to prevent excessive evaporation. Whenever noticeable evaporation had occurred, this was made good by the addition of glass distilled water up to the 10 litre mark.

TREATMENT OF FIELD SNAILS

No serious attempt was made to rear snails in the laboratory for use in the investigation, since the rate at which they were used was too great to warrant added time which would have had to be spent on culturing. Field snails only were, therefore, used and these had to be allowed time to adjust to laboratory conditions before use to ensure reasonable uniformity.

All snails brought into the laboratory were, on arrival, placed under quarantine. This involved their being kept either in their native water, or in standard aquarium water if the risk of physiological shock on transfer from their native water had already been eliminated. During the quarantine period the animals were kept, usually at a higher density than normal, in 7,5 litre polycrate tubs. Ailing and abnormal snails would usually succumb during the first two or three days in quarantine, and the remainder were tested for the presence of *Schistosoma* and *Chaetogaster* during the next two weeks. Thereafter all snails passed as fit for experimentation were transferred to standard culture aquaria as described above.

Each batch of snails was designated a number on arrival in the laboratory. The batch number indicated its origin and from the record kept, its date of collection could be established. The record also showed to what use each batch was subsequently put.

a. *Chaetogaster* inspection: Visual inspection under a dissecting microscope readily revealed heavy infestations of *Chaetogaster*, provided that the snail was totally immersed and actively moving. While one or two worms in the mantle cavity of a snail could and did escape detection, these were not considered likely to affect the experimental results. Only twice during the investigation was it considered necessary to eliminate a heavily infested snail, which had escaped detection, from an experiment. All infested snails were immediately culled.

   While Shongweni snails were often found to harbour *Chaetogaster*, none was recorded from either Krantzkloof or Nelspruit snails.

b. *Schistosoma* infection: Snails were placed in groups of

five, into 50 ml beakers containing the medium in which they were being quarantined, and inspected for the liberation of cercaria. Should cercaria be found with any particular group, the infected snail could be isolated by subsequently testing each individual in the group. All snails were tested three times during the two-week quarantine period.

Only a single infected snail was found in the course of the investigation. This specimen originated in the Palmiet River near its confluence with the Umgeni which was not a regular collecting site.

c. Physiological shock on transfer: Early in the investigation it was noticed that Shongweni snails suffered abnormally high mortality when transferred to standard aquarium water, while Krantzkloof snails did not. Batch SH-1 from Shongweni suffered a 34% mortality during the first two days in standard aquarium water, compared with 3,5% for batch KK-1 from Krantzkloof, collected on the same day and treated identically.

It was necessary to establish whether this abnormally high mortality of the Shongweni snails was due to the change in culture medium, and, if so, whether by suitable acclimation the animals could be brought into standard culture. Survival alone was considered insufficient indication that the animals could be used in physiological experiments. Since the present investigation concerned, among other aspects, the utilization of metabolic reserves, and involved considerations of metabolic rates, the rate of oxygen consumption of snails transferred to standard aquarium water, either directly from their native water or through acclimatory stages, was also considered as an indication of physiological normality.

Further collections from the two localities were made. Samples of water were also collected and composition of the dissolved solids was determined for each. On the basis of these analyses an ionically similar substitute Shongweni water, and two acclimation stages between Shongweni water and standard aquarium water were made up by adding appropriate ions to stock water from the campus

pond. Details of the chemical analyses of these media together with that of the two natural waters, are set out in Table 6.

The Shongweni snails (batch SH-3) were divided into three groups and treated as follows:

Group 1 : transferred from their native water to Shongweni water substitute.

Group 2 : transferred directly from their native water to standard aquarium water.

Group 3 : acclimated in two stages, each of one week duration from their native water to standard aquarium water.

The Krantzkloof snails (batch KK-4) were divided into two groups, one of which was kept in Krantzkloof water, and the other transferred directly to standard aquarium water.

Samples of between 20 and 25 snails were drawn from each group within three hours of their transfer to standard aquarium water, and their rate of oxygen consumption, in 2 ml of the standard medium, was measured in the Warburg respirometer at 25^{0}C. The remainder of each experimental group was kept in standard aquarium water (or the final medium) for a period of two weeks, during which time mortalities were recorded. The rate of oxygen consumption of the survivors from Group 2 of batch SH-3 was measured after the animals had been in standard aquarium water for the two week period. The results of these experiments are shown in Tables 7 and 8. All snails had been starved for 24 hours prior to transfer to the final medium.

It is clear from Tables 7 and 8 that direct transfer of Shongweni snails from their native water to standard aquarium water does subject them to considerable physiological shock. While the mean rate of oxygen consumption is not very much higher than that of acclimated Shongweni snails or Krantzkloof snails, the standard error is considerably greater and the coefficient of variation is more than twice as high, implying more than double the

Table 6 : Results of detailed analyses of natural waters and various artificially prepared media for the laboratory culture of *Bulinus (Physopsis) africanus*.

	Shongweni water	Krantzkloof water	Pond water	Standard aquarium water	Shongweni water	Acclimation water Stage 1	Acclimation water Stage 2
pH	8,3	6,9	7,2	7,6	8,1	7,9	7,7
Conductivity (µmho/cm at 25°C)	837	74	83	223	1273	584	415
Total hardness (ppm $CaCO_3$)	74,5	23,5	27,5	58,5	79,5	61,0	59,6
Alkalinity (ppm $CaCO_3$)	135,5	14,0	22,5	32,0	121,6	69,5	43,5
Ca^{++} (meq/l)	1,27	0,47	0,50	1,13	1,32	1,17	1,13
Mg^{++} (meq/l)	0,21	0,03	0,06	0,04	0,27	0,05	0,06
Na^{+} (meq/l)	5,11	0,04	0,22	0,58	6,14	3,21	2,00
K^{+} (meq/l)	0,20	0,01	0,02	0,02	0,02	0,02	0,02
HCO_3^{-} (meq/l)	2,71	0,28	0,45	0,64	2,43	1,39	0,87
Cl^{-} (meq/l)	3,64	0,17	0,14	0,82	5,10	2,97	2,09
SO_4^{--} (meq/l)	0,34	0,05	0,14	0,15	0,39	0,12	0,14
TDS (ppm)	490	41	65	126	537	308	216

Table 7 : Rates of oxygen consumption of *B. (Physopsis) africanus* on transfer from native water to various artificial culture media.

Batch No.	Number of snails	Treatment	Rate of O_2 uptake (µl/mg dry wt./h)	
			Mean S.E.	V_C*
SH-3	25	Native to Shongweni water substitute	1,3±0,066	25,4
SH-3	25	Native to standard aquarium water (direct)	1,6±0,215	66,3
SH-3	25	Native to standard aquarium water (2-stage acclimation)	1,4±0,070	25,0
SH-3	12	Native to standard aquarium water (survivors 2 weeks after direct transfer)	1,3±0,106	28,5
KK-4	20	Kept in native water as control	1,3±0,070	24,6
KK-4	22	Native to standard aquarium water (direct)	1,3±0,064	23,1

* V_C = Coefficient of variation = 100 x Standard Deviation/Mean

individual variation within the sample. This conclusion is further substantiated by the considerably increased mortality on transfer. Shongweni snails were, therefore, in all instances acclimated to laboratory conditions.

Specimens from batch NPT-1, collected at Nelspruit, were transferred directly to standard aquarium water with no mortality. These snails were tested in the respirometer on arrival, and again after two weeks in laboratory culture. The rates of oxygen consumption were 1,5±0,088 and 1,4±0,073 respectively. Although Krantzkloof and Nelspruit snails did not require acclimation, they were nonetheless kept under standard laboratory culture conditions for at least two weeks before they were used in an experiment.

Table 8 : Mortality of *B. Physopsis africanus* on transfer from native water into various artificial media.

Batch No.	No. of snails	Treatment	Mortality (No. died)		
			Week 1	Week 2	Total
SH-1	88	Native to standard aquarium water (direct)	30	-	-
SH-3	83	Native to standard aquarium water (direct)	29	8	37(46%)
SH-3	74	Native to Shongweni water substitute	2	1	3(4%)
SH-3	85	Native to standard aquarium water (2-stage acclimation)	2	1	3*(4%)
KK-1	57	Native to standard aquarium water (direct)	2	-	-
KK-4	85	Native to standard aquarium water (direct)	2	0	2(2,5%)
KK-4	62	Kept in native water under laboratory conditions (control)	3	0	3(4%)

*Three snails died during acclimation.

PRETREATMENT OF SNAILS PRIOR TO USE IN EXPERIMENTS

While the standard culture conditions, quarantine and acclimation ensured, as far as possible, that the snails used in experiments were in near uniform physiological condition, the nature of the experiments was such that additional safeguards against undue variability were necessary. It was impossible in a mass culture of 25 to 40 snails in an aquarium to know whether any individual was or was not feeding regularly, or whether it had recently laid eggs, both of which could have an effect on the experimental results. To overcome this, snails intended for use in a particular experiment were placed singly in plastic cups containing 400 ml of standard aquarium water. Other than being isolated, these snails were fed and treated in the normal way for a period

of at least ten days before use. During this time they were inspected daily for abnormal behaviour such as remaining stationary for any length of time, non feeding and non production of faeces, and for the presence of eggs.c Any snail judged as being abnormal, or which had produced eggs during the pretreatment, was eliminated from the experiment.

The snails were starved for 24 hours prior to use in an experiment in order to empty the alimentary canal. Carricker (1946) reports the time required for the passage of ingested food from feeding to defaecation as being between 2 hours 20 minutes and 5 hours 50 minutes for *Lymnea stagnalis appressa*. A 24 hour starvation period was therefore considered adequate to nearly, if not completely, empty the gut. On no occasion did any of the experimental animals produce faeces during the respirometry experiments.

7. AESTIVATION IN *BULINUS (PHYSOPSIS) AFRICANUS*

Aestivation in terrestrial Gastropoda, as typified by *Helix pomatia* (Wells, 1944), is characterised by inactivity. The snail remains retracted within its shell which closes by means of a mucus veil, and it does not feed, being dependent upon stored energy reserves. In this state it remains alive during periods of adverse environmental conditions. This ability to survive non-seasonal adversity is shared by other terrestrial Gastopoda. *Natalina quekettiana* (Melvill & Ponsonby), during dry periods, seals its shell with soil and a cementing agent (probably mucus), and a small unidentified snail from the Namib desert has been kept alive in the laboratory without food or water for six months (personal observations). *Sphicterochila boisseri* (Charpentier) which inhabits the desert regions of Palastine may survive the harsh desert conditions which prevail in its habitat for periods longer than a year in an inactive state (Schmidt-Nielsen, Taylor & Shkolnik, 1971).

Aquatic snails, as far as is known, only aestivate when removed from water. Like their terrestrial counterparts, they retract into the shell, which may be closed by an operculum in the operculate Prosobranchia (Meenakshi, 1964; Coles, 1968; Little, 1968), by a mud plug in some Pulmonata, notably *Bulinus (Bulinus) tropicus* (Krauss) (Annecke & Peacock, 1951) or simply by means of a mucus veil, often referred to as the epiphragm, as in *Bulinus (Physopsis) africanus*. Aquatic snails may aestivate on the surface of the bottom of dried up ponds, or they may burrow into mud if it is sufficiently soft, or they may find shelter under logs and stones, or among the roots of marginal vegetation (Annecke & Peacock, 1951; Olivier, 1956; Cridland, 1967). These snails remain alive during the period of exposure, and resume their normal activities after sufficient rain has fallen to re-inundate their habitat.

The aestivating *B. (Physopsis) africanus* is, therefore, characterised by:

(i) being out of water,

(ii) being retracted into its shell and ostensibly inactive,

(iii) the presence of a mucus veil covering the exposed surface of the foot; this may not be readily seen when the animal has contracted deep into the shell, but its presence may often be inferred from a white deposit on the inner surface near the aperture, and,

(iv) being alive; this can be determined by the definite form of the animal within the shell which can be seen when viewing the snail against a strong light, and by the absence of a smell of putrification which is noticeable in a dead snail within a few hours after death, even before lysis of the tissues has set in.

The aestivating *B. (Physopsis) africanus* therefore survives under conditions which, when compared with those obtaining in its normal aquatic environment, are extremely harsh; it is subject to temperature extremes without the buffering effect of water, to starvation, and to desiccation.

8. ACTIVITY IN RELATION TO AESTIVATION IN *BULINUS (PHYSOPSIS) AFRICANUS*

One of the criteria characterising the aestivating *B. (Physopsis) africanus* is inactivity. *Pila virens* (authority not quoted) has been reported by Meenakshi (1964) as remaining active for some time after having been removed from water, the period of activity being determined by the ambient relative humidity. Little (1968) has reported a similar period of activity prior to aestivation in *Pomacea lineata* (Spix) and *Pomacea depressa* (Say). Such pre-aestivation activity has not been reported for aquatic pulmonate snails, but was noticed in *B. (Physopsis) africanus* during the course of the present investigation. It was not altogether unexpected, since aestivating snails have been found under sheltering objects such as stones and vegetation. No activity had, however, been observed in an aestivating aquatic snail after the onset of aestivation; it had been assumed that once the animals finally became immobile, no further movement took place until aestivation was broken by the snail being immersed in water.

A number of *B. (Physopsis) africanus* were collected as aestivators at Shongweni. These snails had gone into aestivation as a result of a considerable change in the water level of the cushion basin of the Vernon Hooper Dam following the temporary opening of the scour valves of the dam. Scouring had taken place four weeks prior to collection of the snails, thus they could reasonably be expected to have been in aestivation for that period. The snails were placed on a bed of wet umgeni sand on arrival in the laboratory, and it was found, on inspection the following morning, that a number of them had burrowed into the sand. Activity was, therefore, possible, not only immediately prior to aestivation, but also once the animals had been in aestivation for some time.

Activity prior to, and during, aestivation has survival value in that it allows a snail, within limits, to seek out a favourable place in which to aestivate. The activity patterns of *B. (Physopsis) africanus* immediately prior to and during aestivation were therefore investigated. The experiments

carried out were essentially of a preliminary nature and merely serve to show what activity may take place under different environmental conditions.

MOVEMENTS OF *B. (PHYSOPSIS) AFRICANUS* ON DRYING OUT OF ITS HABITAT

The aggregation of snails in the deepest part of a pool, as it dries out during the dry season, must perforce result in considerable competition for dwindling food resources and for space during the final stages of drying. As aestivating snails had been found among the exposed bases of the marginal plants in a pool while there was still water in the deepest part, it appeared unlikely that the snails actually follow the water level down as a pool dries. The following experiment was done to determine the behaviour of the animals in relation to changing water level.

Method:

Twenty *B. (Physopsis) africanus* were placed into standard aquarium water in a polycrate trough measuring 35 x 25 x 11 cm, in which a sloping sandy substratum was provided as shown in Figure 2. A mud substratum would in many ways have been preferable, but it introduced additional variables in that it discoloured the water and could have affected the composition of the dissolved solids in the water.

The trough was housed in the snail culture room and the water was allowed to evaporate. The number of snails remaining in the water were recorded daily. The snails were fed in the normal way, and the water temperature was monitored by means of an Amarell maximum-minimum thermometer.

Results:

By the time the water level had fallen from 8 cm to 3 cm in the deepest part of the trough, no snails had permanently left the water. The water temperature ranged between 17°C and 27°C throughout the 22 day duration of the experiment.

The experiment was repeated out of doors, exposing the trough to the full sun. No snails left the water permanently during the first two days while the water level was high and the maximum water temperature was 27°C. After two hot days, during which the water level fell rapidly and the water

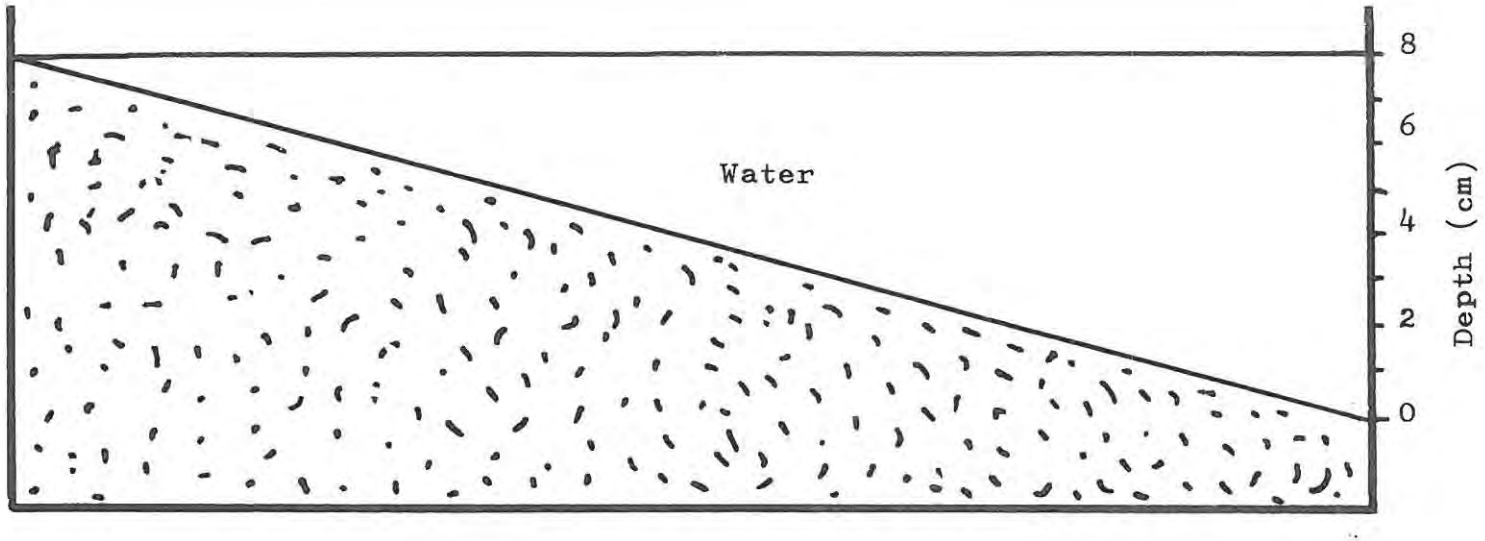

Figure 2 : Trough used to study the response of *B. (Physopsis) africanus* to the drying up of its habitat.

temperature rose to 34°C, all the snails had left the water, and were, apparently, going into aestivation; some had attempted to burrow into the sand. Three snails had re-entered the water by the day following the 34°C water temperature but these left the water again during the course of that day. The results are detailed in Table 9.

Table 9 : Movement of *B. (Physopsis) africanus* when its habitat dries out through exposure to the sun.

Day	Max. & Min. temperature for previous 24 hours (°C)	Depth of water at deepest part of trough (cm)	Number of snails left in water
0	-	8,0	20
1	27/18	7,0	20
2	25/17	6,3	20
3	25/19	5,5	17
4	26/19	4,5	19
5	29/18	3,5	9
6	34/19	2,0	0
7	28/21	1,6	3
8	29/19	0,4	0

It seems, therefore, that high temperatures are, at least in part, responsible for the animals leaving the water.

ACTIVITY OF *B. (PHYSOPSIS) AFRICANUS* ONCE OUT OF WATER

Since activity has been observed in snails after they had been removed from water, it was necessary to know what factors govern the duration of the period of activity.

Method:

Specimens of *B. (Physopsis) africanus*, which had been subjected to normal experimental pre-treatment, were placed on a substratum of wet umgeni sand in individual 400 ml plastic cups. A water table which just reached the surface of the

sand was provided. The position of each snail was indicated by a marker pushed into the sand, next to the animal. The snails were inspected daily to see wether any individuals had changed their position from the marker; whenever such movement occurred, the marker was re-located to the new position of the snail.

A record was kept of the water content of the sand. This was assessed on the following criteria:

(i) if free water appeared on the surface of the sand following a light pressure from a finger applied to the surface, the sand was considered *wet*,

(ii) if the sand obviously contained moisture, as inferred from its colour and cohesion, but no free water appeared after light pressure on the surface, it was considered *damp*, and,

(iii) if the sand was powdery, with no cohesion between particles, it was considered *dry*.

Results:

The results are shown in Figure 3. The snails were active during the initial stages, while the sand was wet. Activity fell off as the sand dried out, and several individuals burrowed into the sand. Unfortunately no experiment was done starting the animals on dry sand. It has, however, been noticed that animals placed on dry filter paper will generally cease all movement during the first three hours, and always within the first six hours.

It seems, therefore, that moisture content of the surface governs the duration of pre-aestivation activity, which supports Meenashki's (1964) observation that ambient relative humidity governs the duration of the period of activity.

ACTIVITY OF *B. (PHYSOPSIS) AFRICANUS* DURING AESTIVATION

Following the observation that *B. (Physopsis) africanus* which had been aestivating for four weeks did, in fact, move, the following experiment was done to confirm the observation, and to determine whether, as in the case of pre-aestivation movement, the activity was governed by the moisture content of the substratum.

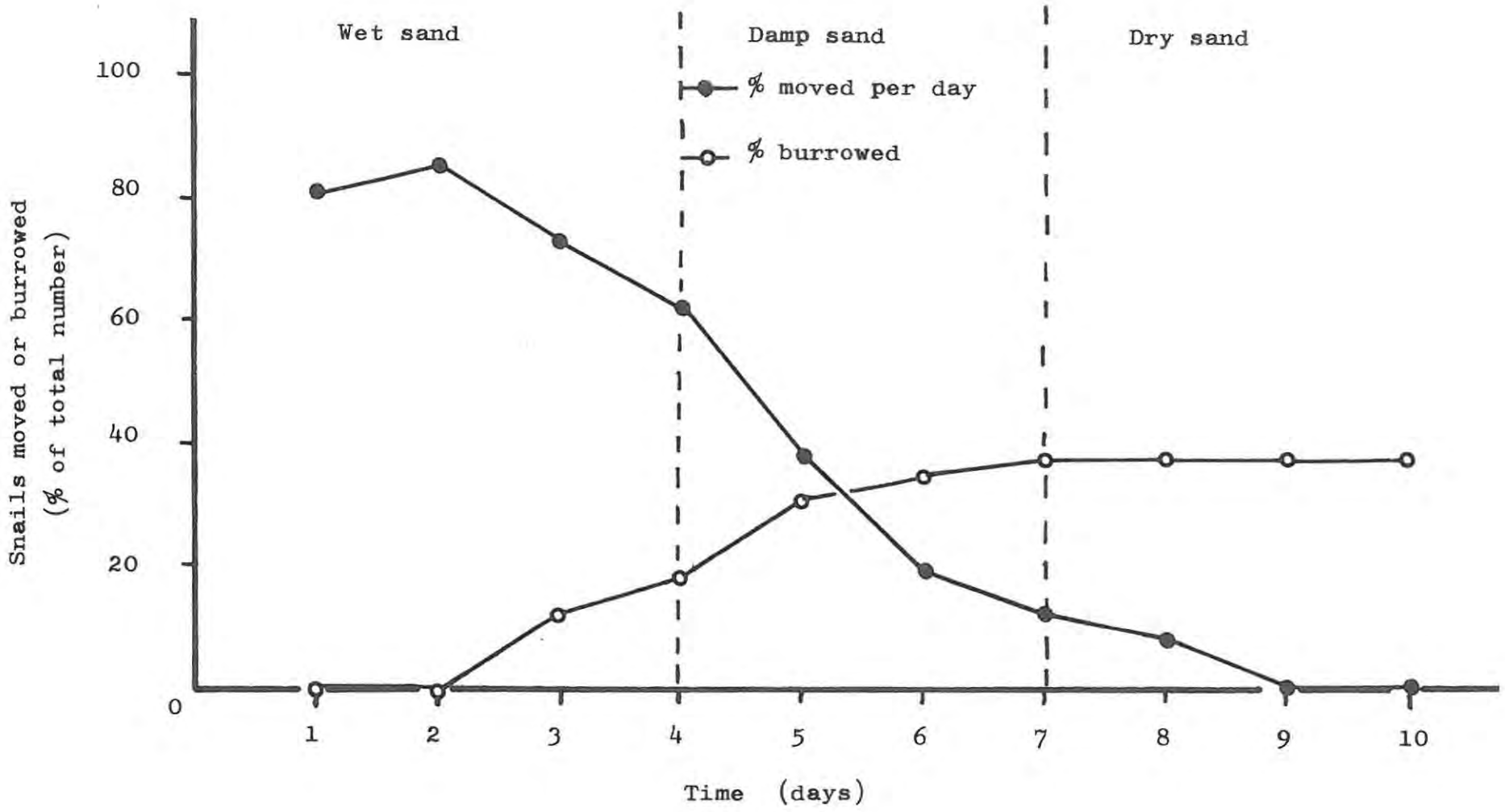

Figure 3 : Activity of *B. (Physopsis) africanus* in relation to moisture content of sandy substratum.

Method:

26 aestivating *B. (Physopsis) africanus* collected at Shongweni were placed individually in 400ml plastic cups containing dry umgeni sand. Each snail was positioned so that the aperture of the shell was in contact with the substratum and its position was indicated by a marker. After a period of four days water was introduced into the bottom of the cup by means of a syringe; sufficient water was provided to adjust the water table to a level just below the surface. Free evaporation was allowed, so that the sand could slowly dry out. The snails were inspected daily throughout the experiment for indications of movement.

Results:

The results, shown in Figure 4 amply confirmed that the animals could and did become active during aestivation, with ten out of a total of 26 individuals showing definite signs of movement during the course of the experiment. Again the water content of the substratum proved to be the factor determining both the onset and termination of the period of activity.

DISCUSSION

The activity patterns immediately prior and during aestivation, demonstrated in the above experiments, could prove to be of considerable importance to *B. (Physopsis) africanus* in surviving periods of drought. With the onset of dry conditions evaporation increases and the weather is characterised by long periods of consecutive sunny days. During the hottest part of the day water temperatures in shallow marginal depressions left at the edge of a pond, when the main water has receded, would be such as to cause the snail populations of these depressions to leave the water and seek out adequately sheltered positions in which to aestivate. Such positions would include the shelter afforded by stones and vegetation, as well as the mud itself. Snails in the main body of the water in such a pond may also leave it under the influence of high temperatures, which would be particularly prevalent in the exposed shallows at the water's edge. These snails may return to the water when it cools down at night, but at least

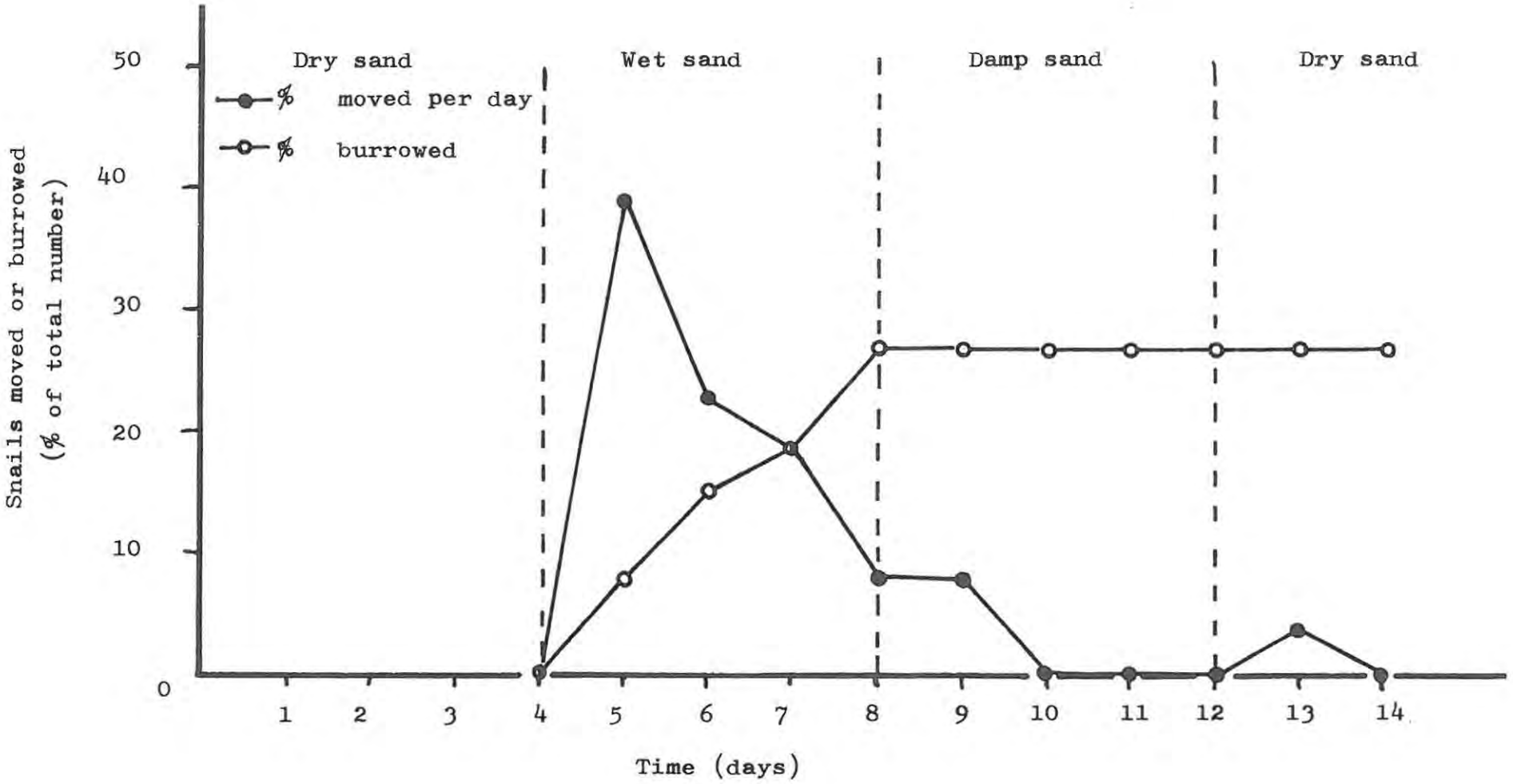

Figure 4 : Activity shown by aestivating *B. (Physopsis) africanus* when placed on wet sand.

a proportion are likely to have encountered suitable shelter in which to aestivate, and thus would not return.

Aestivation clearly cannot be regarded as an "all or nothing" state of inactivity. *B. (Physopsis) africanus* can become active during aestivation, and, during such periods of activity, can change its position to one more favourable for survival. The observed attempts at burrowing are indicative of the latter. These periods of activity during aestivation have their counterpart in hibernating mammals where periodic arousal, often to replenish some dwindling reserve, is not uncommon (Lyman & O'Brien, 1969). It is not known whether, if food were available, the snails would feed during these periods, but it seems at least likely that the internal water store would be replenished.

Clearly the whole question of activity immediately prior to, and during aestivation needs much more detailed investigation. The orientation behaviour of the snails in response to various physical stimuli must be studied if the suggestions put forward here are to be confirmed. While such a study certainly falls within the scope of the present investigation, it was not undertaken, since the more fundamental physiological aspects of aestivation in *B. (Physopsis) africanus* needed to be known to put such orientation behaviour in its proper context.

9. OXYGEN CONSUMPTION IN RELATION TO AESTIVATION IN *BULINUS (PHYSOPSIS) AFRICANUS*

Animals if they are to survive in an environment of fluctuating physical conditions, need to keep themselves within their zone of tolerance for each of the factors concerned. This can, in the short term, be achieved by behavioural means, or by brief sublethal conforming, even into the zone of resistance. Long term adverse change, often seasonal and inescapable, may, for survival, require more stable adjustments, particularly in the case of poikilotherms. Such adjustments often involve the adaptation of vital functions to the environmental change, and include such mechanisms as acclimation, diapause, aestivation and hibernation.

Studies on hibernation in homoiotherms have stressed the importance of depressed metabolism in surviving cold temperate and polar winters (Kayser, 1961; Musacchia & Saunders, 1969). The effect of such a metabolic adjustment is two-fold; it reduces the temperature gradient between the animal and its environment and, by the same token, conserves the metabolic reserves at a time when replenishment is, at best, difficult. Aestivation is essentially an adaptation to a different set of conditions, being a period of dormancy during summer or a dry season. As an adaptation in poikilotherms it is, particularly in warm temperate, sub-tropical and tropical regions, likely to subject the animal concerned to a wide range of physical conditions. These conditions include high temperatures which, among other potentially lethal effects, may cause enzyme inactivation (Prosser & Brown, 1961) which can only effectively be offset by some form of resistance adaptation normally involving a depression of the metabolic rate.

Various vital functions bear a direct relationship to the metabolic rate of an animal. Among these the rate of oxygen consumption, the rate of heart beat, and the excitability of muscle and nervous tissue have each been used as an index of metabolic rate (Precht, 1958; Prosser &

Brown, 1961; Vernberg & Vernberg, 1970). The rate of oxygen consumption has, for this purpose, an advantage over the other parameters in that it minimises handling and, more important, does not involve any operations which mutilate or kill the experimental animal.

Ghiretti (1966) has reviewed our knowledge of molluscan respiration. From this review it is clear that there is among the Pulmonata a considerable range of rates of oxygen consumption, with marked differences even between species of the same genus, such as to make generalization impossible. Respiration in relation to aestivation has been studied in non -marine Prosobranchia (Meenakshi, 1956; Coles, 1968, 1969a), and in terrestrial (Wells, 1944; Eckstein & Abraham, 1959) and aquatic (von Brand, McMahon & Nolan, 1957; Coles, 1969b) Pulmonata. Among the Planorbidae only the Planorbinae have been studied to any extent. The studies of von Brand and his co-workers (von Brand *et al.*, 1948, 1950, 1957) on *Australorbis glabratus* probably constitute the most complete investigation of any single pulmonate species. By contrast, the Bulininae have suffered considerable neglect; apart from a preliminary investigation by Coles (1969b) into the oxygen consumption of aestivating *Bulinus (Physopsis) nasutus* nothing is known about respiration during aestivation in any member of this sub-family.

The present investigation seeks to answer the following questions:

(i) Is metabolism depressed during aestivation in *B. (Physopsis) africanus*? If so,

(ii) how soon does the depressed metabolism manifest itself, and

(iii) does such depressed metabolism extend the zone of tolerance of the animals during aestivation, at least where temperature is concerned?

Von Brand *et al.* (1948) have demonstrated that all species of prosobranch and pulmonate snails investigated by them show a marked reduction in their rate of oxygen consumption when starved. *Australorbis glabratus* showed a similar reduction during aestivation (von Brand *et al.*, 1957). Thus any investigation into the phenomenon of aestivation

needs to take this into account, and for this reason aestivating animals were compared with both fed and starving individuals wherever pertinent.

THE RATE OF OXYGEN CONSUMPTION OF FED, STARVING AND AESTIVATING *B. (PHYSOPSIS) AFRICANUS*.

In order to establish whether any depression of the metabolic rate, as reflected in the rate of oxygen uptake, is characteristic of aestivation in *B. (Physopsis) africanus*, the rates of oxygen consumption of animals which had been in aestivation for a period of 21 days was compared with snails which had been normally fed and snails which had been starved for an equivalent period. A period of 21 days was decided upon since very few were able to survive starvation for a longer period.

Method:

Snails which, after at least two weeks under standard laboratory conditions, had been subjected to normal experimental pre-treatment (see Section 6 - Laboratory Culture Methods), were placed individually in Dickens-Simer RQ flasks containing 2ml of standard aquarium water. Their oxygen consumption was measured over a period of four hours in a Warburg respirometer at 25°C. Using a table of random numbers, each of these snails was randomly allocated to one of three groups, which were treated as detailed below.

Group 1 : Fed snails. Feeding of these snails continued normally, in isolation, under experimental pre-treatment conditions.

Group 2 : Starved snails. Snails kept singly under experimental pre-treatment conditions, but were starved. Care was taken to, as far as possible, eliminate any food source; all faeces were removed from the container while these were being produced, and both the container and water were changed weekly to ensure that no utilizable algal browse developed. However, the possibility that *B. (Physopsis) africanus* is a bacterial feeder cannot be ruled out, in which case at least some food would have been available to the snails, since treatment was not

such as to eliminate bacterial growth in the culture containers.

Group 3 : Aestivating snails. Snails were placed singly on a substratum of umgeni sand in 400 ml plastic cups. A water table reaching the surface of the sand was provided and this was allowed to evaporate leaving the animals dry.

Since groups 2 and 3 suffered a considerably greater mortality than group 1, these were allocated extra individuals so that the final numbers in all three groups would be comparable.

After a period of 21 days under the above conditions, the oxygen consumption of each individual snail was again measured over a period of four hours at 25°C. Aestivating snails were not returned to standard aquarium water for this final determination, but were placed in a dry flask at least 48 hours before the determination, in order to minimise the effect of mechanical disturbance (Coles, 1969b). The snails were killed immediately after the final rate of oxygen uptake had been measured, and their tissues were dried to constant weight. The rate of oxygen consumption could thus be expressed as µl O_2/mg dry weight/h. It must however be remembered that the dry weight of starving and aestivating snails will be less than that of fed individuals. Basing the rate of oxygen uptake on the final dry weight of the snail in each instance, as has been done throughout this investigation, will tend to reduce slightly the difference between fed, starving and aestivating snails. This was, however, unavoidable.

Von Brand *et al.* (1948) showed that size had an effect on the rate of oxygen consumption of *A. glabratus*, smaller individuals having a higher rate. The size range of the snails used in the present investigation was between 145 and 380 mg total live weight (mean 273 mg), which is much narrower than 12 to 2220 mg range for which von Brand *et al.* established a negative correlation. No significant correlation was apparent within the size range of animals used in this investigation ($r = -0{,}037$; $0{,}95 > P > 0{,}90$ for 93 snail sample). Furthermore, what correlation there might have been will, in any event, have been offset by the initial and final rates applying to the same individuals throughout.

No account was taken of the relative importance of pulmonary and cutaneous respiration. Alberts (1966) has shown that *B. (Physopsis) africanus* depends largely on cutaneous respiration. Jones (1961) found that both cutaneous and pulmonary respiration contributed to a constant total oxygen uptake in *Planorbis corneus* L. and *Lymnea stagnalis* L. For both the latter species, the partial pressure of oxygen dissolved in the water determined the contribution made to the total by cutaneous respiration, while pulmonary respiration made up the balance. It seems reasonable to expect that the needs of other pulmonate snails are similarly met, thus the site of respiratory exchange will not affect the results obtained.

Results:

Specimens of *B. (Physopsis) africanus* which had been aestivating for 21 days differed significantly in their rate of oxygen consumption from both fed snails and snails which had been starved for an equivalent period ($0,001 > P$ in both instances). Table 10 shows the initial (i.e. pre-starvation or pre-aestivation) and final rates of oxygen uptake for all three groups.

Table 10 : Comparison between the rates of oxygen uptake of fed, starving and aestivating *B. (Physopsis) africanus* at 25°C.

Treatment	No. of snails	Rate of oxygen consumption (µl/mg dry wt/h)					
		Initial			Final		
		Rate	S.E.	V_c	Rate	S.E.	V_c
Fed	52	1,44	0,035	17,4	1,40	0,024	12,5
Starved 21 days	50	1,44	0,034	16,5	0,81	0,015	12,6
Aestivating 21 days	51	1,37	0,035	18,3	0,47	0,014	21,2

The normal fed individuals have a rate of oxygen consumption at 25°C which, when expressed in terms of wet tissue weight, is comparable with that of *Australorbis glabratus* at 30°C (von Brand, Nolan & Mann, 1948). This is possibly a reflection of the more tropical distribution of the latter species. It is not really possible to compare the rate with that of *B. (Physopsis) nasutus* since Coles (1969b) expresses his results as µl O_2/g total live weight/24h. However, his figure of 182 µl O_2/g total live weight/24h would translate to roughly 15 µl/g tissue wet weight/h at 24°C, compared with 192 µl/g tissue wet weight/h for *Australorbis glabratus* at 30°C (von Brand *et al.*, 1948) and 202 µl/g wet weight/h for *B. (Physopsis) africanus* at 25°C.

Starving *B. (Physopsis) africanus* show a depression in their oxygen consumption rate comparable with that found in *Australorbis glabratus* by von Brand, McMahon and Nolan (1957), but it does not reach the level recorded by von Brand, Nolan and Mann (1948) for the same period of starvation in that species. It is possible that the expression of the rate of oxygen consumption as µl/g wet tissue weight/h, which seems to have been taken over from the clinical pathologists via the mammalian physiologists, may account for this discrepancy, as well as for the variable results obtained by them and by other workers. I have found that the water content of normal active *B. (Physopsis) africanus* ranges from 82% to 93% of the wet weight of the animal minus its shell (mean 87,3%, S.E. ± 0,32 for a sample of 25 snails). Determining wet tissue weight by subtracting the dry shell weight from the total live weight introduces a further variable in the form of water absorbed onto the shell and contained in the mantle cavity. Such extraneous water would have the effect of lowering the measured rate of oxygen consumption.

Aestivating *B. (Physopsis) africanus* also does not depress their rate of oxygen uptake to that of *A. glabratus* (von Brand *et al.*, 1957). The rate for *A. glabratus* fell to 20% of the pre-aestivation level in 21 days, whereas that of *B. (Physopsis) africanus* reached only 34% after the same period. Coles' (1969b) figure of 9% after 6 weeks aestivation for *B. (Physopsis) nasutus* cannot, of course, be compared.

CHANGES WITH TIME IN THE RATE OF OXYGEN CONSUMPTION OF STARVING AND AESTIVATING *B. (PHYSOPSIS) AFRICANUS*.

The previous experiment showed that snails kept out of water were physiologically different from snails which had been starving under otherwise normal conditions. Aestivation could, therefore, be defined in terms other than those set out in Section 7, and the state of aestivation could be successfully induced in *B. (Physopsis) africanus* by the treatment described in the previous experiment.

It was, however, necessary to ascertain whether the snails go into aestivation immediately they are removed from water, which seemed unlikely in view of the pre-aestivation activity shown by many individuals, or whether there is a slow change-over from the active to the aestivating state. Since aestivating snails could be distinguished from active snails by their rate of oxygen consumption, this parameter could be used to follow the changes taking place at the onset of and during aestivation. Starving snails, which also show a depression in the rate of oxygen consumption, could provide a useful control.

Method:

Experimental animals which had been kept under standard culture conditions for at least two weeks, and which had been subjected to normal experimental pre-treatment, were divided into two groups. The allocations were again done by means of a table of random numbers. The two groups were treated as detailed below.

Group 1 : Starving snails. These snails were treated as described for starved animals in the previous experiment, but had their rate of oxygen consumption determined daily at a temperature of 25°C. Between determinations they were kept under strict starvation conditions. Recordings were continued over a period of 20 days.

Group 2 : Aestivating snails. Fully fed snails were removed from water after 24 hours without food. They were dried at ambient humidity for a period of six hours and then placed individually in standard conical Warburg reaction flasks. Their rate of oxygen

consumption at 25°C was determined daily over a period of 20 days, during which time they were left undisturbed in the flasks.

Results:

Figure 5 shows the onset and progression of the depressed rate of oxygen consumption in both starving and aestivating *B. (Physopsis) africanus*. For clarity the coordinates, together with their respective error estimates and coefficients of variation are set out separately in Table 11. The difference between the rates for the two groups becomes apparent after the first five days. It can, however, be seen from Table 11 that the coefficient of variation for the aestivating animals increases markedly after the first five days, while that for the starving animals remains fairly constant. This implies an increase in the variability of the aestivating group to more than four times that of the starving group. However, the results, when considered individually, showed that the animals in the aestivating group could be divided into the following three categories:

(i) those which had steadily depressed their rate of oxygen consumption and, therefore, presumably their metabolic rate, well beyond the level for a starving animal,

(ii) those which, for some unknown reason, were unable to depress their rate of oxygen consumption beyond the starvation level, and,

(iii) those which depressed their rate of oxygen consumption below the starvation rate, and subsequently, possibly in response to mechanical disturbance re-elevated it as reported by Coles (1969b).

Figure 6 shows the progression of an individual from each of the above categories, compared with a starving individual.

In order to relate the above observation to survival, I have grouped together all individuals which survived 28 days aestivation and compared these with those snails which had died during the course of the experiment. The result of this regrouping, which is shown in Figure 7 and Table 12, futher accentuates the difference between starving and aestivating *B. (Physopsis) africanus*. Those individuals which did not

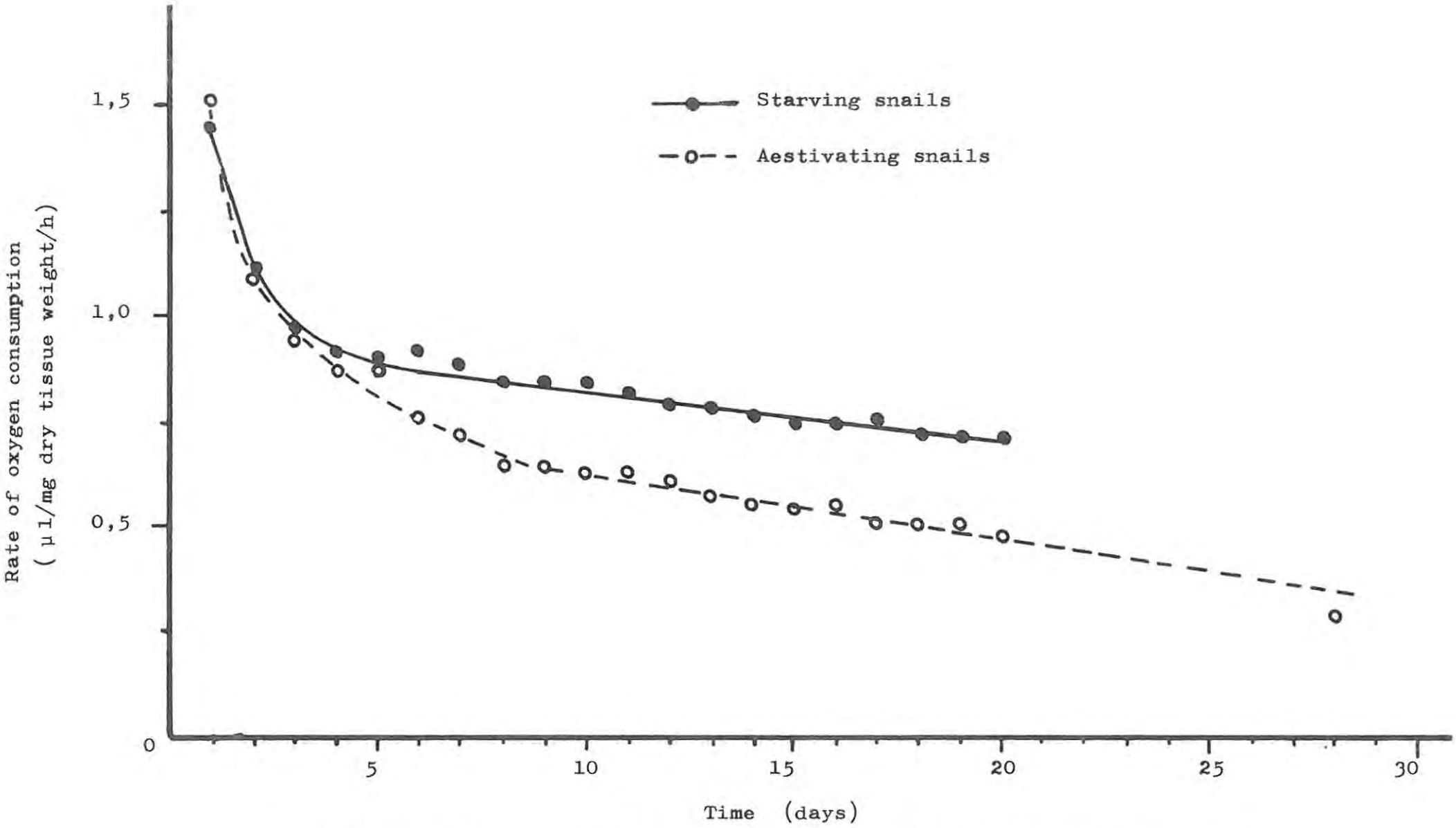

Figure 5 : Mean rates of oxygen consumption of *B. (Physopsis) africanus* recorded daily during progressive starvation and aestivation.

Table 11 : Rates of oxygen consumption of starving and aestivating *B. (Physopsis) africanus* at 25°C recorded daily over a period of 20 days. (For explanation see text).

Day	Rate of oxygen consumption (µl/mg dry tissue wt/h)					
	Starving			Aestivating		
	N†	Rate	V_c	N†	Rate	V_c
1	20	1,44±0,051	15,8	30	1,50±0,042	15,2
2	20	1,10±0,025	10,5	30	1,08±0,016	8,4
3	20	0,97±0,016	7,6	30	0,93±0,012	7,2
4	20	0,92±0,018	8,8	30	0,86±0,014	8,8
5	19	0,89±0,017	8,4	20*	0,87±0,046	24,0
6	20	0,91±0,013	6,5	30	0,76±0,022	15,2
7	19	0,88±0,014	6,9	19	0,71±0,036	21,9
8	19	0,84±0,019	10,1	29	0,63±0,031	26,5
9	18	0,84±0,018	8,8	19	0,63±0,042	28,8
10	18	0,84±0,012	6,2	26	0,62±0,038	31,5
11	18	0,82±0,017	9,2	17	0,62±0,055	36,1
12	17	0,78±0,017	8,9	26	0,60±0,051	43,3
13	17	0,78±0,016	8,5	16	0,56±0,055	39,1
14	16	0,76±0,015	8,2	25	0,54±0,046	42,2
15	16	0,74±0,013	7,2	15	0,53±0,050	36,6
16	15	0,73±0,021	11,1	24	0,54±0,045	40,4
17	15	0,75±0,021	10,8	15	0,50±0,056	42,9
18	15	0,71±0,017	9,4	24	0,50±0,042	40,4
19	14	0,71±0,018	9,2	15	0,49±0,058	46,0
20	14	0,71±0,018	9,5	23	0,47±0,040	41,3
28**				13	0,28±0,021	26,8

†N = Number of animals

*Results from an additional experiment, involving ten aestivating snails, have been incorporated into the results. The rates of oxygen consumption of these snails were recorded only on every *second* day after day five, hence the decrease of ten in the number of snails on alternate days after the fifth day of the experiment.

**Experiment was continued for 28 days for aestivating snails. Dead snails were removed from the experiment daily in order to determine tissue dry weight, but oxygen consumption was only recorded on the final day.

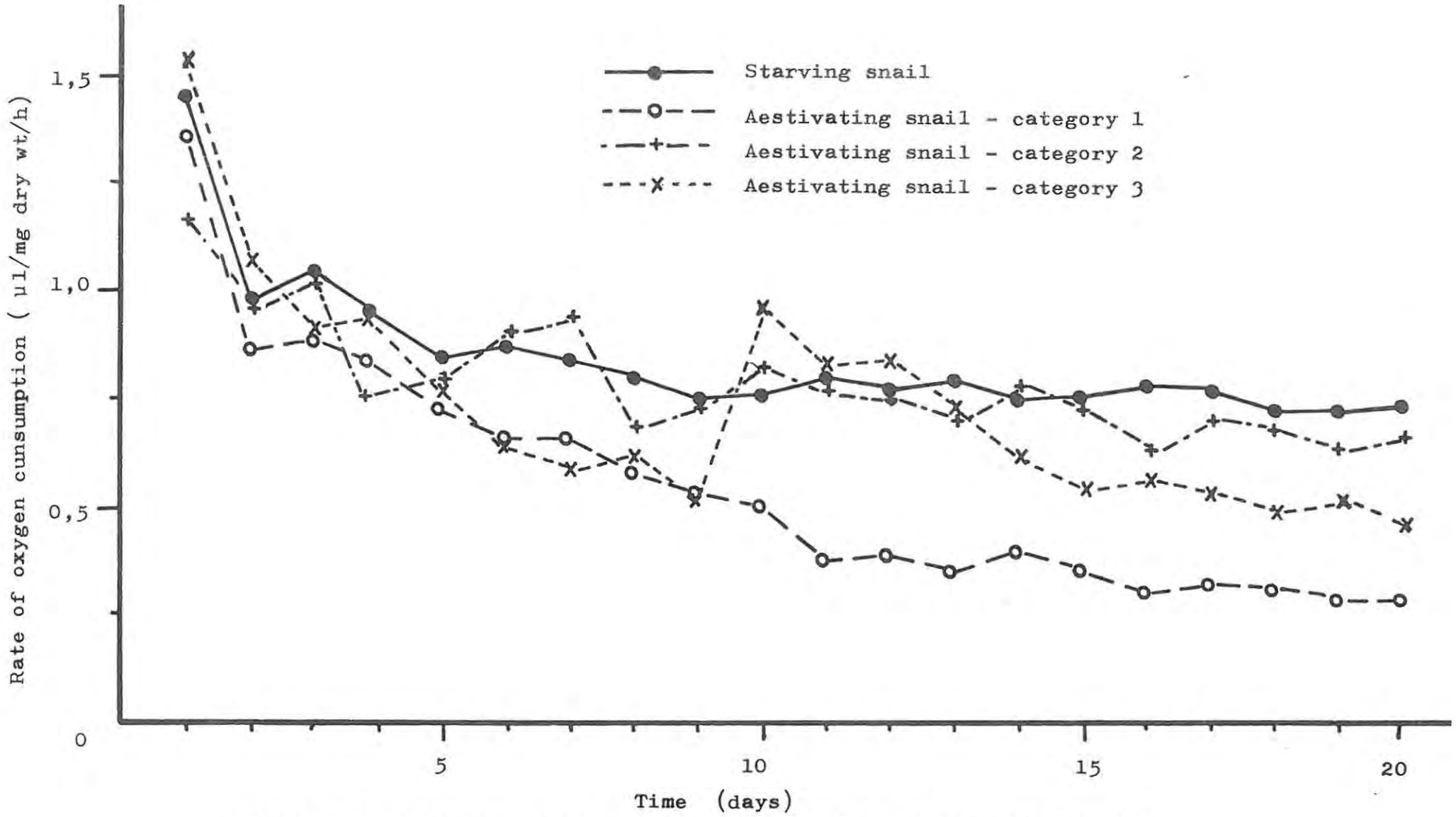

Figure 6 : Rates of oxygen consumption of individual *B. (Physopsis) africanus* recorded daily during progressive aestivation, compared with that of a starving individual. (For explanation see text).

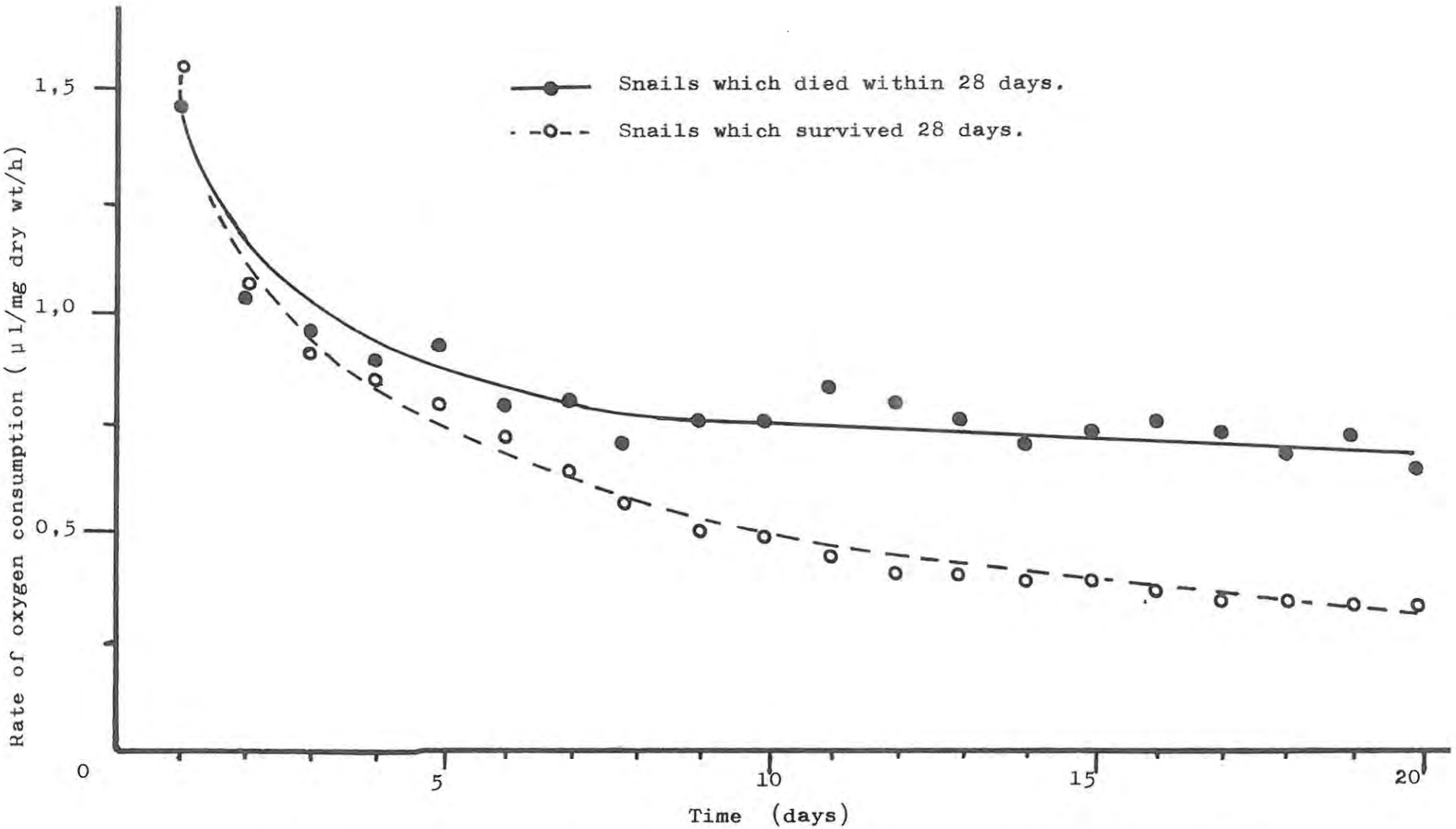

Figure 7 : Mean rate of oxygen consumption of aestivating *B. (Physopsis) africanus* which had survived 28 days aestivation compared with that of those which had succumbed during this period.

Table 12 : Rates of oxygen consumption at 25°C of aestivating *B. (Physopsis) africanus* which had survived 28 days exposure compared with those which had succumbed during this period. (For explanation see text).

Day	Rate of oxygen consumption (µl/mg dry wt/h)					
	Survived			Succumbed		
	N	Rate	V_c	N	Rate	V_c
1	13	1,55±0,062	14,5	17	1,46±0,055	15,6
2	13	1,06±0,029	9,7	17	1,09±0,019	7,4
3	13	0,90±0,017	7,0	17	0,95±0,015	6,5
4	13	0,83±0,018	8,1	17	0,89±0,018	8,4
5	9*	0,78±0,025	9,7	11*	0,93±0,078	27,6
6	13	0,71±0,025	12,8	17	0,78±0,039	20,8
7	9	0,63±0,028	13,5	10	0,79±0,054	21,5
8	13	0,56±0,018	11,4	16	0,69±0,050	29,0
9	9	0,50±0,021	12,7	10	0,75±0,056	23,8
10	13	0,49±0,016	12,1	12	0,75±0,060	27,9
11	9	0,44±0,023	15,9	8	0,83±0,049	16,8
12	13	0,41±0,011	9,7	13	0,79±0,069	31,4
13	9	0,41±0,019	14,3	7	0,76±0,072	25,2
14	13	0,39±0,009	8,4	12	0,70±0,070	34,5
15	9	0,39±0,018	13,7	6	0,73±0,054	18,0
16	13	0,37±0,014	14,0	11	0,75±0,047	20,8
17	9	0,35±0,018	15,6	6	0,73±0,060	20,1
18	13	0,35±0,014	15,1	10	0,68±0,050	22,9
19	9	0,34±0,021	18,4	6	0,72±0,073	24,6
20	13	0,34±0,018	18,8	10	0,64±0,053	26,1
28	13	0,28±0,021	26,8	0**		

* and ** see subscripts to Table 11

survive 28 days exposure could not be regarded as having effectively gone into aestivation, and these served not only to raise the mean values in Table 11 for aestivating snails but, in doing so, were also responsible for the high coefficients of variation. The rate of 0,34 $\mu l O_2$/mg dry weight/h shown by snails which had effectively gone into aestivation represents 22% of the pre-aestivation rate, a figure comparable with that found by von Brand *et al.* (1957) for *A. glabratus*.

THE EFFECT OF TEMPERATURE ON THE RATES OF OXYGEN CONSUMPTION OF FED, STARVING AND AESTIVATING *B. (PHYSOPSIS) AFRICANUS*.

A depression of the rate of oxygen consumption, as shown in the previous experiments, has survival value in that it doubtless conserves the metabolic reserves of the snails. However, the possibility of it being, in addition, a form of resistance adaptation, at least to transient high temperatures which the snails may experience during aestivation, could not be overlooked. Such resistance adaptation would be apparent in changes in temperature coefficients of various rate functions, including the rate of oxygen uptake.

Method:

Experimental animals which had been kept under standard laboratory culture conditions for at least two weeks, and which had been subjected to normal experimental pre-treatment were divided into three groups. A larger number of snails were allocated to the aestivating group as some selection had to be done here to eliminate as many snails which had not effectively gone into aestivation as possible. The three groups of snails were treated as detailed below:

Group 1 : Fed snails. The rate of oxygen consumption of fully fed snails was measured at different temperatures ranging from 5°C to 35°C, at which temperature they showed signs of physiological stress. Two or sometimes three determinations could be made during the course of one day, after which the animals were allowed to feed for two days to recover from the enforced starvation in the respirometer and during pre-treatment. A further 24 hours starvation was, of course necessary before recordings

could be resumed. When recordings were resumed after such a feeding interval, the experiment was started at 25°C for one hour to check for normal respiration; thereafter the water bath was set for the new experimental temperature. One hour was allowed as equilibration time when the bath had reached the experimental temperature, after which the manometers were closed and recording commenced. Activity at high temperatures could have affected the results. Seven snails judged to have shown undue activity were eliminated from the experiment.

Group 2 : Starving snails. The rate of oxygen uptake by snails which had been starved for ten days was determined at different temperatures as described for Group 1. There was, of course, no feeding interval, but in all other respects the treatments of the two groups were the same. Four snails were eliminated from the experiment as they were moving actively at the higher temperatures.

Group 3 : Aestivating snails. The rate of oxygen consumption of snails which had been under aestivation conditions for 16 days was determined at different temperatures ranging from 5° to 50°C. The snails were kept in their respective reaction flasks between recordings to minimise disturbance. All rates of oxygen uptake were recorded over a period of four hours except those at 45° and 50°C where it was measured over two and one hours respectively. Prolonged exposure to these high temperatures could and did cause fatalities. Of 13 snails used in a preliminary experiment three died during four hours exposure to 45°C while a further six died after two hours at 50°C.

In the light of the results obtained in the previous experiment, it would have been desirable to select aestivating snails with a rate of oxygen consumption less than 0,6 µl/mg dry weight/h. This was not possible, since the dry weight was not known before the end of each experiment. Survival to 16

days under aestivation conditions already served to some extent to select for animals which had successfully gone into aestivation. From the survivors only snails with a rate of oxygen consumption less than 0,05 µl/mg total live weight/h, which was estimated to correspond approximately to 0,6 µl/mg dry weight/h, were used in the experiment. Of the 25 snails thus selected, three showed very erratic rates of oxygen consumption; two of these died at 35°C with no measurable oxygen uptake at this temperature, while the third died at 40°C with a rate of oxygen consumption of 2,6 µl/mg dry weight/h. These animals were not included in the results.

Results:

The effect of temperature changes on the rates of oxygen consumption of fed, starving and aestivating *B. (Physopsis) africanus* is shown in Figure 8 and Table 13. The results show that starvation brings about a displacement of the rate-temperature curve to the right, whereas aestivation causes a clockwise rotation. The zone of tolerance is extended by some 15°C, but this must be considered in terms of the actual times of exposure to the raised temperatures. Taking the equilibration times into account, exposure to 45°C and 50°C were, at best, three and two hours respectively, yet some individuals did die at these temperatures during the experiment. These snails increased their rate of oxygen consumption by more than 200% immediately prior to death. In preliminary experiments increases of up to 800% were recorded preceding the death of an animal, but since these experiments were not rigorously controlled, the results may have been caused by factors other than temperature alone.

DISCUSSION

Von Brand *et al.* (1957) tend to minimise the distinction between aestivation and starvation. They do recognise aestivation as a condition different from starvation in water, largely on the basis of the longer survival of aestivating snails and on the effect of desiccation in

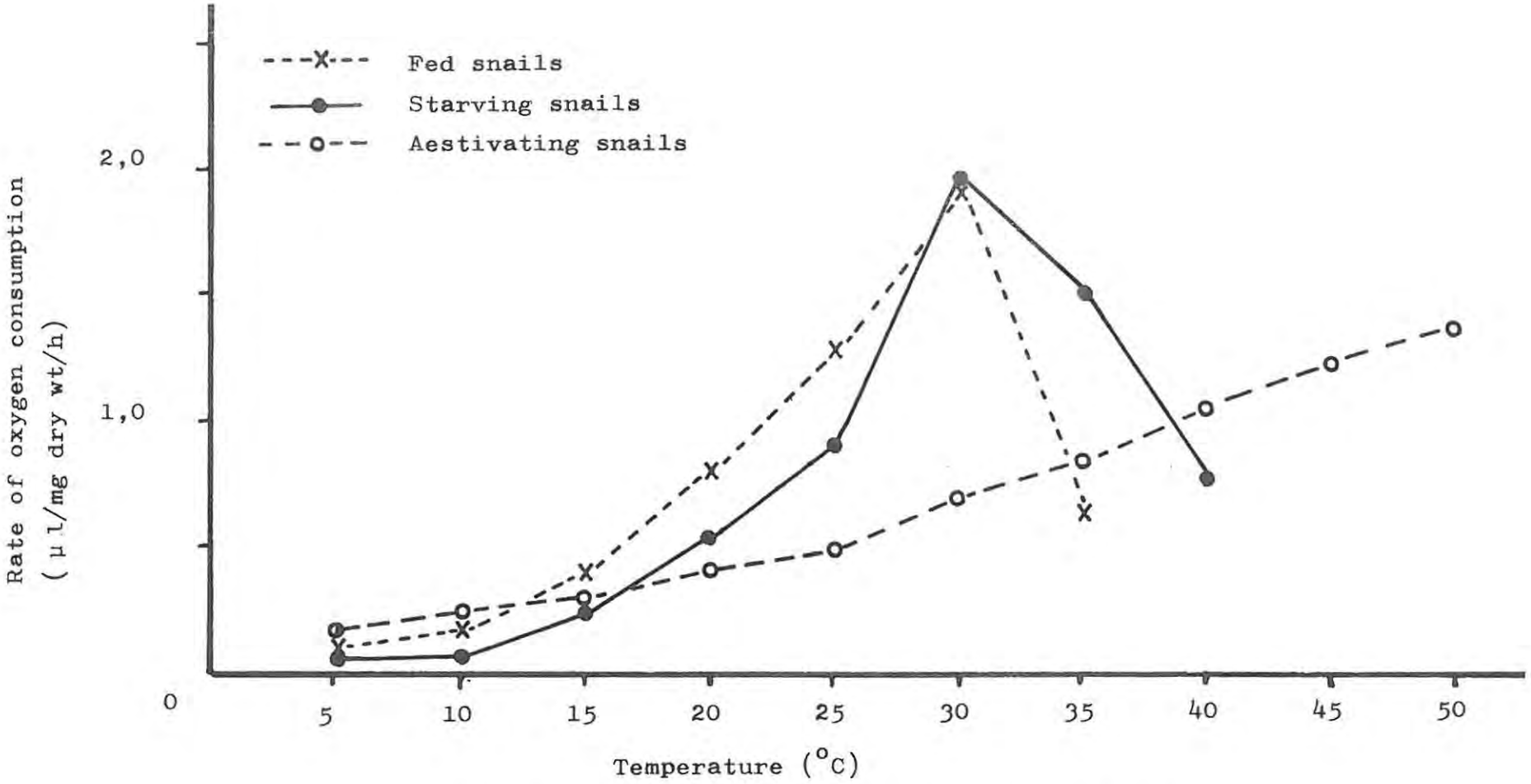

Figure 8 : Mean rates of oxygen consumption of fed, starving and aestivating *B. (Physopsis) africanus* at various temperatures.

Table 13 : Rates of oxygen consumption of fed, starving and aestivating *B. (Physopsis) africanus* at different temperatures.

Temperature	Fed				Starving				Aestivating			
	N*	Mean rate	S.E.	V_C	N*	Mean rate	S.E.	V_C	N*	Mean rate	S.E.	V_C
5°C	22	0,09	0,005	26,3	12	0,06	0,007	38,4	22	0,17	0,011	29,7
10°C	22	0,18	0,007	18,1	12	0,09	0,007	28,3	22	0,23	0,010	20,5
15°C	22	0,40	0,014	16,6	12	0,27	0,008	9,9	22	0,31	0,012	17,9
20°C	22	0,81	0,032	18,2	12	0,52	0,015	9,7	22	0,42	0,017	19,0
25°C	22	1,39	0,047	15,5	12	0,90	0,022	8,5	22	0,50	0,021	19,7
30°C	22	1,91	0,065	15,6	12	1,96	0,047	8,3	22	0,70	0,030	20,0
35°C	22	0,63	0,065	47,2	12	1,52	0,193	44,1	22	0,85	0,037	19,9
40°C					11	0,78	0,117	50,2	22	1,06	0,048	20,7
45°C									21	1,24	0,049	18,0
50°C									20	1,38	0,057	18,8

*N = Number of animals used

depressing the rate of oxygen uptake, rather than on the difference between the rates of oxygen consumption of starving and aestivating snails. Meenakshi (1964), on the other hand, draws a sharp distinction, based on oxygen consumption, between aestivating and non-aestivating *Pila virens*, claiming that aestivation in this species is anaerobic. The results obtained in the present investigation point to aestivation in *B. (Physopsis) africanus* being aerobic, although the presence of anaerobic pathways cannot be excluded; they have been shown to exist in *Australorbis glabratus* (von Brand, 1950). Complete anaerobiosis during aestivation has, to date, only been shown for *Pila virens*. Coles (1968, 1969a) has found aestivation in *Pila ovata* to be aerobic, but the rate of oxygen consumption is depressed as has been shown here for *B. (Physopsis) africanus* and by von Brand *et al.* (1957) for *A. glabratus*. Aestivating *B. (Physopsis) nasutus* are also aerobic, and break aestivation to become active under anaerobic conditions (Coles, 1969b).

The discrepancy between the conclusions of von Brand *et al.* (1957) and the findings in the present investigation is simply one of degree, and therefore more apparent than real. It can to some extent be attributed to the extreme variability in the rate of oxygen consumption of starving *A. glabratus*. While activity in the starving *A. glabratus* cannot be ruled out as a factor contibuting to this variability, the expression of the rate of oxygen consumption in terms of tissue wet weight is also likely to have added to it. Von Brand *et al.* (1948) at times placed more than one snail in a reaction flask when measuring oxygen uptake. If this practice was follwed when investigating aestivating *A. glabratus* (von Brand *et al.*, 1957) and if *A. glabratus* is similar to *B. (Physopsis) africanus* in not all individuals being able to successfully go into aestivation, this would tend to reduce the difference between the oxygen uptake rates of starving and aestivating animals.

The different temperature coefficients for the rates of oxygen uptake of starving and aestivating *B. (Physopsis) africanus* serve to underline the difference between the two states. Since Q_{10} values have comparative application over

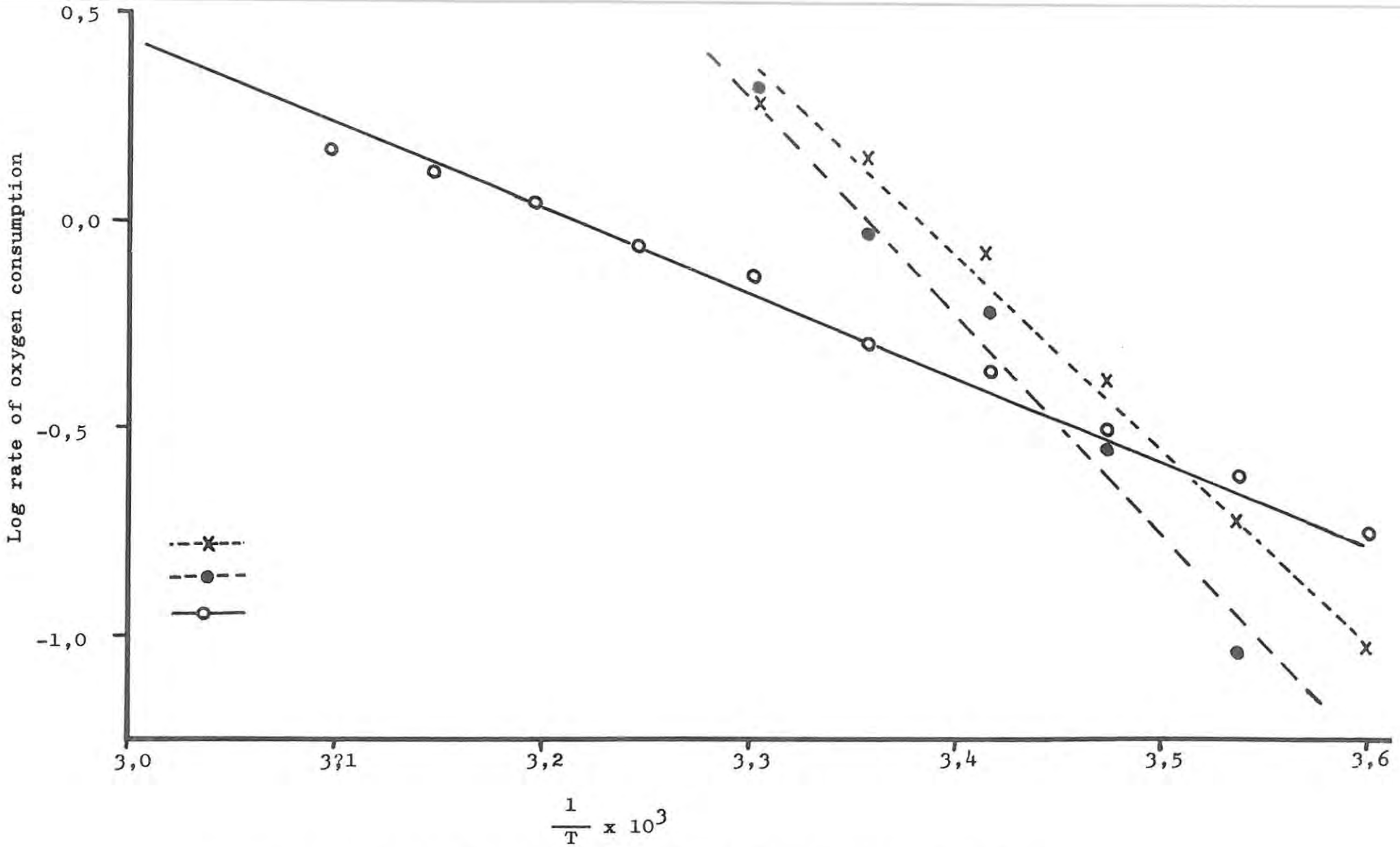

Figure 9 : Arrhenius plot of the rate of oxygen consumption/ temperature relationship in fed, starving and aestivating *B. (Physopsis) africanus*.

only a limited temperature range, an Arrhenius plot of the data in Table 13 is shown in Figure 9. This conversion shows no significant difference between the regression lines for fed and starving animals (0,5 >P >0,1) while that for aestivating animals differs significantly from both (0,001 > P in both instances). Such a change in the slope of an Arrhenius plot implies a difference between the activation energies of at least some components of the enzyme systems governing metabolism under the two sets of conditions. The activation energies for fed and starving snails were found to be 21,2 and 20,5 kcal/mole respectively, while that for aestivating snails was 8,7 kcal/mole. This suggests that aestivation involves a change in the metabolic pathway/s of the snail rather than a simple limitation in either substrate or enzyme concentration, a suggestion which is further substantiated by the extension of the zone of tolerance. The reduced rate of oxygen consumption during starvation would seem to reflect a limitation imposed on metabolism, possibly as a result of a reduction in readily mobilised or even unstored metabolites, since the rates of enzyme catalysed reactions are, according to the Briggs-Haldane-Michaelis theory, determined by the molar concentration of the substrate. A change in the enzyme concentration would, of course, have the same effect. That substrate concentration is possibly the factor responsible for the depressed metabolism finds some support in the rapid decline of the rate of oxygen consumption during the initial stages of starvation; presumably the snails must, after utilizing newly assimilated food, begin to draw on metabolites which need to be mobilised, and the whole system could become limited by the rate at which this mobilisation takes place.

Eckstein and Abraham (1959) have shown an increase in liver succinic dehydrogenase activity after the termination of aestivation in the snail *(Helix) Levantina hieraeolyma* (authority not quoted). While studies on a single enzyme within a complex is, in itself, of doubtful value, the temperature coefficient in the presence of excess substrate could well provide supporting evidence for the change of metabolic pathway suggested above. Eckstein & Abraham's (1959)

results can be explained simply in terms of a difference in the enzyme concentration, thus little could be concluded from them. I have, therefore, attempted to repeat their experiment on *B. (Physopsis) africanus*, but without success. The method involves the reduction of triphenyl-tetrazolium chloride to formazan by the hydrogen atoms, produced in the dehydrogenation of succinic acid to formic acid in the presence of the succinic dehydrogenase, present in the snail liver homogenized in cold phophate buffer at pH 7,5. No measurable dehydrogenase activity could be obtained from either *B. (Physopsis) africanus* or *Helix aspersa* Müller liver, although the reagents used were effective in determining succinic dehydrogenase activity in rat liver.

While the results obtained require confirmation at the biochemical level, it seems safe to say that aestivation in *B. (Physopsis) africanus* involves a depression of the metabolic rate, probably through the substitution of a metabolic pathway different from that which it normally employs. The survival value of this change as a form of resistance adaptation to at least transient high temperatures is clear, and is in keeping with the observations of Cridland (1967) on the temperature extremes which aestivating aquatic pulmonates can survive.

10. WATER LOSS AND THE AESTIVATION PROCESS IN *BULINUS (PHYSOPSIS) AFRICANUS*

Animals living at humidities below saturation must lose water to the environment. Shiff (1960) has shown that *Biomphalaria pfeifferi* and *Bulinus (Physopsis) globosus* will survive on dried mud for periods of 50 and 30 days respectively. The humidity at the surface of the mud ranged from 86% to 94% Relative Humidity (R.H.). These conditions would result in water loss from the snails during aestivation. Wells (1944) has reviewed the early work done on the relationship between body water content and aestivation in *Helix pomatia*. He quotes Fischer and Duval (1925) as showing that an increase in blood osmotic pressure, following water starvation, brings about both aestivation and a reduction in the rate of oxygen consumption in this species. Wells does not agree that water content is a determining factor in aestivation, since Howes and Wells (1934) were able to induce aestivation in *H. pomatia* under conditions of complete saturation, and because hydration of *H. pomatia* takes place only after animals have broken aestivation and emerged from their shells. More recently von Brand *et al.* (1957), Meenakshi (1964) and Little (1968) have all suggested that body water content is implicated in the initiation of the aestivation process of aquatic snails.

Water content is also important to survival during aestivation. Von Brand *et al.* (1957) showed that *Australorbis glabratus* does not survive aestivation well under conditions of low humidity. Shiff (1960) has studied the survival of *Lymnea natalensis* (Krauss), *Biomphalaria pfeifferi* and *Bulinus (Physopsis) globosus* under conditions of experimental desiccation. He showed that under his experimental conditions *B. (Physopsis) globosus* suffered a 75% mortality after 120 hours in an atmosphere of 70% R.H. These findings suggest that aestivation is only possible where some form of shelter against the desiccating effect of sub-saturated atmospheric humidities is available.

Rehydration and the termination of aestivation has been studied by Little (1968) in *Pomacea lineata* and by Coles (1969b) in *B. (Physopsis) nasutus*. Little showed an almost

immediate hydration of aestivating *P. lineata* on being immersed in water, while Coles was able to measure a rapid rise in the rate of oxygen uptake following the hydration of aestivating *B. (Physopsis) nasutus*. Both findings suggest that water is the determining factor in the termination of aestivation.

The present investigation seeks to determine the role of the animal's water content in initiating, maintaining and terminating aestivation in *Bulinus (Physopsis) africanus*. While water loss may be important in determining the onset of aestivation, temperature could not be overlooked as a contributory factor, since the drying out of pools must subject the snail population to considerable fluctuations immediately they are exposed to the extremes of the terrestrial environment.

DESICCATION AND TEMPERATURE AS POSSIBLE FACTORS IN THE INITIATION AESTIVATION IN *B. (PHYSOPSIS) AFRICANUS*.

The conditions under which aquatic pulmonate snails survive aestivation have been shown to favour water loss (Shiff, 1960) and subject them to diurnal temperature fluctuations of considerable magnitude (Cridland, 1967). Diurnal temperature fluctuations are particularly marked during the winter dry season; the winter mean daily air temperature fluctuations at the collecting sites are 8°C and 14°C for Durban and Nelspruit respectively (Schulze, 1965). The temperature maxima in the sun will be considerably higher than those on which the above figures are based, since the temperature recordings for the climatological data were all made with a thermograph housed in a Stevenson screen (Schulze, 1965). Aestivating snails in their natural habitat may, therefore, well experience temperatures above 30°C during the day and even at or near the Natal coast, may be subjected to night temperatures below 10°C. Cridland (1967) records a maximum sun temperature of 50°C and a minimum temperature of 1,6°C in one of his experiments, though it is not likely that these temperatures were recorded on the same day.

In order to assess the respective rôles of diurnal

temperature change and of water loss in the initiation of aestivation, the rates of oxygen uptake were determined for snails which were subjected to constant humidity with temperature fluctuations simulating those which would be experienced in the field, and these were compared with the oxygen uptake rates of snails which had been subjected to a constant temperature of 25°C but at a lowered humidity which would increase the rate of water loss.

Method

Specimens of *B. (Physopsis) africanus* collected from Krantzkloof which had been under standard laboratory culture conditions for 14 days, and which had been subjected to the normal experimental pre-treatment were divided into two groups, which were treated as detailed below.

Group 1 : Subjected to fluctuating temperature. The snails were kept in Warburg reaction flasks and subjected to the following temperature fluctuations:

0900 to 1200 h : 25°C (rate of oxygen consumption was measured over this period)

1200 to 1700 h : 32°C

1700 to 0800 h : 8°C

0800 to 0900 h : temperature raised to 25°C

Temperature changes were effected by changing the temperature of the water bath of the Warburg respirometer; the flasks were kept in the water bath throughout.

The snails were subjected to the above temperature regime over a period of eight days.

The humidity within the flasks would be controlled by the potassium hydroxide used as a carbon dioxide absorbant, and would approximate to 93% R.H. (Solomon, 1951). Since the potassium hydroxide is slowly converted to potassium carbonate by the carbon dioxide released during respiration, the humidity within the flasks would, if anything, increase with time. The potassium hydroxide in the flasks was not replaced during the course of the experiment.

Group 2 : Desiccated snails. Snails were kept in Warburg reaction flasks, and the flasks were kept open in a desiccator at 75,5% R.H., except when the rate of oxygen consumption was being determined. The relative humidity in the desiccator was controlled by means of a saturated solution of sodium chloride (Winston & Bates, 1960). The rate of oxygen consumption of the snails was measured daily over a period of seven days.

The concentration of the potassium hydroxide used as a carbon dioxide absorbant in the flasks was increased from 10% to 20% in order to nearly match the relative humidity in the desiccator.

Results:

Figure 10 shows the effect of desiccation and of diurnal temperature fluctuations on the rate of oxygen consumption during the early stages of aestivation. For comparison the rates of oxygen uptake at approximately 94% R.H. and at a constant temperature, as shown in Table 11 (Section 9) are also included. The coordinates of the curves in Figure 10, together with the respective standard errors are given in Table 14. From these data it appears that a lower humidity, and therefore a higher rate of water loss, does have the effect of depressing the rate of oxygen consumption more rapidly, but it also proves fatal to the snails at an early stage in aestivation. Temperature fluctuations appear to have no effect at all.

THE EFFECT OF DESICCATION ON WATER LOSS AND SURVIVAL IN AESTIVATING *B. (PHYSOPSIS) AFRICANUS*.

Von Brand *et al.* (1957) have shown that desiccation at low humidities results in a very high mortality in *A. glabratus*, and Shiff (1960) has confirmed this finding for *Biomphalaria pfeifferi*, *B. (Physopsis) globosus* and *Lymnea natalensis*. Cridland (1967) has, however, demonstrated *B. (Physopsis) africanus* to have an unexpectedly high survival rate in sandy soil of low moisture content; it was therefore necessary to determine the effect of desiccation at different relative humidities on the rate of water loss

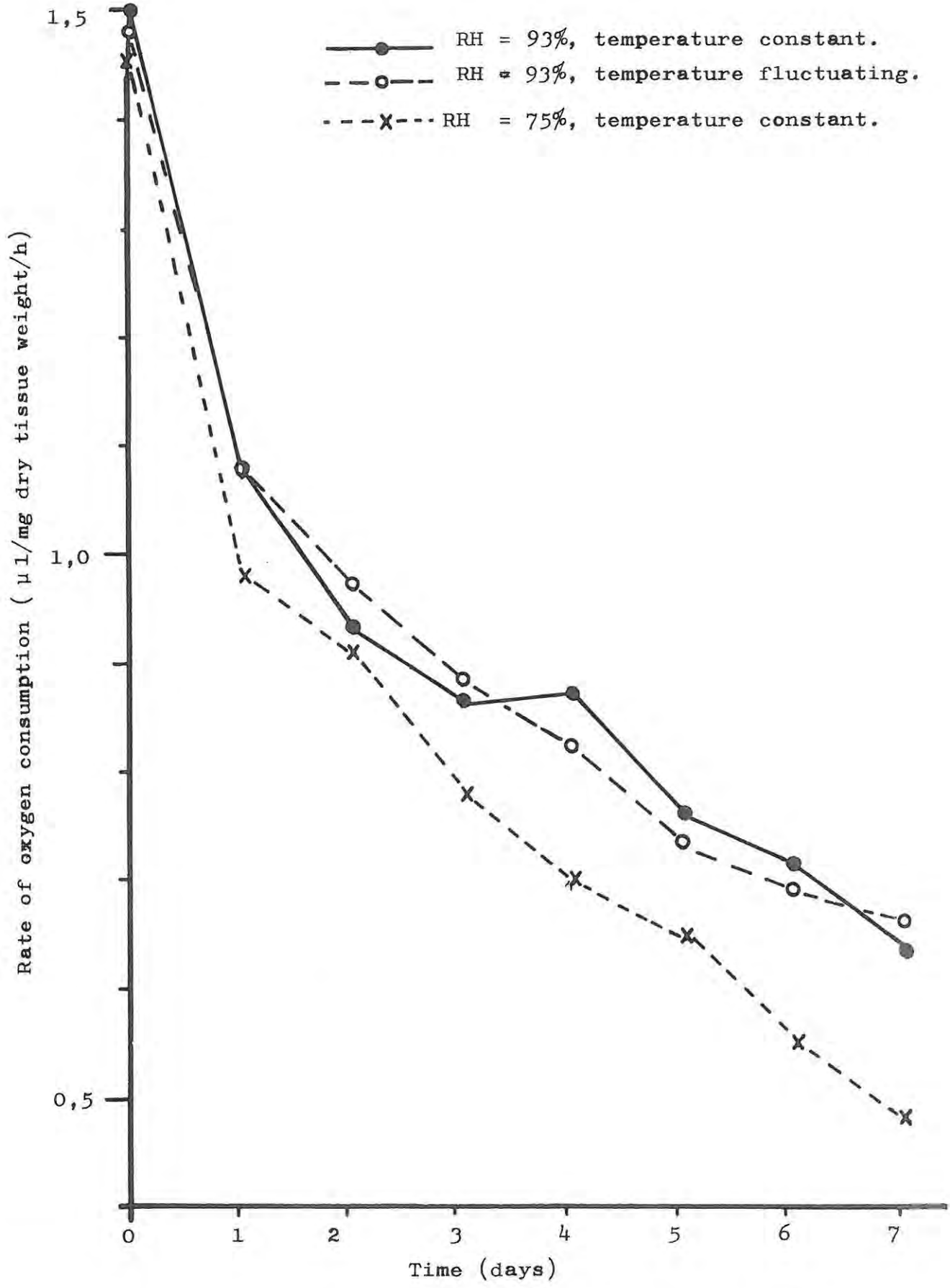

Figure 10 : The effect of atmospheric humidity and of temperature fluctuation on the rate of oxygen consumption of aestivating *B. (Physopsis) africanus*.

Table 14 : Rates of oxygen consumption of aestivating *B. (Physopsis) africanus* subjected to (i) constant temperature at high humidity, (ii) diurnal temperature fluctuations at high humidity, and, (iii) desiccation at 75% R.H.

Day	0	1	2	3	4	5	6	7	8
(i) 93% R.H., temperature constant at 25°C									
Number of snails	30	30	30	30	20	29	19	29	19
Rate of O_2 uptake (µl/mg dry wt/h)	1,50	1,08	0,93	0,86	0,87	0,76	0,71	0,63	0,63
Standard error	0,04	0,02	0,01	0,01	0,05	0,02	0,04	0,03	0,04
(ii) 93% R.H., temperature fluctuating between 8°C (night) and 32°C (day)									
Number of snails	10	10	10	10	10	10	10	10	10
Rate of O_2 uptake (µl/mg dry wt/h)	1,48	1,08	0,97	0,88	0,82	0,74	0,69	0,66	0,63
Standard error	0,07	0,02	0,01	0,02	0,02	0,03	0,03	0,03	0,04
(iii) 75% R.H., room temperature, determination done at 25°C									
Number of snails	10	10	10	8	8	8	6	2	0
Rate of O_2 uptake (µl/mg dry wt/h)	1,46	0,98	0,91	0,78	0,70	0,65	0,55	0,48	-
Standard error	0,05	0,01	0,03	0,03	0,04	0,06	0,06	-	-

and survival of aestivating *B. (Physopsis) africanus*.

Method:

Three groups of snails collected at Krantzkloof, which had been under standard laboratory conditions for 14 days, and which had been subjected to normal experimental pre-treatment, were subjected to desiccation at relative humidities of 98%, 87% and 53% R.H. Humidities of 98% and 87% R.H. may well be experienced by the snails in there natural environment, whereas 53% R.H. would represent extreme desiccation, probably only encountered by snails aestivating in very exposed positions. Desiccation was carried out in 3 litre desiccators, in which the humidity was controlled by means of saturated solutions of the following salts (Winston & Bates, 1960): distilled water, which theoretically should subtend 100% R.H., but, in practice only gives humidity of about 98% in a container of the size used; potassium sodium tartrate - 87% R.H. at 25°C; and sodium dichromate 53% R.H. at 25°C. The relative humidities which saturated solutions of these salts subtend vary but little with temperature in the range 20° to 30°C. The experiments were carried out at room temperature, thus temperature fluctuations would have affected the saturation deficits to which the snails were subjected. However, the temperature fluctuations in the laboratory during the course of the experiment varied only between 20,3° and 24,0°C, which even at the lowest relative humidity used, would have resulted in a maximal fluctuation in the saturation deficit of 2,2 mm mercury.

The snails used in the experiment were weighed prior to desiccation. They were placed in the desiccators, and weighed daily thereafter throughout the eight day period of desiccation. Dead snails were removed from the desiccators every day; these could readily be detected by their smell.

Results:

The decrease in weight and the mortality due to desiccation under different conditions of humidity are shown in Figure 11. Table 15 shows the mean weight loss by each group for each day of the experiment, together with the standard errors. From these results it seems that the *rate* of water loss,

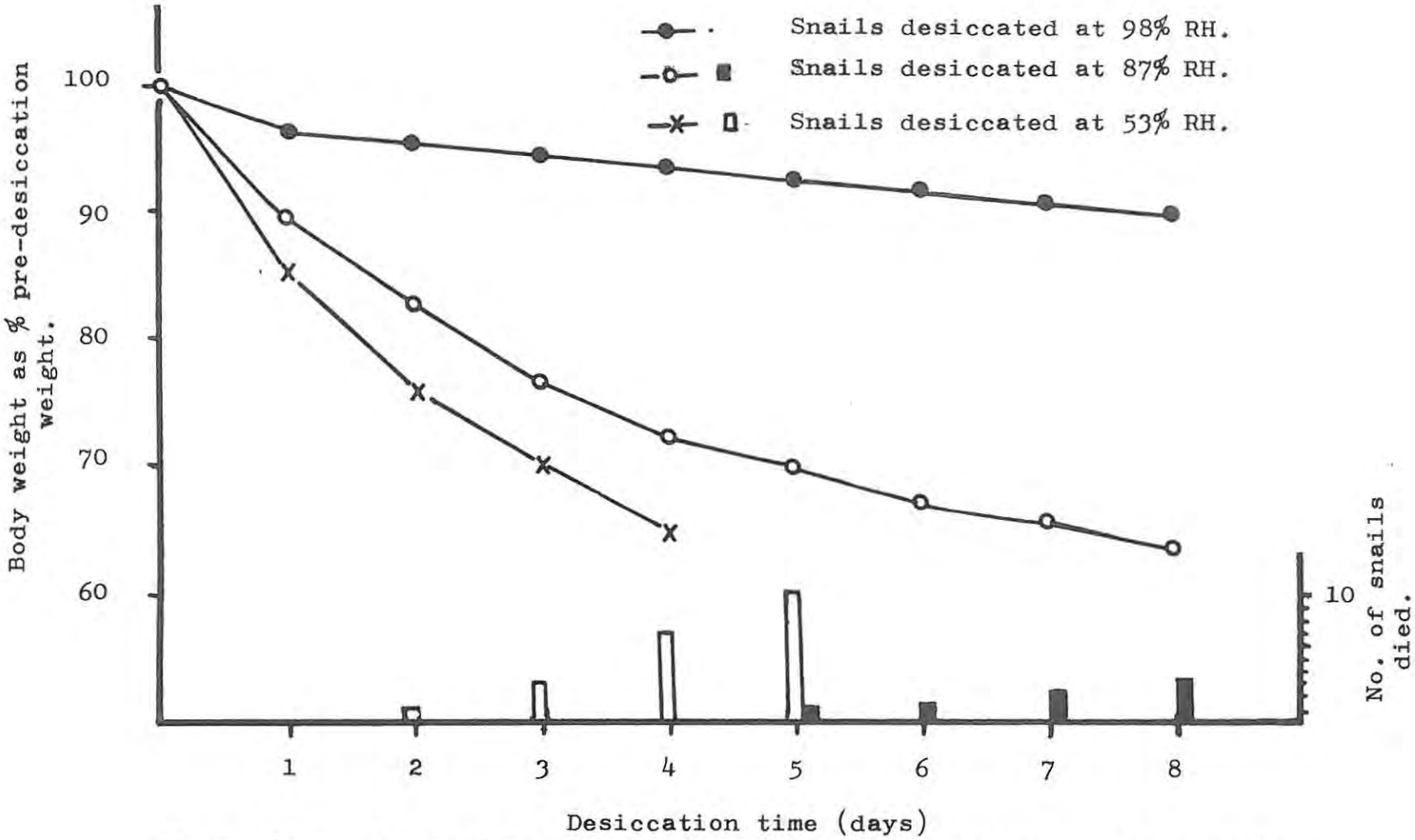

Figure 11 : Weight loss by and mortality of aestivating *B. (Physopsis) africanus* subjected to desiccation at different relative humidities.

Table 15 : Weight loss by aestivating *B. (Physopsis) africanus* subjected to different relative humidities.

Day	1	2	3	4	5	6	7	8
Kept at 98% R.H.								
Mean weight loss (% of initial weight)	3,6	1,0	1,0	1,0	1,1	1,0	1,0	0,9
Standard error	0,26	0,07	0,03	0,05	0,05	0,06	0,04	0,04
Number alive on day	10	10	10	10	10	10	10	10
Kept at 87% R.H.								
Mean weight loss (% of initial weight)	10,3	6,7	6,1	4,3	2,5	2,6	1,8	1,9
Standard error	0,35	0,36	0,16	0,37	0,22	0,26	0,15	0,12
Number alive on day	10	10	10	10	9	9	8	7
Kept at 54% R.H.								
Mean weight loss (% of initial weight)	14,7	9,2	6,0	5,0	-	-	-	-
Standard error	0,83	0,94	0,43	0,49	-	-	-	-
Number alive on day	10	9	6	3	0	0	0	0

rather than the *amount* of water lost, is the important factor determining survival. Snails desiccated at 87% R.H. lost as much water in eight days as did those desiccated at 53% R.H. in four days, yet the mortality in the former experiment was only 30% of that in the latter.

Figure 12 shows the mean daily rate of water loss incurred at the different saturation deficits represented by the relative humidities used in the experiments at the mean laboratory temperature of 22°C.

REHYDRATION AND THE TERMINATION OF AESTIVATION IN *B. (PHYSOPSIS) AFRICANUS*.

Aestivating snails become active when placed into water (von Brand *et al.*, 1957, Shiff, 1960, Cridland, 1967, Coles, 1969b). Such termination of aestivation by returning the snails to water is contrary to the results of Wells (1944) for *Helix pomatia*, where mechanical stimulation by falling rain is said to cause the animals to break aestivation, and rehydration then follows. The finding in the present investigation that water loss does affect the onset of aestivation suggested that rehydration could have the reverse effect of terminating it. This, if true, could well explain the activity which followed when aestivating snails were placed on wet sand. The effect of rehydration on weight, rate of oxygen consumption and activity were therefore investigated.

Method:

The snails which had survived eight days desiccation at 87% R.H. in the previous experiment were placed in a Warburg reaction flask, the side-arm of which contained 1 ml of standard aquarium water. The snails were positioned so that the aperture of the shell was in contact with the bottom of the flask. After 24 hours during which to recover from the effects of any mechanical disturbance, the rate of oxygen consumption of each snail was determined. The water in the side-arm was then tipped into the flask and the rate of oxygen consumption was measured over the first hour, and again over the following three hour period, at the end of which all but one of the snails had emerged from their shells. The snails were dried and weighed, after which they

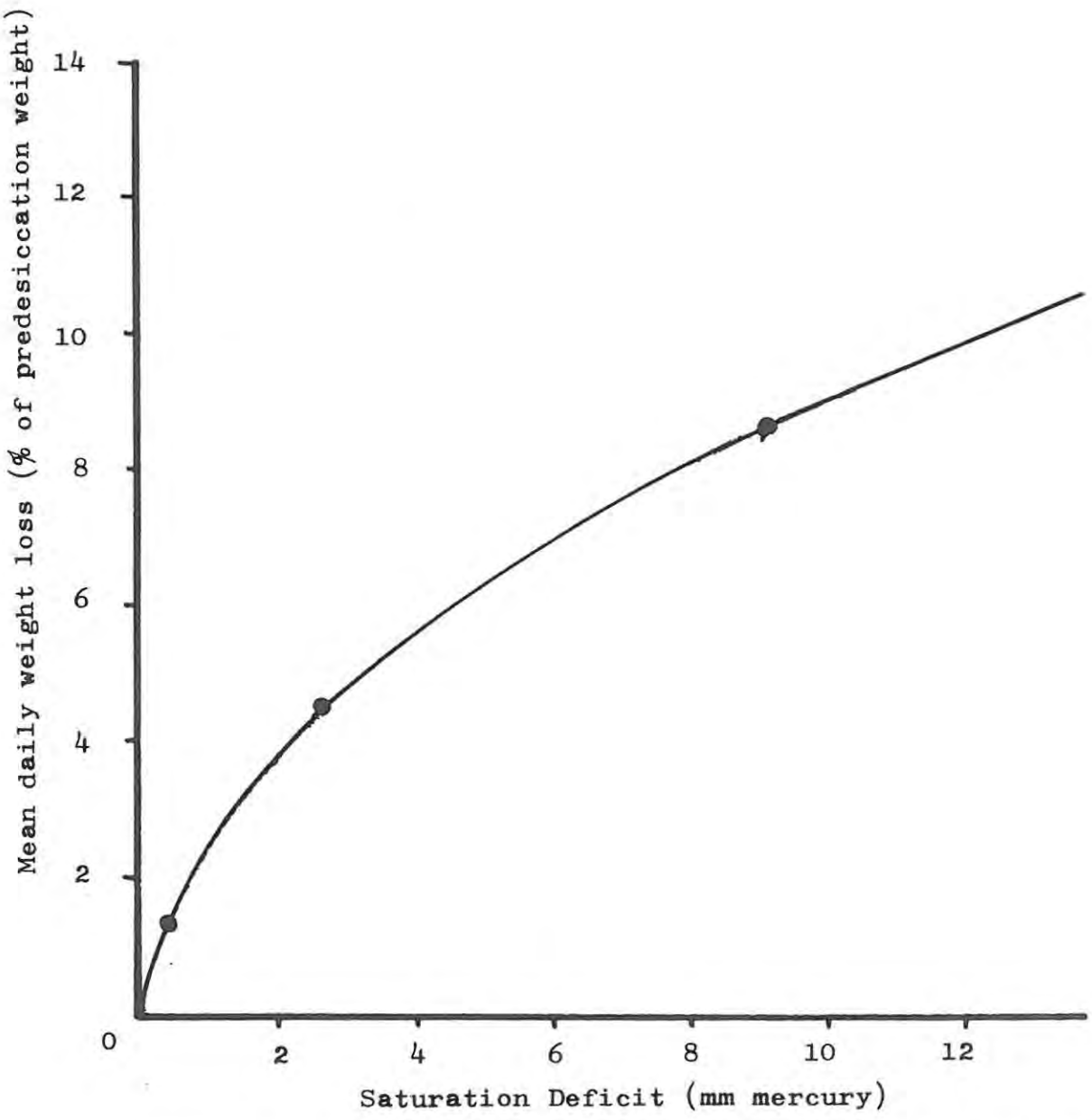

Figure 12 : The relationship between saturation deficit and the mean daily water loss incurred by aestivating *B. (Physopsis) africanus*.

were killed, removed from their shells and their dry weight was determined.

Results:

The results, summarised in Table 16, show that rehydration results in the animals regaining much of the weight lost. The rate of oxygen consumption, after an initial decline, also returns to the starvation level. Coles (1969b) shows a similar decline on adding water to an aestivating *B. (Physopsis) nasutus*, but in the case of that species, the rate of oxygen consumption drops to a negative level, and is then followed by a steady increase to approximately 10 times the aestivation level and a slower decrease to five times the aestivation level. None of the snails used in the present investigation showed this pattern. The considerably greater difference between aestivation and post-aestivation rates of oxygen uptake measured by Coles is probably largely due to his having used snails which had been aestivating for six weeks and longer, and in which the rate of oxygen consumption would be considerably lower than in the present experiment.

CHANGES IN THE BLOOD OSMOTIC PRESSURE OF AESTIVATING *B. (PHYSOPSIS) AFRICANUS*.

Water loss such as that found to be incurred by aestivating *B. (Physopsis) africanus* should reflect itself in an increase in the internal osmotic pressure of the snail, and if water loss is instrumental in the initiation of aestivation, a relationship between blood osmotic pressure, water loss and the depressed rate of oxygen consumption characteristic of aestivation was to be expected. An attempt was, therefore, made to determine the blood osmotic pressure of aestivating snails.

Method:

Snails collected from Krantzkloof, which had been kept under standard laboratory conditions for 14 days, and which had been subjected to the normal experimental pre-treatment, were divided into two groups which were treated as detailed below.

Table 16 : Changes in weight and rate of oxygen consumption in aestivating *B. (Physopsis) africanus* when aestivation is terminated by placing the snail in water.

	Pre-aestivation weight (mg)	Weight after 8 days at 87% RH (mg)	Weight Loss (mg)	Post aestivation weight (mg)	Weight Gain (mg)	Rate of oxygen consumption Aestivation	Post aestivation 1st hour	Post aestivation Subsequent
Mean (7 snails)	356,2	232,0	124,2	337,8	105,9	0,51	0,26	0,87
Standard error	16,0	11,3	6,1	11,7	6,9	0,23	0,01	0,03

Group 1 : Control. Snails were carefully dried immediately after being removed from water, and the foot was rapidly punctured by means of a sharp dissecting needle. This not only punctured the foot, but also caused the snail to retract violently, expelling an appreciable volume of blood which was held in the aperture of the shell. As much as 40 µl of blood could be extruded by a snail, and this was collected in a 50 µl Hamilton syringe. The osmotic pressure of the whole blood thus collected was determined in a Hewlett Packard 302B Vapour Pressure Osmometer.

Group 2 : Aestivating snails. The snails were allowed to aestivate in a desiccator at 87% R.H. for a period of five days.
These were to have been similarly treated to the control group, but no blood could be extracted by the method described above, even after the shell had been broken away carefully to expose the retracted foot. Upon dissection of the snails, what blood was present in the haemocoel was of such a viscosity that it could not be effectively drawn up in a syringe. Attempts to collect blood samples in capillaries for freezing point determinationby the method of Ramsay and Brown (1955), as an alternative to the vapour pressure method, proved equally fruitless. Only three samples of blood were collected from the 12 snails used.

A shortage of snails, resulting at least in part from a removal of aquatic vegetation from the collecting site at Krantzkloof, precluded the inclusion of a starved control group.

The effect of rehydration on the blood osmotic pressure was investigated using five snails from the Umlaas River. These were weighed prior to desiccation at 87% R.H. over a period of five days, after which they were weighed again before and after rehydration. Blood samples could be obtained from the rehydrated snails after four hours in water, and the osmotic pressure of the blood was determined by the

vapour pressure method.

Results:

Table 17 summarised the results obtained from the experiment. Clearly this aspect of the aestivation process needs further investigation. The results for the aestivating snails in particular are highly suspect, since no replicates were done and the three measurements obtained ranged from 201 to 235 mOsmoles which represents a wide range of concentrations. The difference between the controls and the rehydrated snails may be attributable to differences in origin or to the period of desiccation to which the latter were subjected. The experiment does have value in that it does show a change in blood volume, and suggests an increase in blood osmotic pressure during aestivation, which would be expected.

DISCUSSION

While the results suggest a relationship between water loss and the onset of aestivation as has been put forward by von Brand *et al.* (1957), Meenakshi (1964) and Little (1968), the conclusion that desiccation results in high mortality is inescapable, both from the findings of Shiff (1961) and those presented here. However, it does appear that it is the rate of desiccation rather than the absolute amount of water lost which is important in determining survival, a slower rate resulting in a lower mortality. Lockwood (1967) has reviewed cellular osmotic adjustment in various invertebrates. From his review it is apparent that the osmotic adjustment of the cells of those species studied is regulated by variation in the concentration of free amino acids within the cells. Such variations in the amino acid concentration are unlikely to be effected rapidly; rather the animals are likely to require a period of time over which to slowly acclimate to changes in body fluid concentration. In *B. (Physopsis) africanus*an increase in free intracellular amino acid concentration would be required to offset the increase in the osmotic pressure of the body fluids during aestivation. If the osmotic pressure of the body fluids were to change rapidly and continuously, the cellular

Table 17 : Changes in the blood osmotic pressure of *B. (Physopsis) africanus* during aestivation and following rehydration.

	Initial weight (mg)	Weight at end of aestivation (mg)	Weight loss (mg)	Weight following rehydration (mg)	Weight gain (mg)	Blood osmotic pressure (mOsmoles)
Control Number of snails = 12						
Mean	323,2	-	-	-	-	152,8
Standard Error	14,6	-	-	-	-	2,7
Aestivating Number of snails = 12						
Mean	365,7	257,1	108,6	-	-	218,0
Standard Error	18,4	13,3	6,1	-	-	(3 determinations only)
Rehydration Number of snails = 5						
Mean	280,9	170,4	110,5	275,2	104,4	166,0
Standard Error	12,6	14,2	3,5	11,7	4,1	3,2

mechanism for producing free amino acids may not be able to keep pace, resulting in cell damage and death.

If, indeed, body water content, and therefore, by inference, blood osmotic pressure, does provide the trigger for initiating aestivation, it does not necessarily follow that the influence is direct. Coles (1969b) reports the presence of a non-dialyzable, acetone extractible, heat stable substance in the liver of non-aestivating *Pila ovata* which, when added to respiring muscle tissue slices of that species, results in an increase in the rate of oxygen consumption. This principle, tentatively identified as a steroid, is absent from the livers of aestivating snails. He has also been able to show the presence of this, or a similar sunstance in *B. (Physopsis) nasutus* and *Biomphalaria sudanica* (Martens). These findings point to a possible hormonal regulation, which, in turn, may be triggered by the internal osmotic pressure. However, there does appear to be a relationship, either direct or indirect between water balance and aestivation, water being one of the determining factors in both the initiation and termination of the process. It may simply be the need for cellular osmotic adjustment which governs the metabolic change associated with aestivation.

11. THE UTILIZATION OF METABOLIC RESERVES BY *BULINUS (PHYSOPSIS) AFRICANUS* DURING STARVATION AND AESTIVATION

The period of aestivation, like that of hibernation, is one of enforced starvation during which the animal concerned is wholly dependent for its metabolic needs on energy reserves stored during periods of active feeding. Hibernation is a seasonal phenomenon which is, as far as is known, always preceded by a "period of preparation" during which abnormally large reserves of high energy compounds such as carbohydrates and fats are accumulated in the various storage depots in the body. This generalization holds good for vertebrate hibernators (Kayser, 1955) as well as for those Mollusca which are known to hibernate and which have been studied (von Brand, 1931; Baker, Boyd, Clarke & Ronan, 1942). The need for aestivation can, however, arise at almost any time, irrespective of season, and this precludes any set "period of preparation". This is particularly true in regions where a period of drought may occur in the middle of what is normally a rainy season and may recur at irregular intervals. In much of Africa, as elsewhere, such drought periods are not only unpredictable, but may also be of such severity as to cause a complete drying out of shallow water bodies. Aestivation, as a survival mechanism in molluscs inhabiting such environments, should, therefore, effect an economy in the utilization of whatever metabolic reserves the animals may have at the onset of the adverse conditions.

Martin (1961) and Goddard and Martin (1966) have reviewed our knowledge of carbohydrate metabolism in the Mollusca. Glycogen appears to be the main carbohydrate reserve and is known to occur in all classes of the phylum. Galactogen has been found to be associated specifically with the albumen gland or generally with the female reproductive system in many Gastropoda, including Pulmonata. Von Brand (1931) has carried out a detailed study of the carbohydrate content of *Helix pomatia* throughout a full annual cycle. He has demonstrated a very definite pre-hibernation build-up of stored polysaccharide which is slowly utilized during winter hibernation and which is further depleted at a variable rate after

hibernation has terminated. Both von Brand (1931) and Theil (1959) have found the lipid content of *H. pomatia* to fall during hibernation, but changes in this case are very much less marked than the fluctuations in polysaccharide content. The nitrogen content of *H. pomatia* shows fluctuations similar to those for lipid (von Brand, 1931). Schmidt-Nielsen, Taylor and Shkolnik (1971) have followed the fluctuations in the content of stored metabolic substrates in the Palastinian desert snail *Sphincterochila broissieri*. This animal is in an almost constant state of aestivation, since more than a year may pass without rain in its very harsh environment. No clear storage pattern emerged from their results.

Von Brand, McMahon and Nolan (1957) have determined the polysaccharide and lipid content of aestivating and starving *Australorbis glabratus*, the only freshwater species in which this aspect has been studied. Their findings suggest very little difference between the rates of consumption of these substrates under conditions of starvation and aestivation. This, together with the fact that the measured rate of oxygen consumption in both cases exceeds that which would be required for the total oxidation of carbohydrates and lipids metabolized, leads them to suggest that protein forms the main metabolic substrate during prolonged starvation and aestivation.

The above investigations, while showing the rate at which metabolic reserves are utilized and their nature, do not take into account the storage sites from which they are mobilized but refer to the composition of the snail as a whole. Studies on the freshwater mussel *Fusconaia undata* (authority not quoted) have shown the glycogen content of the hepato-pancreas of this species to be three times that of the foot in well fed specimens, whereas in starved animals the foot has a higher glycogen content than the hepatopancreas (Calvin, 1931, quoted in Goddard & Martin, 1966). The present investigation therefore seeks not only to establish the rates at which different substrates are used by starving and aestivating *Bulinus (Physopsis) africanus*, but also to define the specific pool from which the reserves are mobilised, and, if possible, to relate utilization to the foregoing metabolic study.

THE COMPOSITION OF INTACT *B. (PHYSOPSIS) AFRICANUS*

Since all previous studies on the metabolic reserves of pulmonate Gastropoda have expressed the results in terms of total content and not in terms of individual organ systems, it was necessary for comparative purposes to carry out analyses of intact snails. Investigation of separate organ systems involves dissection, and therefore, inevitable loss of part of the body fluids of the snail, thus invalidating to a large extent comparison with the results of other workers.

Method:

Specimens of *B. (Physopsis) africanus* collected from Krantzkloof, which had been in standard laboratory culture for at least 14 days, and which had been subjected to the normal experimental pre-treatment, were removed from water, carefully dried with absorbant tissue paper and weighed. They were killed by dipping them, spire first, into near boiling water. Care was taken that no water entered the aperture of the shell, or in any way came into contact with the tissues. Ten seconds exposure to this high temperature was sufficient to completely immobilize a snail, and the tissues could then be removed from the shell without damage. Failure to kill the snails prior to removal from the shell resulted in violent muscular contraction, which caused rupturing of the mantle and consequently a loss of body fluids. It was often necessary to break the shell at the apical or sub-apical whorl in order to remove the tissues intact; this was done under a stereo microscope, and shell fragments were recovered. The intact soft parts were weighed after gentle drying with absorbant tissue paper. Both the tissues and the shell were dried to constant weight at 60°C, after which the tissues were analysed for total carbohydrate, protein and lipids.

Results:

Table 18 shows the composition of intact *B. (Physopsis) africanus* together with comparable data for two other pulmonate species. While there is good overall agreement, the three species differ in detail, much of which, in the case of *Sphincterochila boisseri*, can be attributed to the different habitat in which it is found. Difference between *B. (Physopsis)*

Table 18 : Composition of *B. (Physopsis) africanus* compared with *B. (Physopsis) globosus*[1] and *Sphincterochila boissieri*[2].

	B. (Physopsis) africanus		*B. (Physopsis) globosus*		*Sphincterochila boissieri*	
No. of snails	10		?		1200	
Total constituents	Mean weight per snail (mg)	% of total weight	Mean weight per snail (mg)	% of total weight	Mean weight per snail (mg)	% of total weight
Shell (dry)	124,6±12,0	46,6±1,1	-	-	2 256	56,0
Tissue (wet)	128,5±15,7	46,7±1,4	-	-	1 805	44,0
Extraneous water	18,1	6,7	-	-	-	*
Total	271,2±28,5	100,0	-	-	4 064	100
Constituents of wet tissue	Mean weight per snail (mg)	% of wet weight	Mean weight per snail (mg)	% of wet weight	Mean weight per snail (mg)	% of wet weight
Water	110,3±13,7	85,6±0,3	-	-	1 462	81
Solids	18,2± 2,0	14,4±0,3	-	-	343	19
Total	128,5±15,7	100,0	-	-	1 805	100
Constituents of dried tissue	Mean weight per snail (mg)	% of dry weight	Mean weight per snail (mg)	% of dry weight	Mean weight per snail (mg)	% of dry weight
Protein	11,31± 1,24	62,3±0,7	-	60,8	195	57,0
Carbohydrate	1,45± 0,20	7,9±0,3	-	4,0**	43	12,5
Lipid	0,98± 0,12	5,4±0,2	-	11,7	5	1,5
Undetermined	4,42± 0,49	24,3±0,7	-	24,5***	100	29,0
Total	18,16± 2,0	100,0	-	101,0	343	100,0

[1]Mozley, 1954
[2]Schmidt-Nielsen *et al.*, 1971
*Wet weight of tissues determined by subtracting shell weight from total live weight.
**Estimated but not actually determined.
***Includes $CaCO_3$, $MgCO_3$, K_2CO_3, KCl, $Ca(PO_4)_2$, iron oxides, alumina and silica, all determined.

africanus and *B. (Physopsis) globosus* may well reflect differences in the nutrition of the animals used; also the carbohydrate content of *B. (Physopsis) globosus* was not quantitatively determined by Mozley (1954) but merely inferred as constituting the remainder after other constituents had been accounted for.

Von Brand, Baernstein and Mehlman (1950) found the total carbohydrate content of *A. glabratus* to constitute between 2,69% and 1,35% of the wet tissue weight, depending on whether the snails came from an aquarium with a plentiful or sparse food supply. These figures are both higher than the 1,13% calculated from the data in Table 18. Several factors may be responsible for this discrepancy. It may reflect an interspecific difference, since von Brand *et al.* (1950) have determined the carbohydrate content of *Lymnaea natalensis* as being 3,55% of the wet tissue weight, while that of *L. stagnalis* is only 1,26%. If unlike the *B. (Physopsis) africanus* used in the present investigation, which had been starved for 24 hours prior to analysis, the *A. glabratus* used by von Brand *et al.* were actively feeding immediately prior to analysis, this could also account for the discrepancy. In actively feeding snails the carbohydrate in the gut contents, which is not necessarily assimilated, will have been included in the determination. Whatever the reason for the difference, it does not lie with the stored polysaccharide content of the snails, for it was subsequently shown that there is good agreement between the percentage polysaccharide present in *B. (Physopsis) africanus* and *A. glabratus*.

THE COMPOSITION OF THE DIFFERENT ORGAN SYSTEMS OF FED, STARVING AND AESTIVATING *B. (PHYSOPSIS) AFRICANUS*.

Products of digestion in pulmonate snails are largely stored in the liver (Hyman, 1967) whence they are distributed to meet the requirements of the animals. The albumen gland constitutes a further storage site concerned chiefly, if not entirely, with reproduction. It was necessary to know from which sources the energy reserves of the animals were drawn, both during starvation and aestivation.

Method:

Specimens of *B. (Physopsis) africanus* from all three collecting sites were used in this experiment. Snails which had been kept under standard laboratory culture conditions for at least 14 days, and which had been subjected to normal experimental pre-treatment, were divided into three groups using a table of random numbers. These groups were respectively fed, starved or left to aestivate on wet sand as described in Section 9. The duration of the experiment was again 21 days, after which period, the snails were killed by dipping them into near boiling water and removed from their shells. The tissues were dissected and divided into three major organ sytems, *i.e.*

(i) the reproductive system, including the hermaphrodite gland and duct, the albumen gland, vas deferens and oviduct, seminal receptacle and prostate gland, but excluding the penis, preputium and penial retractor muscle,

(ii) the alimentary tract, including oesophagus, liver and intestine, but excluding the muscular buccal mass, and,

(iii) the musculature, including the foot and pedal retractor muscles, as well as the penis and associated structures and the buccal mass. Nervous tissue which could not be readily separated from the musculature was included in this fraction.

These individual organ systems were dried to constant weight at 60°C after which quantitative determinations were made of the total carbohydrates, total polysaccharides, lipids and protein present in each organ system.

Two series of experiments were carried out. The organ systems of individual snails which had been fed, starved or in aestivation over the 21 day period were analysed. This permitted the calculation of error estimates for each organ system within each of the categories. It involved twelve analyses per snail, plus replicates where these were required and was thus very time consuming. The data obtained from these individual analyses were, therefore, supplemented by analyses performed on further pooled tissue samples. Here the snails were dissected into their individual organ systems

as before, but the organ systems of up to six snails were pooled for analysis. The results obtained from this series of experiments do not, therefore, lend themselves to statistical analysis, since the value for any particular tissue constituent obtained from such a pooled sample represents the mean value of the animals which had contributed to the sample.

Results:

The results obtained from the analysis of individual snails are shown in Table 19. These include the mean absolute values for protein, total carbohydrate, total polysaccharide and lipid, as well as the respective standard errors. Table 20 shows the results obtained from pooled tissue samples. These confirm the trends apparent in Table 19.

Care needs to be taken in interpreting the results presented in the above tables, since both starvation and aestivation involve multiple changes in composition of the snails. Considered on the basis of the *percentage* of the individual organ systems which each of the respective metabolic substrates comprises, the conclusions would be that:

(i) protein is utilized from the musculature, but not from the alimentary and reproductive systems, during both starvation and aestivation,

(ii) carbohydrate is utilized only from the alimentary system during both starvation and aestivation, but to a much greater extent during starvation where the content drops from 7,9% in fed snails to 2,5%, whereas in aestivating snails it drops to only 5,9%, and,

(iii) lipid is utilized from all three organ systems, with no apparent difference between starving and aestivating snails.

These conclusions would be misleading. Consider as an example the carbohydrate content of the reproductive organs of fed and aestivating snails in Table 19, which are respectively 7,5% and 8,4% of the dry tissue weight of the reproductive organs. The 8,4% which remains in the reproductive organs after 21 days aestivation is, in fact, 8,4% of a considerably *reduced tissue dry weight*. It is apparent from the dry weight values in Tables 19 and 20 that the alimentary and reproductive

Table 19 : Composition of individual organ systems of *B. (Physopsis) africanus* determined from the tissues of individual snails.

	Fed snails			Starved snails (21 days)			Aestivating snails (21 days)		
	N	Mean weight per snail (mg)	% of dry weight	N	Mean weight per snail (mg)	% of dry weight	N	Mean weight per snail (mg)	% of dry weight
Musculature									
Protein	30	6,12±0,39	63,4±0,7	23	5,02±0,36	56,1±1,5	30	5,94±0,26	54,8±0,6
Total carbohydrate	30	0,45±0,04	4,7±0,2	23	0,41±0,03	4,4±0,2	30	0,51±0,03	4,7±0,1
Polysaccharide	30	0,41±0,03	4,3±0,2	23	0,38±0,03	4,1±0,2	30	0,46±0,03	4,2±0,1
Lipid	20	0,17±0,02	1,6±0,2	15	0,10±0,01	1,5±0,2	20	0,20±0,02	1,8±0,1
Dry weight	30	9,6 ±0,58	100,0	23	9,2 ±0,58	100,0	30	10,9 ±0,51	100,0
Alimentary system									
Protein	30	3,48±0,22	51,7±0,7	23	2,3 ±0,14	55,5±1,6	30	3,89±0,16	53,3±0,6
Total carbohydrate	30	0,53±0,05	7,5±0,2	23	0,10±0,01	2,1±0,1	30	0,44±0,02	6,0±0,1
Polysaccharide	30	0,41±0,03	6,0±0,1	23	0,09±0,01	1,9±0,1	30	0,40±0,02	5,5±0,1
Lipid	20	0,61±0,06	8,8±0,3	23	0,22±0,02	5,1±0,6	30	0,36±0,03	4,8±0,3
Dry weight	30	6,8 ±0,46	100,0	23	4,3 ±0,29	100,0	30	7,3 ±0,30	100,0
Reproductive system									
Protein	27	3,36±0,24	75,8±1,1	23	1,51±0,11	68,6±1,7	30	2,35±0,12	75,8±0,7
Total carbohydrate	28	0,40±0,05	7,5±0,7	23	0,18±0,02	7,9±0,3	30	0,26±0,02	8,4±0,2
Polysaccharide	28	0,32±0,03	7,1±0,4	23	0,14±0,01	6,4±0,3	30	0,23±0,02	7,4±0,2
Lipid	17	0,40±0,05	8,9±0,5	15	0,14±0,02	6,8±0,7	20	0,23±0,02	7,4±0,7
Dry weight	28	4,5 ±0,33	100,0	23	2,2 ±0,18	100,0	30	3,1 ±0,16	100,0

Table 20 : Composition of individual organ systems of *B. (Physopsis) africanus* determined from pooled tissue samples.

	Fed snails			Starved snails (21 days)			Aestivating snails (21 days)		
	N*	Mean weight per snail (mg)	% of dry weight	N*	Mean weight per snail (mg)	% of dry weight	N*	Mean weight per snail (mg)	% of dry weight
Musculature									
Protein	48	7,15	62,2	41	5,97	56,3	48	5,22	56,7
Total CHO	48	0,55	4,8	41	0,48	4,5	48	0,45	4,9
Polysaccharide	48	0,51	4,4	41	0,46	4,3	48	0,43	4,7
Lipid	48	0,23	2,0	41	0,15	0,9	48	0,17	1,8
Dry weight	48	11,5	100,0	41	10,6	100,0	48	9,2	100,0
Alimentary system									
Protein	48	4,82	54,2	41	2,63	54,8	48	3,03	52,3
Total CHO	48	0,70	7,9	41	0,12	2,5	48	0,34	5,9
Polysaccharide	48	0,55	6,2	41	0,11	2,3	48	0,32	5,5
Lipid	48	0,86	9,7	41	0,23	4,8	48	0,25	4,3
Dry weight	48	8,9	100,0	41	4,8	100,0	48	5,8	100,0
Reproductive system									
Protein	48	4,28	75,1	41	1,58	71,8	48	2,07	78,1
Total CHO	48	0,50	8,8	41	0,16	7,3	48	0,23	8,7
Polysaccharide	48	0,42	7,4	41	0,15	6,8	48	0,22	8,3
Lipid	48	0,45	7,9	41	0,13	5,9	48	0,15	5,7
Dry weight	48	5,7	100,0	41	2,2	100,0	48	2,7	100,0

* N = Total number of snails used, each pooled sample contained the organs of 4 to 6 snails.

systems in particular are subject to weight loss during starvation and aestivation. Percentages are, therefore, meaningless for comparative purposes and the true reduction in metabolic substrates during starvation and aestivation can only be arrived at by comparing the absolute weights of the substrates.

Comparison of such absolute weights is only possible provided they can be referred to a snail of known weight. Two groups of snails of different sizes may contain the same *percentages* of the substrates under consideration, but the *absolute* values would be different. What is, in fact, required is a "Standard Snail", in which the contribution of each individual organ system to the total dry weight is known. In addition it is necessary to know the weight loss incurred by such a "Standard Snail" during both starvation and aestivation over the experimental period of 21 days, and also how this weight loss is distributed among the individual organ systems. The percentages which the substrates are found, in an experiment, to represent in a particular organ system can then be applied to that organ system in the "Standard Snail", and the absolute values for these substrates utilized by such a snail during starvation and aestivation can be determined.

The only data on which to base the composition of such a "Standard Snail" are those shown in Tables 19 and 20. By retaining the relative proportions arrived at by calculating the mean dry weights of all the snails in each of the respective categories in both tables, and applying these proportions to a fed snail with a total recovered dry tissue weight of 25 mg, the weights of the individual organ systems of a "Standard Snail" of that weight was calculated, as well as its weight loss and composition after 21 days starvation and 21 days aestivation. The composition of such a "Standard Snail" together with the measured data on which it is based, are shown in Table 21. It is conceded that the "Standard Snail" is based only on recovered dry weight, and that in the process of dissection fragments of tissues are lost, or they may even be included with the "wrong" organ system. Such errors are inevitable, and I submit that they would

Table 21 : Proportion of the total dry weight of fed, starving and aestivating *B. (Physopsis) africanus* contributed by each individual organ system extrapolated to a "standard snail" in each category. For derivation, see text.

Organ sytems:	Musculature		Alimenatry system		Reproductive system		Total
	Mean dry weight (mg/snail)	% total dry weight	Mean dry weight (mg/snail)	% of total dry weight	Mean dry weight (mg/snail)	% of total dry weight	Mean dry weight (mg/snail)
Observed values (Tables 19 & 20)							
Fed snails	10,55	45,4	7,85	33,8	4,85	20,8	23,25
Starving snails (21 days)	9,90	59,5	4,55	27,3	2,20	13,2	16,65
Aestivating snails (21 days)	10,05	51,5	6,55	33,6	2,90	14,9	19,50
Standard snail							
Fed	11,35	45,4	8,45	33,8	5,20	20,8	25,00
Starved (21 days)	10,65	59,5	4,89	27,3	2,36	13,2	17,90
Aestivating (21 days)	10,82	51,5	7,06	33,6	3,13	14,9	21,01

probably prove to be largely self cancelling. Their magnitude would, in any event, be small, as every effort was made to recover as much of the tissue as possible during dissection.

Table 22 shows the data presented in Tables 19 and 20 extrapolated to "standard" fed, starving and aestivating snails. The actual losses of metabolic substrates, presumably due to catabolic processes can now be compared. The utilization of metabolic substrates as calculated for a "Standard Snail" does not, of course, lend itself to statistical analysis. It can however, be assumed, provided that the fallibility of the assumption is realised, that the standard errors for the amounts of the substrates in the individual organ systems of a population of "Standard Snails" would not differ to any great extent from those given in Table 19; they would, if anything, be smaller, and would, therefore not attribute a significant difference to the data where it does not exist. These standard errors can thus be used to test the significance of the difference between the substrate contents of fed, starving and aestivating "Standard Snails" shown in Table 22.

(i) Protein. Protein is utilized from the musculature in both starving and aestivating snails ($0{,}001 > P$ in both instances), with no significant difference between the protein contents of the musculature of starving and aestivating animals ($0{,}5 > P > 0{,}1$).

Protein is also utilized from the alimentary and reproductive systems, but to a lesser extent by aestivating than by starving snails ($0{,}001 > P$ in all instances).

A curious anomaly is apparent in the case of the protein content of the musculature, where there is a reduction in the protein content without concomitant reduction in dry weight. This can only be explained in terms of the undetermined inorganic fraction, since the carbohydrate and lipid content of the musculature change but little during starvation and aestivation.

(ii) Carbohydrate. Since the carbohydrate content of both aestivating and starving snails comprises almost

Table 22 : Distribution of metabolic reserves among individual organ systems of "standard" *B. (Physopsis) africanus* and losses of these substrates incurred during 21 days starvation and aestivation.

	Fed snails	Starved snails		Aestivating snails	
	Content (mg/ snail)	Content (mg/ snail)	Loss (mg/ snail)	Content (mg/ snail)	Loss (mg/ snail)
Dry weight					
Musculature	11,35	10,65	0,70	10,82	0,54
Alimentary system	8,45	4,89	3,56	7,06	1,39
Reproductive system	5,20	2,36	2,84	3,13	2,07
Total	25,00	17,90	7,10	21,01	4,00
Protein					
Musculature	7,27	5,63	1,64	6,00	1,27
Alimentary system	4,76	2,62	2,14	3,73	1,03
Reproductive system	3,88	1,66	2,22	2,41	1,47
Total	15,91	9,91	6,00	12,14	3,77
Carbohydrates					
Musculature	0,51	0,49	0,02	0,52	-0,01
Alimentary system	0,68	0,12	0,56	0,42	0,26
Reproductive system	0,47	0,18	0,29	0,27	0,20
Total	1,66	0,79	0,87	1,21	0,45
Lipid					
Musculature	0,16	0,13	0,03	0,20	-0,04
Alimentary system	0,66	0,24	0,42	0,32	0,34
Reproductive	0,44	0,14	0,30	0,21	0,23

entirely polysaccharide (see Tables 19 & 20), and since the data may well exaggerate the difference between total carbohydrate and polysaccharide as the determination of the latter requires its precipitation and re-dissolving which could incur losses, only total carbohydrates are considered in Table 22.

There is no significant difference between the total carbohydrate contents of the musculature of fed, starving and aestivating snails ($0,9 > P > 0,5$).

There is significant utilization from both the alimentary and reproductive systems ($0,001 > P$ in both instances), with aestivating snails metabolizing less carbohydrate from these sources than starving snails ($0,001 > P$).

(iii) Lipid. As in the case of carbohydrates, lipids do not appear to be utilized from the musculature ($0,9 > P > 0,5$). Lipids are utilized from the alimentary and reproductive systems ($0,001 > P$ in both instances). Again the utilization by aestivating snails is less than that by starving animals ($0,001 > P$ and $0,01 > P > 0,001$ for the alimentary and reproductive systems respectively).

It is also possible, from the data in Table 22, to calculate the volume of oxygen, at S.T.P., required for the complete oxidation of the substrates lost during 21 days starvation or aestivation by a "Standard Snail". This is shown in Table 23, in which the calculated oxygen consumption, in ml oxygen at S.T.P., is compared with the actual measured oxygen consumption at S.T.P. shown in Table 11 (Section 9), corrected for the weight of a "Standard Snail". There is reasonably good agreement between the calculated and measured values for starving animals, but in the case of aestivators the agreement is not as good; even if all the snails had effectively entered aestivation, the measured oxygen consumption still exceeds the required value by some 20%.

Table 23 : Oxygen required for the complete oxidation of the metabolic substrates utilized by *B. (Physopsis) africanus* during 21 days starvation and aestivation.

Substrate	Utilized during 21 days (mg)	Oxygen required for complete oxidation	
		Weight (mg)	Volume (ml at STP)
Starving snails			
Protein	6,00	8,16	5,70
Carbohydrate	0,87	0,93	0,65
Lipid	0,75	2,20	1,54
Total			7,89
Measured oxygen consumption			7,66
Aestivating snails			
Protein	3,77	5,13	3,59
Carbohydrate	0,45	0,48	0,34
Lipid	0,53	1,55	1,09
Total			5,02
Measured oxygen consumption			6,12

THE PROGRESSIVE UTILIZATION OF METABOLIC SUBSTRATES BY STARVING AND AESTIVATING *B. (PHYSOPSIS) AFRICANUS*.

Studies on the rates of oxygen consumption in starving and aestivating *B. (Physopsis) africanus* showed the depression in the rate of oxygen uptake, which is characteristic of both conditions, to set in progressively, with the difference between starving and aestivating animals only becoming apparent after the first five days. The utilization of stored metabolites should follow the same pattern. Weekly determinations were, therefore, made on both starving and aestivating snails.

Method:

Snails collected from Nelspruit, which had been under standard laboratory conditions for at least 14 days, and which had been subjected to normal experimental pre-treatment, were used. These snails were all from the same batch (NPT-2), which should minimise the variability and thus compensate for the small numbers used.

The experimental animals were divided into starving and aestivating groups, which were treated as previously described. Samples were withdrawn from both groups after 7, 14 and 21 days, and the protein, carbohydrate and lipid content of their main organ systems were determined as described in the previous experiment. All determinations were done on pooled tissue samples.

Results:

Tables 24 and 25 show the progression of the utilization of the metabolic reserves by starving and aestivating *B. (Physopsis) africanus*. These results, extrapolated for "standard" snails, are set out in Tables 26 and 27. It has been necessary here to interpolate values for the dry weights of the different organ systems at 7 and 14 days aestivation and starvation. This has been done on the basis of the actual measured weight losses over these periods, since it was apparent that the reproductive system was subject to considerable weight loss during the first seven days, and negligible change thereafter. The dry weight changes of the different organ systems are therefore included in the tables. Figures 13 and 14 summarise the data in Tables 26 and 27 for

Table 24 : Progressive changes in the composition of the individual organ systems of STARVING *B. (Physopsis) africanus* (Batch NPT-2) over a period of 21 days.

Period of starvation	0 days		7 days		14 days		21 days	
No. of snails	5		20		23		15	
Content	mg/snail	% of dry weight	mg/snail	% of dry weight	mg/snail	% of dry weight	mg/snail	% of dry weight
Musculature								
Protein	8,17	64,0	6,16	60,4	5,64	57,6	4,86	55,2
Carbohydrate	0,57	4,4	0,50	4,9	0,47	4,8	0,41	4,7
Lipid	0,18	1,4	0,19	1,9	0,17	1,7	0,15	1,7
Dry weight	12,76	100,0	10,20	100,0	9,81	100,0	8,80	100,0
Alimentary system								
Protein	6,05	56,3	3,51	56,6	2,71	54,2	2,17	54,3
Carbohydrate	0,87	8,1	0,37	6,0	0,21	4,2	0,09	2,3
Lipid	1,0	9,3	0,21	3,4	0,13	2,6	0,09	2,3
Dry weight	10,74	100,0	6,22	100,0	5,00	100,0	3,98	100,0
Reproductive system								
Protein	5,42	74,6	1,82	75,8	1,66	72,2	1,52	76,0
Carbohydrate	0,66	9,1	0,17	7,1	0,16	6,9	0,16	8,0
Lipid	0,62	8,5	0,19	7,9	0,14	6,1	0,11	5,5
Dry weight	7,26	100,0	2,39	100,0	2,30	100,0	2,01	100,0

Table 25 : Progressive changes in the composition of the individual organ systems of AESTIVATING *B. (Physopsis) africanus* (Batch NPT-2) over a period of 21 days.

Period of aestivation	0 days		7 days		14 days		21 days	
No. of snails	5		31		24		17	
Content	mg/snail	% of dry weight	mg/snail	% of dry weight	mg/snail	% of dry weight	mg/snail	% of dry weight
Musculature								
Protein	8,17	64,0	6,72	60,0	5,84	58,4	5,44	55,0
Carbohydrate	0,57	4,4	0,49	4,4	0,50	5,0	0,46	4,6
Lipid	0,18	1,4	0,18	1,6	0,23	2,3	0,19	1,9
Dry weight	12,76	100,0	11,21	100,0	10,00	100,0	9.89	100,0
Alimentary system								
Protein	6,05	56,3	3,47	56,0	3,90	56,5	2,65	50,0
Carbohydrate	0,87	8,1	0,40	6,5	0,42	6,1	0,31	5,8
Lipid	1,00	9,3	0,30	4,8	0,26	3,8	0,24	4,5
Dry weight	10,74	100,0	6,20	100,0	6,90	100,0	5,28	100,0
Reproductive system								
Protein	5,42	74,6	2,70	79,4	2,56	77,6	2,29	73,9
Carbohydrate	0,66	9,1	0,29	8,5	0,26	7,9	0,23	7,4
Lipid	0,62	8,5	0,22	6,5	0,14	4,2	0,09	2,9
Dry weight	7,26	100,0	3,40	100,0	3,30	100,0	3,10	100,0

Table 26 : Progressive losses of metabolic substrates incurred by a "standard" *B. (Physopsis) africanus* over a 21 day period of STARVATION.

Period of starvation	0 days	7 days		14 days		21 days	
	Content (mg/snail)	Content (mg/snail)	Loss (mg)	Content (mg/snail)	Loss (mg)	Content (mg/snail)	Loss (mg)
Dry weight							
Musculature	11,35	10,90	0,45	10,83	0,52	10,65	0,70
Alimentary system	8,45	6,05	2,40	5,42	3,03	4,89	3,56
Reproductive system	5,20	2,58	2,62	2,52	2,68	2,36	2,74
Total	25,00	19,53	5,47	18,77	6,23	17,90	7,10
Protein							
Musculature	7,27	6,58	0,69	6,23	1,04	5,88	1,39
Alimentary system	4,76	3,43	1,33	2,94	1,82	2,65	2,11
Reproductive system	3,88	1,96	1,92	1,82	2,06	1,79	2,09
Total	15,91	11,97	3,94	10,99	4,92	10,32	5,59
Carbohydrate							
Musculature	0,51	0,53	-	0,52	-	0,50	0,01
Alimentary system	0,68	0,36	0,32	0,23	0,45	0,11	0,57
Reprodictive system	0,47	0,18	0,29	0,18	0,29	0,19	0,28
Total	1,66	1,07	0,59	0,93	0,73	0,80	0,86
Lipid							
Musculature	0,16	0,20	-	0,19	-	0,18	-
Alimentary system	0,66	0,21	0,45	0,14	0,52	0,11	0,55
Reproductive system	0,44	0,20	0,24	0,15	0,29	0,13	0,31
Total	1,26	0,61	0,64	0,48	0,78	0,42	0,84

Table 27 : Progressive losses of metabolic substrates incurred by a "standard" *B. (Physopsis) africanus* over a 21 day period of AESTIVATION.

Period of aestivation	0 days	7 days		14 days		21 days	
	Content (mg/snail)	Content (mg/snail)	Loss (mg)	Content (mg/snail)	Loss (mg)	Content (mg/snail)	Loss (mg)
Dry weight							
Musculature	11,35	11,05	0,30	10,82	0,53	10,82	0,53
Alimentary system	8,45	7,29	1,16	7,17	1,28	7,06	1,39
Reproductive system	5,20	3,30	1,90	3,25	1,95	3,13	2,07
Total	25,00	21,64	3,36	21,24	4,76	21,01	4,99
Protein							
Musculature	7,27	6,63	0,64	6,32	0,95	5,95	1,32
Alimentary system	4,76	4,08	0,68	4,05	0,71	3,53	1,23
Reproductive system	3,88	2,62	1,26	2,52	1,36	2,31	1,57
Total	15,91	13,33	2,58	12,89	3,02	11,79	4,12
Carbohydrate							
Musculature	0,51	0,48	0,03	0,54	-	0,51	0,01
Alimentary system	0,68	0,47	0,21	0,44	0,24	0,41	0,27
Reproductive system	0,47	0,28	0,19	0,26	0,21	0,23	0,24
Total	1,66	1,23	0,43	1,24	0,42	1,14	0,52
Lipid							
Musculature	0,16	0,18	-	0,25	-	0,21	-
Alimentary system	0,66	0,35	0,31	0,27	0,35	0,32	0,34
Reproductive system	0,44	0,21	0,23	0,14	0,30	0,09	0,35
Total	1,26	0,74	0,52	0,66	0,60	0,62	0,64

Key to Figures 13 and 14 :

———— Aestivating snails.

· – – – – Starving snails.

\+ Total recovered dry weight.

● Musculature.

X Alimentary system.

o Reproductive system.

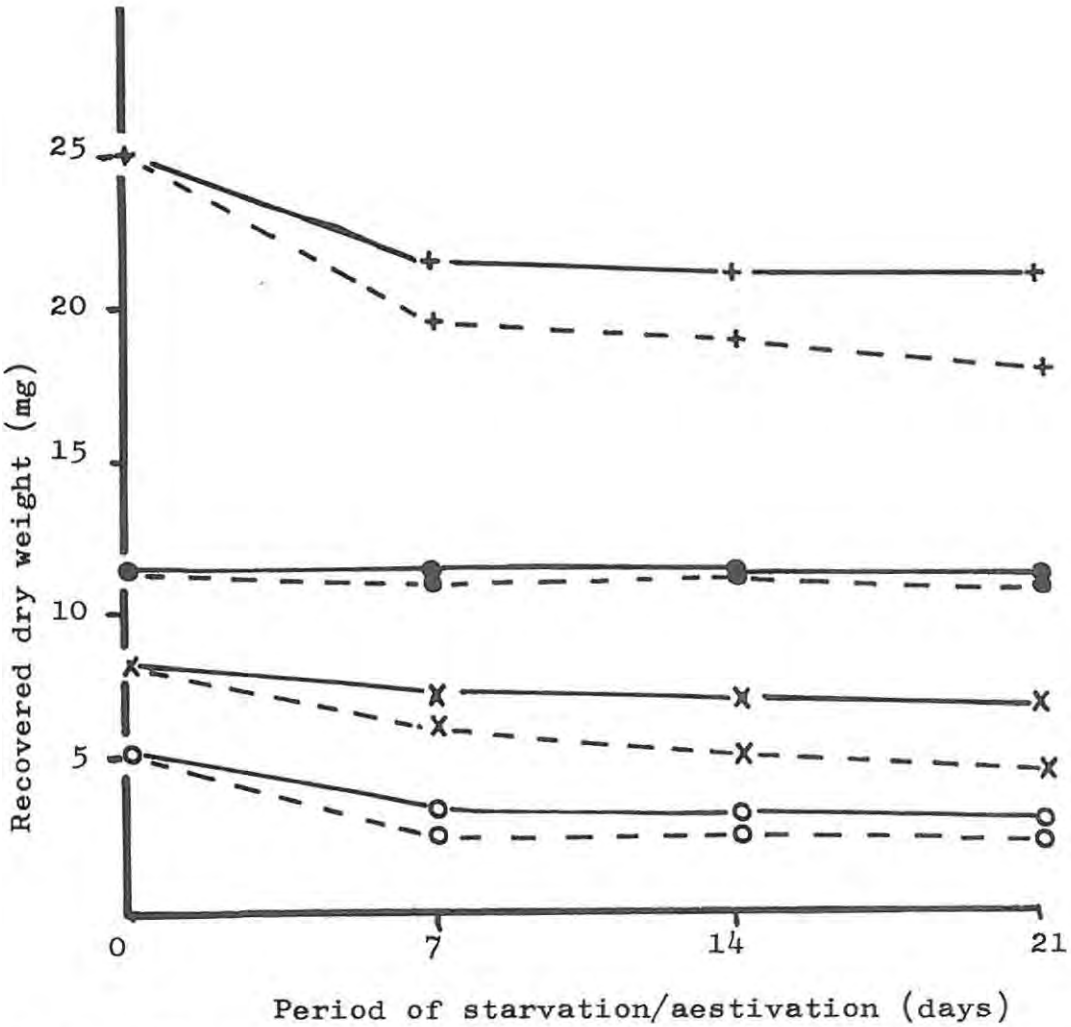

Figure 13 : Changes in the recovered dry tissue weight of a "standard" *B. (Physopsis) africanus* and of its individual organ systems during starvation and aestivation.

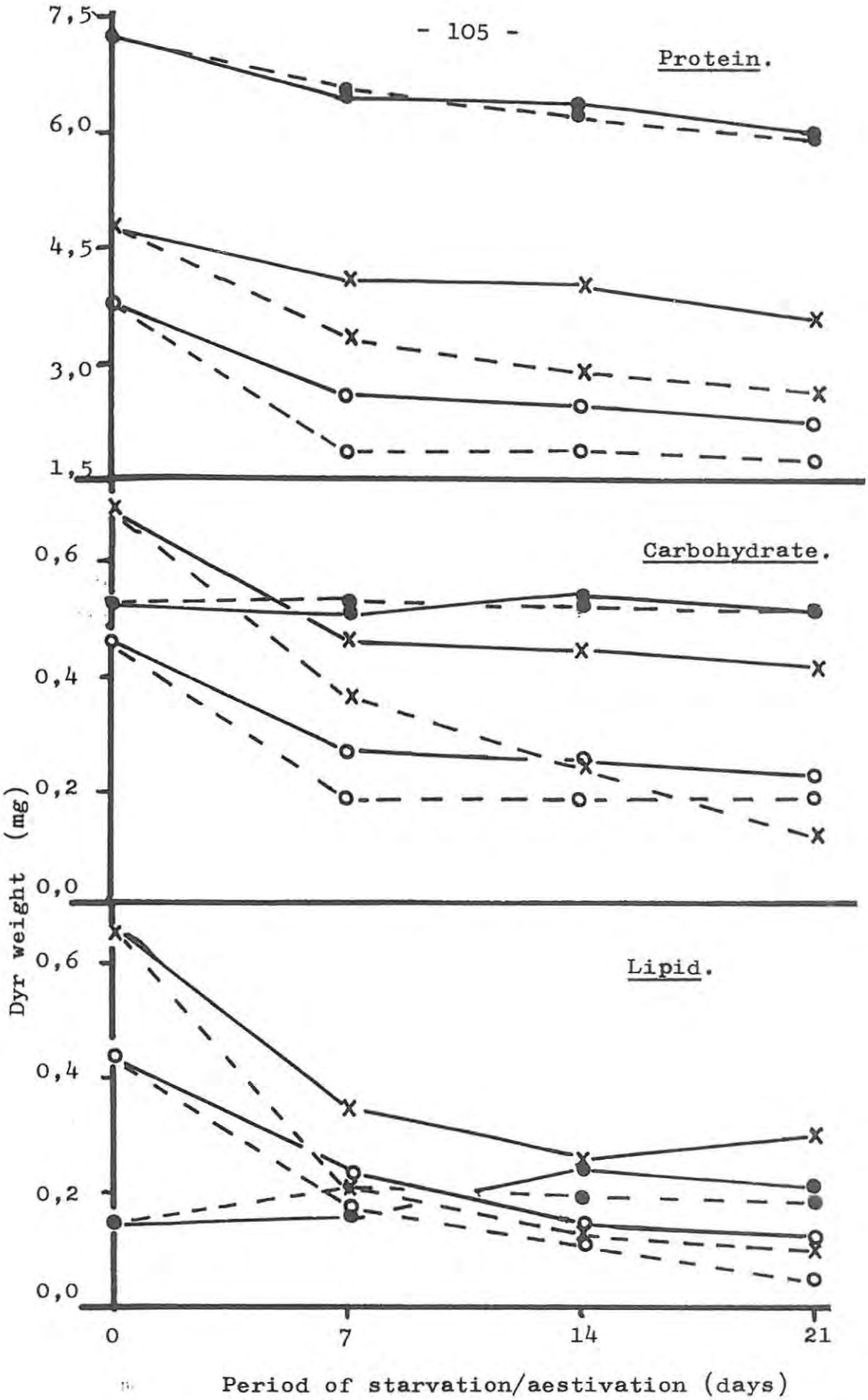

Figure 14 : Changes in the protein, carbohydrate and lipid content of the individual organ systems of a "standard" *B. (Physopsis) africanus* during starvation and aestivation.

easier interpretation.

From the data it is clear that the major loss in dry weight takes place during the first seven days of starvation or aestivation. In both cases, the musculature is little affected, while the alimentary and reproductive systems contribute materially to the loss in weight. Weight loss from the reproductive system is almost entirely confined to the first seven days of starvation or aestivation. In the case of the alimentary system there is a continuous loss in weight throughout the 21 days in starving animals, but the weight loss is curbed after the first seven days in aestivators.

A trend similar to the weight loss pattern is apparent in the loss of metabolic substrates during starvation and aestivation. Losses from the musculature are minimal and constant, whereas in the alimentary and reproductive systems the losses are greatest during the first seven days. In all instances a greater economy is shown by the aestivating snails; this is particularly apparent in the protein and carbohydrate utilization from the alimentary system. Almost all carbohydrate other than polysaccharide is utilized during the first seven days (see Tables 24 and 25).

Table 28 and Figure 15 show the oxygen required for the complete oxidation of the substances used during starvation and aestivation. While the calculated oxygen requirement corresponds reasonably well with the measured uptake over the total period of 21 days, there is a considerable discrepancy between the values for starving animals over the first 14 days of starvation. The only plausible explanation for this discrepancy, other than experimental error, is an incomplete metabolism or partial oxidation of certain substrates in the early stages of starvation. This suggestion finds some support in the presence of lactic acid and "volatile" acids reported by von Brand *et al.* (1957) in *A. glabratus*, but the significance of this is not clear. The fact that the lactic and "volatile" acids disappear from the tissues of aestivating *A. glabratus* after 20 days also fits the above hypothesis.

Table 28 : Oxygen required for the complete oxidation of metabolic substrates utilized by starving and aestivating "standard" *B. (Physopsis) africanus* during progressive starvation and aestivation over a period of 21 days.

Period of starvation/ aestivation:	7 days			14 days			21 days		
Substrate	Utilized* Weight (mg)	O_2 required Weight (mg)	O_2 required Volume (ml at STP)	Utilized Weight (mg)	O_2 required Weight (mg)	O_2 required Volume (ml at STP)	Utilized Weight (mg)	O_2 required Weight (mg)	O_2 required Volume (ml at STP)
Starving snails									
Protein	3,94	5,36	3,75	4,92	6,69	4,68	5,59	7,60	5,32
Carbohydrate	0,59	0,63	0,44	0,73	0,78	0,55	0,86	0,92	0,64
Lipid	0,64	1,88	1,32	0,78	2,29	1,60	0,84	2,46	1,72
Total O_2 required			5,51			6,83			7,68
Measured O_2 uptake			3,05			5,48			7,65
Aestivating snails									
Protein	2,58	3,51	2,46	3,02	4,11	2,88	4,12	5,60	3,92
Carbohydrate	0,43	0,46	0,32	0,42	0,45	0,32	0,52	0,56	0,39
Lipid	0,52	1,52	1,06	0,60	1,76	1,23	0,64	1,88	1,32
Total O_2 required			3,84			4,43			5,63
Measured O_2 uptake			3,38			5,50			6,26

*Utilized weight = weight loss for each substrate as shown in Tables 26 and 27.

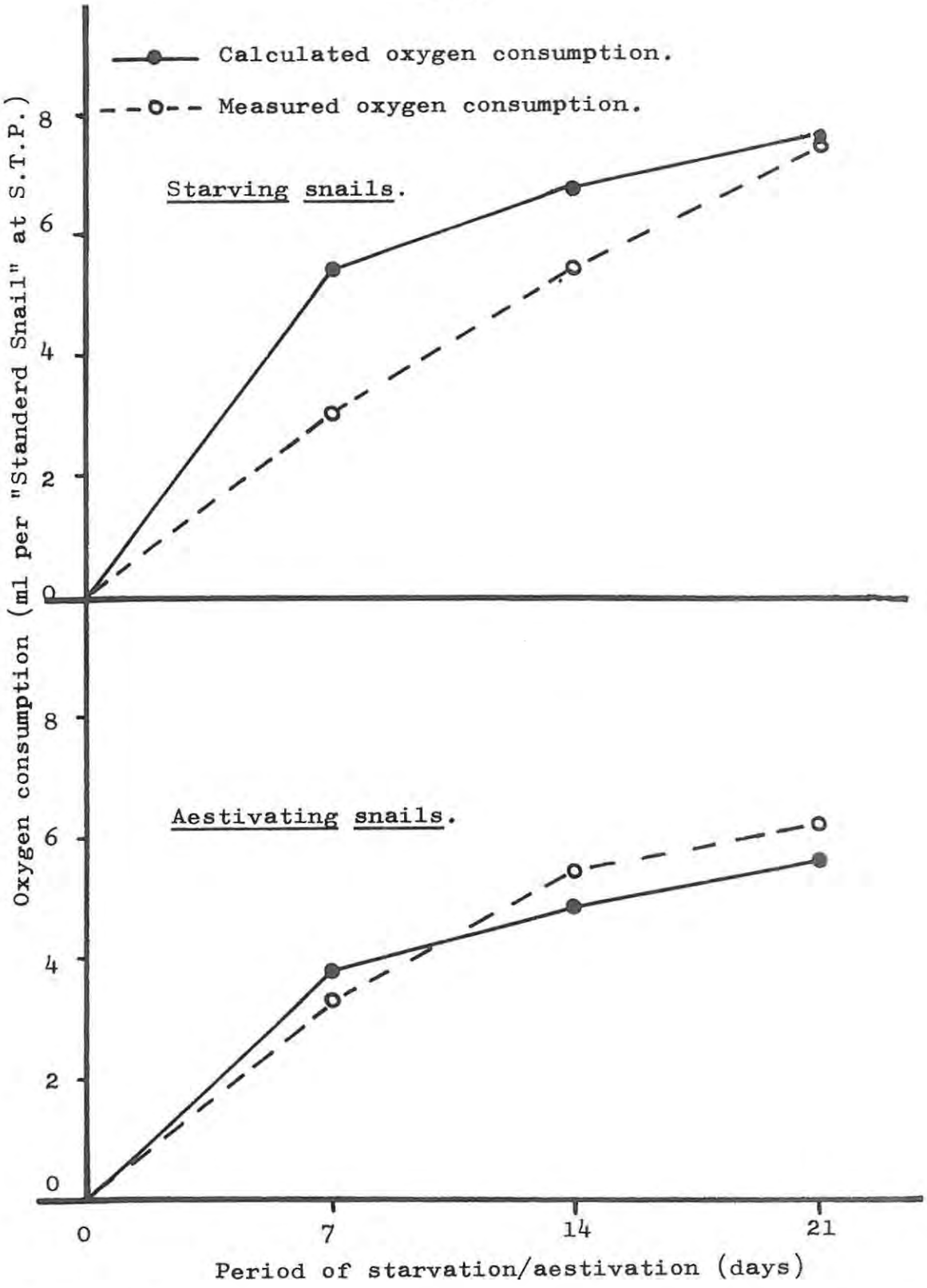

Figure 15 : Oxygen required for the complete oxidation of the metabolic substrates utilized by starving and aestivating "standard" *B. (Physopsis) africanus* over a period of 21 days, compared with the measured consumption.

OXYGEN CONSUMPTION AND THE CARBOHYDRATE CONTENT OF THE ALIMENTARY SYTEM OF AESTIVATING *B. (PHYSOPSIS) AFRICANUS*.

The results of the foregoing experiment showed the carbohydrate content of the alimentary system to be most clearly indicative of the difference between starving and aestivating *B. (Physopsis) africanus*. While protein constitutes the main metabolic substrate during both starvation and aestivation, the *percentage* carbohydrate in the alimentary system is markedly different in the two conditions. Studies on oxygen consumption during starvation and aestivation showed that not all snails could effectively enter aestivation when removed from water, thus the carbohydrate content of the gut should also be indicative of whether a snail was aestivating or merely starving out of water.

Method:

Fifteen snails collected from Krantzkloof, which had been under standard laboratory conditions for a period of 14 days and which had been subjected to normal experimental pre-treatment, were induced to aestivate by placing them on wet sand as described earlier. After 15 days the rate of oxygen consumption of the survivors was measured in a Warburg respirometer at 25^{0}C, and the carbohydrate content of the alimentary system was determined.

Results:

The relationship between the rate of oxygen consumption and the carbohydrate content of the alimentary system in aestivating *B. (Physopsis) africanus* is shown in Figure 16. The carbohydrate content of the alimentary system seems, from these results, to be a very sensitive indicator of the difference between starvation and aestivation. A low rate of oxygen uptake corresponds, in all but one case, to a high percentage of carbohydrate in the alimentary system. The single exception may well be an animal in which aestivation was partially broken at some stage during the experiment.

DISCUSSION

The results show clearly that aestivation effects an economy in the utilization of stored metabolites and that this economy is only effectively apparent after the first

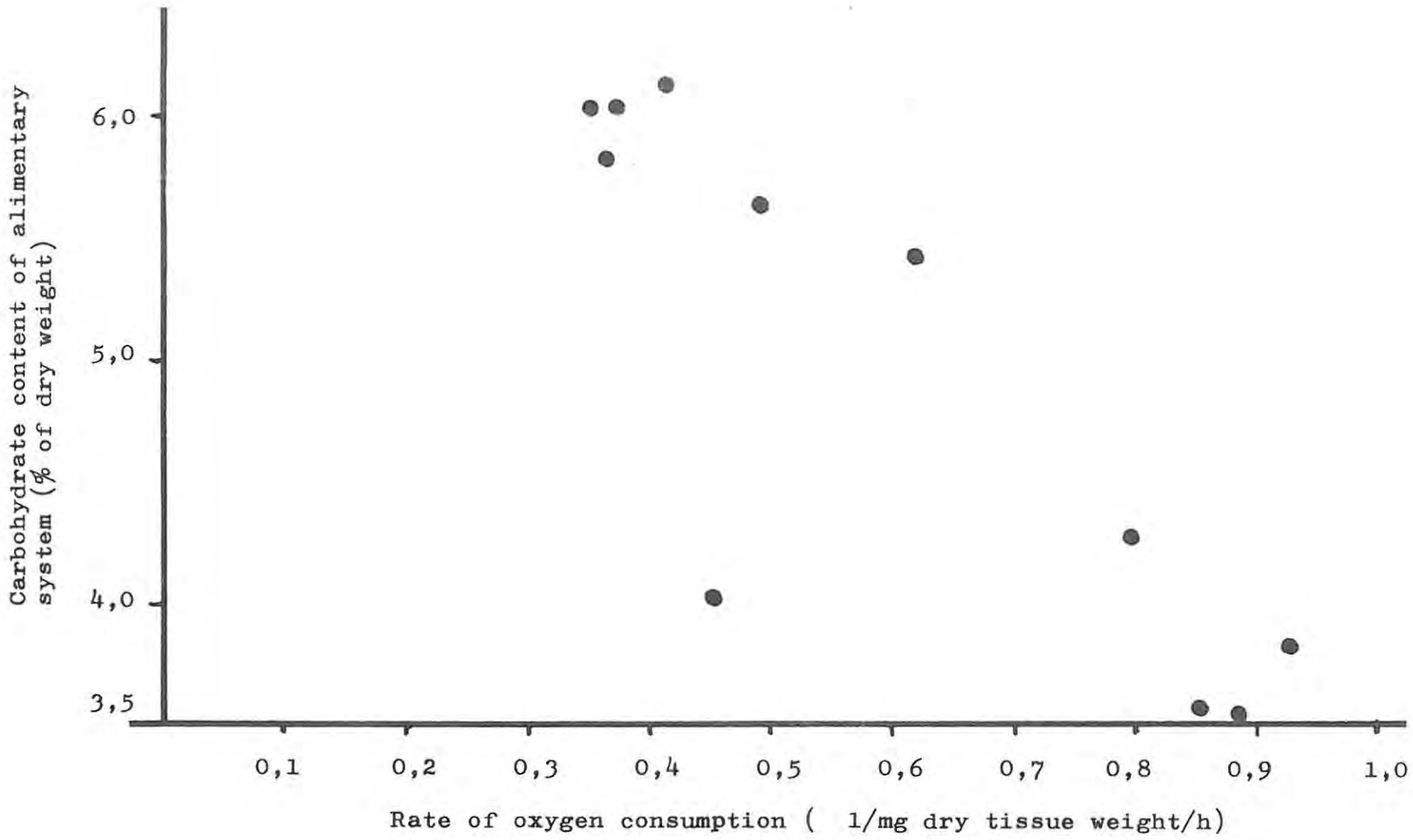

Figure 16 : Relationship between the rate of oxygen consumption and the carbohydrate content of the alimentary system in *B. (Physopsis) africanus* which had been aestivating for 14 days.

seven days of aestivation. This is in keeping with the results obtained in Section 9, where the difference between the rates of oxygen uptake of starving and aestivating snails only became apparent after the first five days. A number of points do, however, arise out of the results which require discussion.

Nearly all the carbohydrate other than polysaccharide is depleted early in starvation and aestivation ; polysaccharide comprises 97% and 96% of the total carbohydrate content of starving and aestivating *B. (Physopsis) africanus* respectively after seven days. Thus the more readily mobilised reserves are utilized first. While the carbohydrate content of the musculature remains fairly constant in both starving and aestivating snails, that of the reproductive and alimentary systems is subject to considerable change, suggesting that these form the more labile carbohydrate stores. In the case of the alimentary system, depletion of the stored carbohydrates follows very much the pattern suggested by the changes in the rate of oxygen consumption, with aestivating snails husbanding the reserve more effectively than starving animals after the first seven days. The withdrawal of carbohydrate from the reproductive system shows a different pattern; in both starving and aestivating animals there is a very rapid fall in the carbohydrate content of these organs during the first seven days, with an almost complete levelling off thereafter. A portion of the carbohydrate stored in the reproductive system is, therefore, conserved under conditions of starvation and aestivation.

Galactogen has been found to occur in the albumen gland of various pulmonate species, including some Planorbidae (McMahon, von Brand & Nolan, 1957). May (1931, quoted in Martin, 1961) has found this polysaccharide to be present in the eggs of *Helix pomatia* as well as in its reproductive system, and showed that the adult is able to metabolise it only under conditions of extreme starvation 20 days after all other carbohydrate has disappeared from the tissues. The carbohydrate remaining in the reproductive organs of *B. (Physopsis) africanus* may, therefore, well comprise largely, if not entirely, galactogen, the more readily utilized

carbohydrates having been metabolised during the first seven days. If indeed the remaining polysaccharide in the reproductive system is galactogen, the actual weight of polysaccharide will be much higher than that shown in Tables 19, 20, 24 & 25. The intensity of the colour developed by galactogen with anthrone is only 54% of that developed by an equal weight of glucose (Morris, 1948). The actual galactogen content could be arrived at by multiplying the figures in the tables by 1,85. Since no qualitative analyses were performed on the polysaccharides present in *B. (Physopsis) africanus* it cannot be said with certainty that galactogen is present; indeed, the presence of glycogen still remains to be confirmed, hence, all the results presented here have been in terms of "equivalent mg glucose". It seems reasonable, however, to suggest the presence of these two polysaccharides in *B. (Physopsis) africanus*, pending confirmation.

Little is known about either the synthesis or the catabolic metabolism of galactogen in those Mollusca in which it is found. The presence or uridine-diphosphate-glucose and uridine-diphosphate-galactose in *H. pomatia* (Goddard and Martin, 1966) shows that a mechanism for the inter-conversion between glucose and galactose exists in that species. No mention is, however, made of the enzyme UDP glucose epimerase, which is essential for the conversion. The stability of galactogen as a carbohydrate store will only be explained when the kinetics of the enzymes involved in both phosphorylation and interconversion of the resultant galactose to glucose have been studied.

The utilization of protein as an energy store is considerable under both starvation and aestivation conditions which confirms the prediction by von Brand *et al.* (1957). The pattern of utilization as shown in Figure 14 is essentially similar to that shown for carbohydrate, with the protein content of the musculature showing relatively little change when compared with the alimentary and reproductive systems. The anomaly of a reduction in the protein content of the musculature in excess of the total weight lost by this fraction is not readily explicable. The inorganic fraction of a whole *B. (Physopsis) globosus* comprises largely calcium

salts, calcium carbonate and calcium phosphate together making up 11% of the dry tissue weight (Mozley, 1954). Calcium is, at least in part, stored in the liver of *Helix spp* (Morton, 1958), and it seems possible that, with the utilization of liver protein, the ability of this organ to retain all the stored calcium salts is diminished, resulting in a re-location of these salts to storage sites in the musculature and its associated glandular organs. The amoebocytes in the blood of *Helix* are known to transport calcium from the liver to the mantle for incorporation into the shell (Morton, 1958). Verification of this suggestion can, however, only be obtained through a detailed study of the calcium content and turnover of *B. (Physopsis) africanus*.

Lipid metabolism calls for little comment. As a substrate it appears to be as labile as carbohydrate and protein in both starving and aestivating snails. The pattern of utilization again points to a constancy in the musculature, with the alimentary and reproductive systems constituting the more labile stores.

The discrepancy between calculated oxygen requirements and the actual measured oxygen consumption merits further study during the early stages of starvation and aestivation. Experimental error is a real possibility, since the snails used in the oxygen determinations are not those used in the substrate determinations. To assume that all carbohydrate metabolised is, in fact, glycogen would make a difference of less than 1% to the calculated oxygen requirements, and cannot, therefore, be responsible for the difference. However, since the discrepancy is only really apparent during the first two weeks of starvation, it may have some significance. Partial oxidation of substrates during early stages of starvation, with a resultant oxygen debt, and subsequent completion of the metabolic process seems to have little funtional significance in terms of our present knowledge of either aestivation or surviving periods of starvation, since oxygen is certainly not limiting. Yet, this seems to be the only explanation for the difference if it is, in fact, real. Von Brand *et al.* (1950) have shown that anaerobic pathways are present in a number of pulmonate snails, but

these are glycolytic pathways and carbohydrates cannot account for the whole difference.

12. GENERAL DISCUSSION

"Knowledge comes but wisdom lingers" (Tennyson)

On reflection, the results presented in this thesis no more than accentuate the problems related to aestivation in the aquatic Pulmonata. Knowledge has been gained about aestivation in *B. (Physopsis) africanus*, and the earlier work by von Brand *et al.* (1957) on *Australorbis glabratus* has largely been confirmed; yet problems remain, although the directions for future research into the aestivation phenomenon may, one hopes, be somewhat more clearly indicated as a result of what has been presented here.

In the absence of all necessary information, aestivation in the Pulmonata can, at this stage, only be rationalized through speculation on what knowledge has been gleaned to date. It is necessary to view it as a phenomenon governed by basic physiological principles and modified by selection to meet the needs of the aquatic pulmonates. Such a physiological process cannot, of course, be considered out of the context of the environment which animals inhabit, for it is only in terms of the physical and biotic factors which impinge on the animals that the importance of aestivation to the success of the freshwater molluscan fauna can be understood.

AESTIVATION REDEFINED

In Section 7 I defined aestivation in *B. (Physopsis) africanus* in terms of the observed characteristics of snails, both aquatic and terrestrial, found under unfavourable conditions. This definition, an extension, perhaps, of that given by Kenneth (1963) was based only on what could be established without recourse to experimentation; a snail which was out of water, inactive, had secreted a mucus veil and was alive, was considered to be aestivating. In this state at least part of a snail population was known to survive both in the field and in the laboratory (Olivier, 1956; Olivier & Barbosa, 1956; Shiff, 1961 and 1964; Cridland, 1967). The results of the present investigation, together with those of von Brand *et al.* (1957) and Coles (1969b) call for a re-

definition of the state of aestivation, at least in the Pulmonata.

Aestivation can validly be recognised as a physiological state which, through a depression of the metabolic rate , not only considerably slows down the utilization of stored metabolic reserves, but also extends the zone of temperature tolerance. In addition, aestivating animals can survive considerable loss of body water. It is, in effect, a form of resistance adaptation. Not all *B. (Physopsis) africanus* successfully enter this state of dormancy, thus a snail found out of water is not necessarily aestivating, but those which do are able to withstand a range of physical conditions and a period of starvation which would prove lethal to the non-aestivating snail.

Inactivity can no longer be regarded as characteristic of the aestivating *B. (Physopsis) africanus*. The aestivating snails can, and do, move when conditions are conducive to such activity, *i.e.* when the substratum is wet, and, in so doing, can select a less exposed site in which to weather adversity should this be necessary.

In terms of the above findings it is possible to redefine aestivation in *B. (Physopsis) africanus* as a dormant state, induced in some members of the population by removing the snails from water, and characterized by a depression in the metabolic rate which differs from that manifest in starving snails by a lower rate of oxygen consumption and a lower temperature coefficient. Such aestivating snails are normally inactive, but aestivation may be interspersed by periods of activity, initiated by an increase in the moisture content of the substratum.

AESTIVATION AS A FACTOR IN THE ECOLOGY OF *B. (PHYSOPSIS) AFRICANUS*.

The applicability of the conclusions drawn from the results of this laboratory investigation to the conditions in the natural habitat of *B. (Physopsis) africanus* remain to be confirmed. Field conditions experienced by freshwater snail populations vary with locality, and while insufficient data has, to date, been gathered on the conditions experienced by any specific population, it is possible from general

climatological and distribution data at least to relate the laboratory observations to conditions which may occur in the field. The need for confirmation does, however, remain.

Figure 17 shows the distribution of the sub-genus *Bulinus (Physopsis)* in the Republic of South Africa. It is absent from the arid western regions of the country, the highveld regions of the central plateau and the southern coastal regions. Two species occur in South Africa. *B. (Physopsis) globosus* has a more tropical distribution; it occurs from the Sahara through west and central Africa, Rhodesia and Mocambique and extends into the northern and eastern Transvaal and the coastal regions of northern Natal. *B. (Physopsis) africanus* has a similar distribution, extending from east Africa through central Africa, Rhodesia and Mocambique, but in the Republic of South Africa its range extends beyond that of *B. (Physopsis) globosus* in that it has been found in such high lying areas as Johannesburg, and along the coastal belt as far south as Humansdorp (Mandahl-Barth, 1958; van Eden & Combrinck, 1966; Brown, 1967).

Annecke and Peacock (1951) and Schutte and Frank (1954) have found *B. (Physopsis) spp* to inhabit both natural running waters and stagnant waters of almost every description. Lakes, irrigation and catchment dams, seepage ponds, "grassy pans" (= seasonally flooded grassland?), and marginal pans of rivers are all known suitable habitats for the snails. In river systems they tend to inhabit backwaters and flood pans, where water movement is minimal, and in lakes and large storage dams they tend to be absent from those shores subject to considerable wave action (Mozley, 1954). Marginal ponds on such shores are, however, a favoured habitat.

The range of *B. (Physopsis) africanus* in southern Africa is, with the exception of the tropical coastal region of Mocambique, characterized by wet summers and dry winters, with the greater part of the rainfall being restricted to the months October to March. Earlier and later rains may fall in the coastal regions (Schulze, 1965). These climatic conditions have a profound effect on many of the habitats of freshwater snails. Many of the smaller streams cease to flow in the dry season, leaving isolated pools which slowly dry up.

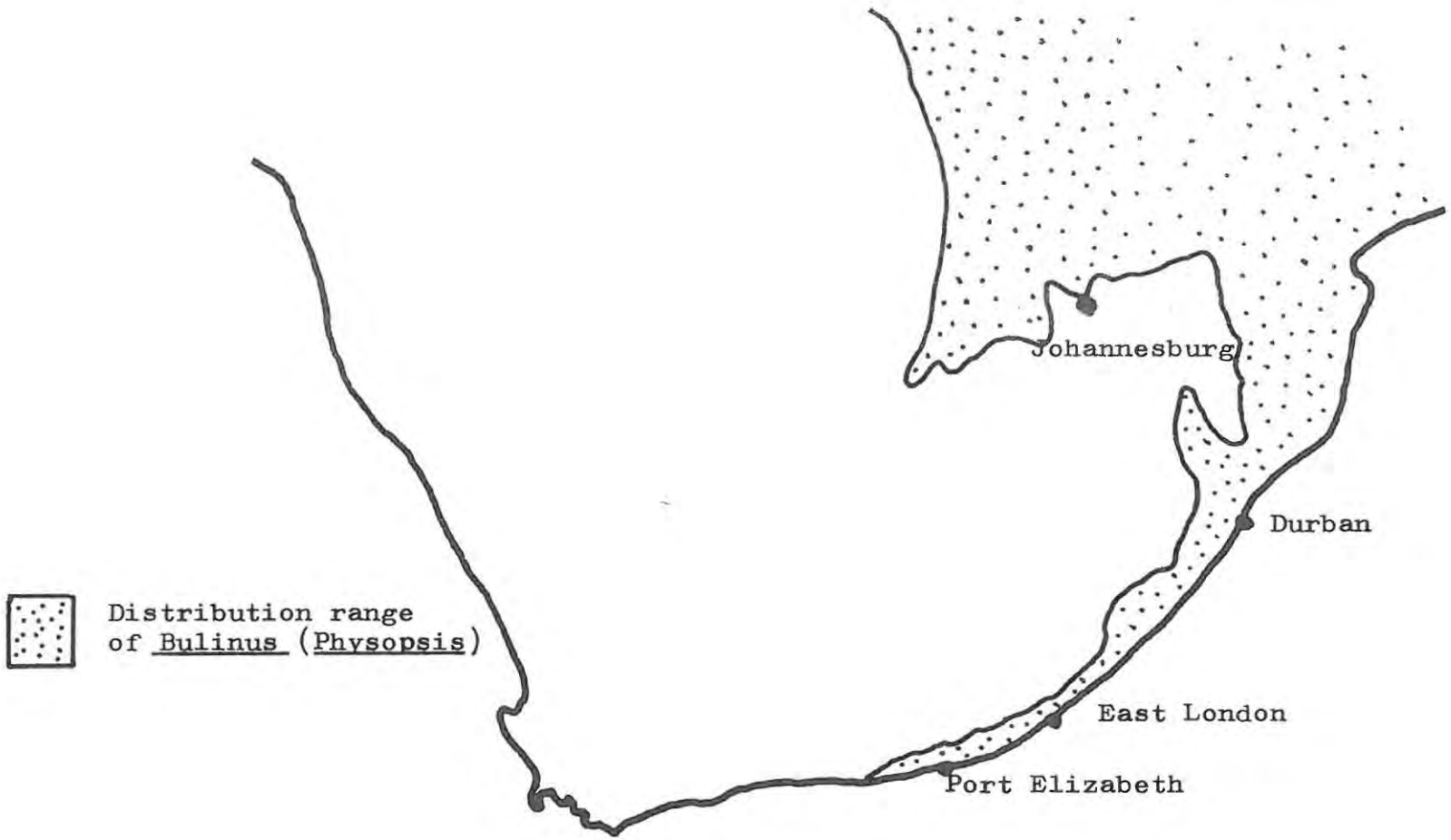

Figure 17 : The distribution of the sub-genus *Bulinus (Physopsis)* in South Africa (after van Eeden & Combrinck, 1966).

Grassy seepage pans disappear early in the season, and, with the fall in river levels, marginal pans become cut off from rivers and may also disappear. Water in catchment dams is utilized during dry months for irrigation purposes. Much of the summer habitat of the snails therefore disappears during winter. The first rains of the season are usually not sufficient to restore these bodies to their summer levels, and some habitats may not receive any permanent water until summer is well advanced; seepage accounts for much of the early rainfall, and only when the ground is saturated and with the advent of summer storms is there sufficient run-off for the habitats to be restored. In a poor rainy season, several water bodies may receive no standing water at all. Thus several months may pass during which the snails inhabiting these habitats will, if they are to survive, have to spend in aestivation. Shiff (1964c) has followed the sequence of water levels together with the fate and population dynamics of *B. (Physopsis) globosus* in such a temporary habitat near Salisbury. This pool became dry in August and only started to fill towards the end of November.

The time of falling water levels is characterised by high day temperatures; the almost complete absence of cloud cover allows for considerable and continuous insolation, while, with the exception of the coastal regions, the low moisture content of the air enhances evaporation. These, then are the conditions with which the snails have to contend as the habitat dries up. Air temperatures and insolation increase steadily from about mid-August, but humidity tends to remain low until the rains set in, thus the conditions which obtain at the time when the habitats are likely to be completely dry are not the most equible.

In the final stages of drying, the shallow isolated water bodies inhabited by snails will be subject to heating. In deeper pools a daily thermocline may be set up, and Shiff (1969) has shown that *B. (Physopsis) globosus* tends to select the higher surface temperature which was found to be near optimal for the greatest intrinsic rate of natural increase. In shallower water temperatures are, however, likely to rise to much higher levels. This would be particularly true for margins of pools which have retreated from the shelter of the

marginal vegetation, and for isolated depressions in which snails may become trapped through falling water levels. Such increases in water temperature will cause the snails either to seek cooler and deeper water, or, in the absence of this, to leave the water altogether as has been shown in the present investigation. Once out of water, conditions could prove more equible; evaporative cooling of the mud would tend to reduce the surface temperature, although the snails will be exposed to increased insolation. Two courses would now be open to the snails; they may, through mediation of the appropriate orientation reactions, seek shelter under vegetation, stones, logs *etc.*, or they may burrow into mud. Only activity has, so far, been shown to occur, and the snail's responses to physical stimuli, particularly to light, urgently require investigation. It is also necessary to establish whether *B. (Physopsis) africanus* habitually burrows, or whether it only does so as a last resort on not finding suitable shelter. While I have found aestivating *Bulinus (Bulinus) tropicus* in mud, the only aestivating *B. (Physopsis) africanus* I have encountered in the field have been under some form of shelter, or even fully exposed.

The above implies that the snails progressively leave the water to go into aestivation, and that only relatively few will remain in the deepest part of the pool immediately prior to final drying up. This requires verification in the field. Such a pattern would, however, have survival value. A concentration of a major part of the population in the deepest part of the pool would result in considerable competition not only for the now rapidly dwindling food supply, but also for suitable aestivation sites. Also, if with the onset of rains, the pool were only partially filled, and such partial filling were to be followed, as often happens, by a dry spell, considerable mortality might result, since the snails will again be exposed to all the hazards of the early stages of aestivation. A progressive evacuation of the pool on drying will result in a progressive recruitment to the population on filling, thus a catastrophe early in the rainy season will have a minimal effect on the population.

The conditions now faced by the aestivating snails are

those of late winter and spring. Air temperature maxima within the distribution range of the snails are high during August to October, and precipitation is, for the most part, less than 5% of the annual average (Schulze, 1965). More important is, of course, the microclimate to which the aestivating snails are subjected. Ground temperatures during this time of the year have been measured at the Roodeplaat Horticultural Research Station, 21 km north of Pretoria, and these may reach 35,5°C under grass cover and 45,6°C under bare ground, both at a depth of 1 cm (Schulze, 1965). Significantly, these are among the highest ground temperatures recorded during the course of a year, Cridland (1967) reports even higher ground temperatures in his experimental trays in Salisbury. The ability to survive transient high temperatures of this order of magnitude has been shown to be manifest in aestivating *B. (Physopsis) africanus*, and this seems to be attributable to the metabolic adjustment which characterises aestivation. Summer drought, which is not uncommon in southern Africa will increase the temperature stress; some temporary seepage pools with dark soils may well reach temperatures of 50°C. There is urgent need for exact temperature monitoring of actual aestivation sites. Bruton (pers. comm.) has measured temperatures up to 38°C in a dried up pool on the shores of Lake Sibayi in which *B. (Physopsis) globosus* and *Biomphalaria pfeifferi* were known to be aestivating. These temperatures were taken under moss covering the aestivating snails, which were also shaded by vegetation. These snails survived in aestivation for more than a year, including a summmer.

Much of the normal aestivation period is characterised by high saturation deficits, resulting from mean daily humidities below 40% R.H. and high day temperatures. We know nothing of the humidity, and therefore saturation deficit at actual aestivation sites, nor has the water loss from aestivating snails in the field been measured. It seems likely that, in the early stages of drying and when the substratum is wet, water loss would be minimal. The pre-aestivation period of activity can thus be extended to enable the snails to select a suitable aestivation site. Burrowing would probably reduce water loss through evaporation during the aestivation period to a minimum,

and could, therefore prove to be an ideal aestivation site from the point of view of curbing water loss. It does, however, create an anomalous situation. Conditions in mud are likely to prove anaerobic, or nearly so, owing to the biochemical oxygen demand. These conditions are likely to persist until the water content of the mud has fallen to the extent that air replaces most of the interstitial water at the level of the snail. Von Brand *et al.* (1950) have demonstrated the presence of anaerobic metabolic pathways in *A. glabratus*, and, if similar pathways exist in *B. (Physopsis) africanus*, which seems likely, the snails should be able to survive in water-logged mud for several days at the expense of their stored carbohydrate. Coles (1969b) has, however, shown that anaerobic conditions have the effect of terminating aestivat -ion in *B. (Physopsis) nasutus*, and it seems likely, therefore, that it would also inhibit the initiation of aestivation. However, much depends on the behaviour of the snails during this initial phase; they may initially simply remain inactive near the surface, sufficiently close to breath, and gradually burrow deeper as the water table falls, continually maintaining contact with the air. This will delay the onset of aestivation, but is less expensive in terms of stored energy reserves than glycolysis. Since we do not fully understand the metabolic pathways, and since an anomalous condition in early stages of starvation has suggested itself in the present investigation, the possibility of responses to low oxygen tensions being different before and during aestivation cannot be ruled out.

Whatever the aestivation site, water loss will initially be minimal; this allows for a slow acclimation to the decrease in body water. Such slow acclimation has been shown to be necessary, and finds support in the work of Klekowski (1959, 1961 quoted in Hyman, 1967), who showed that *Planorbis planorbis* L which had been kept in brackish water prior to aestivation were better able to survive desiccation than those which had not been so treated. The total dissolved solids are bound to rise with the evaporation of water from a pool, thus the chances of successful aestivation would be increased by this acclimation.

During prolonged aestivation water loss would seem to be

the factor most likely to be limiting. At a conservative estimate, based on the utilization of carbohydrate reserves from the alimentary system of a *B. (Physopsis) africanus* with a tissue dry weight of 25 mg ("Standard Snail") this reserve should last 17 weeks of aestivation; in practice this figure will be considerably higher, since the rate of oxygen consumption, and therefore presumably the metabolic rate, continues to fall after three weeks. Metabolic reserves can therefore last for a considerable period of time. Not so the animals water store; even at the rate of water loss incurred at a moderate saturation deficit of 0,4 mm mercury, all body water will have been lost in 20 weeks, and death will have occurred long before. The snails may be subjected to considerable water loss, particularly if they are aestivating on the surface, and this, as has been shown in the present investigation, leads to a marked reduction in the blood volume. Apart from the physiological implication of the actual water loss, this will affect the hydrostatics of the snail. Brown (1964) has shown that extrusion of the foot in *Bullia spp* requires blood to be pumped into the cephalopedal sinus. A drastic reduction in blood volume could, therefore, impair, if not completely preclude, locomotion during aestivation once water had been lost. This is, of course, contrary to the findings presented here, where it has been shown that *B. (Physopsis) africanus* after several weeks of aestivation on the surface and under exposed conditions, can, and do move when placed on wet sand. This, like prolonged survival under conditions favouring water loss, can only be explained by the possibility of at least partial rehydration. Unlike the Helicidae, *B. (Physopsis) africanus* does not cover the foot with the mantle during retraction into the shell. The foot of the aestivating snail is exposed in the shell, and is covered by a mucus veil. The mantle is stretched along the columella, extending to the truncation. It has been noticed that snails placed in water after a period of aestivation often trap a bubble of air in the shell which prevents the foot from coming into contact with the water. Similarly, the volume of water which could be placed in the side arm of the Warburg reaction flasks in the experiments investigating the termination of aestivation

(see Section 10) was insufficient to come into direct contact with the foot, yet the aestivation was broken and the snails became active in all instances. It is tentatively suggested that the extended mantle tissue is the site of water uptake, for it was, in all instances, in contact with the water. Snails placed on wet sand could, if the mantle was in contact with the interstitial water, absorb sufficient moisture to permit activity, and such a mechanism would also permit periodic rehydration from such transient water sources as light rains, which do occur during winter, dew and even water which has evaporated from the snail and which condenses in the shell.

Predation is yet another factor with which the aestivating snails have to contend. Little is known about this aspect of the biology of aestivation. Shiff (1964c) has observed ants attacking exposed aestivating snails, and claims that only those individuals which are able to burrow into the mud escape this predation. Yet, I have found specimens of *B. (Physopsis) africanus* which had been aestivating on the surface for several weeks, apparently untouched. Predation would, of course, vary from locality to locality.

With the filling of the habitat, processes are reversed. The first water to settle in the pool will rehydrate the snails in the deepest part of the pool, and these will break aestivation. The response to anaerobic conditions reported by Coles (1969b) may well act in concert with increased body water content to stimulate the snails to activity. Those individuals aestivating at higher levels will be able to rehydrate to some extent, by absorbing water from the substratum, but could possibly remain dormant as a form of contingency insurance against possible changes in climatic conditions. These will be recruited into the population as the water level rises. Water initially settling in the habitat is likely to be high in dissolved solids compared with the pool when full, thus reverse acclimation is possible. The physiological shock shown by the Shongweni snails on being transferred to a medium of lower dissolved solid content suggests that this is necessary. I have not investigated survival of *B. (Physopsis) africanus* after aestivation has

been broken in standard aquarium water, since in most instances dry weights of the snails needed to be determined immediately.

Mozley (1954) has pointed out the importance of rapid reproduction to the recolonization of the habitat after a period of drying. Such recolonization may be in competition with other species. While aestivation does bring about a reduction in metabolic reserves, it has been shown that the carbohydrate store of the reproductive system is carefully husbanded. This reserve, which will almost certainly prove to be galactogen, enables reproduction to commence almost as soon as secretory activity of the reproductive system is fully restored and without the need for building up reserves for the eggs. Many *B. (Physopsis) africanus* starving under laboratory conditions were found to produce viable eggs which further supports the suggestion that the reproductive organs are kept functional even at the expense of the adult snail. The advantages of this emphasis on reproduction to the population are, of course, tremendous, in such an unstable habitat. A study of the feeding habits of the young snails might well prove their food requirements to be different from those of the adults and it will doubtless prove smaller,thus the young may survive where the adults can not. It does seem surprising that a considerable loss in all reserves is incurred from the reproductive organs. This aspect needs to be studied histologically and histochemically, as it is most likely to be connected with a cessation of secretory activity during aestivation. My *subjective* impression is that there is a reduction in the prostate gland and *bursa copulatrix*, but this needs confirmation.

Copulation appears to be necessary for egg production following aestivation. Ten snails which had been taken from an actively breeding population and induced to aestivate for a period of 28 days, produced no eggs during a 30 day period after the termination of aestivation, in which they were kept individually isolated. On placing the snails in aquaria in pairs, viable eggs were produced after seven days. Shiff (1964c) has shown that breeding commences in the field almost immediately after aestivation has been terminated, with eggs already present in the habitat five days after filling. From

the above preliminary observation it seems that these eggs were not fertilized by sperm stored over the aestivation period, suggesting that the reproductive system is re-activated almost immediately aestivation is broken.

The results of the present study fit in well with the expected field conditions, but detailed field data on a population of *B. (Physopsis) africanus* is lacking. There is urgent need for a study on this species, along the lines of that of Shiff (1964c) on *B. (Physopsis) globosus*, considering however, not only the population ecology of the snails, but also the microclimates, and particularly the biotic factors, which affect the snails in both their active and their very vulnerable aestivating state.

AESTIVATION AS A FORM OF RESISTANCE ADAPTATION

There can be little doubt that aestivation has much in common with other forms of adaptive dormancy. Hibernation, diapause and quiescence all serve to tide the animals concerned over periods of environmental adversity in much the same way as does aestivation. Hibernation and obligatory diapause (Lees, 1955) are seasonal phenomena, while quiescence (Lees, 1955) and aestivation are not, being solely dependent on environmental stimuli for their manifestation. Precht (1958) in discussing temperature adaptation in poikilotherms distinguishes between "genotypic" and "phenotypic" adaptations. In such a classification, hibernation and obligatory diapause would certainly fall into the former category, while aestivation and quiescence would fall in the latter; facultative daipause would possibly bridge the two. Does this, however, mean that the physiological bases of all these adaptive strategies are different? In the absence of detailed biochemical studies, it is impossible to say with certainty, but we do know that basic to all is a depression in the metabolic rate as reflected in the rate of oxygen consumption. To my knowledge, nothing is known about the triggering of the seasonal preparation for hibernation in *H. pomatia*; this may well lie in sign stimuli such as day length or temperature change, and hibernation with its characteristic metabolic change may simply follow through an internal triggering mechanism

once the pre-hibernation storage of metabolic reserves has been completed. Aestivation may well invoke the same metabolic mechanism, but, unlike hibernation, the response would now be directly to the stimulus of unfavourable conditions, possibly through mediation of a different internal control. Thus while "genotypic" and "phenotypic" *may* describe the conditions governing the inactivity and depressed metabolism associated with the two states, their basic mechanism, *i.e.* their ability to adjust, must be genetically controlled in both instances. May not the basic physiological mechanism, therefore, also be common to the two states? Since Natural Selection acts on what an animal has and does, contingencies which the animals have to face will govern the use to which the basic mechanism is put.

The survival value of aestivation to *B. (Physopsis) africanus* is clear, but its origins need to be sought in a more wide-spread phenomenon. Bullock (1955) draws attention to the almost universal occurrence among poikilotherms of adjustments to temperature changes, both seasonal and transient. These adjustments, the best known of which is temperature acclimation, allow eurythermic species to "maintain a certain level of metabolism (and other characters measured as rates) in spite of changes in ambient temperature". Such adjustments may be "genotypic" as in various physiological races of the crab *Uca pugilator* (Vernberg & Costlow, 1966) or "phenotypic" as in numerous examples quoted in Precht (1958). Temperature acclimation is a form of resistance adaptation to temperature extremes (Precht, 1958; Prosser, 1958; Prosser & Brown, 1961); aestivation in *B. (Physopsis) africanus* has the same end result, and can therefore be regarded as similar. The characteristics of temperature acclimation to elevated temperatures involve a metabolic adjustment, as does aestivation. Prosser (1958) has proposed a scheme for the classification of metabolic temperature responses in poikilotherms, based on the rate-temperature curve. Basically he recognises two types of adjustment; *translation* of the curve (Acclimation Type II), *i.e.* a change in the position of the curve without a change in slope, implies altered enzyme activity, while *rotation* of

the curve (Acclimation Type III), *i.e.* a change in the slope of the curve, indicates a change in activation energy, and, by inference, a change in metabolic pathway. Acclimations Types I and IV respectively denote no acclimation and a combination of types II and III. Aestivation in *B. (Physopsis) africanus* would conform to a Type III acclimation in Prosser's scheme. Acclimation does, of course, require that the animal concerned is kept at a higher (or lower) temperature than normal, and that through adjusting its metabolism to the new temperature by one of the above means, it is able to tolerate greater extremes. Aestivation in *B. (Physopsis) africanus* shows the same characteristics, but without the stimulus of a period of exposure to a higher temperature, since the depression of the metabolic rate which is characteristic of aestivation can be achieved at a temperature similar to that obtaining in the aquaria housing active snails.

Temperature acclimation and the increased temperature tolerance which is conferred on *B. (Physopsis) africanus* by aestivation are thus very similar. The underlying physiological mechanism appears to be the same, but the mechanism which initiates the two states is different. Prosser (1955) has reviewed physiological variation in animals, and quotes numerous examples where selection has favoured the formation of physiological races within species, depending on the demands of the environment. The terrestrial environment would certainly demand rapid adjustment to temperature change, and it seems likely, therefore, that selection for a shortening of the acclimation period would have acted very strongly on the terrestrial ancestors of the Planorbidae. Genetic assimilation (Waddington, 1953) has been shown to be capable of rapidly assimilating a "phenotypic" character into the genotype, thus temperature induced changes in the metabolic process could become geared to more rapid change and to an alternative triggering mechanism. Selection would, of course, also act to extend the range of animals to meet the demands of the environment.

Temperature acclimation *per se* has not been studied in *B. (Physopsis) africanus*, and the similarity between the effects of temperature acclimation in other poikilotherms

and aestivation in these snails cannot be regarded as valid reason for precluding temperature acclimation from contributing to their survival of high temperatures during aestivation. It is very likely that raised temperatures over a period preceding the drying up of the habitat may facilitate the changeover to the aestivation pathway once aestivation becomes imperative. Temperature acclimation in relation to both the extension of the zone of tolerance in active *B. (Physopsis) africanus* and survival during aestivation urgently requires study. If, indeed, as appears possible from the results presented here, the two states share at least parts of an underlying mechanism, it is likely that they will reinforce one another.

But it seems aestivation requires, for its successful initiation, the water loss resulting from exposure to a subsaturated atmosphere. Precht (1958) draws attention to the fact that partial dehydration in many plants, and an increase in the internal osmotic pressure of some insect larvae confer an increased resistance to both high and low temperatures. The relationship between water content and resistance adaptation to temperature is not, therefore, peculiar to aestivation. This, too, lends some support to the suggestion that aestivation constitutes a highly specialised form of temperature acclimation.

Water loss can, of course, in its own right prove lethal to the aestivating *B. (Physopsis) africanus*. Indeed, from the results of the present investigation it appears to be the environmental factor most likely to cause the death of the aestivating snail. Cells require to maintain a certain minimal water content for effective functioning. The state of water within cells is not a simple solvent/solute relationship (Wyllie, 1965; Dainty, 1965) and the water retaining properties of cells against an osmotic gradient is, as yet, by no means fully understood (Robinson, 1965). The role of acclimation to reduced body water content has, however, been shown to be linked with an increase in the free intracellular amino acid content (Lockwood, 1967), and the adjustment of the intracellular osmotic pressure may therefore also be partially instrumental in the survival of high temperatures by aestivating *B. (Physopsis) africanus*. Thus the two factors most likely to prove lethal

to the aestivating snail could possibly be countered by mechanisms which are functionally interlinked.

It is apparent from the results obtained in the present investigation that survival of the inevitable water loss which accompanies aestivation is, in itself, dependent upon acclimation, and it seems likely that this acclimation period is necessary for the increase in intracellular free amino acid. There is, however, a limit to this form of control and prevention of excessive water loss seems essential. Two striking features of the effects of desiccation are the drastic reduction in blood volume and the decrease in the rate of water loss with time. Neither phenomenon has been investigated further, but both deserve comment. A shift of water from the intercellular to the intracellular compartment has been reported in some invertebrates, a striking example being the American desert cockroach *Arenivaga*, which on desiccation for periods longer than four days, will move nearly the whole of the water contained in the haemocoel to the intracellular compartment (Edney, 1968). This shift takes place suddenly, apparently in response to the state of hydration of the tissues, and well before excessive water loss has been incurred. The amount of water transferred to the tissues was well in excess of the total water loss. This raises the question of the significance of such a transfer. If such a sudden transfer occurs in *B. (Physopsis) africanus* and if it should coincide with the reduction in the rate of water loss, or with the onset of a reduction in the rate of oxygen consumption, it may well lead to a clearer understanding of either the mechanism of control of water loss, or the initiation of aestivation.

The dormancy which accompanies aestivation in *B. (Physopsis) africanus* is obligate. Active snails are subject to considerable water loss, which can only be curbed by inactivity. The shell is impermeable to water, or nearly so, and thus protects the retracted snail by restricting water loss to the area exposed in the aperture. Evaporation from an active *H. aspersa* takes place over the exposed body; mucus is constantly being secreted by the snail and water is lost from the mucus (Machin, 1964). Inactivity will reduce water loss to that part

of the body which is exposed to the subsaturated atmosphere, which, in the case of *B. (Physopsis) africanus* is part of the foot. The mucus veil, when it dries, may help to slow down the rate of water loss. Machin (1972) has shown that a state of dynamic equilibrium exists between the water content of secreted mucus and the underlying mantle tissue in retracted *Otala lactea*. The mucus is hygroscopic, and appears to overlie a region of reduced permeability. While the full implications of Machin's findings are not yet clear, they do seem to point to the imporatnce of secreted mucus in maintaining water balance, and it may be assumed that the mucus veil in *B. (Physopsis) africanus* has a similar function. *B. (Physopsis) africanus* is, by virtue of its size, very vulnerable to water loss when active out of water, thus activity must be restricted to conditions of near saturation.

There remains the question whether temperature and water loss do, in fact, themselves directly affect temperature acclimation and aestivation, or whether they consitute sign stimuli for the secretion of a mediating hormone. Precht (1958) draws attention to the fact that while an intact animal may show metabolic adjustment on temperature acclimation, cell and tissue respiration in its individual organ systems may not conform to this adjustment; this suggests a hierarchical control, logically through the medium of hormones. Little is known of the endocrine system of the Gastropoda. Lever, Jansen & de Vlieger (1961) have been able to demonstrate that the right pleural ganglion of *Lymnea stagnalis* regulates water balance in this species. To this may be added the isolation of a principle which stimulated metabolic activity from the livers of *Pila ovata*, *B. (Physopsis) nasutus* and *Biomphalaria sudanica* by Coles (1969a). These findings suggest the possibility of an endocrine control, but this possibility needs to be investigated for both acclimation and aestivation if we are to understand these processes and their possible inter-relationship.

AESTIVATION AND THE EVOLUTION OF FRESHWATER GASTROPODA

Morton, as recently as 1958, wrote "Hibernation, aestivation and tolerance of dehydration are achievements of the higher

Stylomatophora." Boltt (pers. comm.) has informed me that in 1953 aestivation in Rhodesian freshwater Mollusca other than *Bulinus (Bulinus) forskalii* (Ehrn.) had still to be confirmed. The confirmation was not too long in coming, and we know today that among the Bassomatophora aestivation is a widespread phenomenon. If, however, the evolutionary history of the Pulmonata is considered, the almost universal occurence of aestivation would not be surprising.

The origin of the Pulmonata is obscure, although there appears to be little doubt that they share a common origin with the Opisthobranchia (Morton, 1958; Hyman, 1967); certainly, the close similarities in the reproductive systems of the two sub-classes seems to point to this relationship. The families Chilinidae and Ellobiidae are considered to be among the most primitive of the Pulmonata, and these are both Bassomatophora. The Ellobiidae are considered to be at the base of pulmonate evolution, although they are preceded in the fossil record by some Stylomatophora (Morton, 1958). The separation of the Pulmonata from their prosobranch ancestors is thought to have taken place during the late Palaeozoic; the Ellobiidae appear in the fossil record as a recognisable family in Jurassic deposits.

Present-day Ellobiidae typically inhabit estuaries and salt marshes; a few species have, however, invaded the freshwater or become terrestrial. Conditions on tidal mud-flats and in salt marshes are such as to favour the evolution of pulmonary respiration, and they are also likely to subject the fauna to considerable temperature changes, periodic drying and flutuations in salinity. Such conditions call for the adaptations which would pre-adapt the animals to terrestrial conditions; both temperature acclimation and osmotic regulation at the cellular or organismic level would be required, and, since the habitat may be subject to periodic drying, escape from extreme desiccation would also have a selective advantage. Among the Stylomatophora selection has been for greater terrestrial specialization, yet the basic adaptations acquired in the ancestral habitat have held the key to their success. If, as seems likely, aestivation is but a highly specialized form of acclimation,

and hibernation is a specialised form of aestivation, geared to weathering winter cold which is seasonal, the success of the Stylomatophora on land, which is surpassed among the invertebrate phyla only by the Arthropoda, is easy to understand. The spread of the order would now depend on the selection pressures exerted on the snails by the environment. *Helix aspera*, as an example, is a species which has been introduced into South Africa from Europe, and has successfully invaded most of the country. In spite of its probably being confined to gardens, it must nevertheless experience extremes of desiccation and temperature never encountered in Europe, and its success can largely be attributed to its ability to aestivate. The same holds for such desert snails as *Sphincterochila boisseri*, whose survival in a very harsh environment must be attributable to its ability to aestivate.

Wells (1944) has shown that *H. pomatia* undergoes a regular hydration and dehydration cycle, in which regular changes in weight and water content occur. The snails hydrate themselves during a period of feeding and dehydration occurs during a period of inactivity which follows. Aestivation is induced during the dehydration phase, reactivation of the snails only following on favourable conditions, and such reactivation is caused not by rehydration, but by the mechanical stimulation of rain falling on the shell. Rehydration then follows reactivation. *Sphincterochila boisseri* retains a possible remnant of the activity cycle shown by *H. pomatia* in that the inactive snail shows what appear to be "bursts" of increased oxygen uptake, but without the activity which would normally be associated with it. Thus the snails appear to be in a state of almost constant aestivation. The Limacidae have adapted in another direction; these slugs are able to rehydrate from water vapour (Dainton, 1954) in spite of having a high rate of water loss when active under subsaturated conditions. Such ability has enabled them to dispense with the protection afforded by the shell, and this will, in all probability, apply to other families of pulmonate slugs as well. The slugs are, however, confined to relatively moist environments, although they can incur considerable water loss without succumbing. Success of the slugs, does, however,

lie in inactivity (aestivation?) when conditions are unfavourable.

The Bassomatophora are thought to be secondarily aquatic. They have, at least in some families, developed a secondary gill for aquatic respiration which could suggest that at an earlier stage in their evolution they led a more terrestrial existence. This may, of course, have been in the ancestral habitat. The nature of the habitat of the Bassomatophora is, however, such that a retention of some of the terrestrial adaptations of their ancestors would be favoured by selection, and aestivation is such an adaptation; the nature of aestivation in terrestrial and aquatic pulmonate snails is the same, only the details differ. Dehydration is still the trigger and rehydration, possibly reinforced by anaerobiosis has become the terminating agent. Yet, Coles (1969b) has shown that aestivating *B. (Physopsis) nasutus* respond to mechanical stimulation by an increase in the rate of oxygen uptake. I have been able to confirm this for a small sample of five *B. (Physopsis) africanus*. Thus the response elicited by mechanical stimulation in *H. pomatia* may well also be manifest in the Planorbidae, but here it requires reinforcing by hydration to achieve termination of aestivation. The differences between aestivation in the Bassomatophora and Stylomatophora thus simply reflect the different demands of their respective environments.

The Prosobranchia have parallelled the Pulmonata in their invasion of the freshwater and terrestrial environment. The presence of the operculum will have conferred at least some advantage on these snails in curbing water loss, and the fact that they are represented by a smaller number of terrestrial species would, it seems to me, simply reflect a shorter evolutionary history in that environment. Aestivation has been shown to occur at least in the Ampulariidae among the Prosobranchia (Meenakshi, 1964; Coles, 1968, 1969a), and it seems likely that the selection pressures with which the Pulmonata have had to contend, will have been met by terrestrial and freshwater Prosobranchia in much the same way. Even the carbohydrate reserve in the reproductive system of the freshwater Prosobranchia is similar to that of the Pulmonata

in that it is a form of galactogen, but its detailed structure is markedly different from pulmonate galactogen (McMahon, von Brand and Nolan, 1957).

RESISTANCE ADAPTATION, CAPACITY ADAPTATION AND THE DISTRIBUTION OF SOUTH AFRICAN FRESHWATER SNAILS.

Resistance adaptation determines the range of physical conditions which an animal can survive, and, depending on the total selection pressures which act on a population, can easily lead to capacity adaptation, *i.e.* the ability of the population to maintain itself through reproduction under a set of physical conditions where previously only tolerance was possible. The difference between the distribution ranges of *B. (Physopsis) globosus and B. (Physopsis) africanus* would seem to indicate differences in capacity adaptation since *B. (Physopsis) africanus* must be capable of carrying out all its life processes over a wider range of conditions than *B. (Physopsis) globosus*. Such capacity adaptation will reflect itself in the intrinsic rate of natural increase under the various conditions, and studies comparable with those of Shiff (1964a, 1964b, 1964c) on *B. (Physopsis) globosus* are urgently needed for *B. (Physopsis) africanus*. Such investigations need to be carried out in conjunction with more extensive studies on the physiological adaptations of both species. Only in this way will the factors governing the distribution of these two important species be established. Other freshwater snails cannot, of course, be neglected; apart from their economic importance, physiological studies on such species as *Biomphalaria pfeifferi, Bulinus (Bulinus) tropicus* and *Lymnea natalensis* could well, through their different life habits, bring us closer to an understanding of some of the fundamental factors which govern freshwater gastropod distribution.

Further physiological studies on freshwater snails will also doubtless have implications in the epidemiology and control of the various trematode parasites to which they are host. Van Eeden (1966) has drawn attention to the fact that not all strains of host snail are equally susceptible to *Schistosoma* infection and that the distribution of bilharziasis

is more restricted than the range of the host snails. He suggests that this may be accounted for by the snails being "in a process of adaptation to changing conditions" and I would suggest that such adaptation is physiological. The balance between parasite and host is a delicate one; for survival of the parasite, the host must survive, yet the parasite must draw on the reserves of the host. I have been able to measure the rate of oxygen consumption of a single infected *B. (Physopsis) africanus*, which was 2,1 µl O_2/mg dry weight/h. It was unable to depress its rate of oxygen uptake below 1,31 µl/mg dry weight/h even after four days out of water. At this stage it died. Coles (pers. comm.) claims that *B. (Physopsis) nasutus* can survive aestivation with a sporocyst in the foot, but not if it is located in the liver. Since the incidence of sporocysts in the foot is uncommon relative to the liver, aestivation may well serve to cull many infected snails from temporary waters.

It, is however, the balance between the active snail and the parasite which is important. Snails adapting to changes in environmental conditions, and which more frequently have to resort to resistance adaptation than their capacity adapted counterparts, may well prove less capable of accomodating parasites such as schistosomes and therefore succumb as a result of the infection. If this is true for aestivation then it may well prove equally true for temperature acclimation. The need for resistance adaptation will, however, sooner or later give over to capacity adaptation, and this will ultimately result in a further spread of the parasite.

Thus fundamental research on the physiology of the freshwater molluscan fauna is not an academic luxury, but an urgent need. Rational control can only be carried out in the context of sound ecological investigation, and this includes the study adaptation. Concerted co-operative effort by epidemiologists, systematists, ecologists and physiologists are required to fianlly solve the problems of molluscan ecology and the spread of trematode parasites. The inadequacy of a single-pronged attack on the problem is only too evident in this thesis.

Peccavi! Caret initio et fine!

13. SUMMARY

Freshwater snails are said to go into aestivation when their habitat dries up and they are exposed to the full rigours of temperature and desiccation obtaining in the terrestrial environment. The present investigation seeks to define the aestivation process more closely in the aquatic planorbid snail *Bulinus (Physopsis) africanus*.

1. A review of the present state of our knowledge of aestivation in the Gastropoda has shown that while much is known about the survival value of aestivation in the pulmonate Gastropoda, studies on the physiological basis of the aestivation process have been few. Only a single comprehensive study on the nature of the aestivation process in an aquatic pulmonate species has been undertaken prior to the present investigation.

2. The methods used in the present investigation involved well established techniques. These were evaluated and, where necessary, modified for use on molluscan material.

3. The specimens of *B. (Physopsis) africanus* used in the investigation originated from three localities, the cushion basin of the Vernon Hooper Dam at Shongweni, the Mpolweni River at Krantzkloof and the Bilharziasis Research Unit at Nelspruit.

4. All snails were kept under standard laboratory culture conditions and were subjected to standard pretreatment prior to use in an experiment. All field snails were inspected for the presence of *Chaetogaster sp.* and for *Schistosoma* infection before being admitted to laboratory cultures. Where physiological shock on transfer from the natural habitat to laboratory culture conditions was found to be a risk, the snails were acclimated to laboratory culture conditions in stages.

5. Activity before and during aestivation was investigated. *B. (Physopsis) africanus* was found to leave the water when the water temperature was high. Activity was found to continue after the snails had left the water, provided

that the substratum was wet; activity diminished with the drying out of the substratum. *B. (Physopsis) africanus* was also found to become active during the course of aestivation if placed on a wet substratum, thus activity was possible during the period of aestivation.

6. Both starving and aestivating *B. (Physopsis) africanus* showed a marked depression in their rate of oxygen consumption, with aestivating snails depressing the rate of oxygen uptake further than starving animals. Not all snails removed from water were found to depress their rate of oxygen consumption to the aestivating level, therefore not all snails found alive out of water are necessarily in a state of aestivation. Snails which were effectively aestivating could tolerate a wide temperature range and their rate of oxygen uptake was found to have a different temperature coefficient from that of fed or starving individuals.
7. Water loss was found to be, at least in part, responsible for the onset of aestivation in *B. (Physopsis) africanus*, while temperature fluctuations had no measurable effect on the initiation of the process. Desiccation was found to result in increased mortality in aestivating snails if it was rapid; a slow rate of water loss increased the absolute water loss which the snails could survive. Rehydration was found to terminate aestivation. Desiccation had the effect of drastically reducing the blood volume of the snails, and this was replenished on rehydration.
8. Aestivating and starving *B. (Physopsis) africanus* were found to metabolise largely protein, although carbohydrates and lipids were also utilized in appreciable amounts. All metabolic reserves were conserved more effectively by aestivating snails than by starving snails. Both starving and aestivating snails were found to conserve an appreciable fraction of the carbohydrate content of the reproductive system; this probably consists largely, if not entirely, of

galactogen which is known to be an energy source in the eggs of gastropod molluscs. The utilization of metabolic reserves broadly follows changes in the rates of oxygen consumption in both starving and aestivating *B. (Physopsis) africanus*, although there is a suggestion that only partial oxidation of some substances may take place during the early stages of starvation.

9. Aestivation in *B. (Physopsis) africanus* has been redefined in terms of the above findings, and discussed in relation to other forms of resistance adaptation, with which it shares considerable common ground. It is also considered in the context of other Mollusca and of the environment in which the snails are found. Suggestions are made for future research on the aestivation phenomenon, and related physiological adaptation which affect the survival and spread of the freshwater Mollusca.

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