STUDIES ON THE RESPIRATORY AND HYDROSTATIC FUNCTIONS OF THE MANTLE CAVITY IN TWO FRESHWATER PULMONATE SNAILS

T H E S I S

for the

Degree of Doctor of Philosophy

in the

University of Glasgow

by

Andrew E. Henderson, B.Sc. (Edin.)

April, 1961.

TABLE OF CONTENTS

I. INTRODUCTION

Having reverted to an aquatic habitat, the pulmonate gastropods of the "higher" Basommatophora have a mantle cavity without ctenidia, and clearly modified for aerial respiration. Early descriptions of these animals assign to this cavity the function of a "lung", whose gas content is periodically renewed by the snails during their visits to the surface of the water. As noted by Roszkowski (1914), Lymnaeids with water-filled mantle cavities live continuously submerged in the depths of Lake Geneva, and this first threw doubt on the universal application of such a simple explanation to all the limnic Basommatophora. Subsequent work by Precht (1939) showed that aerial respiration is abandoned by Lymnaea stagnalis at water temperatures below 5°C. Hunter (1953a & b) reports populations of Lymnaea peregra and Physa fontinalis living in both deep and shallow water in Loch Lomond with water-filled cavities, and also populations apparently living continuously submerged but retaining a gas bubble in the mantle cavity. All stocks, from no matter what habitat, could take up surface-breathing immediately after disturbance of even a minor kind (Hunter, 1953b).

It is clear from this that the respiratory function

of the mantle cavity gas bubble is auxiliary to the cutaneous respiration, which may be responsible for the major part of the total respiratory exchange. Thus, it seems possible that in regular diving the mantle cavity gas bubble's function as a "lung" is a less important factor in the snail's behaviour than its other potential uses. Among the other possible functions are those of the gas acting as a physical gill (as occurs in certain aquatic insects (Ege, 1918)), or as a hydrostatic support operating by the reduction of the specific gravity of the whole or part of the animal while it is submerged. The latter function is inevitable if the volume of gas in the mantle cavity is large enough.

The work described in this thesis has been aimed at investigating whether the hydrostatic or the respiratory function of the mantle cavity gas bubble dominates in the behaviour, under laboratory conditions, of two species of pulmonate snails, Planorbarius corneus (L.) and Lymnaea stagnalis (L.); and, more specifically, which function is concerned in the stimulus which drives these animals to the water surface.

The original work can be conveniently divided into six main parts (Sections III to VIII of this thesis). First, a survey of the gas volumes in the mantle cavities

is described. This is followed by a section dealing with the distribution of weight among the component parts of the snail body, and thirdly, by an account of observations made on the weights of the snails under water (V). The next parts describe chemical analysis of the gases in the mantle cavity (VI), and recordings of the pressure conditions within it in the living animal (VII). Finally, Section VIII deals with long-term recordings made of the snails' visits to the surface, and variations produced by alteration of the overlying atmosphere. All these results are then discussed in relation to the observations of other workers on the distribution and behaviour of pulmonate snails.

II. MATERIALS AND METHODS

All the snails used in these studies were adult specimens maintained in the laboratory in large tanks where they were fed on lettuce. They were supplied by L. Haig, Newdigate, and were not used experimentally until they had been in the stock tank for at least one week. Nor were they ever used more than three months after their arrival in the laboratory. Fresh batches appeared to come from the same, or a very similar, stock, being closely similar in the relations of shell to tissue weight, shell length or diameter to width, and in range of total weight. In the text, references to shell size refer to the maximum length of the shell for L. stagnalis and to its maximum diameter for P. corneus measured from the extreme anterior edge of the shell in both. The Planorbarius corneus were all var. rubra (Oldham), i.e. possessed dissolved haemoglobin as a blood pigment.

To avoid harmful effects produced by Glasgow tap water, water for the experiments was brought into the laboratory from Loch Lomond, stored in carboys, and aerated thoroughly before each experiment. The gas analyses were carried out in the Scholander micro-gas-analysis apparatus (Scholander, 1947) as supplied by W.G. Flaig Ltd. Other apparatus and methods developed are described before each set of experimental data.

III. VOLUMES OF GAS IN THE MANTLE CAVITIES

a) Methods

Strong stimulation of the foot of any of the fresh-water pulmonates by chemical or mechanical means will always result in the partial or complete withdrawal of the animal into its shell. This is usually accompanied by the discharge of a large part of the gas contained in the mantle cavity (Jordan, 1922), P. corneus being noticeably more sensitive in this respect than any of the Lymnaeids examined. Unfortunately, this sort of treatment leads to the discharge of large amounts of mucus on the surface of the foot, and into the mantle cavity to a considerable extent. The mucus tends to trap the remaining gas in the cavity in the form of minute bubbles, making accurate assessment of the total volume of gas originally present, difficult.

However, a number of measurements were made by trapping all the released gas bubbles in a funnel blown on a piece of fine-bore capillary tubing. By means of an 'Agla' micrometer syringe attached rigidly to the other end, all the component bubbles were measured by moving them successively past a zero line scribed on the capillary part of the apparatus, each one being sucked up into the body of the syringe after measurement. The syringe

is calibrated in 0.0002 c.c., and measurement of the bubbles to the nearest cu.mm is straightforward, subject only to the error introduced by the curvature of the ends of the bubbles. This error is cumulative in any one sample, but is minimised by using 0.1 mm internal bore capillary tubing. Temperature effects were largely eliminated by carrying out the measurements in a constant temperature room at 10°C with appropriate precautions against local warming of the capillary. Each sample was allowed to equilibrate for fifteen minutes at this temperature after collection from the snail. During this time it was in contact with air-saturated water with possibly widely different gas tensions, and changes in volume over the fifteen minute period of up to 3% were observed. Changes of this magnitude were felt to be unimportant, and were therefore disregarded. The reading was only recorded if constant when repeated five minutes later. All the volumes quoted here are corrected to the water temperature.

The animals used in these measurements had all been kept for at least three weeks in the same large tank at a temperature ranging from 16 to 19°C. In it, they fed on lettuce floating on the surface, and sampling of their bubbles was carried out directly from this environment. For this reason, mechanical stimulation with a glass rod

was used in preference to any chemical methods which would have contaminated the tank. Immediately after an undisturbed surfacing, each snail was deflated under water by holding its shell with one hand and probing the ventral surface of its foot with a blunt glass rod. The escaping gas bubbles were allowed to float up and accumulate in the belled capillary. Each snail was then inspected, still under water, in the beam from a high-intensity microscope lamp, when in most, any remaining large volumes of gas showed up as dark masses. Smaller volumes (< 30 cu.mm) could escape this inspection, and without dissection of the snails it is impossible to be sure of extracting all the gas. Where repeated samplings on the same snail are required, this is obviously not feasible. Each snail, after the first sampling, was labelled in a pre-determined colour coding with small spots of cellulose paint on its shell. Only results where the snail seemed to be completely deflated are recorded here.

b) Results

After the first few such measurements, it was apparent that individuals of approximately the same size could, even when sampled immediately after surfacing, and in water of the same temperature, contain very different volumes of gas (see figs 1-3). This was true for both species

investigated, implying either that the size of the mantle cavity bears no relation to the size of the animal (as measured by a shell dimension), or that it is on occasion not fully inflated.

The effect of deflation is to produce an "over-inflation" of the mantle cavity at the next surfacing. This was shown by making repeated use of an individual, when the increased level of gas uptake was almost exactly maintained (Table 1). This suggests that under these conditions the snails respond by inflating the cavity to near its maximum extent.

Table 1

Volumes of gas extracted after successive surfacings (cu.mm)

L. stagnalis (shell length = 31.2 mm)	342	558	543	560	549
P. corneus (shell diameter = 19.3 mm)	289	516	496	504	

The usual degree of inflation was always found to be considerably below the maximum possible. The wide range of volumes collected from any particular size of animal can easily be accounted for by different degrees

of inflation of the mantle cavity, without postulating any structural variation in the individual size of the mantle cavity.

In a few cases, it was possible to carry out a series of samplings at weekly intervals on the same individuals (Table 2). These were kept at a constant temperature (17°C) in a constantly aerated tank, and were fed on lettuce. Sampling was again done directly from this environment.

Table 2

Volumes of gas in individual snails on different occasions

	Shell size (mm)	Volumes of gas (cu.mm)
L. stagnalis	43.2	842 - 710
	41.0	622 - 512
	35.5	425 - 317 - 186
	30.5	355 - 231 - 164
	25.5	205 - 153 - 135
P. corneus	31.5	514 - 372 - 368
	25.0	391 - 323 - 256
	21.3	345 - 311 - 241
	16.8	297 - 203 - 186

The largest volume yielded by each snail is given first. The overlap between sizes of snails is clear. The results are not in the order in which they were obtained. The

effect produced by deflation is lost during each week's interval in these experiments. Few snails survive deflation, most dying within two or three days, presumably from damage sustained during the process. Since all of them died with air in their mantle cavities, they are not suffering from inability to refill the cavity from the deflated condition. The likely cause is damage to the thin-walled sinuses of the cavity when they receive blood under pressure from the haemocoel of the foot during extreme contraction. In L. stagnalis the colourless nature of the blood makes it difficult to detect in the presence of mucus. A few P. corneus were seen to discharge small amounts of blood from the mantle cavity immediately before refilling it with air. All these animals died within two days.

These results show the great individual variation which can occur in the degree of filling of the mantle cavity. Therefore direct measurements of gas volume as above are of limited significance.

IV. WEIGHTS OF SHELL, VISCERAL MASS, AND FOOT

a) Introduction and method

In considering hydrostatic functions, two possible biological advantages to the snails can be assessed. The hydrostatic function of the mantle cavity gas might be to provide buoyancy for the whole snail, enabling it to float to the surface simply by letting go of the substrate. Such behaviour occurs in the laboratory in L. stagnalis and P. corneus only as a result of "accidental" detachment from the substrate. Other authors (references below, p 108) have observed several species carrying out this manoeuvre, but only Physa fontinalis does so with any regularity in the present writer's experience. On the other hand, a smaller volume of gas could provide buoyancy for the shell and visceral mass alone, as distinct from the head and foot which are in contact with the substrate.

Measurements were made of the weight of the visceral mass and shell together, and of each snail's total weight. While this was being done, the shell weight alone was also recorded. Each living snail was carefully dried with filter paper and weighed. As soon as the snail re-emerged from the shell, that part of it ventral to the female reproductive opening was cut off. This leaves about half the columella muscle attached to the shell in both species.

The detached shell and visceral mass were weighed, together with the blood escaping from them which had been trapped in filter paper. Each shell was then carefully dissected off, dried, and weighed.

b) Results

The results for the two species are shown in Tables 3 and 4. L. stagnalis is seen to have a smaller proportion (64%) of its weight outwith the foot than P. corneus (74%). L. stagnalis has, too, a much lighter shell (22%) in relation to its total weight than P. corneus (45%). Müller (1943) gives a figure for L. stagnalis which agrees with this. On the other hand, means calculated from Füsser and Krüger's (1948) results are both much lower: 13.45% for L. stagnalis and 22.27% for P. corneus. Wide infraspecific variation in shell weight is possible. Experiments with Helix (Oldham, 1934) showed that in one of two groups a higher calcium diet could produce a four-and-a-half times heavier shell after one year. There is no reason to suppose that such variation is not possible in Basommatophora. However, all results show that in L. stagnalis the shell contributes a smaller proportion to the total weight than it does in P. corneus. Further, in L. stagnalis the proportion of the total weight represented by the shell and the visceral mass together, is generally less than that proportion in P. corneus.

Table 3

Weight relations of parts of body

Lymnaea stagnalis

Total weight (g)	Shell + visceral mass as %age of total	Shell weight as %age of total
3.66	63.7% (2.33)	25.7% (0.94)
2.05	75.6% (1.55)	19.5% (0.40)
4.08	58.6% (2.39)	24.3% (0.99)
1.42	69.0% (0.98)	25.4% (0.36)
3.46	69.7% (2.41)	19.7% (0.68)
0.61	59.0% (0.36)	23.0% (0.14)
0.79	63.3% (0.50)	21.5% (0.17)
0.88	69.3% (0.61)	19.3% (0.17)
1.45	66.9% (0.97)	17.9% (0.26)
1.22	68.9% (0.84)	18.9% (0.23)
0.43	65.1% (0.28)	20.9% (0.09)
0.40	52.5% (0.21)	22.5% (0.09)
0.34	64.7% (0.22)	23.5% (0.08)
0.13	56.0% (0.07)	15.4% (0.02)
0.28	64.3% (0.18)	21.4% (0.06)
0.38	55.3% (0.21)	26.3% (0.10)
0.35	65.7% (0.23)	22.9% (0.08)
0.20	70.0% (0.14)	20.0% (0.04)
0.19	68.4% (0.13)	26.3% (0.05)
0.40	65.0% (0.26)	24.7% (0.10)
	Av. = 64.55%	Av. = 21.97%

(Figures in brackets are actual weights in grams)

Table 4

Weight relations of parts of body

Planorbarius corneus

Total weight (g)	Shell + visceral mass as %age of total	Shell weight as %age of total
1.64	68.2% (1.12)	37.8% (0.62)
1.80	71.6% (1.29)	46.1% (0.83)
0.72	83.3% (0.60)	51.3% (0.37)
1.79	75.6% (1.36)	44.7% (0.80)
2.11	67.7% (1.43)	38.3% (0.81)
1.13	82.3% (0.93)	53.0% (0.60)
1.71	72.5% (1.24)	50.9% (0.87)
2.72	81.6% (2.22)	64.3% (1.75)
2.22	83.3% (1.85)	45.0% (1.00)
2.73	63.1% (1.72)	27.8% (0.76)
0.90	74.4% (0.67)	34.4% (0.31)
3.25	75.4% (2.45)	51.4% (1.67)
3.21	76.0% (2.44)	44.2% (1.42)
0.22	72.3% (0.17)	54.5% (0.12)
2.42	77.3% (1.87)	49.2% (1.19)
	Av. = 74.97%	Av. = 46.39%

(Figures in brackets are actual weights in grams)

V. WEIGHT OF THE SNAILS UNDER WATER

a) Introduction

In accordance with the Principle of Archimedes, the weight of the snails while submerged will be reacted against by a force equal to the mass of water they displace. Even in the absence of any gas in the mantle cavity, this can result in a weight reduction of at least 90% in the weights of the two species so examined. When a bubble of air is enclosed in the mantle cavity, the upthrust force will be increased by 1 g/c.c. of gas. In terms more suitable to the present studies, where the volumes of gas are never more than 1 c.c., each cu.mm of gas enclosed will inevitably result in an upthrust on the submerged animal of 1 milligram. The mass of the gas can be disregarded, being less than 0.2% of the mass of the water displaced. The change in volume caused by the removal of the gas from under some five centimetres of water with a consequent decrease in pressure, is too small to be significant. Normally the gas in the mantle cavity is also subjected to a positive pressure by the snail itself (see below) but again this is not large enough to make any appreciable difference between the volume of gas when it is in the mantle cavity and when it is measured at atmospheric pressure.

Alterations in the snails' weights under water, if recorded in milligrams, will thus express variations in the enclosed bubble volumes to the nearest cubic millimetre with an accuracy of ± 0.2%. An accurate picture results of volume changes occurring inside the mantle cavity, whether these are due to active discharge of part of the contents or as the result of some physiological activity on the part of the snails.

b) Determination of the gas volumes by displacement

Initially, this principle was used as the basis of a more rapid and accurate method than direct measurement of the volumes of gas in the mantle cavity.

1) Method

The animals used came from an aquarium tank where they had lived and fed for a month prior to the experiments, and where the temperature ranged from 16 to 19°C. All the experiments were carried out during a seven week period in May and June, each snail being isolated for at least four hours previously in a vessel of fresh loch water maintained in an aerated condition and at a steady 17°C. In this way the snails experience as nearly as possible uniform pre-treatment, and it was therefore hoped that they might respond in a constant way.

A thin metal-foil platform immersed in the experimental vessel was suspended by a fine wire from the arm of a torsion balance of 1 g total capacity. Since the platform weighs about 200 mg under water, the combined weight of it with an adherent snail is sufficient to allow measurement of the snail's underwater weight whether buoyant or not. The snail, within a five minute period of its latest surfacing and without outside interference, moved on to this platform as it rested on the bottom of the vessel. Then the platform was lifted gently by raising the torsion balance on an adjustable stand until it was clear enough of the bottom to allow free weighing.

After deflation, using mechanical stimulation and checking visually as before, the snail was replaced on the platform and re-weighed. During the process of deflation, a large amount of mucus was discharged, but this was found to be of insignificant mass under water. The difference between the two weights is an accurate measurement of the volume of gas originally present, and as a difference, is unaffected by the temperature and diffusional changes during direct measurement of volume.

The present technique was devised independently, but Jacobs (1941) had previously recorded similar measurements of the underwater weights of snails moving on a suspended

vertical sheet. However, Jacobs was not concerned with assessing gas volumes, and briefly studied the effects of loading the shells with small weights.

2) Results

The results of a series of measurements made in this way are shown graphically in figures 1-3, in which bubble volume in cu.mm is plotted against sizes of shell. Results for L. stagnalis are shown in figure 1, for P. corneus in figure 2, and for comparison, from Lymnaea palustris in figure 3. Duplicate samples from individual snails are joined in the figures by vertical broken lines.

As with the direct measurement of volume, the results show very wide variations in the size of bubble contained by each size of snail, and by the same snail on different occasions. While it is impossible to be sure of extracting the total volume of gas present, these variations seem much greater than could be produced by any retention of gas. This was checked by deflating ten animals of each species in the same way as in the volumetric experiments, and then dissecting open their mantle cavities. In three L. stagnalis and five P. corneus, no visible gas bubbles were left, and the greatest volume of residual gas found in any was 12 cu.mm. This cannot account for the wide variation in results obtained, which may therefore be regarded as real differences in the volumes of gas present.

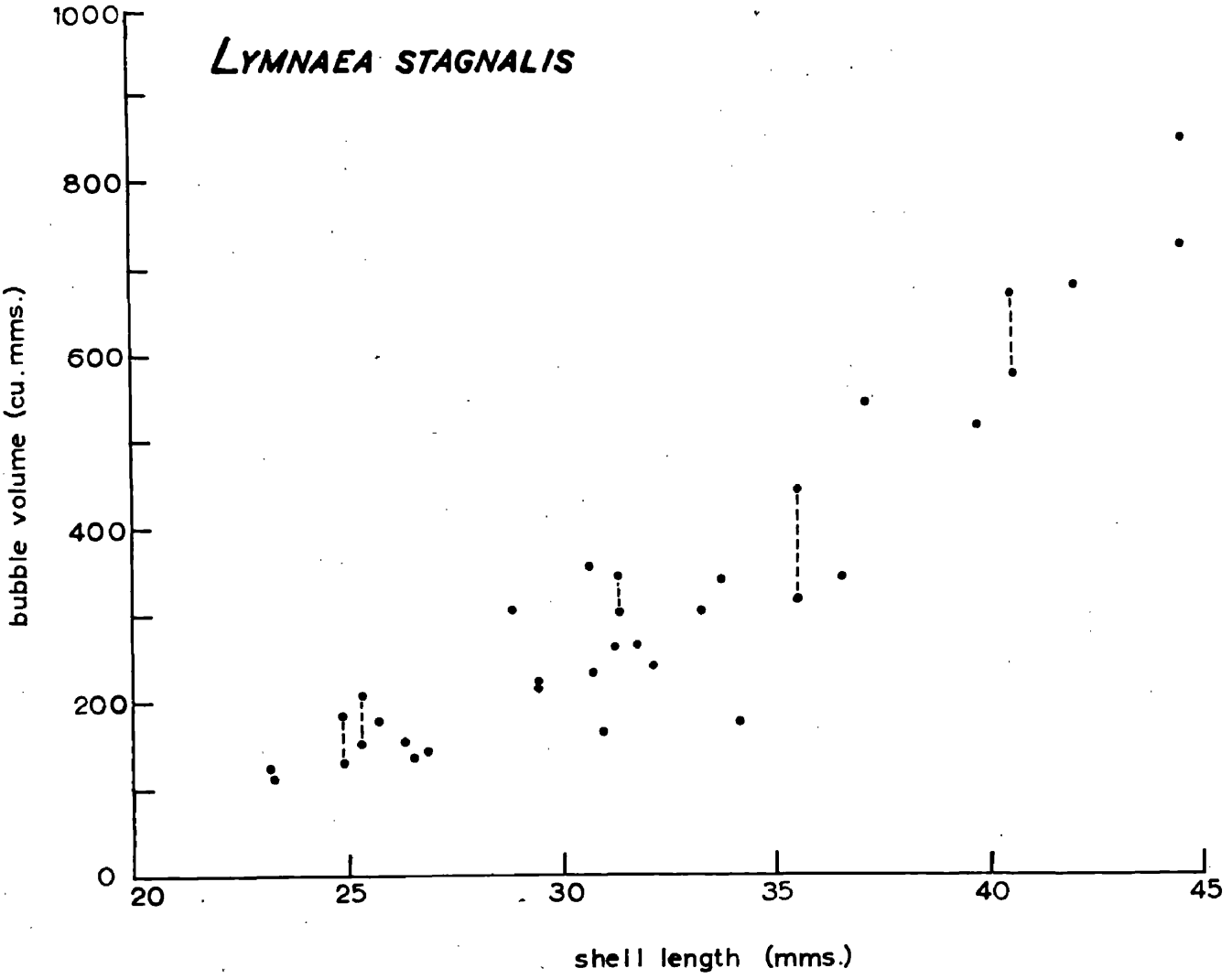


Figure 1. Volumes of gas (in cu.mm) extracted from L. stagnalis plotted against shell length (in mm); duplicate records from individual snails joined by vertical broken lines.

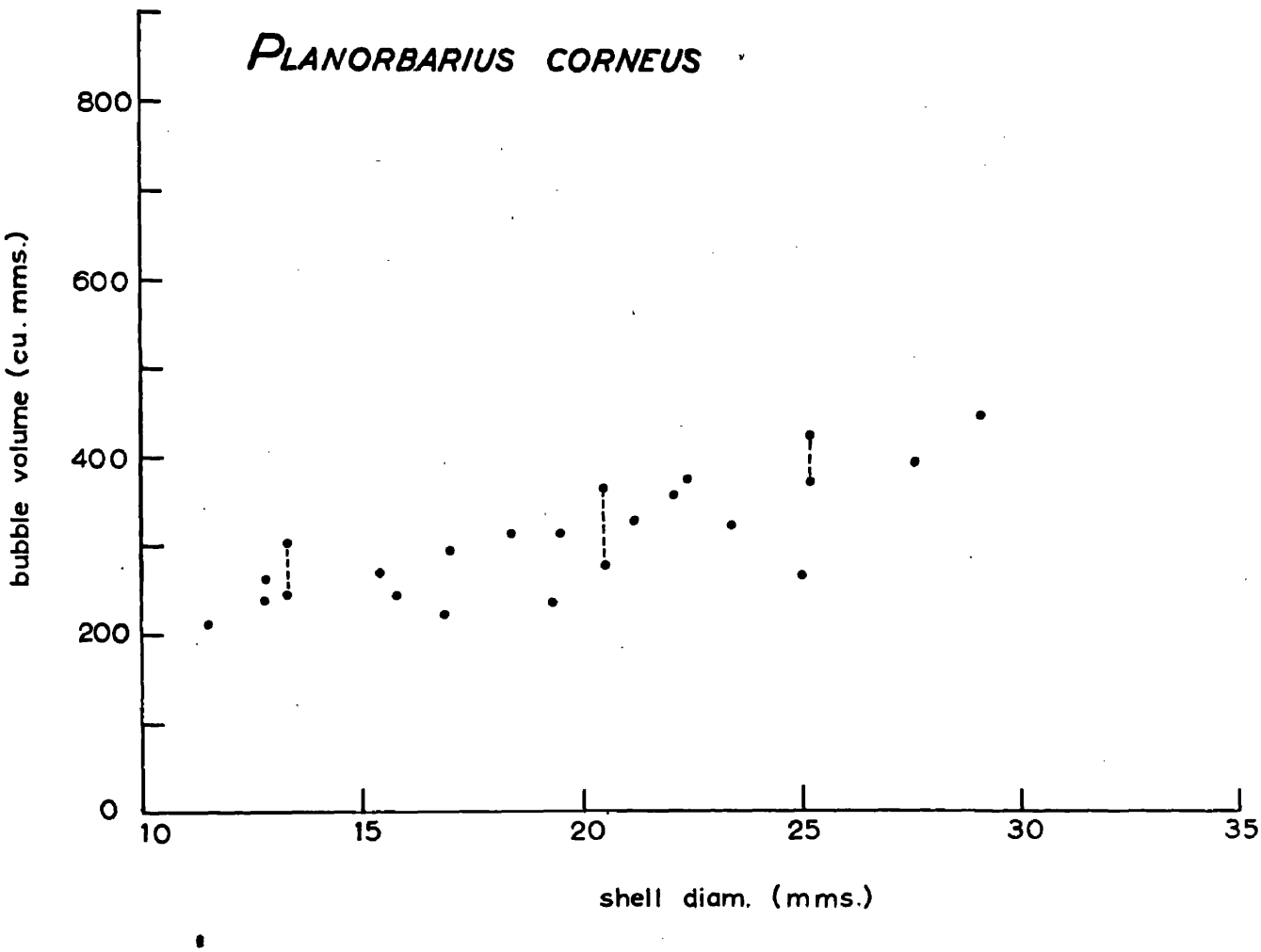


Figure 2. Volumes of gas (in cu.mm) extracted from P. corneus plotted against shell diameter (in mm); duplicate records from individual snails joined by vertical broken lines.

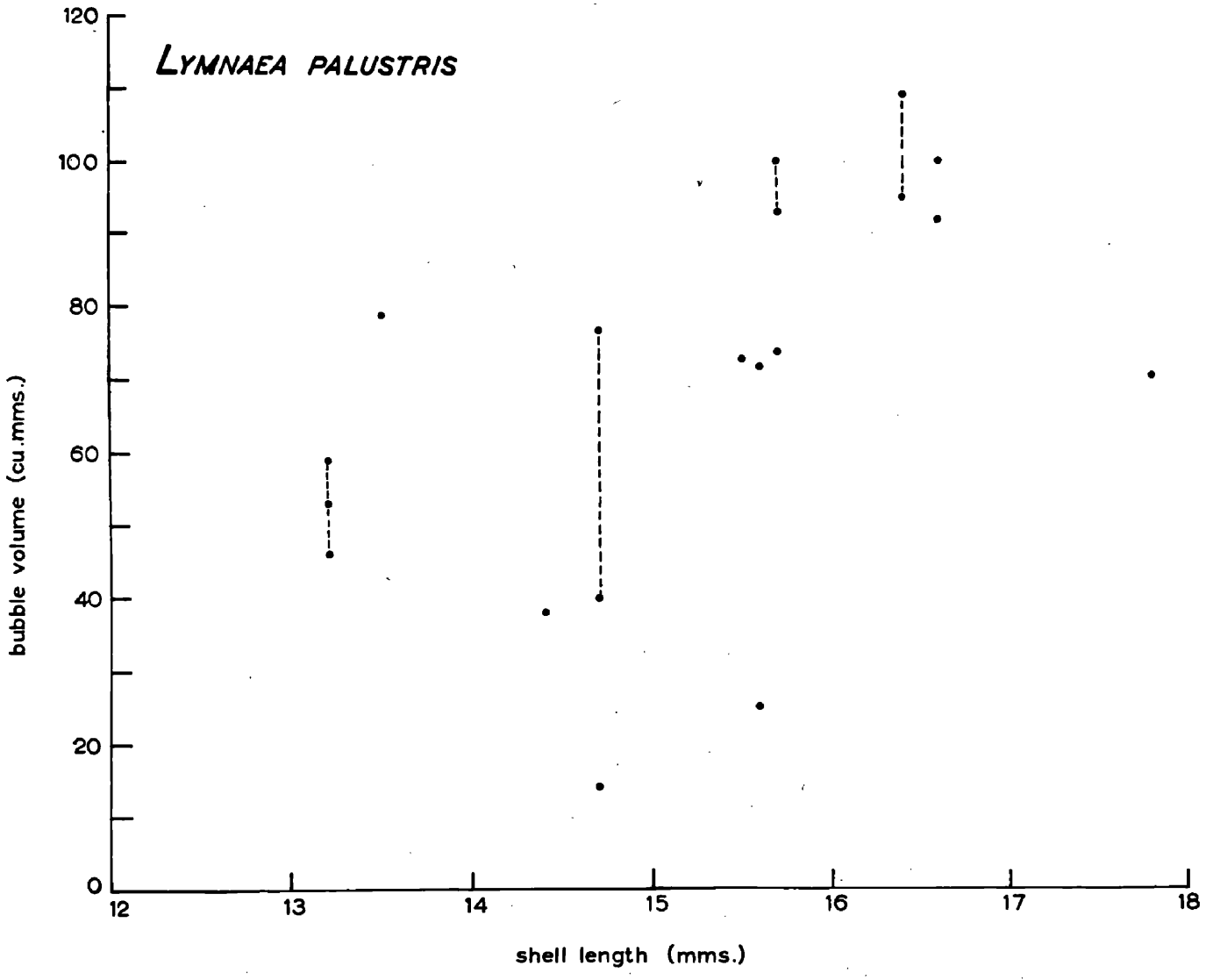


Figure 3. Volumes of gas (in cu.mm) extracted from L. palustris plotted against shell length (in mm); duplicate records from individual snails joined by vertical broken lines.

In general, the larger individuals can contain more gas than the small ones, but do not necessarily do so. A few snails sampled for the amount of gas uptake soon after deflation yielded again the increased volume characteristic of this condition. It might be possible to relate this increased volume to some shell dimension since it represents an approach to a maximum value for each individual. However, the degree of stimulation necessary to produce this can only rarely occur in nature.

These figures confirm the impression gained from the results of direct measurement of volume that the snails normally exist with only a proportion of the potential volume of the mantle cavity actually occupied by gas. The degree of filling, even in water at a constant temperature and with uniform pre-treatment of the animals, varies widely, and apparently without relation to any of the environmental factors under control.

In spite of this "under-filling" of the cavities, all the L. stagnalis examined at this temperature (17°C) were considerably buoyant, their adhesion to the weighing platform lightening the latter by between 40 and 100 mg (mean from 34 snails = -58 mg) (the minus sign indicates "negative weight" of the snail, i.e. its degree of buoyancy). Most of the P. corneus too were buoyant, but

only to a maximum of -45 mg, while a few (5 out of 23) were heavier than water to a maximum extent of 10 mg (mean from 23 snails = -31 mg). The specimens of L. palustris examined showed a range of conditions from a buoyancy of -32 mg to a positive weight of 11 mg (mean from 19 snails = -11 mg).

By taking in 10 to 12% more gas, any of those snails heavier than water could have made themselves buoyant. An increase of at least 20% is always achieved by snails of any of these three species when they have been subjected to deflation. Thus failure to achieve buoyancy in the case of a few animals was not due to an inability to hold enough gas. The degree to which the cavity was filled with air at the surface could be affected by the amount of gas already present, or by changes in this volume and hence in the snails' buoyancies during the previous period of submersion. In order to explore this aspect of the problem, a method was needed which allowed serial weighings at regular intervals over an extended period.

c) Serial weighing after surfacing into air

1) Method

Using the technique just described, or that of Jacobs (1941), weighing at regular intervals over a long period

is impossible because of the snails' tendency to move off the weighing platform. Any interference, such as lifting the snails back on to the platform, will lead them to attempt to reach the surface at the first opportunity, and may even cause them to discharge part of the bubble from the mantle cavity. Such disturbance of their behaviour is undesirable in any experiments concerned with long-term natural processes.

A system was therefore sought where the snails could be weighed at convenient intervals, could be allowed or denied access to the surface, and would be in contact with a volume of water large enough to be reasonably stable with regard to temperature and aeration. The arrangement arrived at is shown diagrammatically in figure 4.

The snail is enclosed in a light, perforated container, large enough to allow the animal to move about freely on the inside. In conjunction with the torsion balance to which it is attached by a length of polythene thread, the container can be raised or lowered through the water in which it is immersed. After trying a variety of containers of suitable mass under water, the best proved to be a type made from a commercially available, hollow PVC sphere, two-and-a-half inches in diameter externally, and with walls an eighth-of-an-inch thick (originally designed for use

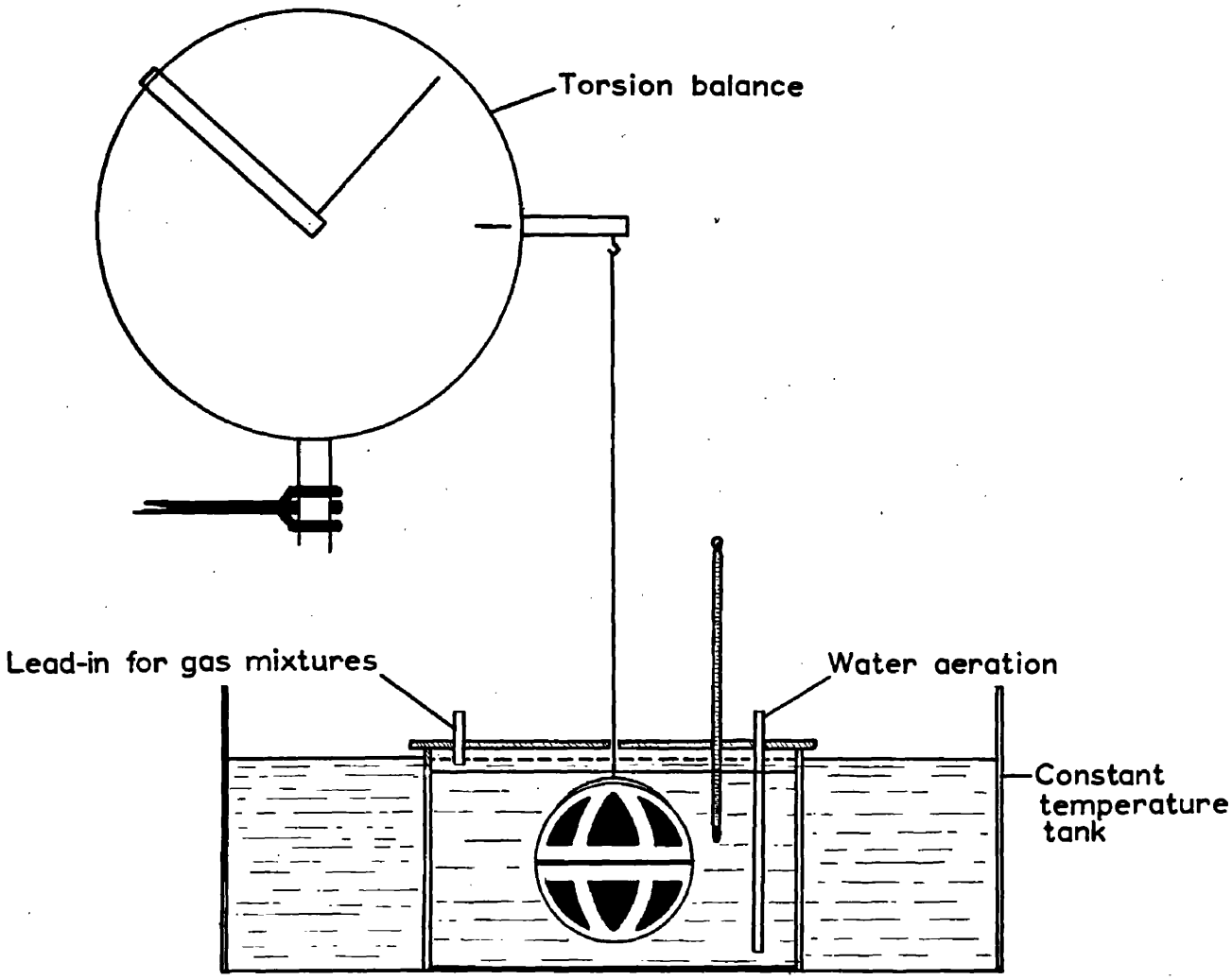


Figure 4. Diagram of apparatus for serial weighing; for further details, see text.

as a practice cricket ball). This allowed even the largest individuals of either species complete freedom of movement on the inside. Perforations in its walls, shaped to the curvature of the sphere and occupying rather more than one-third of its surface area, ensured an adequate exchange between the water inside and outside. This sphere was slit open round its equator, a snail introduced into it, and the two hemispheres clipped together again using nickel silver wire clips to hold the edges of adjacent perforations. The PVC is intrinsically buoyant, and to counteract this, small glass counterweights were attached until the submerged weights of the spheres were in the region of 400 mg. Used in conjunction with the 1000 mg torsion balance, such capsules could easily accommodate the variety of buoyancy conditions found in the two species examined.

The water in which the capsule was submerged was contained in a one-litre glass vessel fitted with a suitably drilled perspex lid. This container was itself almost completely submerged in six litres of water whose temperature was under the control of a "Techne Tempunit". This could hold the temperature of the undisturbed experimental water to within 0.5°C over a twenty-four hour period working within 2°C of room temperature. In all the following experiments, the temperature of the water in the experimental

vessel was adjusted to the temperature of the stock tank before the snails were transferred. This temperature varied according to season and time of day. Slight temperature changes ($< 1^{o}C$) were introduced by aeration with warm air. These were disregarded, but results from experiments with temperature changes $> 1^{o}C$ over the twenty-four hour period were discarded. Similarly, results from those experiments in which the behaviour of the snails was abnormal were rejected. All the experiments whose results are quoted here were carried out on healthy snails immersed in fresh, aerated loch water, renewed for each experiment. While submerged in the capsule, the snails are subject to complete starvation, but this was assumed only to be likely to influence the results of experiments lasting more than twenty-four hours.

A period of four hours was allowed before an experiment began, during which time the sphere was two-thirds submerged in the water. The overhanging internal surface of the sphere enables the snail to reach the surface with its pneumostome, and gives the snail complete freedom of movement for its shell during its surfacing movements. After the four-hour period, the sphere was lowered and the total weight of the animal and sphere measured and recorded. Raising the sphere to the surface again usually resulted in

the snail's opening its mantle cavity to the atmosphere, after which the sphere was again lowered and re-weighed. The time interval between these two weighings was never more than two minutes, and the difference in weights, joined in all the following graphs by a broken vertical line, is equivalent to the volume of gas actually inhaled by the snail during the process of surfacing. Serial weighings of the snails at convenient intervals were then carried out with the animal at a depth of five to seven centimetres under the surface. On occasion, the weights of the animals were also watched continuously over periods of two or three minutes to detect any short-term variations.

The zero weight line marked on all the graphs is the empty weight of the container under water, measured before and after each experiment. It represents the point at which the whole snail has the same specific gravity as water. In all the following figures, time is plotted on the abscissa in convenient units, and weight on the ordinate in milligrams (in 100 mg units on the reduced scale graphs). In the reduced scale diagrams the mean temperature only is given, the range being quoted in appropriate parts of the text.

2) Results

i) Changes over twenty-four hours' submersion

The first series of observations made with this

apparatus measured the underwater weights of individual L. stagnalis and P. corneus of different sizes during twenty-four hours' continuous submersion after a period of undisturbed surfacing into air. The results of thirty representative experiments are shown graphically in figures 5 and 6, the upper half of each figure being occupied by a large-scale, more detailed, version of the first in each series.

As a result of surfacing, all the snails in these experiments have made themselves buoyant at the time of submergence. The subsequent measurements show that their weights have increased with time. By observing the apparatus continuously over periods of five or ten minutes at different stages in this process, it was seen that the reduction in gas volume responsible for this increase in underwater weight is brought about smoothly. Further, there is no visible release of gas from the pneumostome, although both species were observed to bring the pallial gas into contact with the surrounding water by partial protrusion and opening of the pneumostome under water.

The rate of weight increase is initially rapid, being equivalent to a reduction in the volume of gas of between 40 and 60 cu.mm in the first hour of submersion. Thereafter, the rate falls off progressively with a marked

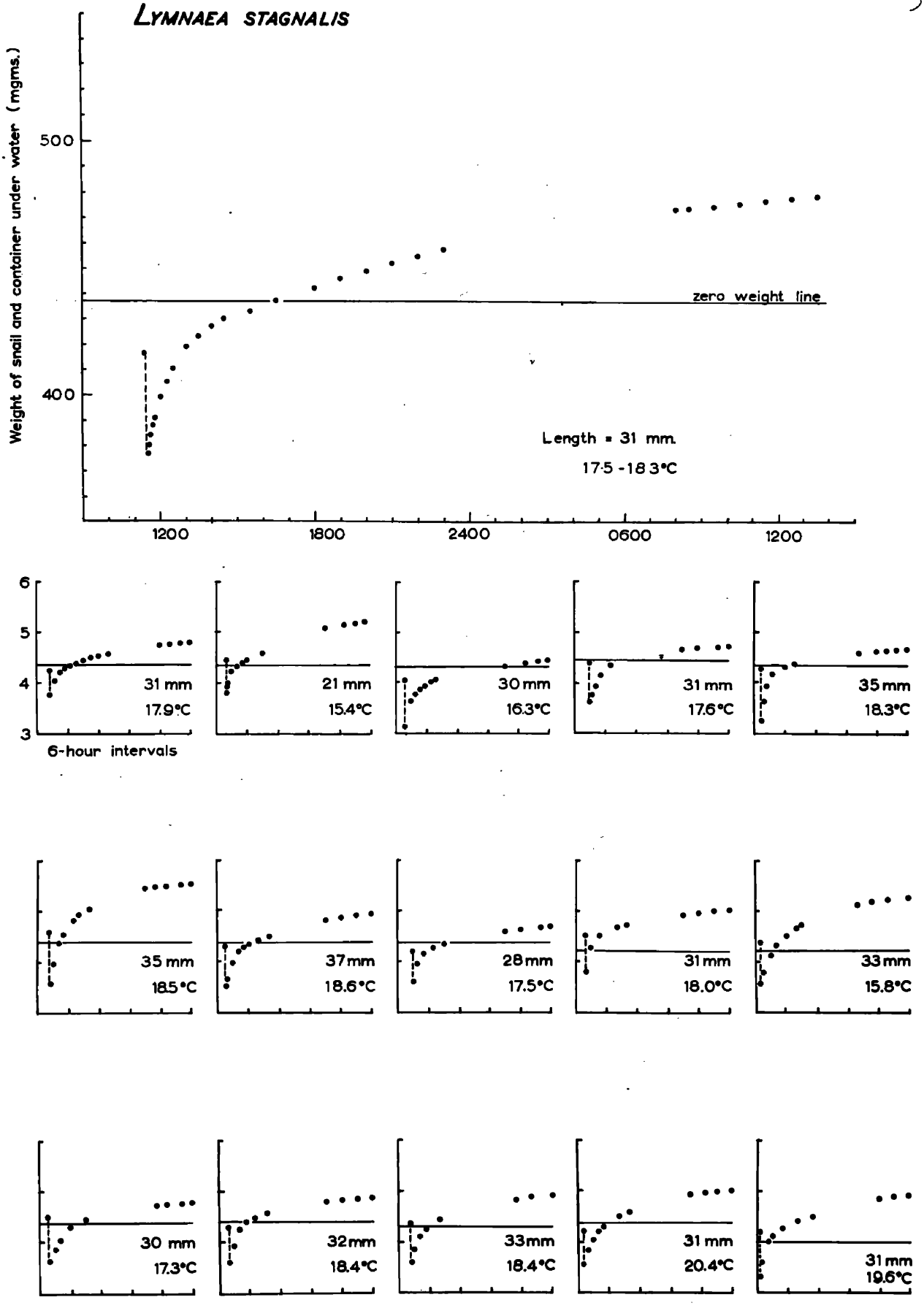


Figure 5. Underwater weights of L.stagnalis over twenty-four hours' submersion. (The first experiment of the series shown enlarged in upper part of figure.)

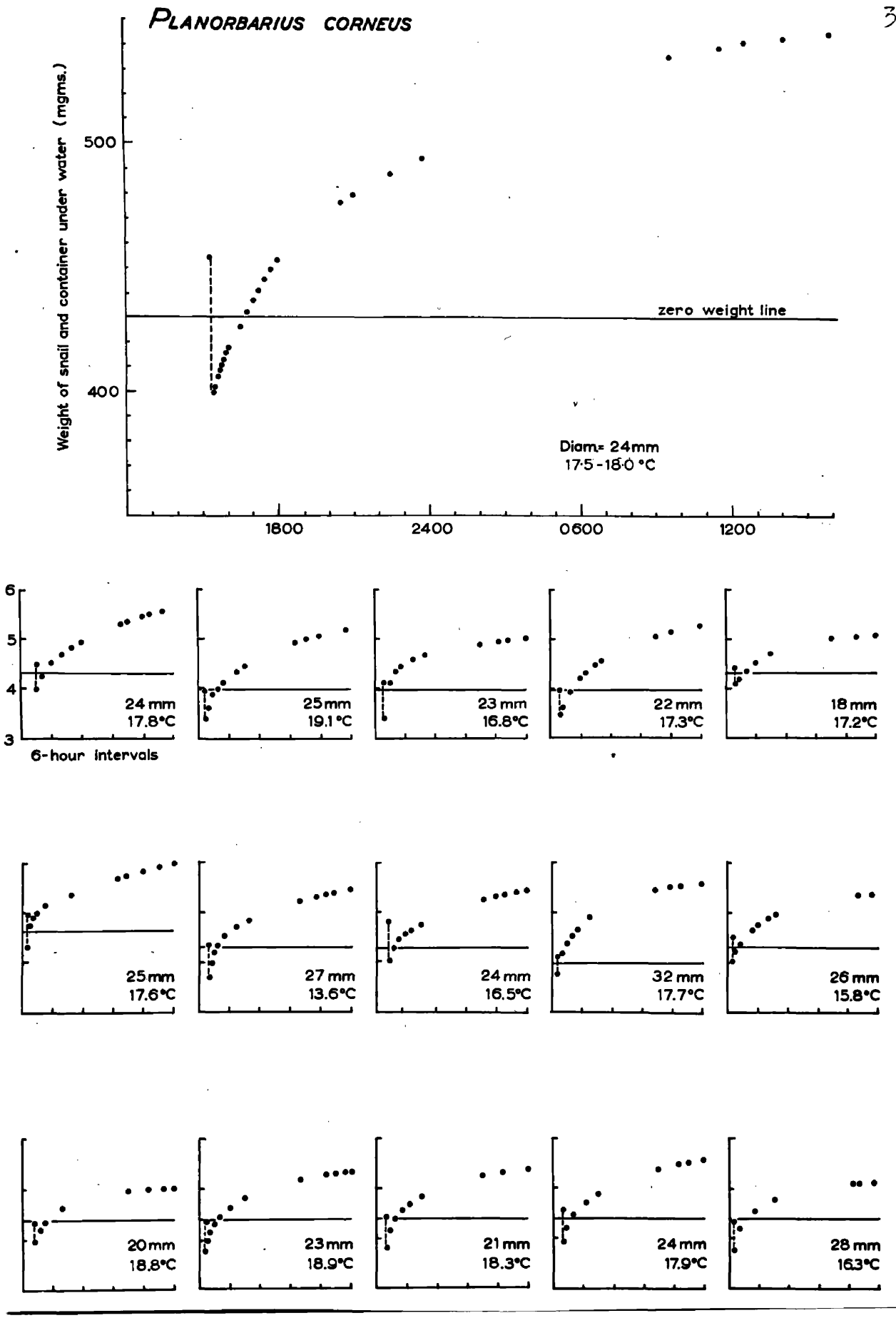


Figure 6. Underwater weights of P.corneus over twenty-four hours' submersion. (The first experiment of the series shown enlarged in upper part of figure.)

change occurring between two and four hours after submergence. The figures also show that during this period L. stagnalis may still be buoyant while P. corneus is already much heavier than water. This has proved a characteristic difference between the two species in all the weighing experiments carried out on snails with air-filled cavities.

After twelve hours without access to the air, the snails were still losing gas at between 5 and 10 cu.mm per hour, but at the end of each twenty-four hour period, this rate had fallen to between 2 and 4 cu.mm per hour. Finally, each snail was forced to give up the gas remaining in its mantle cavity. By immediately re-weighing under water, the volume of gas remaining finally could be calculated. This volume, added to the volume used up during the period of submersion, gives a measure of the original quantity of gas present at the beginning of the dive. From this calculated measure of the volume of gas present at the beginning of a dive, a volume of gas equivalent to the weight decrease at surfacing can be subtracted. This yields a figure for the original quantity of gas present before the experimental surfacing.

In Tables 5 and 6, the absolute changes in quantity of gas during the twenty-four hour period are given. The

Table 5

Volume changes before and during a 24-hour period of submersion

L. stagnalis

Size of snail (mm)	Temp. (°C)	Vol. before surfacing	Vol. taken in at surface	%age increase	Vol. at start of dive	Vol. reduction after 24 hours	%age reduction
31	17.9	240	42	17.5	282	102	36.2
21	15.4	185	68	36.8	253	140	58.9
20	16.3	154	92	59.7	246	139	56.5
31	17.6	129	78	60.5	207	121	58.5
35	18.3	228	101	44.3	329	146	44.4
35	18.5	201	100	50.0	301	197	65.4
37	18.6	335	76	22.7	411	150	36.5
28	17.5	153	62	40.5	215	105	48.8
31	18.0	205	71	34.6	276	131	47.5
33	15.8	142	79	55.6	221	172	77.2
30	17.3	121	87	71.9	208	117	56.3
32	18.4	119	70	58.8	189	137	72.5
33	18.4	186	77	41.4	263	146	55.5
31	20.4	178	73	41.0	251	150	59.8
31	19.6	201	88	43.8	289	166	57.4
				Mean = 45.3 ±14.1			Mean = 56.7 ± 11.2

Table 6

Volume changes before and during a 24-hour period of submersion

P. corneus

Size of snail (mm)	Temp. (°C)	Vol. before surfacing	Vol. taken in at surface	%age increase	Vol. at start of dive	Vol. reduction after 24 hours	%age reduction
24	17.8	263	54	20.5	317	170	53.6
25	19.1	270	62	23.0	332	178	53.6
23	16.8	230	90	39.1	320	163	50.9
22	17.3	201	55	27.5	256	176	68.8
18	17.2	207	40	19.3	247	100	40.5
25	17.6	256	73	28.5	329	172	52.3
27	13.6	275	66	24.0	341	180	52.8
24	16.5	237	78	32.1	315	148	46.9
32	17.7	363	32	8.8	395	185	58.9
26	15.8	275	52	18.9	327	137	41.6
20	18.8	241	41	17.0	282	103	36.9
23	18.9	240	63	26.3	303	155	51.2
21	18.3	175	78	44.6	253	162	64.0
24	17.9	220	76	34.5	296	168	56.8
28	16.3	330	82	40.2	412	142	54.5
				Mean = $\overline{26.9 \pm 9.5}$			Mean = $\overline{52.2 \pm 9.4}$

quantity of gas uptake is quoted first, followed by a figure representing the percentage increase in gas volume due to this intake of air at the surface. After the column giving the absolute volume reduction over the twenty-four hours, the Tables show these as percentage reductions in the volume of gas originally present. The means and standard deviations for these two percentage changes are quoted for each species in the Tables. Variations from the mean figure are large. Neither the absolute quantities of gas consumed nor the percentage changes which they represent can be correlated with the temperature or with size of snail in either species.

In spite of such wide variations, the mean percentage reductions for the two species over twenty-four hours' submersion both represent losses of a little over half of the gas originally present. On the other hand, these two species under standard conditions show markedly different percentage increases in the gas volume due to intake of air at the surface. On this evidence, L. stagnalis increases the volume of its air store to a much greater extent than P. corneus. The difference between the two mean percentage increases (18.4) is four times the standard error of the difference ($\pm$ 4.35). This shows a real difference between the two species, suggesting that under the same conditions L. stagnalis adapts itself for a dive lasting

twice as long as that of P. corneus. In L. stagnalis the volume is sufficient to last for some twenty hours' submersion, without change in subsequent surfacing behaviour. In contrast, after such a period P. corneus would have to double the percentage increase in order to restore the volume to its original value.

Differential sensitivity to handling even after four hours, leading L. stagnalis to continue to take in more air while P. corneus has returned to a normal level, cannot be excluded. If this occurs, the species difference in gas uptake may not be significant under natural conditions. Inspection of the Tables also shows that the interspecific contrast is much complicated by wide variations in the responses of individual snails.

ii) Changes over five days' submersion

A number of experiments were extended to cover five days' submersion. Working at slightly higher temperatures, the thermostatic tank varied by $< 1^{o}C$. Figures 7 and 8 show graphically the results obtained from three animals of each species when subjected to such prolonged submersion. The volume changes equivalent to the weight increases and the percentage reductions which these represent in the volume originally present are quoted in Table 7.

These six snails, in spite of being enclosed for this

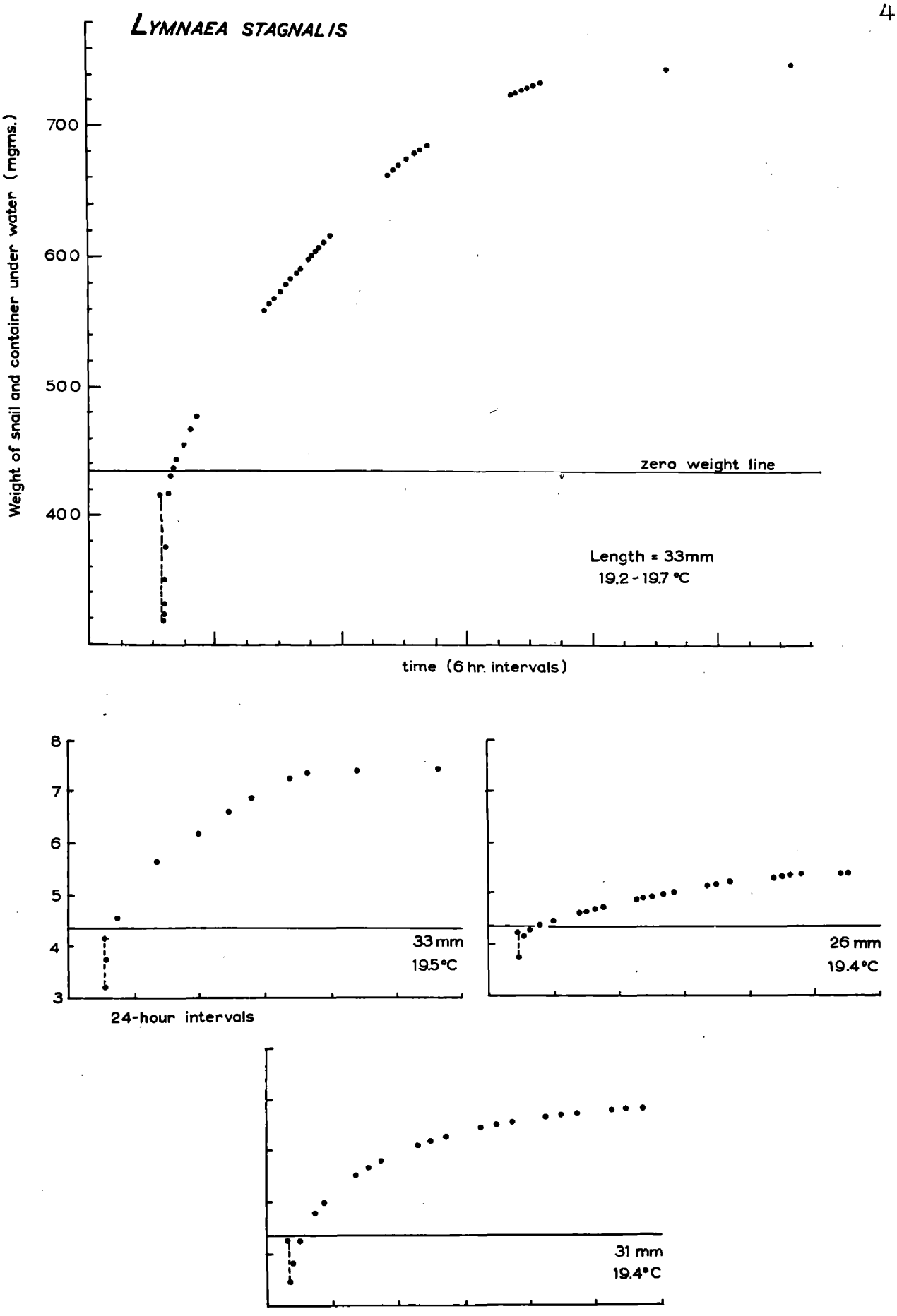
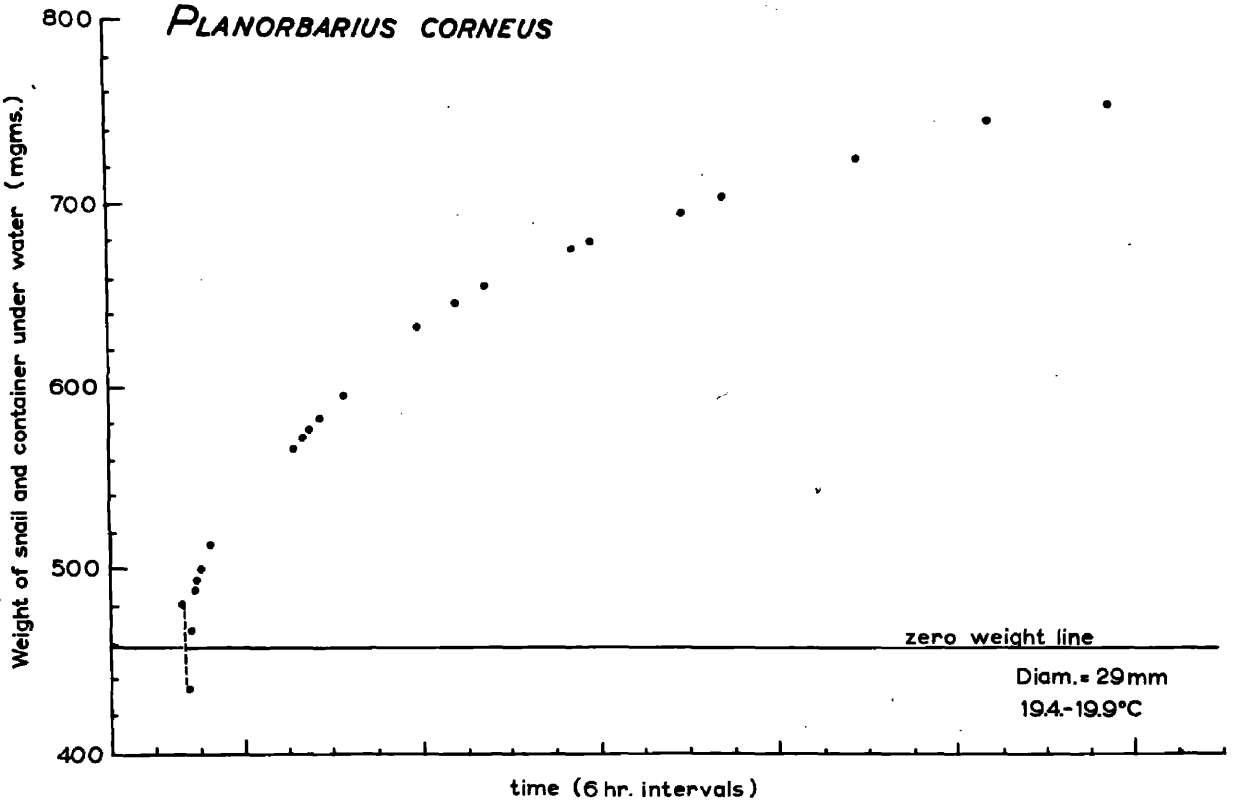


Figure 7. Underwater weights of L. stagnalis over five days' submersion.



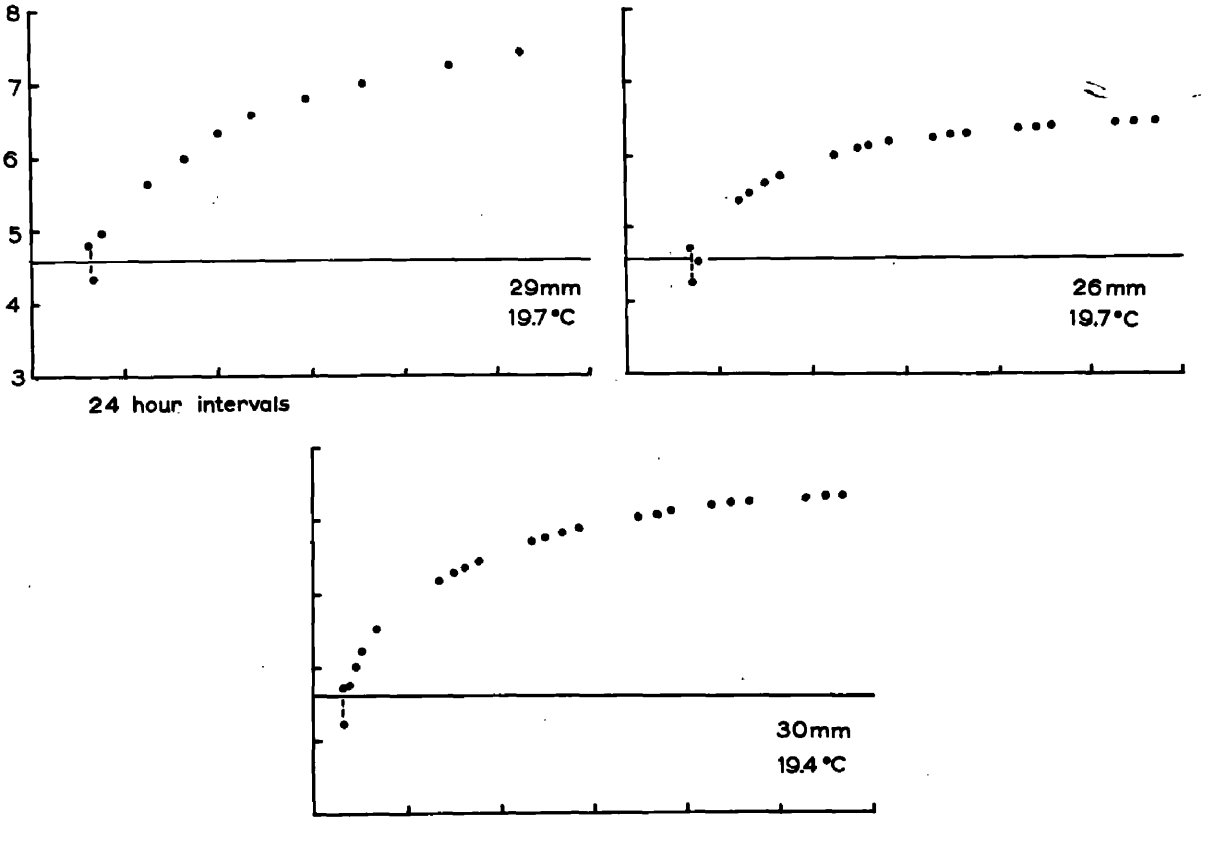


Figure 8. Underwater weights of P.corneus over five days' submersion.

period without food and unable to reach the air, were still active at the end of the experiments, moving about freely and almost continuously on the inside of the capsule. Other experiments had to be discontinued because of the adverse effects the submersion was having on the snails concerned, many of which lost adhesion and lay inactive on the bottom of the capsule. The time required to produce this effect, even under identical conditions of aeration and temperature, varied from one to four days. Smaller snails seemed as subject as larger individuals. All of these snails still had gas in their mantle cavities when they were removed from the capsule. Most recovered completely when placed in shallow aerated water.

After the first twenty-four hours in the six experiments the snails had consumed some 50% of their pallial gas. At the end of the five-day period, each snail was removed from the capsule, its mantle cavity dissected, and the contents examined. It was found that only about 10% of the original gas volume remained, but that none of the snails had admitted water into the cavity. This absence of water was also noted in twenty snails whose records were interrupted within one to four days of submergence. There is no evidence in the graphs of the snails' being able to adjust their buoyancies, and it is unlikely that the gas volume is maintained at reduced pressure. (See

Table 7

Gas volume changes during 5 days' submersion

Lymnaea stagnalis

Length of shell (mm)	Temp. (°C)	Reduction over first 24 hours	%age reduction	Reduction over 5 days	%age reduction
33	19.5	239 cu.mm	53.4	436 cu.mm	97.3
26	19.4	77 cu.mm	46.7	158 cu.mm	93.8
31	19.4	220 cu.mm	58.9	335 cu.mm	89.5
		Av. =	53.0%	Av. =	93.5%

Planorbarius corneus

Diameter of shell (mm)	Temp. (°C)	Reduction over first 24 hours	%age reduction	Reduction over 5 days	%age reduction
29	19.7	137 cu.mm	41.2	322 cu.mm	94.1
26	19.7	140 cu.mm	53.6	215 cu.mm	82.0
30	19.4	184 cu.mm	58.7	283 cu.mm	89.8
		Av. =	51.2%	Av. =	91.9%

also Section VII below.) Therefore the decrease in gas volume must be accommodated by the partial apposition or contraction of the cavity walls.

The rate of consumption of the gas at the end of the five-day period had fallen to a very low value, amounting to 2, 2, and 3 cu.mm per hour for individual L. stagnalis, and 4, 6, and 18 cu.mm per hour for P. corneus.

This length of submersion at the temperatures indicated and at a depth of five to seven centimetres does not seem to influence the snails of either species to convert to a purely aquatic type of respiration with a water-filled cavity. The major part of the respiratory exchange in the later stages must therefore be carried out cutaneously with little involvement of the mantle cavity. Freshwater pulmonates can clearly survive much longer periods of submersion. There is abundant field evidence for prolonged submersion in the smaller species such as L. peregra (see Hunter, 1953b and references therein). In the laboratory, the larger L. stagnalis appressa has been maintained for several generations without access to air (Noland and Reichel, 1943; Noland and Carriker, 1946).

The graphs show a simple extension of the changes already noted during the twenty-four hour periods. The weight increase is again smooth and devoid, even in the

later stages, of any sudden shifts which might indicate respiratory distress. In these experiments, and in all those performed on snails with initially air-filled cavities, there has never been any reversal of the weight trend. There is thus no evidence to suggest maintenance of the gas volume by secretion into the mantle cavity in either species.

Over the same periods, P. corneus and L. stagnalis show closely similar percentage changes. Thus there appears to be no difference in the utilisation of the air-store by the former species with haemoglobin, and the latter without. However, on the fifth day, P. corneus shows slightly faster weight increase, and this could reflect oxygen utilisation possible only with haemoglobin.

iii) Rates of change over four hours' submersion

To assess the hydrostatic significance of the mantle cavity contents, it is worth attempting to predict the rate at which any snail reaches the point at which it has the same specific gravity as water. The rates of weight change over this period so far reported vary widely and so hapharzadly that any such prediction is impossible. The graphs of figures 5 to 8 show that the greatest changes in rate of weight increase occur in the early stages, especially during the first four hours of

submersion. During this period both the whole snail and that part of it enclosed in the shell have become heavier than water. Thus it is at this time that any hydrostatic stimulus might be expected to exert its influence.

A number of recordings were therefore made over the four-hour period immediately following a surfacing into air, some being repeated on individual snails. The results of these are shown in Tables 8 and 9; the snails yielding repeated results are grouped at the top of each Table. Such replicate experiments were carried out at different temperatures where possible. When they were made, an interval for recovery of at least one week was allowed to each snail. Those results marked with an asterisk in Tables 8 and 9 (L. stagnalis 1a, 1b, 10 and 42; P. corneus 2a, 4a and 5b) are from the early stages of experiments which were extended over longer periods (see Table 10). As in the earlier experiments, a number of recordings were discontinued because of abnormal behaviour of the snails. All the figures quoted here represent weight changes in healthy snails moving almost continuously and adhering to the inside of the capsule.

Averaged rates of weight increase over four successive hours are remarkably similar for the two species even over the size and temperature ranges shown (bottom of Tables 8

Table 8

Lymnaea stagnalis					Weight increase in 4 successive hours			
Date	Wt. (g)	Length (mm)	No.	Temp. (°C)	1	2	3	4
16/7	3.1	35	1a	14.8-15.6*	67	29	25	16
18/9	3.1	35	1b	19.4-20.0*	73	25	14	13
27/7	1.9	30	2a	19.0-20.0	41	28	8	7
8/8	1.9	30	2b	16.3-17.0	48	15	9	9
8/7	3.2	31	3a	20.0-20.5	82	24	18	13
28/7	3.2	31	3b	18.2-19.0	69	23	12	10
10/8	3.2	31	3c	16.5-17.3	76	29	12	12
26/7	3.2	37	4a	19.2-20.1	81	15	8	8
17/1	3.3	37	4b	17.8-18.2	61	22	-	-
23/9	2.0	36	5a	18.2-19.0*	40	25	14	16
19/10	2.0	36	5b	17.1-17.5	53	16	8	8
25/5	3.3	36	6	18.2-19.3	81	22	13	11
22/7	3.0	33	7	20.2-20.4	82	31	17	5
23/7	3.0	36	8	18.1-18.7	75	28	-	-
9/12	3.0	34	9	18.8-19.4	74	24	12	12
9/7	3.0	35	10	18.5-19.0*	69	23	12	10
3/8	2.9	36	11	17.2-17.6	73	18	10	9
8/10	2.8	37	12	15.3-16.1	71	24	13	11
7/7	2.8	35	13	15.8-17.0	76	31	14	13
8/9	2.8	35	14	16.9-17.2	78	24	-	-
4/2	2.7	30	15	18.1-19.3	61	21	10	11
9/6	2.7	35	16	20.0-20.2	69	21	11	10
10/10	2.7	34	17	17.8-18.2	77	19	9	-
8/3	2.6	33	18	16.3-17.3	49	19	12	10
24/7	2.5	31	19	18.1-18.5	74	17	11	7
14/7	2.5	33	20	17.8-18.2	63	24	-	-
4/3	2.3	31	21	15.3-16.1	36	16	10	-
21/7	2.3	31	22	15.8-16.1	37	10	-	-
25/7	2.2	30	23	18.2-18.6	35	13	7	6
6/2	2.2	34	24	18.5-19.0	43	15	7	4
15/7	2.2	31	25	18.0-18.3	31	15	9	7
12/2	2.1	33	26	17.1-17.6	29	12	-	-
5/3	2.1	34	27	18.0-18.6	43	11	6	-
6/10	2.1	30	28	14.2-14.7	34	11	-	-
5/8	2.0	34	29	16.3-17.7	34	19	11	8
11/12	2.0	34	30	17.0-17.3	27	12	-	-
27/9	2.0	32	31	18.1-18.9	48	17	10	8
6/8	1.9	31	32	17.9-18.6	44	14	8	7
13/10	1.9	32	33	19.1-19.6	35	17	11	6
4/8	1.9	31	34	17.3-18.1	45	18	9	9
9/10	1.8	31	35	17.2-17.9	25	11	7	5
21/1	1.6	31	36	15.0-15.5	24	13	6	6
16/10	1.5	31	37	17.2-18.0	39	24	10	10
6/7	1.5	32	38	17.3-17.7	49	11	10	8
28/9	1.4	29	39	14.9-15.6	29	12	7	-
3/2	1.3	30	40	15.8-16.2	19	7	2	2
30/1	1.2	30	41	15.3-15.9	17	6	2	3
12/8	1.2	27	42	16.2-16.9*	16	9	5	5
Average weight increase (mg/h)					52.12(48)	18.54(48)	10.15(40)	8.75(36)
Average weight increase/g whole animal (mg/g/h)					21.37	7.83	4.38	3.67
Average gas consumption/g tissue (cu.mm/g/h)					25.64	9.40	5.25	4.40

Table 9

Planorbarius corneus					Weight increase in 4 successive hours			
Date	Wt. (g)	Diameter (mm)	No.	Temp. (°C)	1	2	3	4
10/12	3.2	27	1a	13.2-15.8	53	20	12	8
9/2	3.2	27	1b	18.1-19.1	67	39	17	5
8/12	2.9	28	2a	17.4-18.0*	73	24	9	3
11/2	3.0	28	2b	17.6-18.3	72	20	9	4
3/8	2.8	30	3a	15.2-16.4	36	18	6	2
29/9	2.8	30	3b	17.8-19.1	42	12	7	2
7/10	2.9	30	3c	15.7-16.3	38	24	9	3
7/8	2.5	31	4a	14.9-15.9*	27	19	12	6
5/10	2.5	31	4b	16.9-17.5	34	11	11	5
17/9	2.5	31	4c	17.0-17.5	36	12	7	4
8/5	3.1	28	5a	16.8-17.3	53	16	10	5
26/5	3.1	28	5b	20.0-19.7*	59	10	9	5
2/8	3.2	30	6	17.1-18.2	63	23	12	4
5/2	3.1	31	7	13.9-14.7	59	33	14	9
23/1	2.9	28	8	15.0-15.8	58	27	15	7
5/12	2.8	26	9	15.2-16.1	51	23	10	6
31/7	2.8	25	10	17.3-18.3	56	19	9	3
13/5	2.7	30	11	19.1-20.0	68	21	-	-
15/5	2.6	27	12	17.9-18.5	56	26	12	5
30/9	2.5	21	13	16.0-17.1	50	22	11	4
11/8	2.5	31	14	19.2-20.1	73	18	-	-
11/10	2.4	21	15	18.9-19.7	37	10	5	2
14/9	2.2	24	16	18.6-19.0	40	11	8	4
12/10	2.1	22	17	19.1-20.0	49	12	6	3
7/5	2.1	23	18	18.0-19.3	49	16	8	4
10/8	2.0	20	19	19.2-19.9	33	8	5	3
12/3	2.0	21	20	16.7-17.8	43	17	8	3
14/5	1.9	20	21	16.3-16.5	41	19	-	-
7/6	1.6	19	22	18.9-19.1	44	11	-	-
1/8	1.5	19	23	19.8-20.2	33	9	9	3
29/7	1.5	17	24	19.1-19.8	43	14	7	3
Average weight increase (mg/h)					49.54(31)	18.19(31)	9.59(27)	4.25(27)
Average weight increase/g whole animal (mg/g/h)					19.79	7.12	3.70	1.64
Average gas consumption/g tissue (cu.mm/g/h)					39.58	14.24	7.40	3.28

and 9). The rates of gas utilisation over the first three hours are therefore approximately the same. Both species consume during the first hour of submersion three times the amount used in the second, or six times that used in the third. In the fourth hour, the rate at which gas is used by P. corneus is again halved, while the rate for L. stagnalis is only reduced by about 20%. There are wide variations from these means, both in the rates at which the gas is being consumed, and in their relationships over successive hours. These cannot be consistently related to the size of the snails or to the temperatures. In the replicate experiments each snail gained weight under water at similar rates on successive occasions, even when these were separated by intervals of a week or more. Individual snails of P. corneus consistently show a slightly higher rate of weight increase at higher temperatures, but the rate is not predictable for any size of snail. No results from L. stagnalis show any consistent response to altered experimental temperatures.

Also included in Tables 8 and 9 are the mean weight increases per gram of intact animal per hour, obtained by dividing the weight increase in each hour by the wet weight in air of the snail concerned, and averaging all the results so obtained. Variations introduced by the different sizes of the snails are eliminated by this

process, and the results still show the same ratios of weight increase in successive hours, and for the two species.

Allowing 50% of the total weight as the shell weight in P. corneus, and 20% in L. stagnalis, these mean rates can be converted into rates of gas utilisation per gram of living tissue. These show that P. corneus in the first hour is using more gas per gram of tissue than L. stagnalis. The difference is greater than could be caused by error in the estimation of tissue weight. It becomes much less in the second and third hours, and the relationship is reversed during the fourth hour, when L. stagnalis is seen to be using gas at a faster rate than P. corneus.

iv) Rates of change over prolonged submersion

Table 10 gives the weight increases over a few days for some of those snails which remained active. These support the earlier conclusion that P. corneus can make fuller use of mantle cavity gas in the later stages of extended dives. All P. corneus gained considerably more weight per day than L. stagnalis on the fourth and fifth days.

Reduction of the volumes of gas noted, of 50% after

Table 10

Lymnaea stagnalis					Weight increase	
Date	Wt. (g)	Length (mm)	No.	Temp. (°C)	1	2
16/7	3.1	35	1(a	14.8-17.6	267	131
18/9	3.1	35	1(b	19.4-20.2	272	141
9/7	3.0	35	10	18.5-20.1	262	102
12/8	1.2	27	42	16.2-17.4	99	25
23/9	2.0	36	5(a	18.2-19.3	221	103

Planorbarius corneus					Weight increase		
Date	Wt. (g)	Diameter (mm)	No.	Temp. (°C)	1	2	3
8/12	2.9	28	2(a	17.4-18.2	162	46	24
7/8	2.5	31	4(a	14.9-15.9	137	62	31
26/5	3.1	28	5(b	19.7-20.1	149	60	33

twenty-four hours and 90% after five days could not result from oxygen uptake alone. There must in addition have been loss of the other gases (N_2, CO_2 etc.) into the surrounding water as a result of increased partial pressures. The gas in the mantle cavity therefore functions as both air-store and physical gill (in the sense of Ege, 1918). Thus conversion of the gas volume changes directly into oxygen consumptions is impossible since the proportion of change due to diffusion may vary.

v) Buoyancy level at successive surfacings

Berg and Ockelmann (1959) have shown that the aquatic respiratory rate in pulmonates declines with the tension of oxygen in the water. Similarly Krüger (1948) showed that the respiration of L. stagnalis and P. corneus in damp air is related to the partial pressure of oxygen. Uptake of oxygen from the mantle cavity is only part of this respiratory exchange, but its rate will depend on the partial pressure of oxygen in the mantle cavity. Füsser and Krüger (1951) found that the total respiration of these two species was higher in moist air than when surfacing in water. In air, diffusion of oxygen is more rapid at any given partial pressure than it can be in water. Since the pneumostome is frequently open in damp air, greater availability of oxygen may explain the increased respiration.

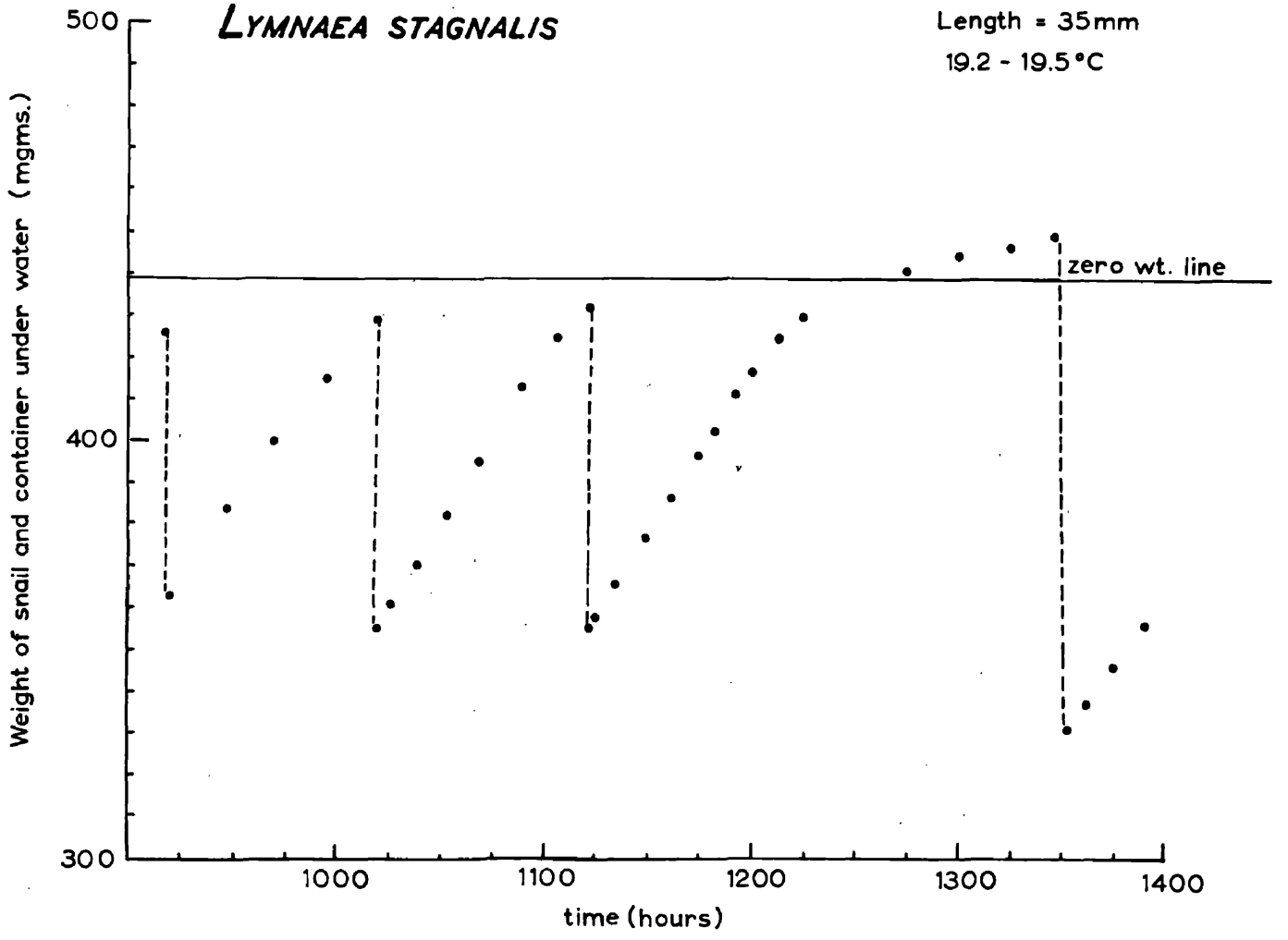
In the same way, the weight increases in these buoyancy experiments reflecting falling rates of gas consumption must be affected by the declining oxygen partial pressures within the mantle cavity. With any given size of snail and rate of oxygen consumption, the smaller the original volume of air, the more quickly will the partial pressure of oxygen be reduced. The surveys of bubble volume reported above show it to be very variable. Using the torsion balance apparatus, the normal behaviour could be imitated, permitting access to air at appropriate intervals and then determining the buoyancy reached.

In each case, after a period of undisturbed surfacing into air, intake of air was measured in this way, and then the snail was kept submerged for about an hour at constant temperature. At the end of this period, the capsule was gently raised to allow the snail to reach the surface as naturally as possible. Water aeration, otherwise continuous throughout submersion, was stopped. Rapid lifting of the capsule to the surface resulted in an increase in the volume of gas taken in. Any slight vibration of the apparatus was again enough to produce such increased uptake. For this reason, under laboratory conditions, a succession of completely undisturbed surfacings can only be achieved on rare occasions. Only seven series were completed in fourteen months of

buoyancy recording. In each, the snail ventilated normally at the water surface which had approached, but not touched it. Figures 9 and 10 present the results from these experiments, showing stable levels of buoyancy on successive occasions. At each surfacing, the snail took in a quantity of air sufficient to restore the buoyancy to its original value. Measured variations amount only to $\pm$ 5 mg, equivalent to variations in the gas volumes of $\pm$ 2%.

A forced extension of the period of submersion is an easily imitated disturbance of the surfacing behaviour, which could occur in nature. As the graphs show, the snails respond to this treatment by increasing the quantity of air they take in at the next surfacing. In most cases this can be achieved by opening the pneumostome once only. However, one P. corneus carried out three surfacing movements (stepped vertical broken line, top of figure 10). Here, as in all cases of repeated surfacings, the major increase in gas volume takes place during the first. Animals of both species can remain submerged for about an hour without alteration in gas uptake at the next surfacing.

An extension of the dive leads both species to take in more air. Only one graph (bottom right, figure 9)



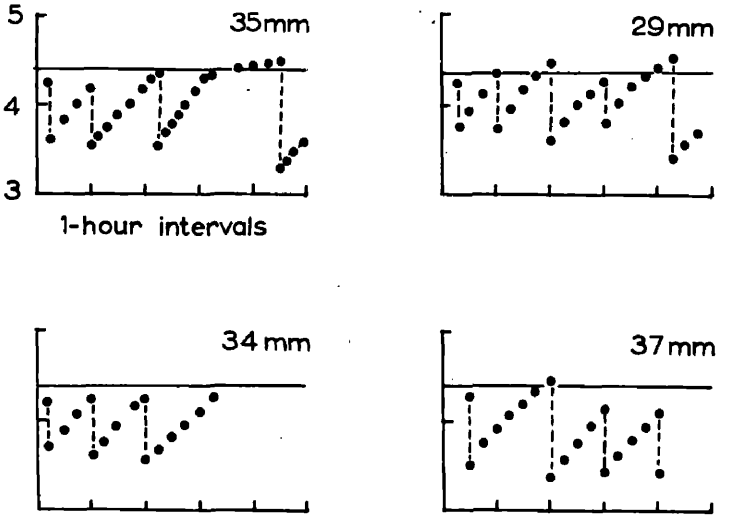


Figure 9. Underwater weights of L.stagnalis after successive surfacings.

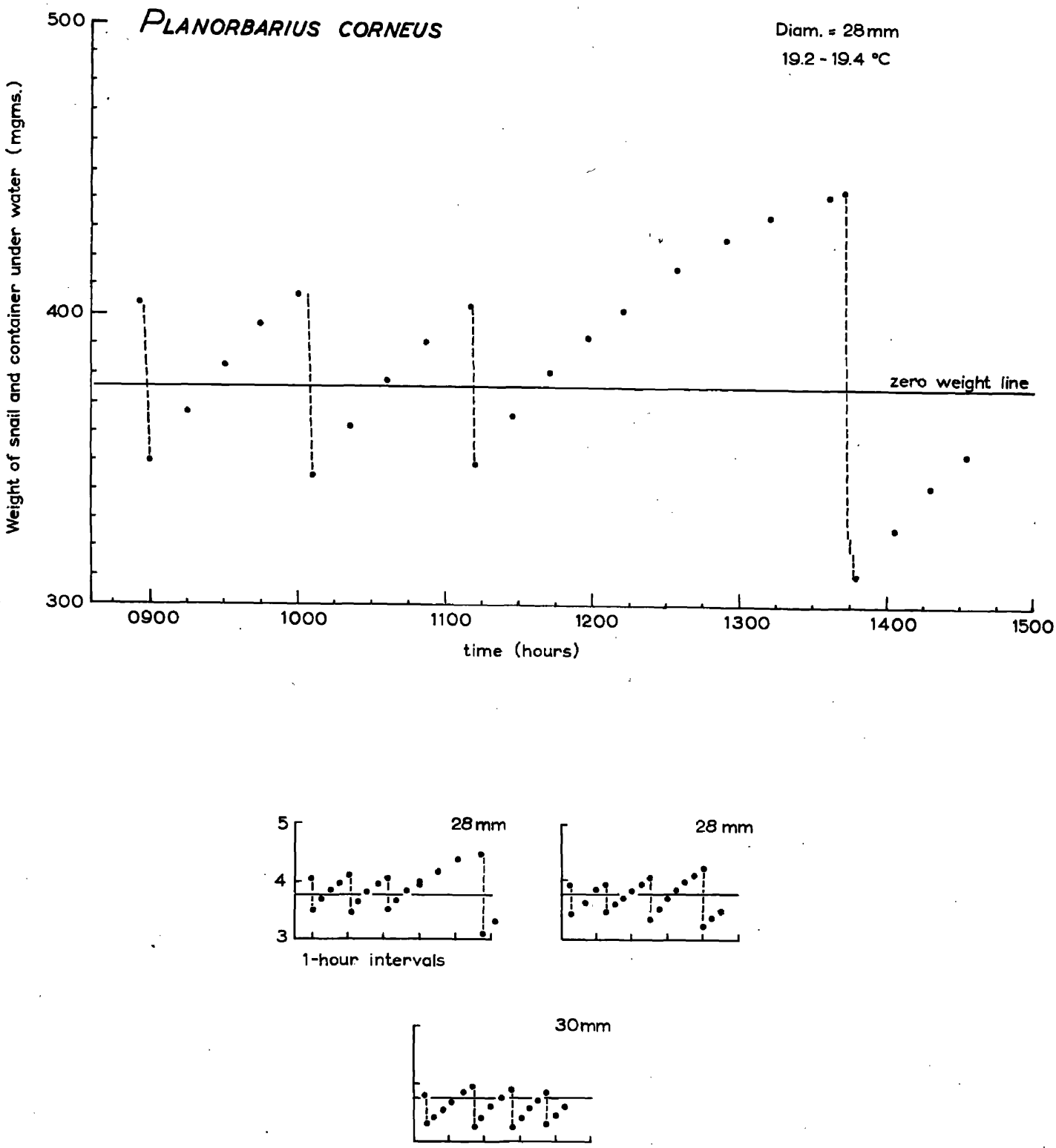


Figure 10. Underwater weights of P.corneus after successive surfacings.

shows how this increased gas uptake is repeated at subsequent surfacings. During many experiments where disturbance had occurred, such increased uptake lasted for up to twelve hours.

Any disturbance, either before or during the surfacing process, will lead to this increase in volume of gas taken in. Because of this, experimental investigation on the effects of such factors as temperature and water oxygenation is impossible. Therefore the rate of gas utilisation under standard physical conditions is not predictable. Thus from such weighing experiments alone, it is not possible to determine whether decreasing supply of oxygen from the bubble, or the buoyancy level as such, releases the normal pattern of surfacing behaviour.

d) Serial weighings after alteration of oxygen pressures and tensions

1) Method

As in the earlier experiments, each snail was permitted hourly surfacings into air until its buoyancy on successive occasions approached a stable value. Five minutes before the next surfacing was due, the experimental vessel was sealed off from the air. Aeration of the water was stopped, and a current of oxygen or nitrogen was passed through the space between the water surface

and the lid. To avoid disturbance of the water surface, the gas flow was kept low (250 c.c. per minute into a space of about 300 c.c.). After five minutes, the capsule was gently raised to allow each snail to surface naturally into the artificial atmosphere. This ventilation is the first in each of the graphs in figures 11 to 14 and is arrowed, with appropriate gas symbol, in the large scale versions.

As soon as the underwater weight was recorded with the capsule re-submerged, the gas flow was stopped. Compressed air was passed over the water surface, and aeration of the water resumed. This effectively restored the water gas tensions to normal. The weights of the snails were then serially recorded. After an interval of two to three hours, the snails were given access to the air. Their air uptakes and subsequent underwater weight increases were then recorded. The experiments were all conducted at constant temperature ($\pm$ 0.2°C), which usually lay between 19 and 20°C.

2) Results

i) Oxygen-rich atmosphere

Figures 11 and 12 show the results of a series of such experiments using oxygen as the replacing gas. Surfacing into this, the gas uptake of the snails is not significantly

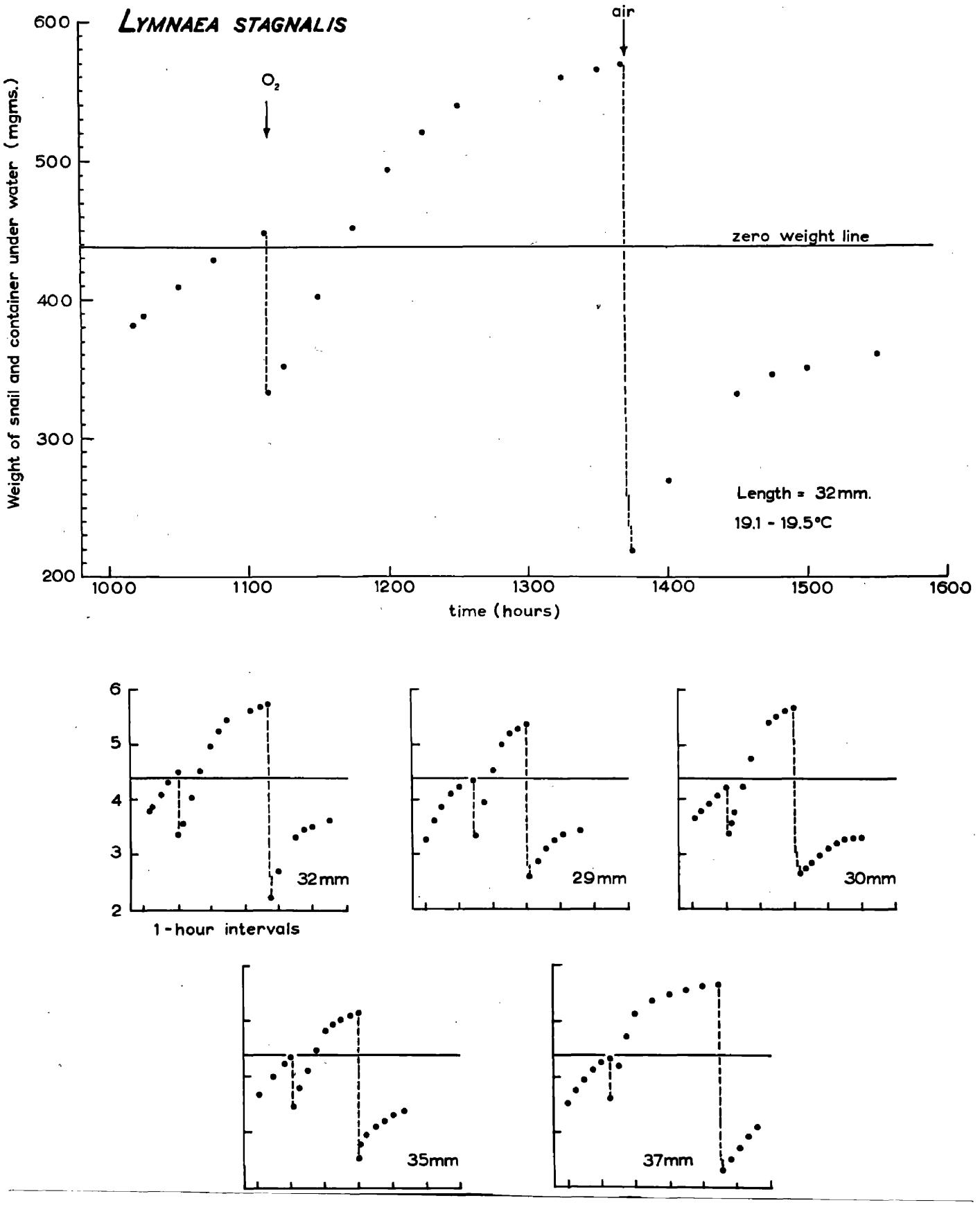
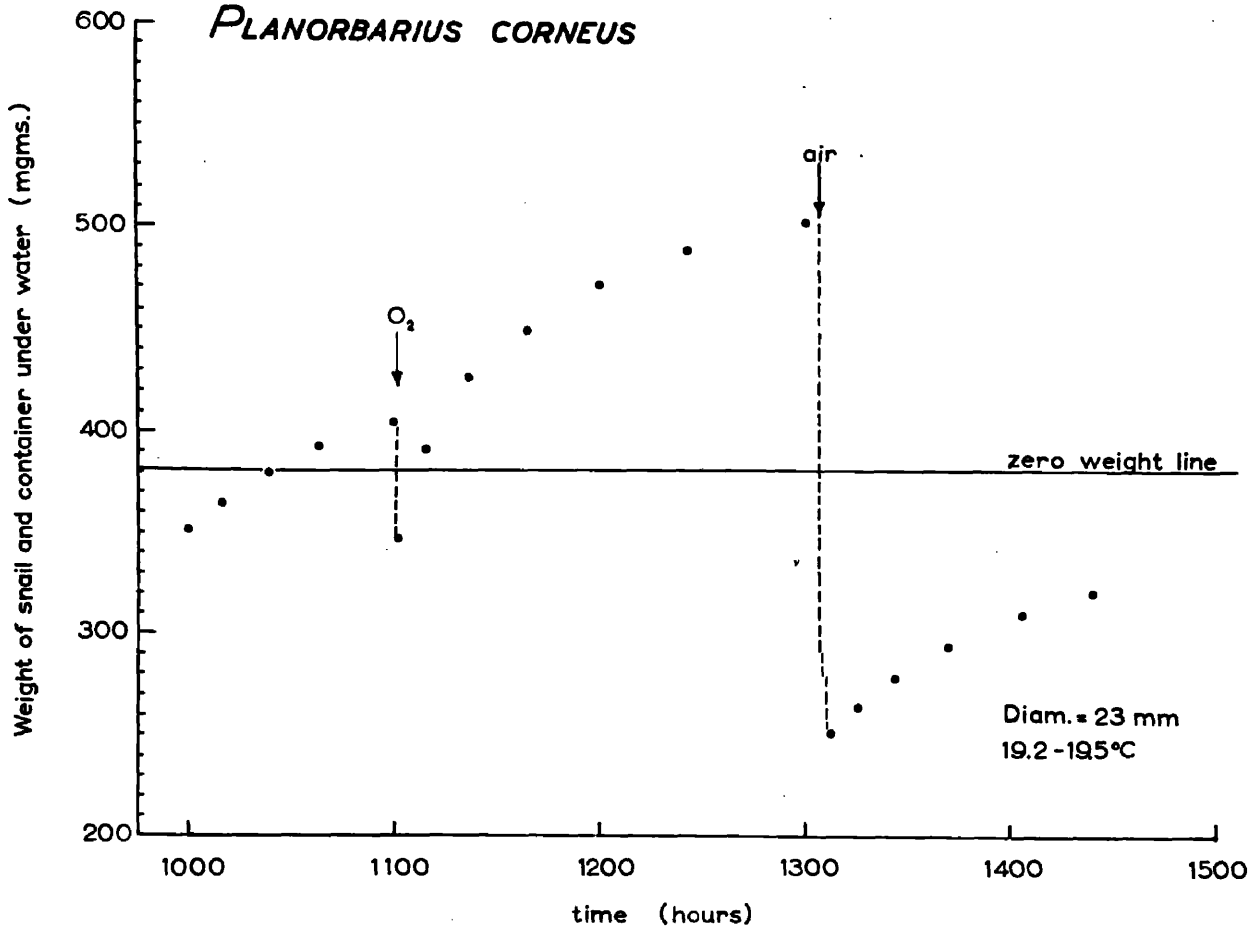


Figure 11. Weight changes of L.stagnalis on surfacing once into an oxygen-rich atmosphere, and subsequently into air.



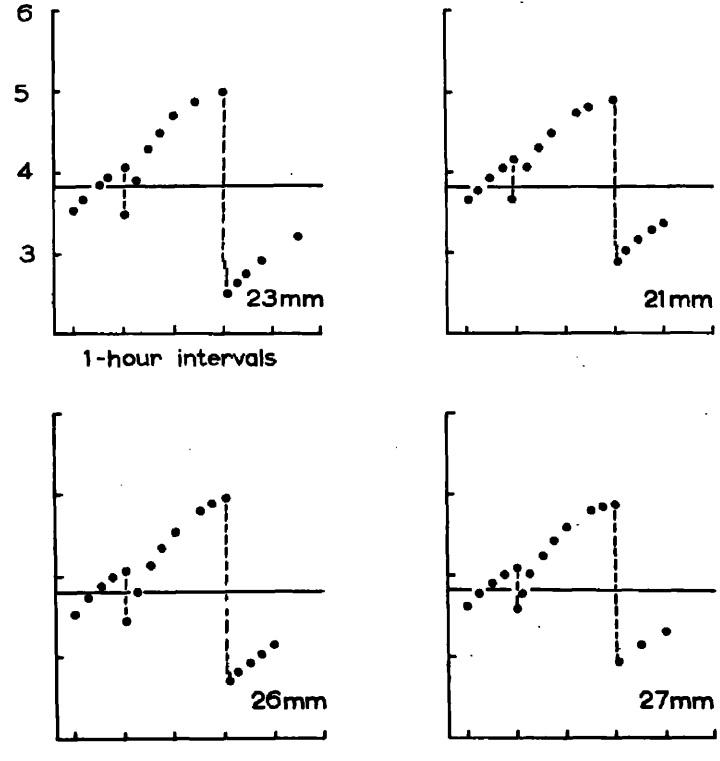


Figure 12. Weight changes of P. corneus on surfacing once into an oxygen-rich atmosphere, and subsequently into air.

different from the value on previous occasions. In all cases, the rates of underwater weight increase are greatly enhanced by this treatment. Over the first hour of submersion, the rates of gas consumption producing these are approximately twice the average rates for animals with air-filled cavities. This must be brought about largely by an increase in the oxygen consumption of the snails, but diffusion of oxygen into the water could also be faster under these artificial conditions. In the absence of information on the continued presence and areas of the gas/water interface it is impossible to separate these two functions. Quantitative analyses of the rates of gas loss are not attempted here.

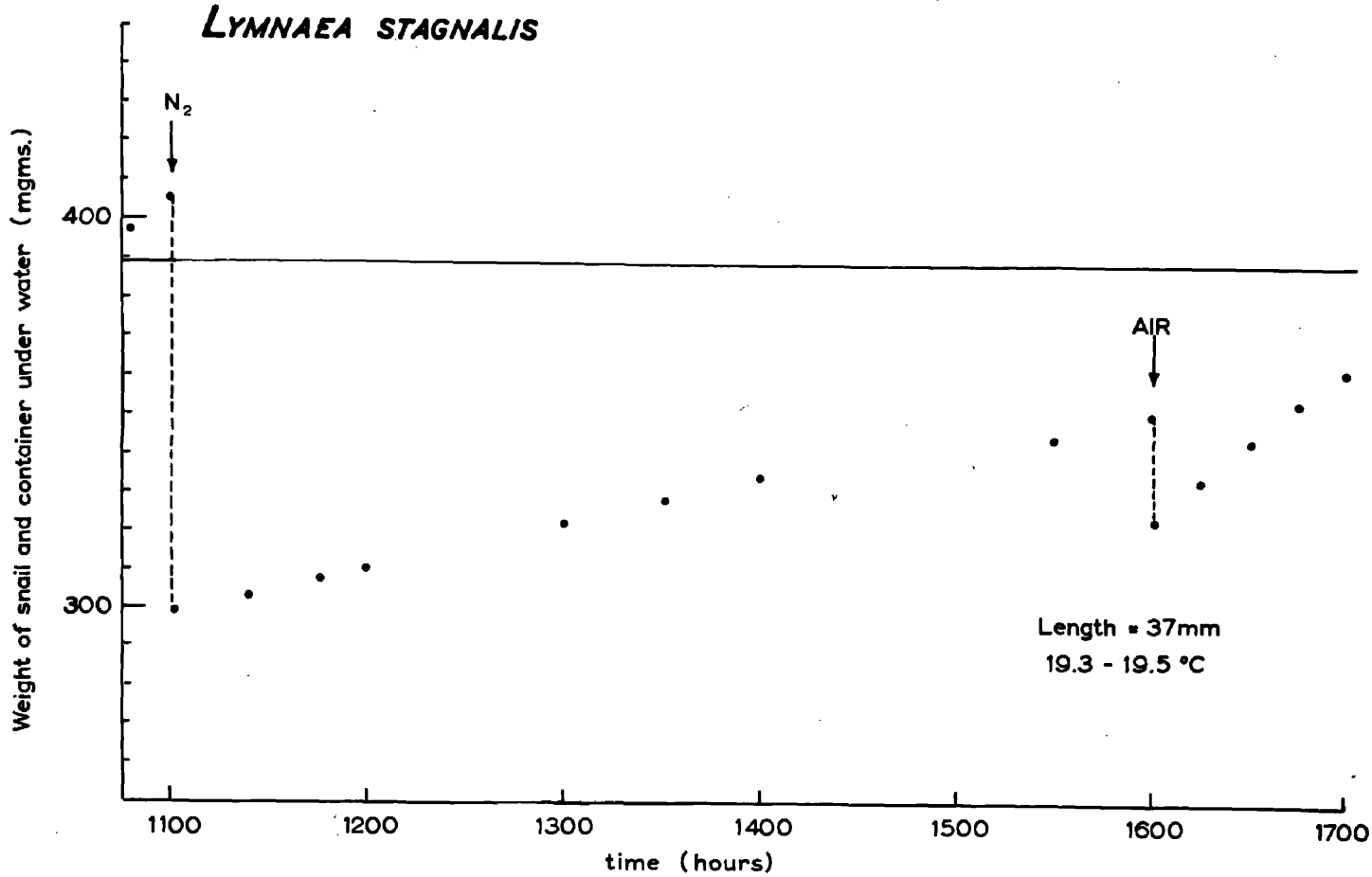
After some two hours' submersion, when snails of both species were much heavier than water, each was allowed to surface into air. A greatly increased uptake resulted in each case, the major part of it taking place during the first ventilation as in previous experiments. This is followed by reversion to the rates of gas consumption before experimental treatment.

If the surfacing behaviour of the snails is released by a hydrostatic stimulus, acceleration of the process of underwater weight increase will speed the onset of this behaviour. Forced ventilation into an oxygen-rich

atmosphere produces such acceleration. It must also delay the onset of oxygen lack by giving the snails a larger initial supply of oxygen than they could have in the same volume of air.

ii) Nitrogen-rich atmosphere

An identical technique was used to substitute nitrogen for air in the mantle cavities. Once more, precautions were taken against disturbance of the snails' behaviour prior to, and during, the inhalation of the nitrogen, and all measurements were made at constant temperature between 18.5 and 19.5°C. Figures 13 and 14 show graphically the results of such experiments. The uptake of nitrogen is indicated in the figures by an arrow on the large scale graphs for the two species. In the small scale versions occupying the lower part of each figure, the first vertical broken line indicates the surfacing into nitrogen, and the second indicates the uptake of air. The volumes of uptake of nitrogen showed no alteration from the volumes of air which the snails had taken in during two or more previous hourly surfacings into air. From direct observation there appears to be no disturbance of their behaviour when the snails surface into an atmosphere of nitrogen. All of them closed the pneumostome after the normal time (10-20 secs. in both species at 15-20°C), and moved normally downwards from the surface.



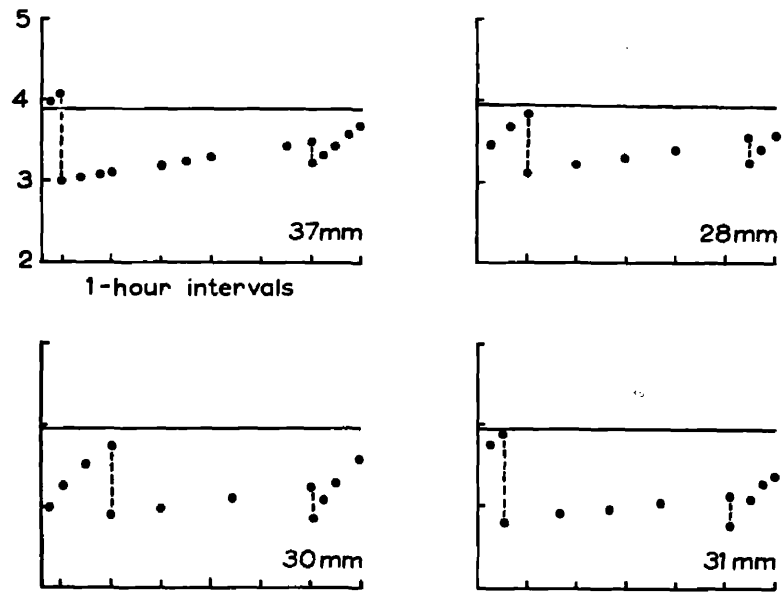


Figure 13. Weight changes of L.stagnalis on surfacing once into a nitrogen atmosphere, and subsequently into air.

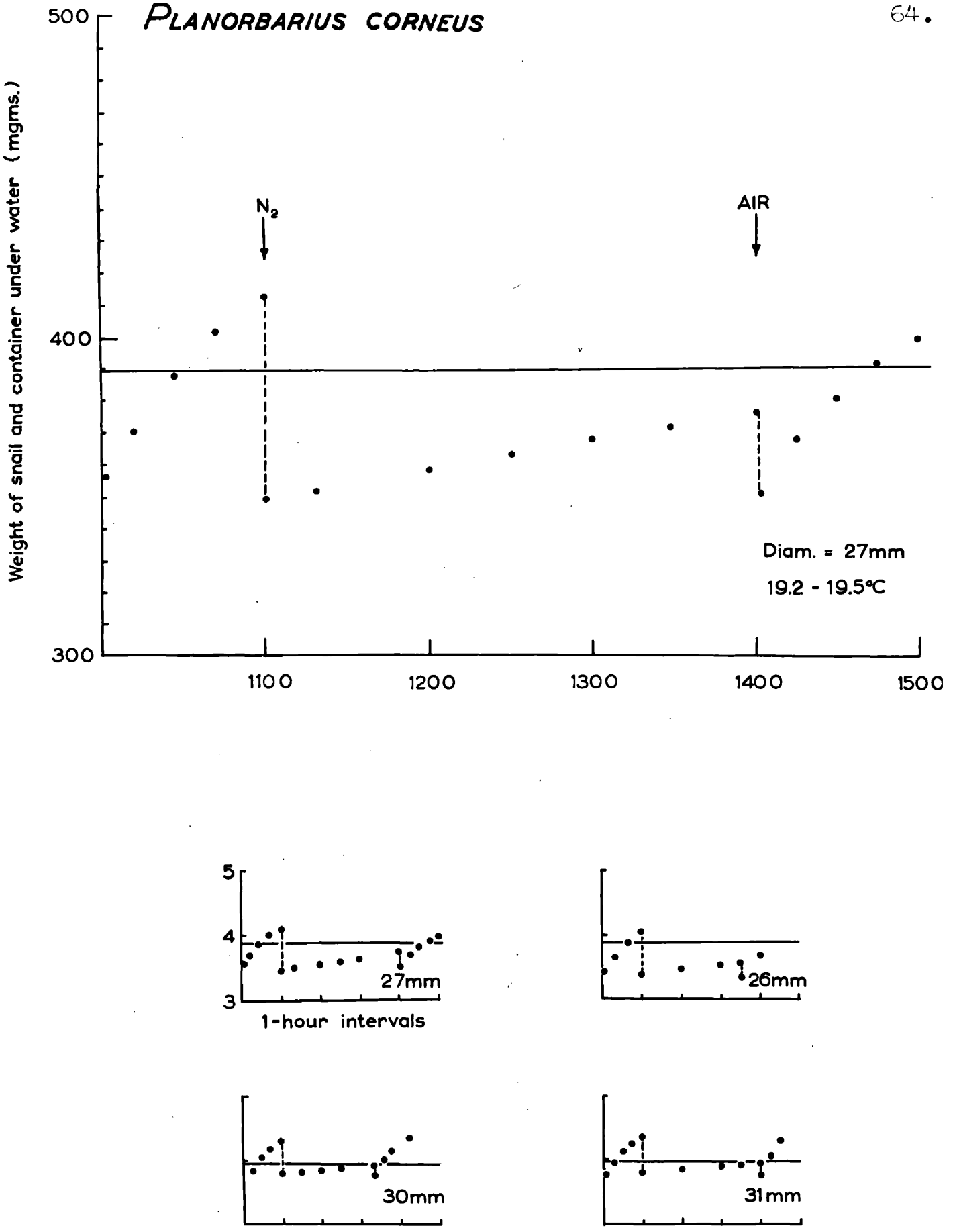


Figure 14. Weight changes of P.corneus on surfacing once into a nitrogen atmosphere, and subsequently into air.

The increase in underwater weight after the mantle cavity has been filled with nitrogen can be used as evidence for physical gill function. The partial pressure of nitrogen in the mantle cavity at the beginning of submersion may be taken as 1 atmosphere, and that of oxygen as nil. (Six analyses showed the atmosphere overlying the water to have, on average, 99.6% N_2.) The tension of nitrogen in the water surrounding the snail which is air-saturated will be 0.8 atmosphere; that of oxygen will be 0.2 atmosphere. Applying the method described by Crisp and Thorpe (1948), $(pN_2 - tN_2) = 0.2$ atmosphere and $(tO_2 - pO_2) = 0.2$ atmosphere. In the case of each gas, the gradient along which they may diffuse is the same. Since the invasion coefficient (Ege, 1918; Crisp and Thorpe, 1948) of oxygen is twice that of nitrogen when both face the same pressure gradient, initially oxygen must diffuse from the water into the nitrogen-filled mantle cavity more rapidly than the nitrogen can diffuse out. This alone would result in an increase in the volume of the mantle cavity gas, and a corresponding decrease in the animal's underwater weight. Weighing shows that the volume of gas is decreasing, implying that part of the oxygen diffusing into the cavity through the pneumostome is being removed by the snail for respiratory purposes. The cavity is therefore functioning as a physical gill maintained at constant pressure but decreasing volume.

With their large, and steadily decreasing, volume and small area of interface in relation to the respiratory rate, the air-filled mantle cavities of these snails come into the category of air-stores as they are defined by Crisp and Thorpe. The rates of diffusion of the gases would thus be directly proportional to the area of interface. The interface at the pneumostome, on most occasions when visible, appears as a fine slit which varies in width during submersion. Its area is neither fixed nor easily subject to measurement. Therefore the results of these experiments can only be used qualitatively in subsequent discussion, as those obtained from the contrasting observations on snails with oxygen-filled cavities.

At the end of about three hours' submersion with the nitrogen-filled cavities, the snails were allowed to surface into air. In spite of the prolonged submersion, and the initial absence of oxygen from the mantle cavity gas, the uptake of air was not increased: the buoyancy is simply restored to its original level. The following period of submersion showed a return to the normal rate of weight increase. The restoration of the air-filled condition in each mantle cavity must therefore be complete, although the percentage increase in gas volume was small. Diffusion through the open pneumostome must be responsible for most of the restoration of the normal gas partial pressures.

iii) High oxygen tension

A further series of qualitative experiments on the relationship between the mantle cavity gas and the surrounding water involved increasing the oxygen tension in the water. Some 45 minutes after each snail had made an undisturbed surfacing into air, the aeration by compressed air was stopped, and oxygen was bubbled through the water instead. This oxygenation of the water then continued for some 30 minutes; the exact period is indicated by two arrows on each graph. All the experiments were carried out at constant temperature between 19 and 20°C.

The results are shown graphically in figures 15 and 16. Within five minutes of the start of oxygenation, the snails had ceased to gain in underwater weight. After a further ten minutes without significant change, each began to lose weight. This signifies an increasing mantle cavity gas volume, and the graphs show this continuing for as long as oxygenation is maintained. It also continued for some time after oxygenation was stopped and ordinary aeration restored (second arrow in figs 15 and 16). In all snails examined, shortly after this weight increase begins again, and the rate of increase for an appreciable time is almost twice that recorded immediately before the first application of oxygen. In fact, this increased rate closely approaches that shown by snails which have surfaced into pure oxygen (cf. figs 11 and 12). This indicates

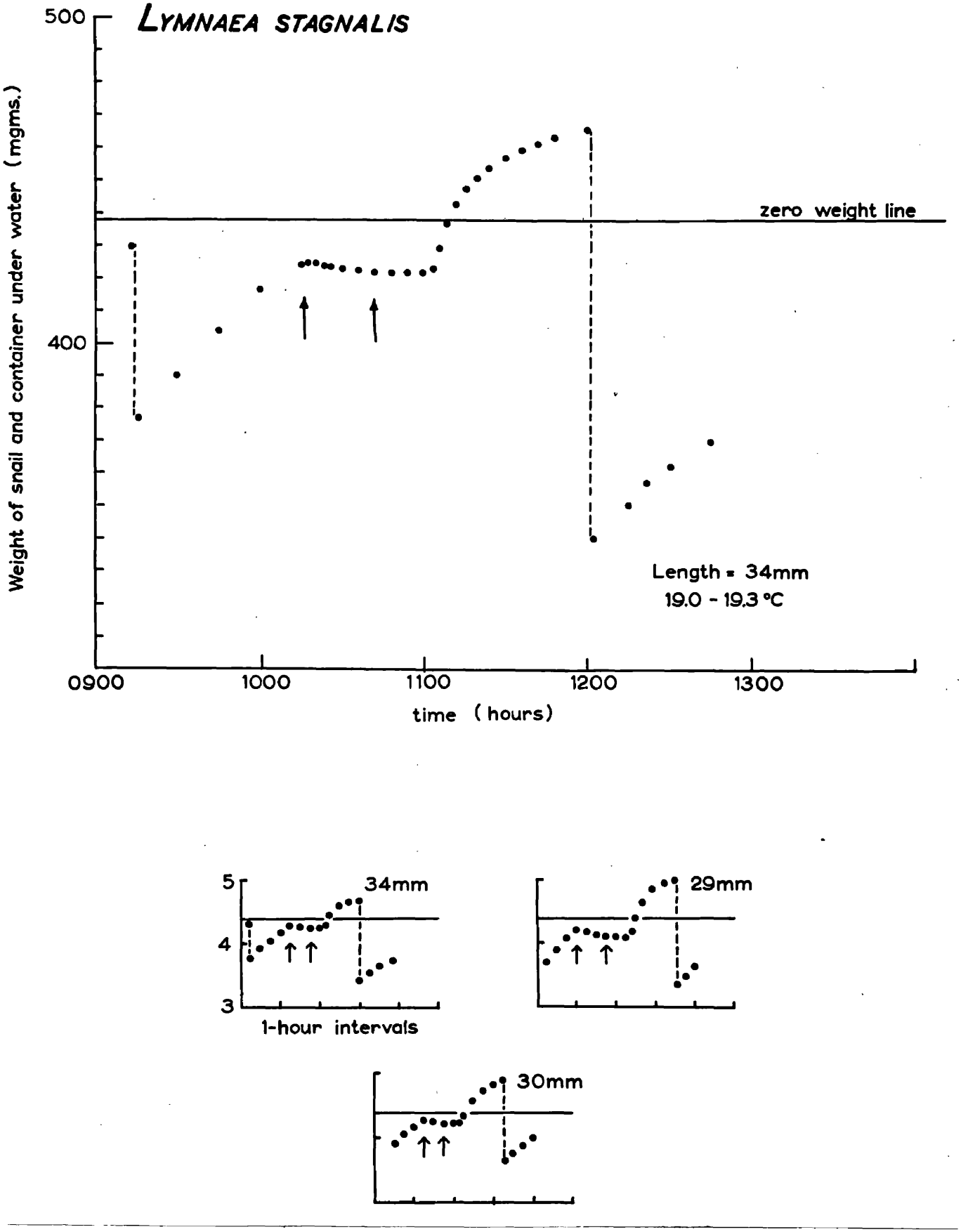


Figure 15. Weight changes of L. stagnalis in response to oxygenation of the water.
(Arrows indicate beginning and end of application of oxygen.)

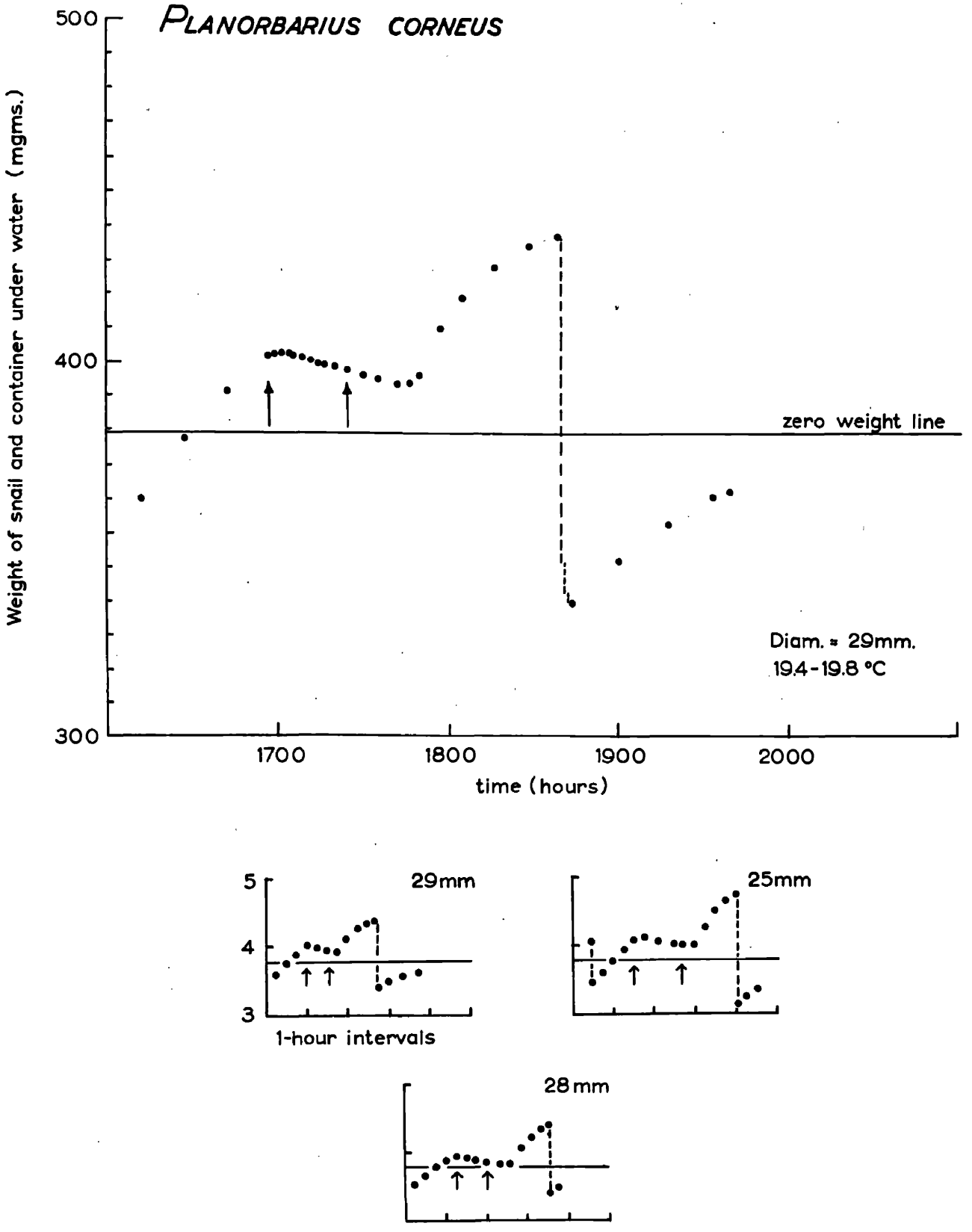


Figure 16. Weight changes of P.corneus in response to oxygenation of the water. (Arrows indicate beginning and end of application of oxygen.)

an increase in the partial pressure of oxygen in the mantle cavity gas, produced by the diffusion into it of oxygen from the water. Therefore these graphs are further evidence of the mantle cavity gas acting as a physical gill. The decreased volume after submersion resulting from this treatment is compensated for, at the next surfacing, by the snails' taking in a greater quantity of air.

VI. COMPOSITION OF THE MANTLE CAVITY GAS

a) Method

A number of workers have analysed the gases contained in the mantle cavities of freshwater snails (Precht, 1939; Hazelhoff, quoted by Jordan, 1922, 1930a & b; Hunter, 1953b). Both Precht and Hazelhoff extracted gas bubbles in contact with water and so diffusional processes may have altered the partial pressures of the constituent gases. Hunter's field analyses were from the smaller pulmonates Lymnaea peregra and Physa fontinalis and may not be relevant to the larger L. stagnalis and Planorbarius corneus under laboratory conditions. A new technique was developed to take samples from individuals of these two species; the apparatus appears in figure 17.

Analyses of the gases were carried out in the Scholander Micro-gas-analyser (Scholander, 1947). The sampling pipette of this apparatus (capacity 0.5 c.c.) was modified as shown in the upper part of figure 17. A short length of rubber tubing with two screw-clamps A and B was attached to its upper end. Slight release of A, with B closed, was used to draw a drop of "acid rinse" solution into the lower part of the pipette. This solution (Scholander, 1947) consists of an acid solution of sodium sulphate to which glycerol and potassium dichromate are

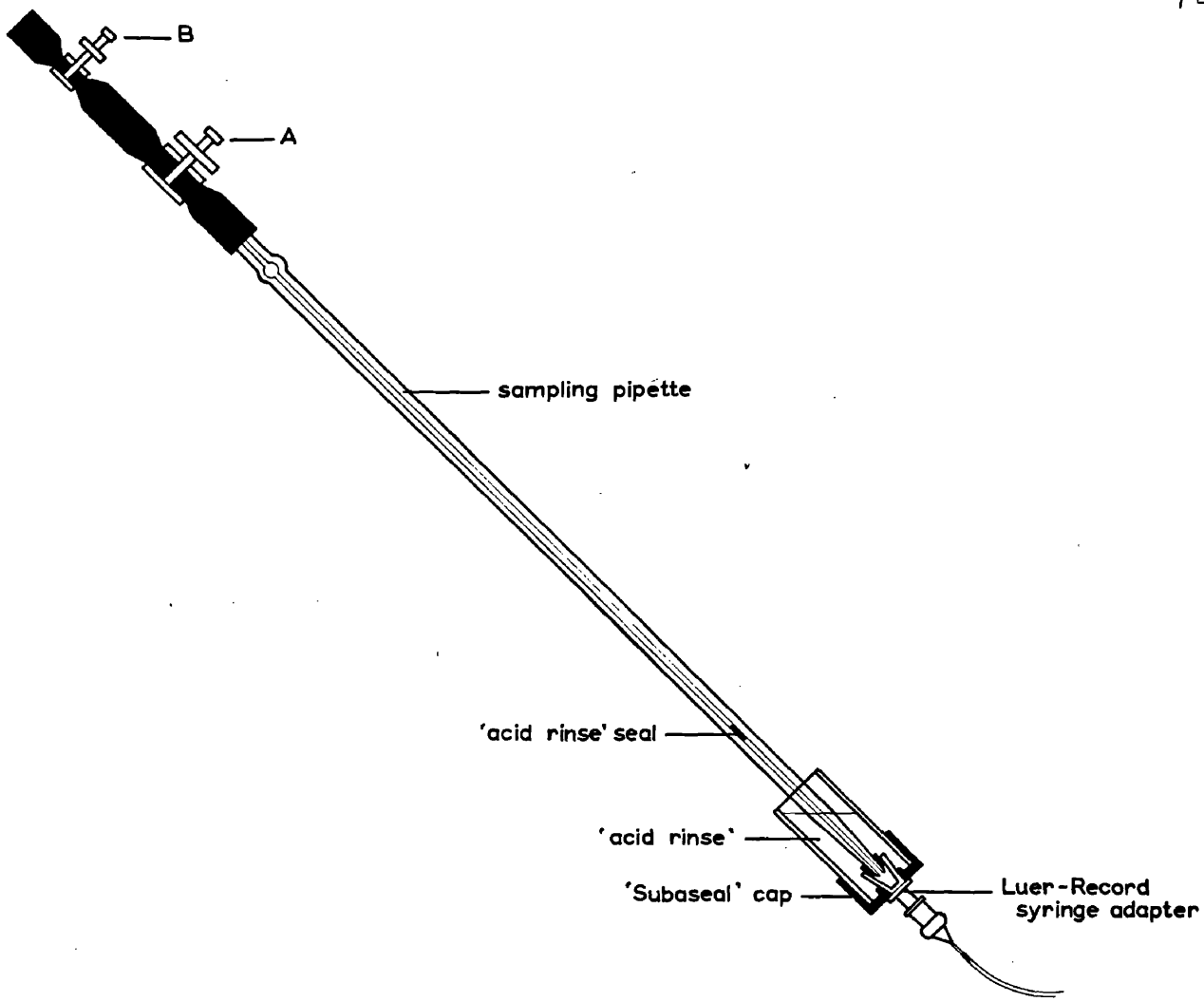


Figure 17a. Scholander sampling pipette as modified for gas sampling from snails.

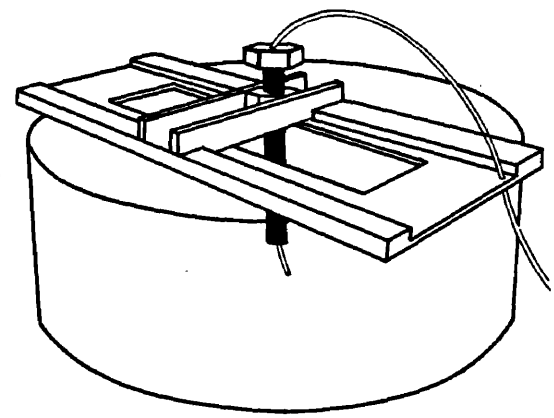


Figure 17b. Device used for introducing and holding sampling tube in mantle cavity.

added. The coefficients of solubility of nitrogen, oxygen and carbon dioxide in it are so extremely low, that diffusion losses can be disregarded. The tip of the pipette was then seated through a small rubber collar into the wide end of a syringe adapter. This, with a "Subaseal" rubber cap and a small glass collar, formed a cup which was filled with acid rinse solution. The air space in the pipette was thus continuous through this solution with the cavity of a hypodermic needle attached to the adapter. This connected with fine polythene tubing (1 mm bore), which was introduced into each snail by means of the apparatus shown in the lower part of the figure.

The snails were each fastened down by their shells in Plasticine with the aperture of the shell pointing upwards. Lowering the water surface until it touched the mantle rim inside the edge of the shell caused the pneumostome to open. The polythene tube could then be gently inserted by sliding it down through an axial hole drilled in the vertical bolt shown in the figure. The polythene tube had an inherent curvature, and this was used to keep the tip of the tube from touching the siphon or the roof of the cavity. If inserted and held steady in this way, the tube caused no retraction, and when the water level was once more raised the pneumostome closed smoothly around it.

By this means, the gas in the mantle cavity (some

500 cu.mm) was continuous with the gas in the sampling pipette and tubing (30 cu.mm up to the acid rinse seal). As soon as the pneumostome closed on the tube, the sealing droplet started to move up and down the sampling pipette, obviously under the influence of pressure changes in the mantle cavity. This was a very valuable guide as to whether the tube remained clear or not. Frequently, and particularly if the tube had been inserted too far, drops of mucus would clog its tip. The slightest obstruction of this nature could be detected by its stopping the movement of the acid rinse seal.

After 30 or 60 minutes, clip A was slowly released, drawing the acid rinse seal up the sampling pipette, and following it, a part of the gas from the mantle cavity as a continuous column. The gas extending into the sampling pipette during the period of submersion can be assumed to have the same composition as that of the mantle cavity. Before withdrawal of the sample, the volume within the apparatus had been small compared with that in the cavity. The movements already mentioned must have resulted in a thorough mixing, in addition to the diffusional processes which alone could remove any gradients in the gas. Before the acid rinse seal reached the dilation in the sampling pipette, the rubber tipped end of the pipette was withdrawn from the syringe adapter under the acid rinse. Slight

further release of clip A brought a drop of acid rinse in behind the sample. The withdrawn sample was thus enclosed between virtually impermeable seals. It could then be transferred to the gas-analyser, and its percentage composition determined. The analyser employs a closed system where volume reductions in the sample, produced by successively absorbing carbon dioxide and oxygen from it, are compensated by displacement of mercury into the system by a micrometer plunger. The solutions for absorbing the gases are introduced without alteration of the total fluid content of the system, and are balanced in regard to vapour pressure.

b) Results

Tables 11 and 12 are the results of such experiments on L. stagnalis after 30 minutes' and 60 minutes' submersion respectively. Tables 13 and 14 present similar results for P. corneus. The percentages quoted are corrected to the second place of decimals, but the means are calculated from results to the third place of decimals which is the accuracy of which the Scholander apparatus is capable. Many of these results are from repeated experiments on individual snails, and these are grouped in the order they were carried out. The temperatures shown in brackets are those of the stock tank immediately before experiments.

Table 11

Analyses of mantle cavity contents

Lymnaea stagnalis

After 30 minutes submersion:

Animal no.	Shell length (mm)	Temperature (°C)	CO_2	O_2	N_2 etc. %ages
1	27.5	17.9 (19.6)	0.56	16.13	83.31
		17.9 (19.6)	0.58	14.41	85.01
		16.9 (21.9)	0.63	14.86	84.51
2	28.0	16.8 (19.6)	0.26	17.29	82.45
		18.5 (21.8)	0.55	15.37	84.08
3	29.0	20.0 (20.2)	0.82	12.43	86.76
		16.9 (19.3)	0.72	15.86	83.41
		20.0 (20.4)	1.17	12.46	86.38
		20.0 (20.0)	0.81	10.71	88.48
4	29.0	18.4 (17.6)	0.58	11.09	88.33
5	29.5	20.1 (19.3)	0.89	13.98	85.13
6	30.0	19.8 (18.6)	0.29	14.59	85.12
7	30.0	19.6 (19.0)	0.37	12.65	86.99
8	31.0	18.0 (19.7)	0.37	15.36	84.27
		19.6 (19.6)	0.99	11.55	87.46
		16.8 (17.9)	0.82	16.71	82.48
9	31.0	18.1 (19.6)	0.71	12.30	86.99
		19.0 (19.5)	0.90	10.23	88.87
		16.3 (19.0)	0.51	14.21	85.28
10	31.0	16.4 (17.0)	0.66	14.93	84.41
11	31.5	18.6 (20.4)	1.43	12.33	86.24
12	32.0	16.7 (18.9)	0.44	17.27	82.29
		18.0 (19.6)	0.52	15.91	83.56
13	32.0	17.7 (19.8)	0.71	12.75	86.55
		20.0 (20.0)	0.54	14.70	84.76
14	32.0	15.4 (19.9)	0.35	16.29	83.36
		20.4 (21.4)	0.66	12.32	87.02
15	32.5	20.0 (20.6)	0.49	13.87	85.64
		20.0 (20.5)	0.72	12.11	87.17
		19.0 (20.5)	0.98	11.65	87.37
		16.3 (17.9)	1.01	12.88	86.11
16	33.0	18.9 (19.7)	0.85	13.59	85.57
17	33.5	20.0 (19.6)	0.60	12.00	87.40
		16.8 (17.0)	0.51	12.31	87.18
		20.0 (19.8)	0.30	14.35	85.35
		17.9 (19.6)	0.63	11.86	87.51
		18.6 (20.0)	0.99	9.78	89.23
		21.4 (21.5)	0.72	11.11	88.17
18	33.5	20.0 (20.0)	1.33	12.40	86.27
		20.4 (20.0)	0.98	10.78	88.24
		20.0 (20.8)	1.04	13.87	85.09
		20.0 (20.9)	0.87	13.73	85.39
		20.0 (20.6)	0.89	9.12	89.99
		20.0 (20.6)	0.99	11.06	87.96
19	33.5	20.0 (19.9)	0.58	12.97	86.45
		16.4 (18.9)	0.62	12.71	86.67
		20.0 (20.5)	0.72	12.11	87.17
		19.7 (20.0)	0.52	14.88	84.59
			CO_2	O_2	N_2 etc.
Averages of 40 analyses:			0.71(2)	13.28(8)	86.00(0)

Table 12

Analyses of mantle cavity contents

Lymnaea stagnalis

After 60 minutes submersion:

Animal no	Shell length (mm)	Temperature (°C)	CO_2	O_2	N_2 etc. %ages
1	28.0	18.3 (20.5)	0.68	10.70	88.62
2	28.5	15.4 (20.0)	0.32	13.88	85.80
		20.0 (21.6)	0.56	13.24	86.19
3	29.0	18.4 (17.6)	0.58	11.09	88.33
4	30.5	17.8 (19.7)	0.64	5.30	94.06
		16.8 (18.0)	0.84	10.56	88.60
		18.2 (19.4)	0.85	3.87	95.29
		20.0 (19.6)	0.62	8.90	90.48
5	32.0	20.0 (19.6)	0.79	7.68	91.53
		20.0 (19.6)	0.71	8.19	91.10
		20.0 (19.2)	0.45	10.68	88.87
6	32.0	18.0 (18.9)	0.53	10.52	88.95
			1.22	6.49	92.29
7	33.5	18.0 (20.4)	0.53	9.04	90.43

	CO_2	O_2	N_2 etc.
Averages of 14 analyses:	0.66(6)%	9.29(6)%	90.03(8)%

Table 13

Analyses of mantle cavity contents

Planorbarius corneus

After 30 minutes submersion:

Animal no.	Shell diam. (mm)	Temperature (°C)	CO_2	O_2	N_2 etc. %ages
1	22.5	19.8 (19.7)	0.68	15.63	83.69
		21.0 (20.0)	0.99	11.93	87.08
		18.3 (17.7)	0.87	14.32	84.81
2	22.5	19.0 (18.3)	0.46	10.63	88.91
3	23.0	18.1 (17.8)	0.56	16.39	83.05
		18.7 (16.9)	0.63	13.77	85.60
		18.2 (17.7)	0.78	16.35	82.88
		19.0 (18.0)	0.82	12.98	86.19
4	25.0	16.7 (19.3)	0.62	15.78	83.60
		17.2 (19.5)	0.83	13.67	85.50
5	27.5	16.8 (20.2)	0.88	17.83	81.29
		17.3 (18.7)	0.96	14.92	84.12
		20.1 (19.1)	0.67	16.32	83.01
6	28.5	17.2 (19.9)	0.53	13.92	85.55
7	29.0	19.0 (20.1)	0.44	15.77	83.80
8	29.0	18.9 (19.3)	0.83	16.12	83.06
9	30.5	18.9 (20.0)	0.44	17.43	82.13
		19.0 (19.8)	0.53	11.69	87.78
		19.6 (20.0)	0.38	12.45	87.17
10	32.0	16.7 (19.8)	0.77	16.36	82.87

	CO_2	O_2	N_2 etc.
Averages of 20 analyses:	0.68(3)%	14.71(3)%	84.60(4)%

Table 14

Analyses of mantle cavity contents

Planorbarius corneus

After 60 minutes submersion:

Animal no.	Shell diam. (mm)	Temperature (°C)	CO_2	O_2	N_2 etc. %ages
1	23.0	18.8 (19.8)	0.74	13.36	85.90
2	23.0	18.4 (17.6)	0.42	10.95	88.63
3	24.5	17.9 (20.1)	0.76	11.25	87.98
4	26.0	20.1 (20.0)	0.67	14.32	85.02
		19.8 (20.0)	0.82	12.96	86.22
5	27.5	17.3 (18.7)	0.74	12.54	86.72
6	29.0	19.1 (19.0)	0.87	10.66	88.47
		20.1 (20.0)	0.42	10.05	89.54

	CO_2	O_2	N_2 etc.
Averages of 8 analyses:	0.67(9)%	12.01(1)%	87.31(0)%

The experimental water temperature, though not controlled, remained within half a degree of that indicated, but was not matched to that of the stock tank. It is more difficult to keep the sampling tube clear in P. corneus, since its mantle cavity has a number of longitudinal folds in roof and floor.

The results show that both species of snails are consuming oxygen from the mantle cavity, but the oxygen percentage falls more rapidly in L. stagnalis than in P. corneus. As shown in the Tables, any individual can consume very different percentages of oxygen over 30 or 60 minutes, even at the same temperature. This does not change consistently with order of experiments, so procedure during the series is not producing this variation. In the earlier weighing experiments, the snails showed variable uptake of gas at the beginning of each submersion. The initial gas volume has been shown to depend on the pre-treatment of each snail, disturbance always causing them to take in more air. This variation in the total amount of oxygen available at the beginning of each submersion obviously affects the time taken to reach any given percentage level. In these gas analysis experiments, the handling of the snails causes increase in their air-store, and therefore these percentage figures at analysis are

certainly higher than would be the case for undisturbed snails. When the present results are compared with those of Precht and Hazelhoff, this argument is confirmed (Table 15).

Table 15

Species	Source	Temp. (°C)	O_2 percentage in cavity a) After 30 min.	b) After 60 min.
L.s.	Hazelhoff	20	12%	7%
	Precht	18.5	5.5%	3%
	"	22.4	3.5%	2%
	Present	15-20	13.3%	9.3%
P.c.	Hazelhoff	20	13%	11.5%
	Present	17-20	14.7%	12.0%

These figures have been extracted from the graphs in Jordan (1922), and Precht (1939) and from Tables 11 to 14 above. Unfortunately Precht does not give comparable figures for P. corneus. For L. stagnalis under the undisturbed conditions which Precht describes, the oxygen percentages have fallen more rapidly than those of the present work, and those of Hazelhoff. The detailed conditions of Hazelhoff's experiments were not recorded by Jordan, but it seems likely that his, like the present snails, had been disturbed before gas uptake.

The pneumostome closes round the sampling tube in an apparently intimate seal. Carbon dioxide accumulation in the mantle cavity would seem more likely under these than under natural conditions, where the gas can be periodically exposed to the water. Despite this, neither species showed any marked accumulation over the 60-minute period. It should be noted that the snails remained moderately active throughout the sampling processes, so there is little likelihood of reduced metabolism affecting the results.

VII. PRESSURE CONDITIONS WITHIN THE MANTLE CAVITY

Variations in gas pressure in the mantle cavity had been noted during the course of the gas analysis experiments. These were recorded and measured using the same technique for inserting fine polythene tubing into the mantle cavity of a snail. The tube was connected in this case to a fine manometer filled with a saturated solution of potassium iodide and mercury biniodide. This has a specific gravity of 2.87, and damped the volume changes of the mantle cavity during observations. The snail in its dish was enclosed in a small pressure chamber, where the ambient pressure could be increased in simulation of increasing depth. Pressure in the chamber was also recorded by a small manometer. Figure 18 shows diagrammatically how the images of these two manometers, one with a red fluid, the other with a green fluid, were simultaneously projected through the slit of a moving-film recording camera loaded with blue sensitive film. Above both manometer menisci, the light can fully expose the film; green light projected alone produces a greyish image on the film; where the red and green are superimposed, the film remains unexposed. Below the manometer traces, a time-marker was used simultaneously to record five second intervals.

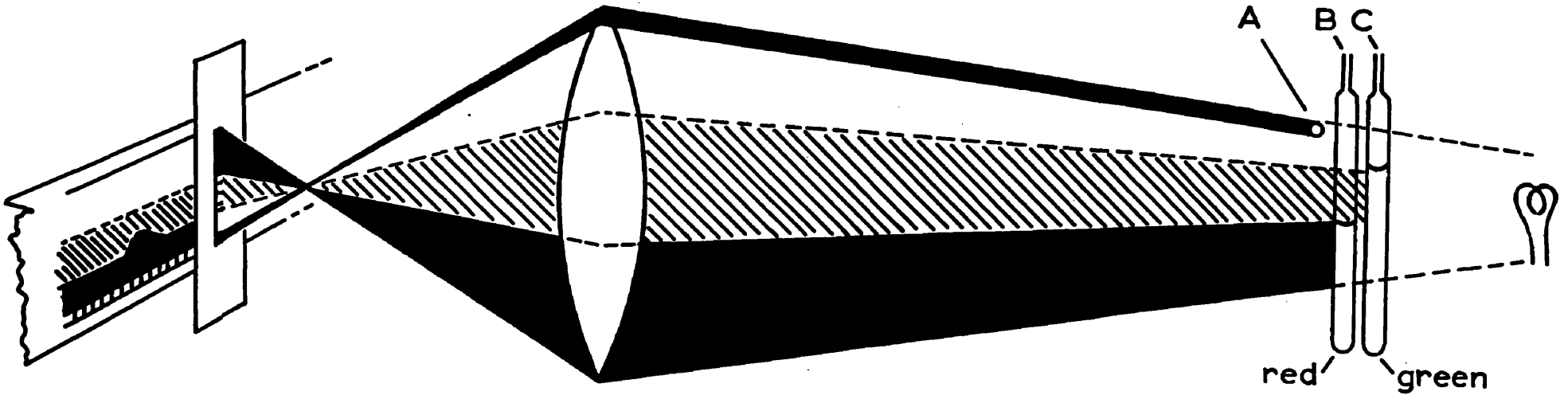


Figure 18. Diagram illustrating principle of pressure recording.
A Time marker arm.
B Proximal limb of one manometer recording external pressure.
C Proximal limb of other manometer recording mantle cavity pressure.

In the upper halves of figures 19 and 20 appear photographs of pieces of developed film (fig. 19 from L. stagnalis; fig. 20 from P. corneus). In these figures, the external pressure is indicated by the upper margin of the grey band; that in the mantle cavity by the white image of the meniscus above the black band. In the tracings forming the lower half of each figure the pressures are plotted in centimetres (of water) from a common base line. The snails always maintain a slight positive pressure in the mantle cavity. Therefore the uppermost pressure record is that from the mantle cavity while the lower, smoother trace is that of the ambient pressure applied. The time scale is the same for both film image and tracing, but the former is graduated in five second intervals and the latter in units of thirty seconds.

The mantle cavity pressures show constant irregular fluctuations, most of them associated with movements of the foot. The snails normally have the gas in their mantle cavities under a minimum positive pressure of some 2 cm (of water) above that of their surroundings. The pressure in the mantle cavity of L. stagnalis can be as much as 7 cm (of water) above the ambient but this is only if strong stimulation of its foot has caused complete retraction into the shell. In P. corneus under similar conditions, a positive pressure of 4 cm (of water) can

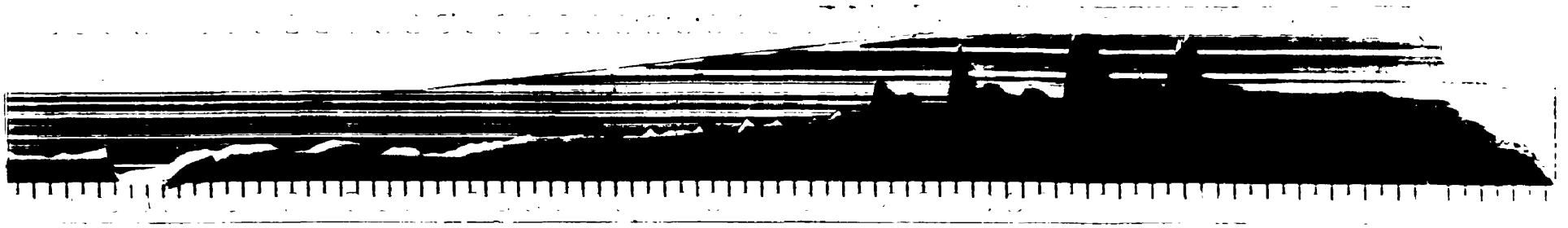


Figure 19a. Photograph of film record of pressure changes in the mantle cavity of L.stagnalis.
(Upper grey trace - external pressure;
lower black trace - mantle cavity pressure)

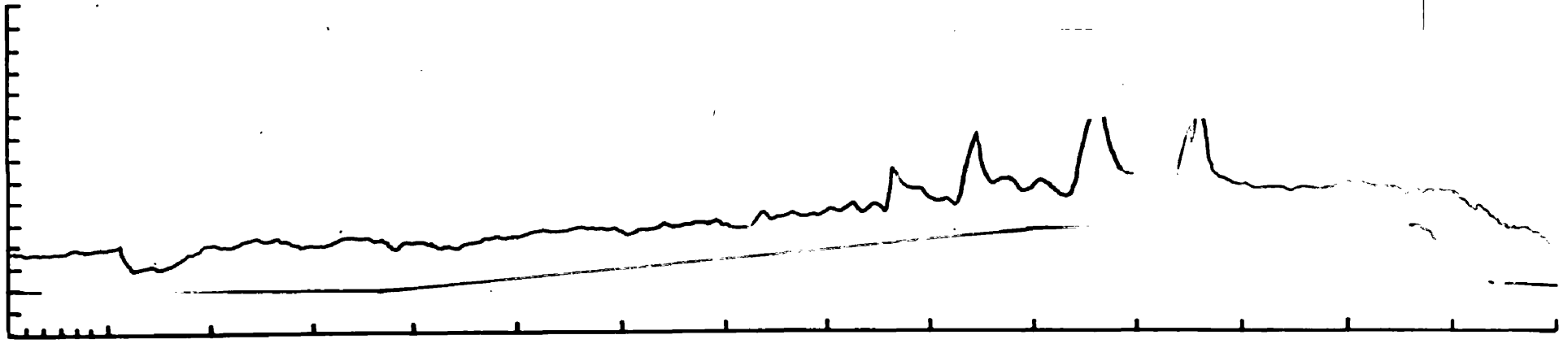


Figure 19b. Tracing of film record of pressure changes in the mantle cavity of L.stagnalis.
(Upper trace - mantle cavity pressures;
lower trace - external pressure)
Ordinate: pressures in cm of water.
Abscissa: 30 second intervals.

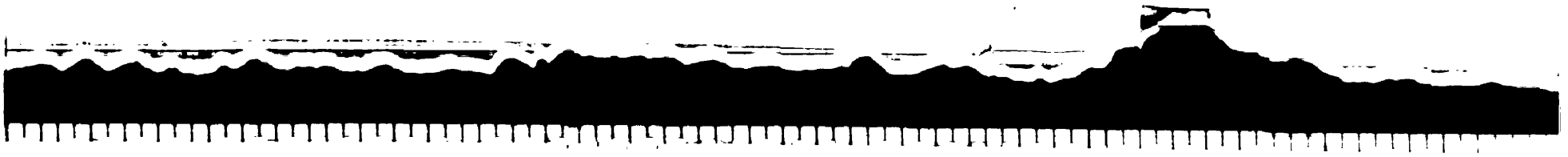


Figure 20a. Photograph of film record of pressure changes in the mantle cavity of P.corneus.
(Upper grey trace - external pressure;
lower black trace - mantle cavity pressure)

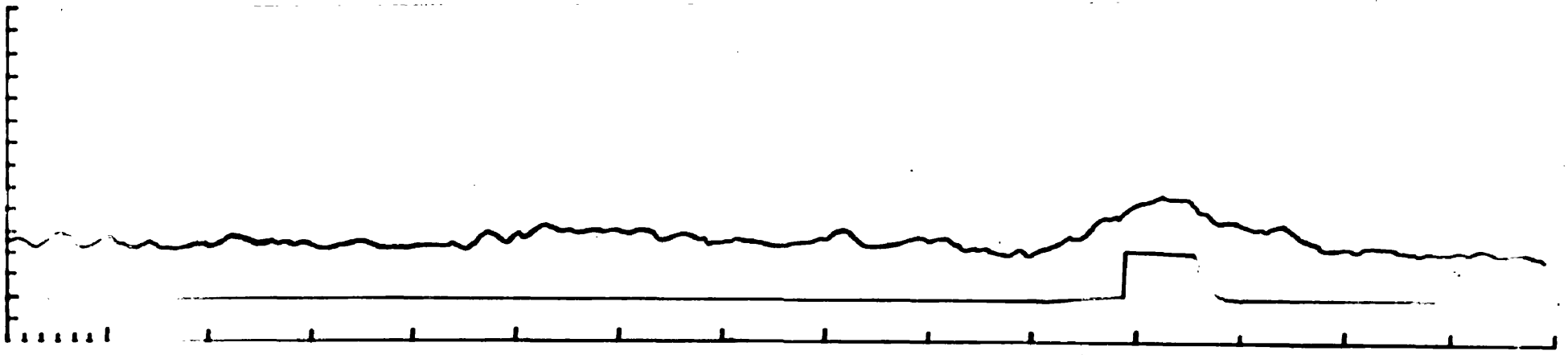


Figure 20b. Tracing of film record of pressure changes in the mantle cavity of P.corneus.
(Upper trace - mantle cavity pressures;
lower trace - external pressure)
Ordinate: pressures in cm of water.
Abscissa: 30 second intervals.

occur, but gas is usually discharged from the siphon before this level of compression is reached.

This apparatus could only record the effects of small pressure changes. Similar experiments, using small rubber diaphragms and mechanical levers to record on a kymograph drum, involved applied pressures equivalent to depths of four feet. These showed that the small excess of pressure was still maintained.

The degree of compression found in neither species is sufficient to alter the buoyancy of the snails. This confirms the underwater weighing results, in which the weight increase was always smooth and uninterrupted.

VIII. SURFACING RHYTHMS

a) Introduction and method

The frequency over a long period with which any snail comes to the surface can only be assessed by recording its behaviour when access to the surface is unhindered. The rates of underwater weight increase of snails' surfacing into an oxygen-rich or oxygen-poor gas phase were found to be predictably increased or decreased (Section V above). Several authors have claimed that under uniform conditions, P. corneus remains submerged twice as long as L. stagnalis (Probst, 1934; Jordan, 1922; Precht, 1939). The details of these experiments are not recorded. Because of sensitivity to handling shown by such snails, it is possible that this species difference reflects experimental conditions rather than a natural difference.

An apparatus was designed to record the surfacing behaviour of snails over long periods, in near natural conditions. Generally, the shallowness of the dish used to house the snails is the most artificial condition met with in laboratory observations. In the field, P. corneus and L. stagnalis have been recorded as occurring in five metres of water in Furesø, Denmark (Wesenburg-Lund, 1917 & 1939), though such a depth would be very unusual in Britain. Such depths are not readily available in the

laboratory but it is desirable to give the snails the maximum vertical range possible if the results are to be related to field conditions.

The experimental tank, made in quarter-of-an-inch perspex, is four feet deep, with an internal section ten inches square. The top of the tank is narrowed and ends in a small chamber four inches high. This chamber measures ten inches by two inches and is further narrowed by a pair of internal plates. These form a steeply pitched inverted-V within which a variable width of air/water interface ten inches long can be contrived. This interface is the only surface available to the snail. A modified high-intensity microscope lamp is arranged to produce a light beam half-an-inch in diameter along the length of this interface. The beam is focussed at the far end on to a phototransistor cell (Mullard OCP 71).

The emitter side of this cell is connected to the +ve side of a 12V dry battery; its base is left unconnected. A 3000 ohm relay (specially made for the purpose by Standard Telephone and Cables Ltd) is connected in series between the collector side of the phototransistor and the -ve pole of the dry battery. A diode (OA 70) is connected in parallel with the relay winding. The current passed by the phototransistor cell, when illuminated by the

uninterrupted light beam was 15 mA. Occlusion of the light beam (even in bright daylight) cut this to 4 mA. The relay only remained closed at an applied current of over 8 mA. The secondary contacts of the relay were used to switch a circuit actuating a barograph pen writing on a kymograph drum, which allowed 36 hours of continuous recording.

By adjustment of the width and vertical position of the light beam, the apparatus could be made to record only those occasions on which a snail came within 2 mm of the interface. Such records do not reveal the actual opening of the pneumostome at the surface. However, the snail cannot ventilate without breaking the light beam and operating the relay. Observation has shown that any approach to the surface which operates the relay is almost always followed by an opening of the pneumostome. The principal disadvantage of the apparatus lies in its inability to deal with more than one animal at a time.

Continuous aeration involved siphoning the water from the bottom of the tank into a filter chamber attached to its side, and air-lifting it back into the top, using standard aquarium equipment. This circulation was arranged so as to leave the recording area undisturbed, and the snail was prevented from entering the necessary holes in the tank sides by plugging them loosely with glass

wool. The tank was filled with water brought from Loch Lomond, and analyses showed that the aeration could keep this water oxygen-saturated for over six months.

b) Results

The results of long-term recordings carried out with this apparatus are shown in figures 21 to 24. The vertical axis in each graph is graduated in hours, periods at the surface being plotted above the horizontal axis, and periods of submersion (dives) below. The figures show the proportion of time spent by any snail in surfacing under different conditions. An inspection of these will reveal the great variety in the lengths of time spent submerged and at the surface, even under unchanging conditions. It must be emphasised that the length of the downward lines represents elapsed time, and does not correspond to any measure of depth of dive as such. These three species can easily reach the bottom of the tank (four feet two inches below the interface) in 20 minutes, while frequently some of the longer dives were spent by the snail moving about on the walls of the tank some twelve inches from the surface. Times spent at the surface do not imply any difficulty in surfacing for the snails inside the angled plates. For each, the width of the air/water interface was carefully adjusted, so that it could freely

bring the pneumostome into contact with the air. Frequently, the snails, having reached the surface, carry out numerous ventilation movements during this time before diving again. Usually the next dive was immediately preceded by one such ventilation.

Radiant heat from the microscope lamp has a very strong attraction for these pulmonates. During preliminary trials of the apparatus, snails frequently spent a whole day in its beam, moving as close as possible to the source. This behaviour was eliminated by placing a Chance ON 22 heat filter glass 3 mm thick in the beam. This cuts out 85% of radiant heat, but passes enough of the visible spectrum to operate the phototransistor.

When a snail is first put into the tank, it usually shows a period of short dives and frequent surfacings. This is shown particularly clearly by one record from L. stagnalis. With this snail (fig. 21), a period of about two days with dives of short duration was followed, after two lengthy surfacings, by a series of dives each of much greater duration ($<$ 18 hours). This series, lasting about a week, encompassed a complete exploration of the depths of the tank. The long surfacings preceding this period mark a clear change in the snail's behaviour, and must be regarded as to some extent preparatory for it.

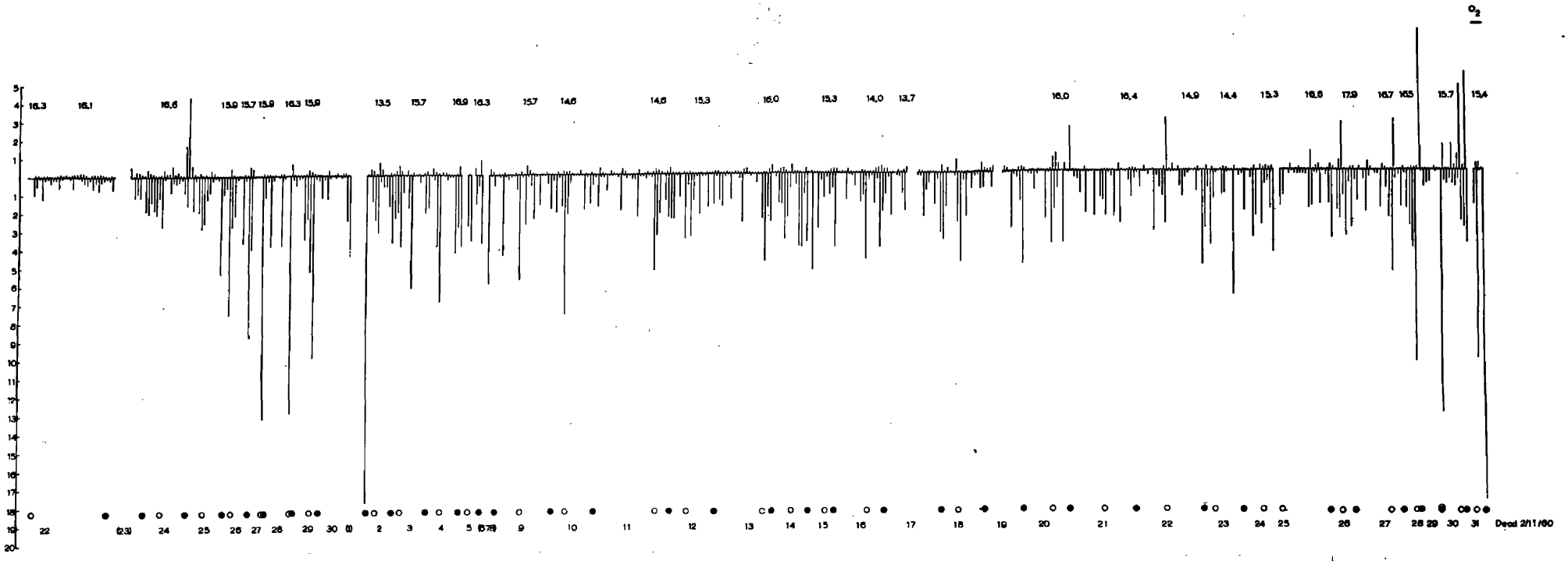


Figure 21. Surfacing/dive recording from L.stagnalis. Duration of surfacings is plotted above, and of dives below, a horizontal base-line. The vertical axis is graduated in hours. Temperature (°C) above record; dates below. (Closed circles - midnight; open circles - noon)

Oxygen administered over period indicated by horizontal line (31 Oct. - 1 Nov.).

It has been shown (figs 12, 13 & 14) that most of the volume increase in the mantle cavity gas at the surface takes place during the first ventilation. Subsequent ventilations add little to this volume, but may, by continually raising the oxygen partial pressures within the cavity, enable the snail to carry out very thorough oxygenation of its tissues preparatory to these prolonged dives.

After exploration of the tank (24 September-2 October), the snail's dives shortened markedly but remained variable and unrelated to time of day. During this part of the recording, the snail was feeding on a growth of filamentous green algae on the tank walls. Subsequently (20 October onwards), as the period of daylight shortened, the algal growth was dying off and the animal was forced deeper into the tank to find food. The algae were also sparser, and for the same amount of food the snail had to move further. In consequence, the duration of dives over this period (22-30 October) became extended. Occasional long surfacings occur during this period. During these, the snail may be involved in repayment of an oxygen debt.

The final part of this record (31 October-2 November), shows the snail's behaviour when the atmosphere into which it surfaced was oxygen-rich. This was contrived by

enclosing the top of the tank in a polythene envelope (ca. 800 c.c. capacity) into which oxygen was released at 250 c.c./minute over the period indicated by the horizontal line in figure 21. Aeration of the water was continued during this period in order to keep the oxygen tension of the water at normal, air-saturated, level. After a dive of about two hours' duration, the snail carried out two lengthy dives, the second of which ended with its death on the bottom of the tank. A relatively small part of each dive was involved in ascent to the surface, although observation of the carriage of the shell and visceral mass showed them to be heavier than water. Over the earlier part of the dive, this snail did not respond to its more rapidly increasing underwater weight. Thus the hydrostatic stimulus was not effective, and therefore it seems that the determining factor for the surfacing pattern is a respiratory one.

Figure 22 shows the results of a second experiment on L. stagnalis. This followed soon after the last, and the food supply had fallen to a low level. The dives of this second snail are initially longer than those of the snail introduced into a tank with a rich algal growth. Over the first day (23-24 December), the dives became progressively shorter as the water temperature rose, and lengthened again

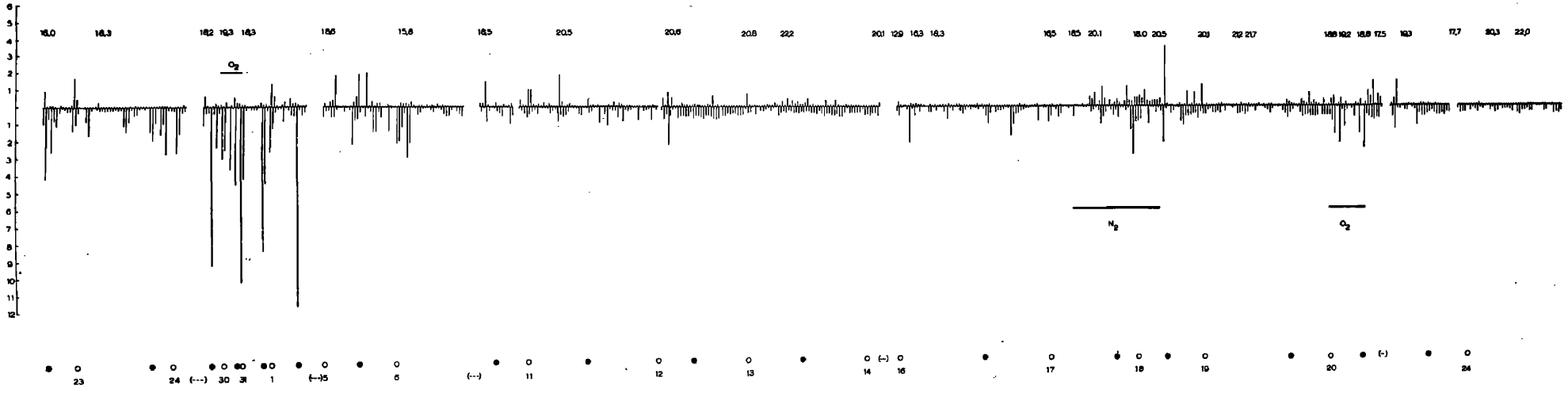


Figure 22. Surfacing/dive recording from L.stagnalis. Vertical axis graduated in hours. Temperatures and dates plotted as in figure 21. Oxygen and nitrogen administered over periods indicated by horizontal lines.

as the temperature fell at night. An oxygen-rich atmosphere over the period indicated by the horizontal line (30-31 December) produced no shortening of the dives during what is clearly still part of the exploratory phase of the snail's behaviour. Indeed, Table 16 shows that the percentage time spent submerged is slightly greater than that before administration of oxygen.

Table 16

Date	Extent of period (minutes)	Gas phase	%age of time submerged	Temp. (°C)
30-31 Dec.	1210	Air	91.36	19.3
31 Dec.	1485	O_2-rich	92.53	19.3
16-17 Jan.	1533	Air	92.10	13.2-18.5
17-18 Jan.	1660	N_2-rich	53.93	18.5-20.9
18-19 Jan.	1644	Air	60.63	20.5-21.7
19-20 Jan.	720	Air	67.94	21.5-18.8
20 Jan.	729	O_2-rich	79.78	18.8-19.2

Food was introduced through the wall of the tank (2 p.m. on 6 January) some twelve inches below the water surface. A shortening of the dives occurred immediately (fig. 22, 6 January). This is not entirely due to the shorter distance between surface and food. Only a small

proportion of total time is spent in ascent and descent, and shorter dives are more likely to result from an increased rate of activity and respiration under these improved conditions of feeding (see Berg and Ockelmann, 1959). At higher temperatures (20 to 22°C on 12-14 January), the lengths of dive approach a more constant value (about 35 minutes). This is still longer than has been suggested before for L. stagnalis.

Similar observations (17-18 January), using a nitrogen-rich atmosphere, showed that while the lengths of dives are not obviously affected, the time spent at the surface is increased. This result shows in Table 16, where the percentage of time spent submerged is markedly less than during similar periods before and after application of the nitrogen. The long surfacing following this treatment seems to imply some respiratory adjustment. Later, application of an oxygen-rich gas phase (20 January) resulted in longer dives, the relevant percentages being quoted in Table 16.

From these results, decreasing buoyancy does not alone initiate surfacing in L. stagnalis. Similar experiments have not yet been conducted on P. corneus, but figure 23 shows a short record from this species under ordinary conditions. It was made after the first L. stagnalis

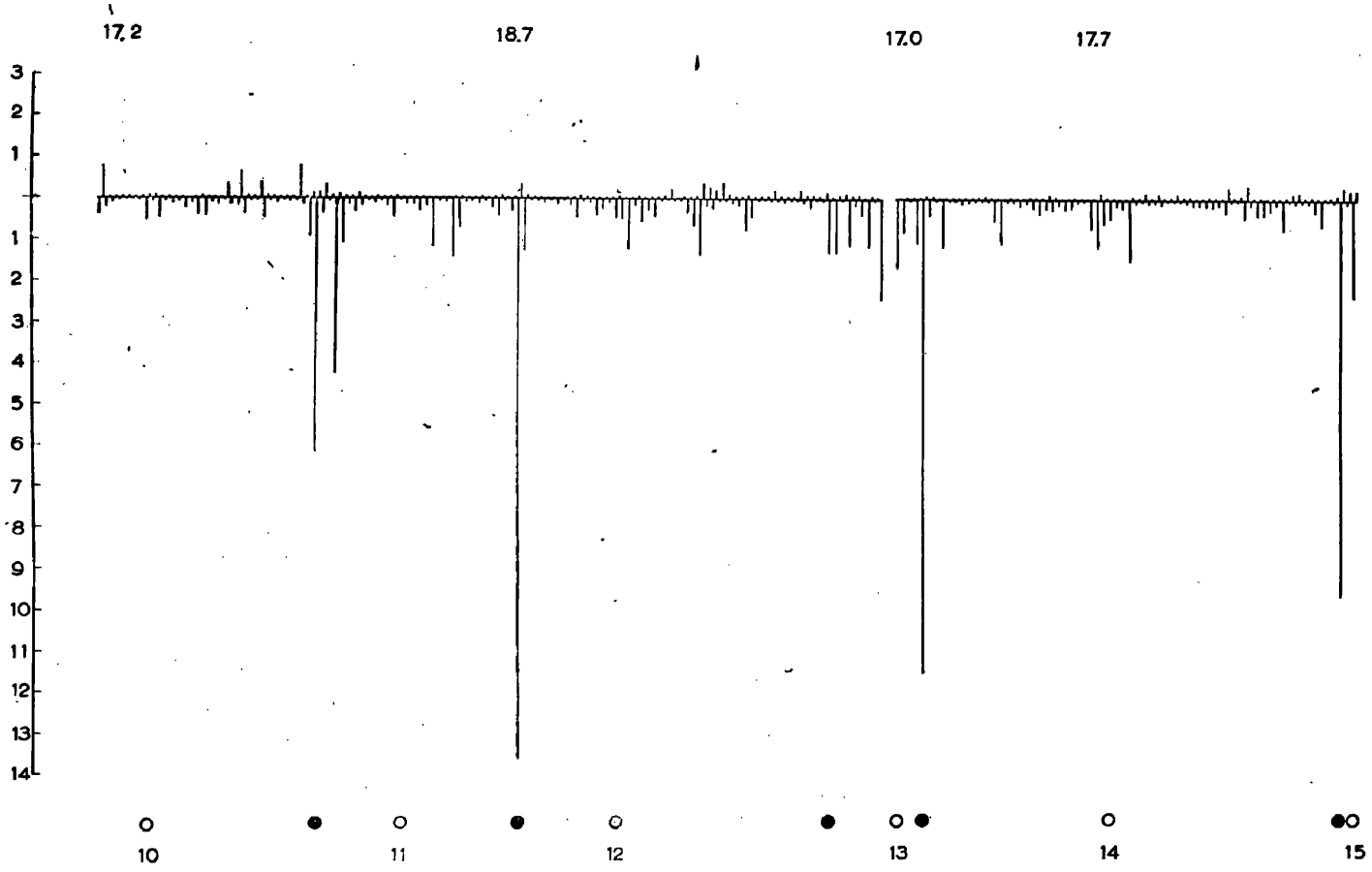


Figure 23. Surfacing/dive recording from P.corneus. Vertical axis graduated in hours; other symbols as in figures 21 and 22.

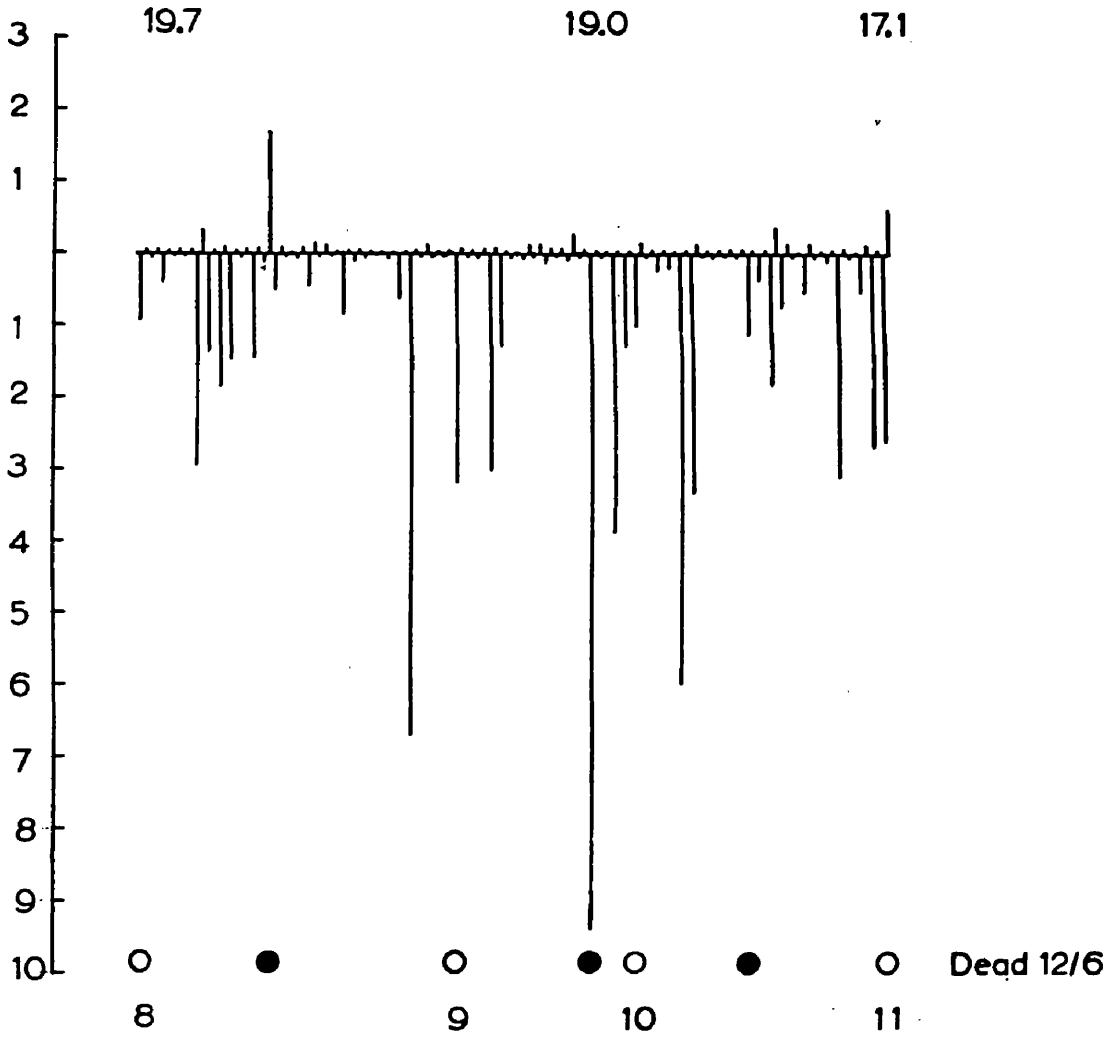


Figure 24. Surfacing/dive recording from L.peregra. Vertical axis graduated in hours; other symbols as in figures 21 and 22.

quoted here, and therefore under poor conditions of feeding. Its dives are shorter than those of L. stagnalis even under these conditions, but it showed a tendency to remain submerged for lengthy periods during the hours of darkness. The great variations in duration of dives make any inter-specific comparisons difficult. A short record obtained from L. peregra (fig. 24) shows that such variations are present here too.

c) Observations on behaviour

Throughout these experiments a number of observations on the behaviour of the snails under water and at the surface have been noted. Opening of the pneumostome is stimulated by contact of the free mantle edge in the region of the pneumostome with a water surface. Other stimuli have been suggested as evoking part of the behaviour at the surface, e.g. deformation of the tentacles by the surface film (cf. Noland and Carriker, 1946; Hunter, 1953b). This mantle stimulus has been used in experiments to open the pneumostome (see SectionVI a) by lowering the water surface on to a snail in the inverted position. On all occasions in these experiments when snails were moving freely, the mantle edge in the pneumostome area was seen to touch and pass through the water surface before opening of the pneumostome started.

The pneumostome of the freshwater pulmonates is a highly mobile tubular structure. At the surface, while closed and still closely applied to the inner side of the mantle, it becomes inflated. Then, sudden release of this pressure is used to penetrate the surface and bring the pallial gas into contact with the atmosphere.

On those occasions when a snail floats to the surface after losing adhesion to the substrate (Section IVa), its pneumostome and shell aperture are still under water, directed downwards. Even without external attachment for the foot, L. stagnalis, L. peregra, L. palustris, and Physa fontinalis can all bring about "rolling" on the longitudinal axis of their shells, until ventilation is possible. This movement is caused by displacement of the mantle cavity gas towards the pneumostome, causing a visible bulging of the mantle cavity floor in this area. In similar circumstances, P. corneus can revolve about a transverse axis to bring the shell aperture to the surface.

Observations were made on several species in the small pressure chamber already mentioned (Section VII). It was found that if the pressure is increased immediately after a normal surfacing of L. stagnalis, L. palustris, L. peregra, Physa fontinalis or Planorbarius corneus, they respond by returning to the surface and re-ventilating. Experiments

by Precht (1939) on L. stagnalis only had already demonstrated this reaction. The pressure increase which was involved in the present work was small (+10 cm Hg) and increased solution of gas in the water need not be considered. The response could be elicited up to five minutes after a surfacing, but later, even high pressures (+40 cm Hg) failed to produce it. These observations were made on snails in two inches of water, and therefore the applied pressure increment can be regarded as constant, which it would not be if the snails were diving into deeper water. If, under increased atmospheric pressure, any snail has been allowed to surface and begin a dive, later release of this pressure when it starts to ascend, does not lead to interruption of its surfacing behaviour. This remains as the fixed and stereotyped movement described by Hunter (1953b).

Normally, for the first fifteen or twenty minutes of a dive, the pneumostome of L. stagnalis, L. palustris, and L. peregra is kept tightly closed. After this first period, especially in small volumes of water with no artificial aeration, the Lymnaeid pneumostome can protrude beyond the edge of the mantle. When protruded it presents a fine slit-like opening on its outer face, through which pallial gas is in contact with the water. The pneumostome of P. corneus is seldom visible under conditions of natural

movement, being hidden between the shell and foot. However, in experimental snails fastened by their shells with the aperture pointing upwards, the pneumostome was frequently seen open under water.

In all species, the area of interface varies during submersion, by extension of the pneumostome and also by changes in the width of the slit. In L. stagnalis, the pneumostome is not continuously open under water, but observed under these laboratory conditions is more often open than closed. In other species under field conditions, Hunter (1953b and unpublished work) noted the pneumostome open under water frequently in L. peregra and L. auricularia, but only very rarely in Physa fontinalis. The stimuli governing the variation in degree of exposure of the pallial gas, and thus its use as a physical gill are unknown. In both L. stagnalis and P. corneus, the rim of the pneumostome and the edge of the mantle in this area are ciliated, and so may be able to create a water current over whatever area of gas is exposed. This will increase the efficiency of the physical gill (de Ruiter, Wolvekamp, Van Tooren, and Vlasblom, 1951).

IX. DISCUSSION

In such relatively slowly moving benthic animals as the pulmonate snails, the degree of penetration to depths in fresh waters will depend on their success in becoming independent of access to the atmosphere. It is clear that a wide variety of natural populations of freshwater pulmonates do not surface regularly to breathe air (Hunter, 1953b, 1957). Further, at low water temperatures in the field (Hunter, 1953a), and in the laboratory (Precht, 1939), aerial respiration is usually abandoned by those pulmonates which surface at higher temperatures. In spite of this, all adult pulmonate snails of any origin can take up rhythmic surfacing under laboratory conditions. Thus knowledge of the stimulus which forces them to the surface is of considerable importance.

Precht (1936) has shown that Planorbid spat are first driven to supplement entirely aquatic respiration and start surfacing by three factors. These are, the size of the snail, the temperature of the water, and the tension of dissolved oxygen. Subsequent surfacings he found to be more frequent at higher temperatures and amongst larger individuals. In this work, and in subsequent more detailed studies, principally on L. stagnalis (Precht, 1939; Precht and Otto, 1948), he has maintained that two factors, the "Füllungsdruck" of the cavity, and the decrease in

oxygen in it, are responsible for terminating dives. Precht uses the former term - Füllungsdruck: the degree of fullness - to mean the extent of inflation of the mantle cavity, which he notes as falling during each dive. As noted in Section VIIIc above, experiments on L. stagnalis, L. peregra, L. palustris, Physa fontinalis and Planorbarius corneus have shown that increasing atmospheric pressure immediately after a surfacing leads them to return to the surface. This agrees with Precht's (1939) observations on L. stagnalis. Both (1934) noted a similar phenomenon in Notonecta glauca, and he ascribed it to perception of increasing specific gravity. The present writer has been unable to confirm Precht's observation on L. stagnalis which he caused to interrupt a surfacing movement by reducing the external pressure as soon as ascent began.

Precht maintained that this behaviour is due to detection of the "Füllungsdruck". He found that attachment of weights to the shell of a snail immediately after surfacing did not lead it to the surface again. Equally, removal of weights during submersion once the snail had turned towards the surface, did not prolong its dive. He rejects the possible hydrostatic stimulus of the submerged gas on these grounds. However, Jacobs (1941) found that attachment of weights to the shell of L. stagnalis did lead this animal to take in more air in compensation. His brief results

suggest that this adjustment is not necessarily immediate, but is perhaps only completed after a number of successive ventilations.

Under laboratory conditions, the present work shows that L. stagnalis and P. corneus are buoyant at the beginning of a dive to such an extent, that if they lost attachment to the substrate, they would immediately float to the surface. Such behaviour has been noted both in laboratory and field studies (Walter, 1906; Dawson, 1911; Alsterberg, 1930; Cheatum, 1934; Jacobs, 1941). In the field the present writer has not seen it in any species other than Physa fontinalis, but in the laboratory it occurs in both L. stagnalis and P. corneus (see Section IVa). In the case of these two species it results from "accidental" detachment from the substrate caused by a snail's moving on to a substrate of light, small, particles. Free ascent of this nature in a large body of water is hazardous, even if attached to the bottom by a thread of mucus in the way described by Dawson (1911) and Cheatum (1934). It could only be indulged in under special conditions, and then only by a minority of pulmonates. Since significant compression of the bubble is not possible in either species (Section VII), buoyancy adjustment by this means, either to prevent flotation from the bottom or to enable a snail to descend

through water without attachment for its foot, is impossible.

Apart from the possibility of providing buoyancy for the whole snail, a smaller gas bubble could provide buoyancy for the shell and visceral mass alone, as distinct from those parts of the snail in contact with the substrate. In both L. stagnalis and P. corneus the gas bubble occupies the most dorsal two-thirds of the outermost (body) whorl of the shell, almost symmetrically about the sagittal plane of the animals. This position is well suited to provide a lifting force which will keep the shell and visceral mass from pressing down on the posterior end of the foot. It is perhaps surprising that P. corneus with a larger proportion of its weight which could thus be supported (Tables 3 and 4), has a lower level of buoyancy. Both these species move with considerably more difficulty when they lack gas in their mantle cavities. Especially on vertical surfaces the visceral mass and shell are dragged after the foot. Spasmodic muscular efforts pull the shell into its normal position with the anterior edge of the aperture immediately above the head region. With increasing weight of the shell and visceral mass after a period of submersion, the necessary adhesive force between the foot of the snail and the substrate must also increase. After treatment at the surface with an oxygen-rich atmosphere, the snail whose surfacing behaviour is recorded in figure 21 had lost its buoyancy by the end of the third dive, and was

unable to crawl upwards on the wall of the tank for more than six inches.

The consistent level of buoyancy maintained by the snails in the experiments above (figs 9 and 10), and the accurate adjustment of the buoyancy in response to loading shown by Jacobs' (1941) observations, imply an accurate sensory appreciation of the buoyancy. In the absence of Jacobs' results, the constant buoyancy could be explained simply as a consequence of a standard extent of inflation of the mantle cavity with air. His results, though few, are convincing, and this simpler explanation cannot be admitted. Detection and adjustment of the buoyancy level could best be carried out by mechano-receptors situated on or near the columella muscle, i.e. in the relatively narrow connection between the foot, adherent to the substrate, and the shell and visceral mass floating in the water. Hunter (1953b) describes in detail the very characteristic pattern of surfacing movements which L. peregra and Physa fontinalis execute before opening the pneumostome to the atmosphere. These decriptions are equally true for L. stagnalis and Planorbarius corneus. The movements involve movement of the shell away from the foot in a sagging motion with considerable stretching of the columella muscle, until the shell and visceral mass are floating at the surface, largely unsupported by the snail's foot. (It was to give freedom

for this movement, that the weighing capsule was made large and kept two-thirds submerged during surfacing of the snail in the weighing experiments.)

Brief histological investigation in the region of the columella muscle using a modification of Schiff's reagent, have so far failed to reveal any sensory apparatus, and no studies seem to have been published. During the present work, attempts were also made to record the action potentials in nerves in this area, but no evidence of sensory action in response to stretching of the columella muscle has been found.

Disturbance during surfacing or prolongation of the time of submersion, will lead to an increased uptake of air (see Sections III and V). This is in agreement with the findings of Precht and Otto (1948). Such an increased volume of air-uptake before a dive, has been shown significantly to increase the time taken for the buoyancy to fall to any given level (see Section V). Also, the increased uptake can significantly increase the amount of oxygen available for this part of the snail's respiration (see Section VIb). The response to agitation of any kind is an uptake of air, a biologically appropriate adjustment where an animal may have to spend a longer time under water than normal.

It is probable that a similar nervous mechanism is

concerned in the initial transfer to aerial respiration which occurs during the growth of many aquatic pulmonates. These snails all hatch with fluid-filled mantle cavities and in suitable habitats, change before reaching adult size (Precht, 1936; Noland and Carriker, 1946; Hunter, 1953a & b). In several habitats, fully-grown adults with water-filled mantle cavities are found, and if disturbed, will adopt surfacing behaviour (Hunter, 1953b). A nervous mechanism sensitive enough to evoke increased air-uptake after a prolonged dive, is even more likely to be concerned in this shift to aerial respiration by adult snails.

Under undisturbed conditions, it is clear that both species investigated regularly restore their buoyancy to a constant level by a standard volume of air-uptake at the surface. As discussed above, from the present results and those of Jacobs (1941), it is most likely that the buoyancy of the shell and visceral mass determines this level of gas-uptake. The subsequent loss of buoyancy is, in part, produced by oxygen absorption for respiratory purposes. This respiration is only part of the total respiratory uptake. Any freshwater pulmonate snail, with a gas bubble in its mantle cavity, can obtain oxygen from the environment in three ways: by aquatic respiration (general cutaneous uptake); by use of the gas bubble as a physical gill; and by direct absorption from the bubble of oxygen taken in at the surface.

Earlier accounts (references below) of respiration in freshwater pulmonates are incomplete and mutually contradictory.

Many workers (Joel, 1919; Borden, 1931; Raffy & Fischer, 1933; Müller, 1943; Füsser & Krüger, 1948; Krüger, 1948; Krywienczyk, 1952) have measured the oxygen uptake by pulmonate snails from their surroundings, the majority being measurements of the total respiratory exchange without attempts to separate the cutaneous and pallial contributions. Borden (1931) gives an average figure for oxygen consumption for P. corneus at 15°C which, if adjusted to allow for the shell, would amount to 52 cu.mm/hr./g of tissue. These were small animals experimented on in 2 c.c. of water under constant agitation, conditions which amount to measurement in moist air. Raffy and Fischer (1933) and Füsser and Krüger (1948) agree that respiratory rates in L. stagnalis and P. corneus are considerably higher when measured in moist air rather than when submerged (L. stagnalis: 136 cu.mm/hr. in air, 26 in water; P. corneus: 96 in air, 15 in water). Therefore Borden's figures are high, so that the store of oxygen in the haemoglobin may last longer than she suggests. Thus significant oxygen storage, denied by Leitch (1916) does occur.

Wide variations in oxygen consumption per gram of tissue occur in the work of Joel (1919), Müller (1943), and

Krywienczyk (1952). For example, Müller records L. stagnalis as consuming from 49.8 to 100 cu.mm/g tissue/hr. Only two sets of workers, Raffy and Fischer (1933), and Füsser and Krüger (1948), have given absolute data on the uptake of oxygen from the mantle cavities alone (in distinction to percentage changes in the gas composition). These figures for actual oxygen consumed, again vary widely, even within individual snails, and range from 10 to 64 cu.mm/hr. for L. stagnalis and from 2 to 36 cu.mm/hr. for P. corneus. The variations are unrelated to the size of the animals (Füsser and Krüger, 1948). Raffy and Fischer (1933) state that the oxygen consumption of L. stagnalis is lower per gram of tissue than that of P. corneus. However, Füsser and Krüger (1948) claim that the respiratory exchange of P. corneus is only some 60% of that of L. stagnalis for animals of the same weight. Recalculation of their figures, allowing 20% for the shell weight of L. stagnalis and 50% for that of P. corneus (see Section IV) reduces the difference between the two species. However, it still appears that P. corneus has the lower rate of oxygen consumption.

Krüger (1948) has shown that oxygen uptake in air for these two species is directly dependent on oxygen pressure in the environment. Only in P. corneus at very low oxygen levels is there any evidence of respiratory independence, i.e. a respiratory rate maintained in spite of falling oxygen

partial pressures. The work of Fournier and Bunschoten (quoted by Jordan, 1930a & b) also seems to show respiratory independence in P. corneus, when the mantle cavity gas has between 17 and 6% of oxygen. Berg and Ockelmann (1959) have measured the oxygen consumption of some freshwater pulmonates with water-filled mantle cavities, and have found most of them to be dependent on the oxygen tension in the water. Some evidence of a critical oxygen level above which there was respiratory independence occurs in Lymnaea auricularia, Myxas glutinosa and Physa fontinalis at between 11 and 13% of the oxygen level produced by air saturation. All these workers comment on the great difficulty in obtaining any standard value for the respiratory rate in pulmonates, which is peculiarly labile under differing environmental conditions such as starvation and mechanical disturbance (references above, see also Precht, 1939; Berg, 1952; Berg, Lumbye and Ockelmann, 1958).

In the course of the underwater weighing experiments (Section V above), it was found that the snails showed gas volume decreases (or weight increases) per hour, which are close to the figures for the known uptake of oxygen from the cavity measured directly. They can be taken as partially produced by this respiratory process. The variations in the rates of gas utilisation shown by weighing, must reflect these similar variations in total oxygen consumption. The

weighing experiments were carried out in controlled conditions, and the variations found seem to originate in differences of individual response. While most of the increase in weight under water may be due to the uptake of oxygen from the cavity, some part of it will be produced if nitrogen diffuses out into the water while the snail is using the bubble as a physical gill. It is difficult to tell to what extent this latter process may be taking place although its occurrence has been demonstrated for both species.

Thus, the work of Section Vd has shown the diffusion of oxygen into the mantle cavity and its subsequent extraction for respiratory purposes (see Results iii).

Also, after ventilation into a nitrogen atmosphere, both species have been shown to use the gas as a physical gill (see Results ii). Further and unequivocally, the long-term weighing experiments (Section Vc) have shown volume changes which can only be explained by loss of nitrogen to the water during use of the gas as a physical gill. Precht (1939) demonstrated the physical gill function of the gas bubble of L. stagnalis by noting an increase in its nitrogen content during submersion after the snails had surfaced into an oxygen atmosphere. Hunter (1953b) has shown that gas-bubbles in some populations of L. peregra and Physa fontinalis

could be used as physical gills, and notes that the pneumostomes of these two species and of L. auricularia can be open under water.

On the other hand, the low oxygen percentages in some examples of L. stagnalis and P. corneus determined by the present writer (Section VI) and Hazelhoff, and Precht's even lower figures for L. stagnalis, suggest that such gas bubbles had been sealed off from the water. Some concentrations of carbon dioxide found during the present studies in L. stagnalis and P. corneus, while not as high as those found by Hunter (1953b) in field analyses from Physa fontinalis, are larger than could occur in gas which had been in contact with water. It has also been shown (Section Vc) that continued use of the gas bubble as a physical gill would lead eventually to its loss. Hunter's discovery of some populations of snails living continuously submerged, yet retaining gas-filled mantle cavities, means that these must have been sealed off from the water. The use of the mantle cavity gas bubble as a physical gill is therefore not obligatory. The factors governing its use are not known, but some of the mechanics of its control have already been noted (Section VIIIc, underwater opening of pneumostome).

Zaijer and Wolvekamp (1958) have shown that L. stagnalis and P. corneus extract oxygen from water to a similar extent.

Their graph of uptake of oxygen from water shows that, over the first three hours, L. stagnalis with air in its cavity uses less oxygen from the water than when it has a water-filled cavity. This period has been noted as the period of most rapid pallial gas consumption (Section V), and during it the gas is being used principally as a direct source of oxygen. The variable amounts of gas present at the beginning of dives are produced by "inhalations" (Tables 5 and 6) greater than any previous authors have suggested (Jordan, 1922 & 1927; Alsterberg, 1930; Precht, 1939).

Analyses of the gas changes expressed as percentage changes during submersion are of only limited value because of the initial volume variation. However, a number of workers have recorded the oxygen percentages in the mantle cavities of these snails at the moment of turning to the surface. P. corneus is recorded as reducing the oxygen level in its store more thoroughly than L. stagnalis before turning to the surface (Probst, 1933; Hazelhoff, 1922). Probst's figures are lower than those of Hazelhoff, but unfortunately neither set of data includes details of temperature or water oxygenation. Precht (1939) has shown that L. stagnalis turn to the surface with a higher oxygen percentage in their mantle cavities in summer than in winter. In winter, he and Otto (1948) have shown that L. stagnalis carries out longer dives and takes up more air at the surface.

This increased uptake seem paradoxical at the lower winter temperatures, but the present studies have shown this to be inevitable since increased uptake is always provoked by extended submersion (Section V). The different oxygen percentages present in the mantle cavities at the moment of turning to the surface in summer and winter, mean a more thorough use of the oxygen in the mantle cavity in summer. Also it implies a greater sensitivity in summer than in winter, to oxygen lack from the mantle cavity.

The surfacing records (Section VIII) show that under the conditions described, the duration of dives by L. stagnalis, L. peregra, and P. corneus is very variable. Viewed against this evidence, the allegation (Jordan, 1922, and subsequent authors) that P. corneus habitually stays submerged for longer periods than L. stagnalis cannot be accepted in such a simple form. The release of surfacing behaviour is complex and must be assumed to be governed centrally. The "surfacing centre" is clearly subject to the influence of many environmental factors. The hydrostatic significance of the gas bubble to L. stagnalis and P. corneus at the surface has been demonstrated. Both species can detect and use the buoyancy level of the shell and visceral mass to determine the volume of air-uptake.

The surfacing observations on L. stagnalis have shown

that more rapid underwater weight increase with an oxygen-filled mantle cavity need not compel this species to surface more frequently (figs 21 and 22). From these latter experiments it is clear that response to the stimulus of buoyancy loss during a dive is not obligatory in L. stagnalis.

Von Brand, McMahon and Nolan (1955) have noted varying concentrations of anaerobic metabolites in snails under normal, aerobic, conditions. They believe these were caused by "some unrecognised factor in handling the snails prior to the experiments (aeration of the aquaria, food?)". In the present experiments, application of an oxygen-rich atmosphere may thus be expected to have a profound effect on the metabolic state of the tissues. Normally, the buoyancy loss in the course of each dive releases, through the surfacing centre, the onset of the surfacing pattern, but the threshold for this response can vary. As a hypothesis, it is suggested that the response is inhibited by more thorough oxygenation at the beginning of each dive. Thus, thorough oxygenation of the tissues produced by repeated surfacing into an oxygen-rich atmosphere could inhibit the onset of the surfacing behaviour in spite of more rapid underwater weight increase (fig. 22). A nitrogen-filled mantle cavity produces a more persistent buoyancy, but in this case, the lower level of oxygenation of the tissues may make the surfacing centre more immediately sensitive to the hydrostatic

stimulus.

Also acting on the surfacing centre will be such drives as the necessity for finding food. Responses to the interaction between this and the respiratory needs have already been demonstrated in L. peregra, which can be forced away from its food supply temporarily during high summer temperatures (Hunter, 1953a).

Under natural conditions, the hydrostatic stimulus arises regularly during each dive, but its releasing action on the surfacing centre may be inhibited by the supply of oxygen along the cutaneous and pallial routes. In P. corneus, the cutaneous supply is helped by the presence of the secondary "gill" with its complete afferent and efferent circulation (Pelseneer, 1895; Boettger, 1944 & 1948). The apparent greater resistance to forced submersion shown by P. corneus compared with Lymnaeids (Alsterberg, 1930; Raffy and Fischer, 1933; Precht, 1939) could reflect very different metabolic responses to anaerobiosis, rather than greater ability for extraction of oxygen from the water (von Brand, Baernstein, and Mehlman, 1950; von Brand, and Mehlman, 1953; Zaijer and Wolvekamp, 1958).

Under conditions of poor water oxygenation, the greater part of the suppression of the action of the surfacing centre will depend on the pallial supply. If the gas bubble in the

mantle cavity is exposed to the water to act as a physical gill, gas escapes into the water, and both the underwater weight and the stimulus resulting from it will increase more rapidly. When the water is well oxygenated, it is possible that the gas bubble is not so used, and that the effects of smaller buoyancy changes of a closed cavity are more easily inhibited in their action on the surfacing centre by the oxygen supplied by the cutaneous route alone. Such semi-permanent inhibition must be the condition in snails living continuously submerged with gas-filled mantle cavities.

In the later stages of dives, extraction from the cavity is unlikely to contribute a significant supply of oxygen, because of the low efficiency of the blood pigments. These can only supply oxygen to tissues which are themselves at low oxygen tensions (Redfield, 1933; Munro Fox, 1945; Zaijer and Wolvekamp, 1958).

As snails move into increasing depths, the gas in their mantle cavities is compressed (Section VII) and their buoyancy decreased. This might be expected to evoke the surfacing pattern, but if the oxygen supply is maintained at a high level, this behaviour can be inhibited. If continued oxygenation of the tissues involves the use of the gas bubble as a physical gill in addition to the cutaneous supply, the gas bubble will eventually be lost. Consequently the

water-filled condition of the mantle cavity could be adopted. The effects of the stimulus of buoyancy loss on the surfacing centre will continue to be inhibited by an ample supply of oxygen from the water. Either falling oxygen tensions in the water or an increase in the hydrostatic stimulus which may be caused by mechanical disturbance, will lead to onset of surfacing behaviour. Increased oxygen demand, for instance during reproductive activity (Berg, Lumbye and Ockelmann, 1958), may also upset the respiratory balance and thus remove the inhibition of the hydrostatic stimulus to surfacing.

In such a multifactorial system, it is impossible for investigation of only one of the factors to yield a complete explanation of the variable behaviour and consequent distribution of these snails. This is especially true of laboratory studies on their respiratory rates. The present study has shown the pallial contribution to the total respiratory exchange to be very variable and easily disturbed. In view of this, any general theories based on data from respiratory rates of these animals must be qualified by the extreme variability shown. In many laboratory studies on respiration, earlier workers have noted such variations, but have ignored them in subsequent discussion of their results. Variability of response seems to be characteristic of the Basommatophora. In natural populations of these pulmonates, field studies have shown physiological variations occurring

in respiration (Hunter, 1953b, 1957), and in growth and reproduction (Hunter, 1961a & b). Such physiological and behavioural plasticity must be of great biological advantage in the relatively unstable environment provided by most bodies of fresh water.

X. ACKNOWLEDGEMENTS

These studies have all been carried out in the Department of Zoology of the University of Glasgow and I am grateful to Professor C.M. Yonge, C.B.E., F.R.S., for the facilities and encouragement he has given me. During the course of the research and the production of this thesis I have received much encouragement and valuable guidance from Dr W. Russell Hunter for which I am very grateful.

Over the period October, 1955 to December, 1956, I received the Vans Dunlop Scholarship in Natural History and Zoology from the University of Edinburgh, which was augmented by a Research Scholarship from the Carnegie Trust for the Universities of Scotland. To both these bodies I must express my thanks for their help.

My thanks are also due to Mr J. Geekie of the Department of Zoology for carefully fabricating to my design, the perspex tanks used in the surfacing experiments.

XI. SUMMARY

1. A study has been made of the function of the gas bubble in the mantle cavities of Lymnaea stagnalis and Planorbarius corneus by various techniques including gas analysis and underwater weighing.

2. The volumes of enclosed gas are variable, but under laboratory conditions, are always sufficient to make snails of both species buoyant immediately after they have ventilated at the surface.

3. P. corneus, with a proportionately heavier shell, is less buoyant than L. stagnalis under the same conditions.

4. Both species lose their buoyancy rapidly over the first three hours of submersion by a process reducing the gas volume. Gas analysis has shown this to be due, in part, to the extraction of oxygen for respiratory purposes. There is no evidence of significant accumulation of carbon dioxide in the cavity of either species.

5. Fluctuations in gas pressure within the cavity are too small to produce variations in any snail's buoyancy.

6. The use of the cavity as a physical gill under certain conditions has been confirmed by experiment and observation.

7. It appears that the buoyancy of the shell and visceral mass determines the amount of gas-uptake during ventilation at the surface.

8. Any prolongation of dive, and any interference with the snails, leads to an increased uptake of gas at the next surfacing.

9. Decreasing buoyancy need not determine the onset of the surfacing pattern in L. stagnalis.

10. Both species have an equal capacity for lengthy periods of submersion. Great variation in the duration of dives is shown under laboratory conditions, and they can be much longer than has previously been suggested.

11. It is concluded that the stimulus evoking the surfacing behaviour to end a dive is a hydrostatic one, but the expression of this is dependent on its interaction with other factors, including the pallial and cutaneous respiratory supplies.

XII. REFERENCES

ALSTERBERG, G., 1930. Wichtige Züge in der Biologie der Süsswassergastropoden. Lund.

BERG, K., 1952. "On the oxygen consumption of Ancylidae (Gastropoda) from an ecological point of view." Hydrobiologia, 4, 225-267.

BERG, K., LUMBYE, J. & OCKELMANN, K.W., 1958. "Seasonal and experimental variations of the oxygen consumptions of the limpet Ancylus fluviatilis (O.F. Müller)." J. exp. Biol., 35, 43-73.

BERG, K. & OCKELMANN, K.W., 1959. "The respiration of freshwater snails." J. exp. Biol., 36, 690-708.

BOETTGER, C.R., 1944. "Basommatophora" in Tierwelt Nord- und Ostsee, 9, 241-478. Leipzig.

---------- 1948. "Die Luftatmung der Basommatophoren in Hinblick auf die Verwandtschaftbeziehungen dieser Tiere." Verh. dtsch. zool. Ges., Kiel 1948; in Zool. Anz. Suppl. 13, 213-219.

BORDEN, M.A., 1931. "A study of the respiration and of the function of haemoglobin in Planorbis corneus and Arenicola marina."
J. mar. biol. Ass. U.K., 17, 709-738.

BOTH, M.P., 1934. "Die Regulation des Luftschöpfens bei Notonecta glauca L."
Z. vergl. Physiol., 21, 167-175.

BRAND, T. von, BAERNSTEIN, H.D. & MEHLMAN, B., 1950. "Studies on the anaerobic metabolism and the aerobic carbohydrate consumption of some fresh water snails."
Biol. Bull., Woods Hole, 98, 266-276.

BRAND, T. von & MEHLMAN, B., 1953. "Relations between pre- and post-anaerobic oxygen consumption and oxygen tension in some fresh water snails."
Biol. Bull., Woods Hole, 104, 301-312.

BRAND, T. von, McMAHON, P. & NOLAN, M.O., 1955. "Observations on the post-anaerobic metabolism of some fresh water snails."
Physiol. Zoöl., 28, 35-40.

CHEATUM, E.P., 1934. "Limnological investigations on respiration, annual migratory cycle, and other related phenomena in fresh-water pulmonate snails."
Trans. Amer. micr. Soc., 53, 348-407.

CRISP, D.J. & THORPE, W.H., 1948. "Water-protecting properties of insect hairs." Trans. Faraday Soc. Discussions, 3, 210-220.

DAWSON, J., 1911. "The biology of Physa." Behav. Monogr., 1, No. 4.

EGE, R., 1918. "On the respiratory function of the air stores carried by some aquatic insects (Corixidae, Dytiscidae and Notonecta)." Z. allg. Physiol., 17, 81-124.

FOX, H. Munro, 1945. "The oxygen affinities of certain invertebrate haemoglobins." J. exp. Biol., 21, 161-165.

FÜSSER, H. & KRÜGER, F., 1951. "Vergleichende Versuche zur Atmungsphysiologie von Planorbis corneus und Limnaea stagnalis (Gastropoda Pulmonata)." Z. vergl. Physiol., 33, 14-52.

HAZELHOFF, E.H., 1922. Unpublished work quoted by Jordan (1922), q.v.

HUNTER, W. Russell, 1953a. "On migrations of Lymnaea peregra (Müller) on the shores of Loch Lomond." Proc. roy. Soc. Edinb. (B), 65, 84-105.

---------- 1953b. "The condition of the mantle cavity in two pulmonate snails living in Loch Lomond." Proc. roy. Soc. Edinb. (B), 65, 143-165.

HUNTER, W. Russell, 1957. "Studies on freshwater snails at Loch Lomond."
Glasg. Univ. Publ., Stud. Loch Lomond, 1, 56-95.

---------- 1961a. "Annual variations in growth and density in natural populations of freshwater snails in the West of Scotland."
Proc. zool. Soc. Lond., 136, 219-253.

---------- 1961b. "Life cycles of four freshwater snails in limited populations in Loch Lomond, with a discussion of infraspecific variation."
Proc. zool. Soc. Lond., 137, (in press).

JACOBS, W., 1941. "Die hydrostatische Bedeutung der Atmungsorgane von Wassertiere."
S.B. Ges. Morph. Physiol. München., 50, 45-52.

JOEL, A., 1919. "Über den Einfluss der Temperatur auf den Sauerstoffverbrauch wechselwarmer Tiere."
Hoppe-Seyl. Z., 107, 231-263.

JORDAN, H.J., 1922. "Ueber Tiere mit inkonstanter Alveolärer Gasspannung."
Bijdr. Dierk., 22, 125-132.

---------- 1927. "Übungen aus der Vergleichenden Physiologie."
Springer Verlag, Berlin.

JORDAN, H.J., 1930a. "Le réglage de la consommation de l'oxygène chez les animaux à "tension gazeuse alvéolaire inconstante"."
Arch. néerl. Physiol., 15, 198-212.

---------- 1930b. "Tiere mit 'inkonstanter Lungengasspannung' und die Bedeutung limitierender Faktoren."
Naturwissenschaften, 18, 607-613.

KRÜGER, F., 1948. "Zur Physiologie des Haemoglobins wirbelloser Tiere."
Verh. dtsch. zool. Ges., Kiel 1948; in Zool. Anz. Suppl. 13, 213-219.

KRYWIENCZYK, J., 1952. "Körpergrösse, O_2-Konsum, und Kriechgeschwindigkeit bei Wasserpulmonaten."
Z. vergl. Physiol., 34, 14-19.

LEITCH, I., 1916. "The function of haemoglobin in invertebrates with special reference to Planorbis and Chironomus larvae."
J. Physiol., 50, 370-380.

MÜLLER, I., 1943. "Die Abhängigkeit des Stoffwechsels von der Körpergrösse und der Zusammenhang von Stoffwechseltypen und Wachstumtypen."
Riv. Biol., 35, 48-95.

NOLAND, L.E. & REICHEL, E., 1943. "Life-cycle of Lymnaea stagnalis completed at room temperature without access to air."
Nautilus, 57, 8-13.

NOLAND, L.E. & CARRIKER, M.R., 1946. "Observations on the biology of the snail Lymnaea stagnalis appressa during twenty generations in laboratory culture."
Amer. Midl. Nat., 36, 467-493.

OLDHAM, C., 1934. "Further observations on the influence of lime on the shell of snails."
Proc. malac. Soc. Lond., 21, 131-138.

PELSENEER, P., 1895. "Prosobranches aériens et Pulmonés branchifères."
Arch. Biol., Paris, 14, 351-393.

PRECHT, H., 1936. "Beginn der Luftatmung bei Planorbiden und deren Abhängigkeit von äusseren Faktoren."
Zool. Anz., 115, 113-125.

---------- 1939. "Lungenatmung der Süsswasserpulmonaten."
Z. vergl. Physiol., 26, 696-739.

PRECHT, H. & OTTO, E., 1948. "Hat die Lunge der Süsswasserpulmonaten hydrostatische Bedeutung?"
Verh. dtsch. zool. Ges., Kiel 1948; in Zool. Anz. Suppl. 13, 213-219.

PROBST, G., 1933. "Über den Sauerstoffverbrauch in der Lunge von Planorbis corneus und Limnaea stagnalis und die Bedeutung des Haemoglobins fur Lungenatmung von Planorbis."
Verh. schweiz. naturf. Ges., 114, 386-387.

RAFFY, A. & FISCHER, P.H., 1933. "Utilisation de l'oxygène gazeuse et de l'oxygène dissous chez les Pulmonés aquatiques."
C.R. Soc. Biol., Paris, 112, 782-784.

REDFIELD, A.C., 1933. "The haemocyanins."
Biol. Rev., 9, 175-212.

ROSZKOWSKI, W., 1914. "Contributions à l'étude des Limnées du Lac Leman."
Rev. suisse Zool., 22, 457-539.

RUITER, L. de, WOLVEKAMP, H.P., VAN TOOREN, A.J. & VLASBLOM, A., 1951. "Experiments on the efficiency of the "physical gill" (Hydrous piceus L., Naucoris cimicoides L., and Notonecta glauca L.).
Acta physiol. pharm. néerl., 2, 180-213.

SCHOLANDER, P.F., 1947. "Analyzer for accurate estimation of respiratory gases in one half cubic centimetre samples."
J. biol. Chem., 167, 235-250.

WALTER, H.E., 1906. "Behaviour of the pond snail Lymnaea elodes, Say."
Cold Spr. Harb. Monogr., 6, 1-35.

WESENBURG-LUND, C., 1917. "Furesøstudier. En bathymetrisk-botanisk-zoologisk Undersøgelse af Mølleaaens Søer."
K. danske vidensk. Selsk., 8th Series, 3, 1-208.

---------- 1939. "Biologie der Süsswassertiere (wirbellose Tiere)." Berlin.

ZAIJER, Joh. J.P. & WOLVEKAMP, H.P., 1958. "Some experiments on the haemoglobin-oxygen equilibrium in the blood of the ramshorn (Planorbis corneus L.)."
Acta Physiol. pharm. néerl., 7, 56-77.