Oxygen consumption and responses of the freshwater snail *Bulinus* (*Physopsis*) *globosus* to gradients of different oxygen tensions

W.J. van Aardt and B.J. Frey
Department of Zoology and Snail Research Unit of the SAMRC, Potchefstroom University for CHE, Potchefstroom

A gradient or choice apparatus, based on the 'fluvarium' principle and suitable for testing the responses of the freshwater snail *Bulinus (Physopsis) globosus* to different partial oxygen tensions was used. In a gradual oxygen gradient established with this apparatus, *B. (P.) globosus* shows a significant preference for oxygenated water. In experiments with a choice between low and high partial oxygen tensions, *B. (P.) globosus* shows a greater preference for oxygenated water. Oxygen consumption rates vary considerably among individuals and decreases when lowering the P_{O_2} in the water.

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Die gedrag van die varswaterslak *Bulinus (Physopsis) globosus* in 'n suurstofgradiënt en 'n keuse tussen lae en hoë suurstofspanning is in die laboratorium ondersoek. Die 'fluvarium'-metode is hiervoor gebruik wat die voordeel het dat die gradiënt reghoekig te lê kom met die rigting van die watervloei. Die resultate toon dat hierdie metode nie net geskik is om suurstofvoorkeur by die slak te toets nie, maar dat *B. (P.) globosus* 'n duidelike voorkeur het vir hoë suurstofspanninge in die water. Ook is die suurstofopname-koers vasgestel by verskillende P_{O_2}-waardes in die water. Dit is gevind dat suurstofverbruik slegs beïnvloed word by suurstofspanninge wat laer as 60 mm Hg is.

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Among the various problems concerning the reactions of freshwater snails to environmental factors, the response and behaviour to oxygen differences in the water is of special interest. Knowledge of this physical factor on the snail will contribute to the ecological understanding of the snail's distribution pattern in a particular habitat or to learning more of its geographical distribution. These pulmonates take up oxygen not only through the body surface but also through the well vascularized mantle cavity by way of the enclosed air bubble. Furthermore some basommatophorans such as *Bulinus* (*Physopsis*) *globosus* have a conspicuously high haemoglobin concentration in their blood as well as the added advantage of an extensive skin fold, the prosobranch, to enhance the gaseous exchange between the animal and the surrounding water.

Experiments testing the reactions of aquatic animals to gradients and to choices of water of different dissolved oxygen content have been carried out by several workers (Gamble 1971). Two basic methods were employed to create stable gradients and oxygen choices. The fluvarium method proposed by Höglund (1951) was used in this study for it has several important advantages over other methods (Gamble 1971).

As far as we can ascertain from the literature no such gradient work has been carried out in the laboratory for *B.* (*P.*) *globosus.* To evaluate the results obtained from the gradient and choice experiments, measurements were also made on the oxygen consumption rate and dive frequencies at different oxygen tensions of the water.

Materials and Methods

The snails used were obtained from domesticated stocks reared by the Bilharzia Field Unit of the SAMRC at Nelspruit, Transvaal. Batches were forwarded to Potchefstroom on a regular monthly basis during 1973-1974. Before starting the experiments the snails were kept in the laboratory for at least one week in well aerated snail aquaria by the methods described by Van der Schalie and Berry (1972) modified by Jennings (1976). The temperature of the water was regulated at 26 °C (±1 °C). In all subsequent experiments snails were exposed to this temperature. The exposure of *B.* (*P.*) *globosus* in the laboratory to oxygen gradients and oxygen choices was done by

W.J. van Aardt*
Department of Zoology, Potchefstroom University for CHE, Potchefstroom 2520, South Africa
and B.J. Frey
Snail Research Unit of the South African Medical Research Council, Potchefstroom University for CHE, Potchefstroom 2520, South Africa
*To whom all correspondence should be addressed

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the fluvarium method (Höglund 1951) where the direction of the water flow is at right angles to the oxygen gradient. The advantages of this method compared to other methods were discussed by Gamble (1971) who used the method in his experiments on marine amphipods. He gave a detailed description of the construction of the oxygen gradient and choice boxes. In our experiments similar boxes were made out of perspex. All the dimensions were the same as those given by Gamble except the height of the box which was enlarged from 1,25 to 2,50 cm. Similarly the gradient and choice chambers (each 23 cm long) were enlarged from 1,25 to 2,50 cm square. In Fig. 1 the gradient apparatus is diagrammatically depicted to show the direction of the oxygen gradient in comparison with the direction of the water flow.

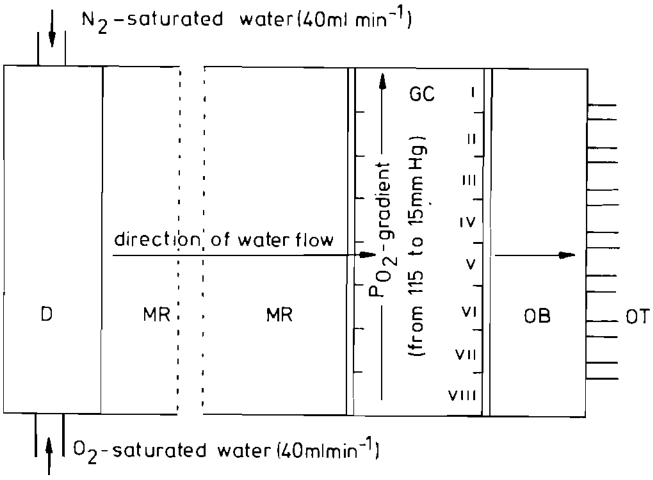

Fig. 1 Top view of gradient apparatus. D{ Distributor, showing arrangement of the two supply tubes. GC: Gradient chamber. MR: Mixing region filled with glass balls. OB: Outflow baffle, showing arrangement of outflow tubes, OT. Snails were placed between zones IV and V before securing the water-tight lid.

The gradient and choice chambers were, for each box, divided into eight equal zones (I – VIII) to assist the positioning of the snails during the tracking observations. The de-oxygenated and oxygenated water streams each flowed at 40 ml per minute into the distributor part of the box. These were prepared by using a counter-flow system of gases (N_2 and air) and water through two vertical perspex columns with an internal diameter of 4,0 cm and 120 cm long. They were filled with ceramic Rachigrings to enable thorough mixing of the gases with water. Gas rotameters and waterpumps were used to control the water flow (40 ml min^{-1}), nitrogen (8 l min^{-1}) and air (1,5 l min^{-1}) through the columns. The water used during the experiments was taken from the aquaria where the snails were kept. The oxygen consumption of full-grown snails was measured at four P_{O_2} tensions of the water medium. The apparatus used was a Gilson respirometer (Model GR. 14, Gilson Medical Electronics, USA). The respiration chambers were modified to measure the oxygen consumed at four experimental oxygen tensions in the water (Fig. 2). The glass vial was filled with 5 ml aquarium water and aeration was applied for 30 min whereafter the gas supply was closed. After an adaptation period of 30 min for temperature and pressure equilibrium, the respiration chambers were opened to the manometers to start the measurements on oxygen consumption.

The decrease in the oxygen tension in a closed water volume

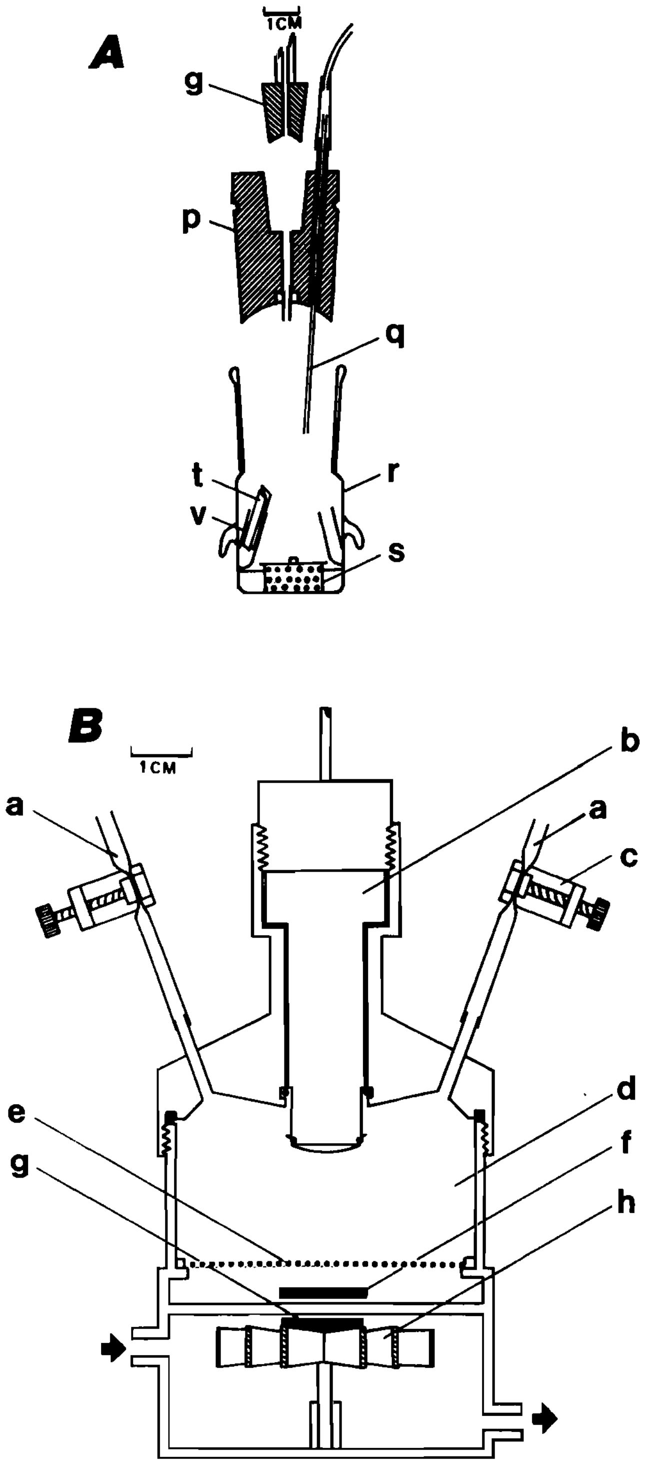

Fig. 2 Oxygen consumption measurements.

A – Exploded view of the respiration chamber used to measure the oxygen consumption rate of snails at various O_2 tensions of the water.

g: Glass coupling from the Gilson-respirometer.
p: Perspex bung to accommodate the respiration vial and bubbler.
q: Bubbler.
r: Respiration vial with two cups (v) supporting the KOH-impregnated filter paper.
s: Perforated container with lid to confine the snail to be measured.

B – Diagram of the respiration chamber used to measure oxygen consumption of snails in a closed-off volume of water.

a: Water refilling and bleeding ports.
b: Clark O_2 electrode.
c: Clamp.
d: Animal chamber.
e: Wire-mesh for support of the snails.
f: Stirrer.
g: Magnet
h: Water turbine.

caused by a group of snails, was determined by means of a Clark-oxygen electrode (Radiometer, Denmark) fixed into a gas-tight perspex container that could be filled completely with 44,5 ml aquarium water (Fig. 2). Continual stirring of the water was accomplished by using a magnetic stirrer driven by a water turbine. To prevent the snails from coming into contact with the stirrer a stainless steel wire mesh was inserted above the stirring bar. The sealed-off chamber, enclosing most of the electrode, was submerged in a water-bath and an immediate recording was made of the oxygen consumption rate with a pen recorder.

Experiments

Oxygen gradient experiments

Forty snails were taken from the aquaria and divided into two equal groups. Snails from Group A were allowed to fill their mantle cavities with air and two snails at a time were carefully placed in the gradient chamber between zone IV and zone V. The lid was tightly secured and the snails allowed to crawl in any direction. For three hours the position of each snail was noted at 30 s intervals. An accurate estimate of the time spent in a particular zone was obtained by counting the number of 30 s occurrences in each zone. This was repeated for Group B. However, their gas bubbles were removed under water by light mechanical stimulation of the foot surface. Snails that did not show any crawling activity were discarded. Most snails, however, moved freely and more than 80% reached zones I or VIII during the three hour observation period. The results are depicted in Fig. 3. Snails of both groups had a very distinct preference for zones V through VIII having a P_{O_2} gradient from about 60 to 115 mm Hg. For instance the total staying time for the three hours was 55% for snails without a gas bubble and 59% for snails with gas in the mantle cavity. The histogram (Fig. 3) shows no real differences in preference for a particular zone. It is noteworthy that snails of both groups, when moving from zones of high to low oxygen tension (VIII to V) moved much more slowly. During their stay in these zones crawling was also slow. This behaviour was reversed when snails moved from lower to higher P_{O_2} tension zones.

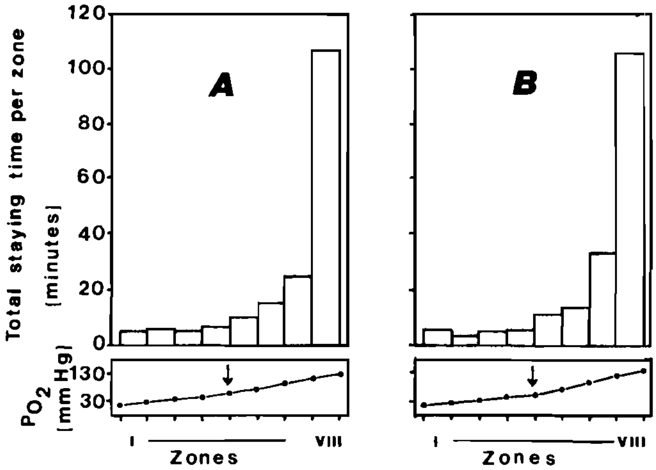

Fig. 3 Oxygen gradient chamber experiments with *B.* (*P.*) *globosus* at 26 °C.

Graph A — Twenty snails with air in the mantle cavity tested for oxygen preferences.

Graph B — Twenty snails tested without an air bubble in the mantle cavity.

Each histogram represents the staying time of 20 snails per zone during a three hour period.

Oxygen choice experiments

The influence of a one-hour exposure of snails to high (60 — 115 mm Hg) and low (15 — 60 mm Hg) oxygen tensions on their oxygen preferences in the choice chamber was investigated. Forty snails, divided in two groups and with their mantle cavities filled with a gas bubble were subjected to this treatment. In one group two snails at a time were kept for one hour in the low tension zones (V — VI) with the aid of a removable perspex partition inserted between zones IV and V. The partition was removed and the track positions noted every 30 s for two hours. A similar experiment was performed with the other group by keeping them for one hour in the high oxygen tension zones. The results are presented in Fig. 4. Snails kept in the low tension zones invariably ventured into the high tension area and stayed in this part of the gradient for the duration of the experiment. If they crawled back and came into contact with the tension demarcation line they immediately responded by turning back to the high tension side of the choice chamber. Snails kept for an hour at the high tension side usually ventured into the low tension side for short periods after which they returned to the high oxygen tension side.

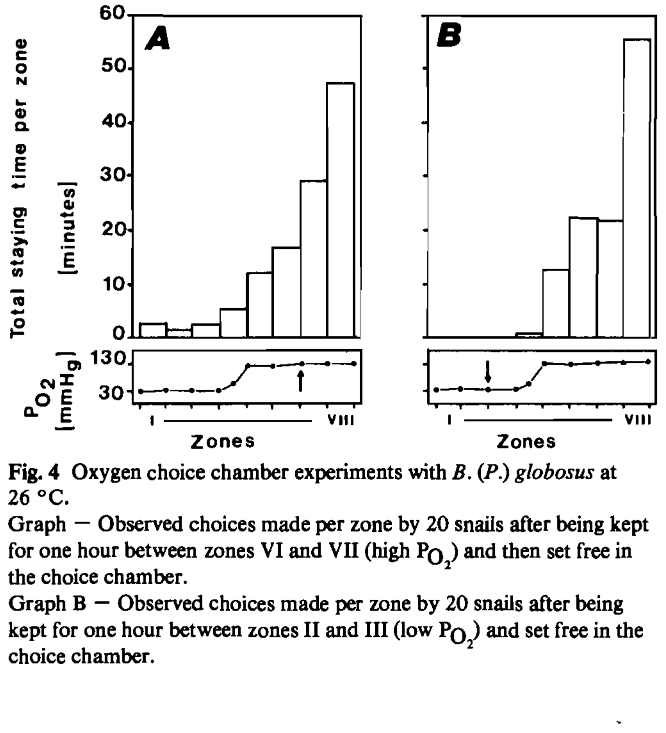

Fig. 4 Oxygen choice chamber experiments with *B.* (*P.*) *globosus* at 26 °C.

Graph — Observed choices made per zone by 20 snails after being kept for one hour between zones VI and VII (high P_{O_2}) and then set free in the choice chamber.

Graph B — Observed choices made per zone by 20 snails after being kept for one hour between zones II and III (low P_{O_2}) and set free in the choice chamber.

Oxygen consumption experiments

Respirometer measurements

Thirty snails were tested individually for one hour at one of four oxygen tensions *viz.* 8, 27, 54 and 132 mm Hg, starting with a water P_{O_2} of 8 mm Hg. The height of the water medium (5 — 6 mm deep) in the respiration vial enabled the snails, confined in their perforated perspex containers, to replenish the air bubble in the mantle cavity, if necessary. The total duration of the experiment per snail was eight hours: 30 minutes gas mixture water equilibration, 30 minutes for thermo-barometric stabilization and one hour measurement at a particular oxygen tension. It means that each snail was exposed for one hour to one of the four P_{O_2} water tensions before commencing the oxygen consumption measurements. The results are presented in Fig. 5. It is evident that a steep decline in the oxygen consumption rate

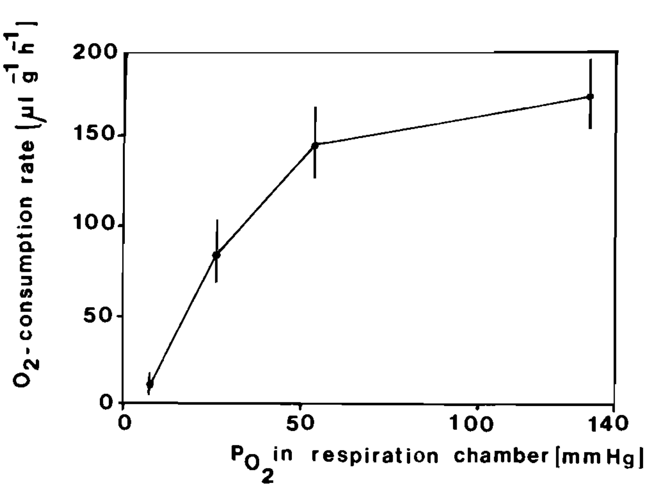

Fig. 5 Oxygen consumption rates for *B.* (*P.*) *globosus* measured using a Gilson-respirometer. A decrease in the P_{O_2} in the respiration chamber lowers the consumption rate of the snails. Vertical bars denote 2 × standard error.

occurs below a P_{O_2} of 50 mm Hg while the rate of oxygen consumed does not differ markedly between the range of 60 and 132 mm Hg tensions. This points to a regulation ability for aerobic respiration at different partial pressures of oxygen. The carbon dioxide as metabolite was removed from the water according to the standard method but other metabolites were not. This may, in part, explain an 'aerobic shutdown' effect below a P_{O_2} of 5 mm Hg in the water.

According to the arguments outlined by Jones (1972) the weight exponent in metabolism for this snail species was taken as 0,75 for the equation, $M = W^b$, where *M* is oxygen uptake in unit time, *W* is body weight (without the shell) *k* and *b* are constants with *b* the exponent of the slope of a straight line. Some insects and pulmonate snails however have oxygen consumption rates that fall on regression lines with a slope closer to 1,0. Such a value of the slope means that the rate of oxygen consumed is directly proportional to the body weight (Schmidt-Nielsen 1975).

In Table 1 oxygen consumption rates were calculated for *B.* (*P.*) *globosus* as a function of both $M = kW^1$ and $M = kW^{0,75}$. Oxygen consumption rates among individual snails varies a great deal. This variation was of the same magnitude in the different partial tensions of the water being tested. This seems to be a universal feature in molluscs (Berg & Ockelman 1959; Prosser & Brown 1961; Ghiretti 1966; Henderson 1963). One explanation is that in pulmonate snails the air volume in the mantle cavity could change, thus indirectly affecting the oxygen exchange rate in the tissues. A more acceptable explanation is the effect of age and condition of the snail which reflects directly the amount of oxygen consumed.

Table 1 The oxygen consumption rate at 26 °C of 30 individuals of *B. (P.) globosus* at four different oxygen tensions of the water (±2 × standard error)

	Oxygen consumption rate ($\mu l\ O_2\ g^{-1}h^{-1}$)	
PO_2 (mm Hg)	$M = kW^1$	$M = kW^{0,75}$
132	276,3 ± 35,9	176,2 ± 21,4
54	226,6 ± 39,2	147,5 ± 21,2
27	136,1 ± 26,6	85,9 ± 15,4
9	7,1 ± 4,6	4,5 ± 2,8

Close system measurements

The decrease in the oxygen tension of the water in a closed volume (44,5 ml) was demonstrated by using a group of 25 full-grown snails of similar size (average body mass 0,29 g). Five individuals were simultaneously placed on the wire mesh in the closed-off chamber. Previously all the air was removed from the mantle cavities so that bubbles, escaping during the experiment, did not affect the oxygen electrode readings. The results are presented in Fig. 6. It is clear from the graph that at the higher oxygen tensions a linear relation exists between the amount of oxygen consumed at a time. This is a further indication that a partial regulation of the consumption of oxygen occurs in water of reduced oxygen tension. Between 33 and 49 mm Hg a deviation in the linearity of the curves of the four groups was found. This means that the amount of oxygen extracted per time unit was no longer a constant proportion. This value in the partial pressure is called the critical oxygen tension (Berg 1952; Bayne & Livingstone 1977). An average value of 42 mm Hg was found for the five snail groups tested.

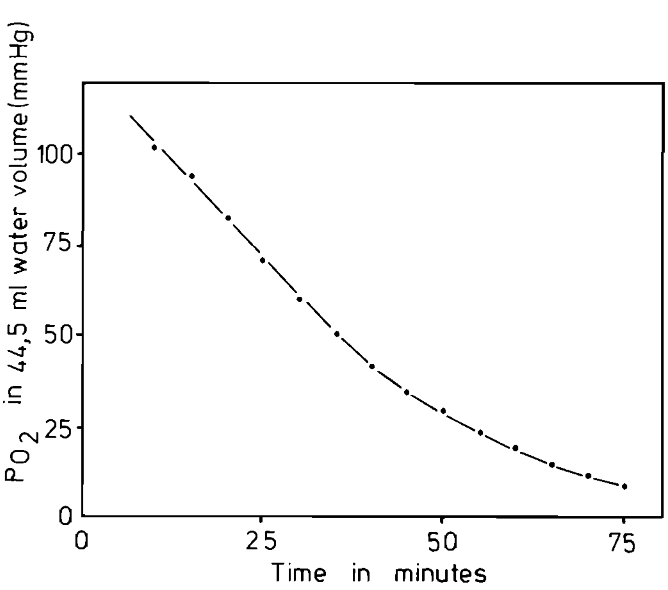

Fig. 6 The oxygen consumption ability of 25 individuals of *B.* (*P.*) *globosus* measured in a closed-off water volume of 44,5 ml. The average body mass of the snails (without the shell) was 0,29 g with a standard deviation of 0,03 g.

Diving experiments

The gas bubble renewal and dive frequency for snails were determined for three hours in water having a P_{O_2} of 132, 43 and 14 mm Hg. Three one-litre glass beakers 22 cm high were divided into 17 horizontal divisions 1 cm apart (starting from the bottom). The snails, with their air bubbles removed, were placed in pairs in the beakers. The air bubble renewal rate for each snail was determined directly after the first filling of the mantle cavity with air. Renewal of a bubble by opening the pneumostome lips does not always take place before a dive. Some snails, particularly at low P_{O_2} tensions, stay at the air/water interface for long periods where replenishing of air takes place. For this reason a distinction was made between the frequency of a dive and the frequency of the opening of the pneumostome. The beakers were kept in a water bath and the water renewed after every three-hour observation period for each of the snail pairs

Table 2 Air bubble renewal and dive frequency of six individuals of *B. (P.) globosus* kept in glass containers with different values of the P_{O_2} in the water. The results were obtained during a 2,5 h observation period. The first figure in paranthesis represents the shortest time in minutes between two renewals and the second figure the longest time

	Air bubble renewal rate/h							Total air bubble renewals/2,5 h	
	Snail No.								
P_{O_2} (mm Hg)	1	2	3	4	5	6	% dives to the bottom	followed by a dive	not followed by a dive
132	2,1	1,4	3,5	—	2,9	3,4	41,8	53	0
	(13:61)	(9:57)	(10:38)	—	(3:39)	(5:30)			
43 – 54	6,3	5,7	7,7	7,1	5,2	6,3	10,9	54	16
	(5:14)	(1:86)	(1:25)	—	(4:23)	—			
14 – 21	9,2	8,9	10,2	9,0	9,7	6,6	0,9	6	100
	(1:12)	(1:15)	(3:11)	(1:15)	(2:14)	(2:23)			

under observation. The results are presented in Table 2. It is evident that snails kept in water saturated with oxygen dived frequently. Nearly half of their dives reached the bottom of the vial. No snails with access to air at this P_{O_2} of the water opened their pneumostomes without a diving cycle. In contrast to this, very few snails kept in the low range (14 – 21 mm Hg) water tension made a dive to the bottom (0,94%). They usually stayed at the surface where the gas bubbles were frequently renewed.

Discussion

The fluvarium method used for the present experiments had not previously been used on this African freshwater snail. The very clear responses found, indicate the value of this method for measuring not only oxygen preferences, but the effect of other physical and chemical properties of the water. The gradients and choices of oxygen produced were very constant during the measurements. But, as was also experienced by Gamble (1971) the zero P_{O_2} and fully saturated extremes at the two opposite sides of the gradient chamber could not be reached. The elaborate and perhaps better design of such an apparatus as proposed in the work of Höglund (1951) could improve the extreme tensions found at zones I and VIII. However, the overall responses found in these experiments could not have been influenced by a shortfall of a 15 mm Hg partial pressure at both the I and VIII zone ends.

According to the tracking data in both the gradient and choice chambers *B.* (*P.*) *globosus* unequivocally prefers water having a high oxygen tension. This view is supported by the dive experiments. More oxygen in the water enhanced the dive frequency while less oxygen increased the open and closed response of the pneumostome for better ventilation of the mantle cavity.

The reasonably fast reaction of the individuals to the submitted gradients and particularly to the choice of low and high oxygen tensions points to the reaction of the animal to an external stimulus by receptors situated somewhere on the body surface. This phenomenon was particularly well illustrated in the case of snails kept at the high partial tension side. When they encountered the transition line towards low oxygen all the snails tested turned abruptly towards the water having a higher oxygen content. It is hard to believe that the responses described above originate solely via receptors sensitive to oxygen levels inside the snail. To substantiate this, the oxygen consumption rate in a closed volume shows that, for these snails, the rate stays at a constant value whereafter it gradually decreases when the P_{O_2} of the water appraoches the 60 mm Hg value. This means that little stress on the respiration ability was encountered towards this low partial pressure value. Yet in the choice and gradient experiments the preference is clearly for the oxygen saturation side.

Much work has been done on closed chamber experiments to mege of this type of experiment is that no CO_2 and other metabolites are removed from the surrounding water which is used for respiration. Mangum and Van Winkel (1973), however, showed that in a wide variety of invertebrate animals, detectable increases in acidic metabolites do not occur above 25 mm Hg partial pressure of O_2 in the water. But they do not rule out the possibility that at the point of non-linearity of the curvature for oxygen consumption rate and at near anoxic conditions, both aerobic (Krebs-cycle) and anaerobic pathways may operate together.

Results of the dive experiment provide some evidence of the gas bubble acting as an oxygen transfer system between air and the epithelium of the mantle cavity. The gas bubble renewal rate is much enhanced at lower P_{O_2} tensions of the water. The frequency of the pneumostomal opening in animals which did not dive rises sharply in poorly oxygenated water. The functional lay-out of the circulatory system in the vicinity of the mantle cavity is, according to Bekius (1972) ideally suited for transporting oxygen to other parts of the body. He found in *Lymnaea stagnalis* (L) that all the snail's blood must pass via the circulus venosus, through the capillary network of the mantle cavity. In doing this the blood comes into very close contact with the enclosed bubble. Thus, with the haemoglobin acting as an auxiliary oxygen carrier (Jones 1972) maximal amounts of oxygen can be transported via the vena pulmonalis to the heart from where it is distributed further.

The oxygen consumption rate in the respirometer chambers where the CO_2 produced by the snails is removed, decreases considerably when the P_{O_2} of the water is in the vicinity of 50 mm Hg. This value coincides with the values obtained for the closed off chamber where it was found that the critical oxygen tension lies between 30 and 50 mm Hg. Furthermore the data obtained from the oxygen gradient and oxygen choice-chamber experiments also show that, judging from their crawling behaviour, *B.* (*P.*) *globosus* avoids P_{O_2} values below 60 mm Hg. Generally speaking it seems that this snail initially responds behaviourally to oxygen decreases in the water and physiologically when P_{O_2} declines further. If it cannot avoid near anoxic conditions in its habitat, it relies on its physiological adaptability for survival. It is not known for how long *B.* (*P.*) *globosus* can endure such anoxic conditions.

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