A Scholander-type respirometer designed for measuring both aerial and aquatic respiration

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Received 31 October 1986; accepted 23 July 1987

An inexpensive respirometer designed in accordance with the measuring principles of the Scholander constant pressure respirometer is described. An advantage of this respirometer is that gas leakages during the measurements of both aquatic and terrestrial animals are completely prevented. This was achieved by using a magnetically induced rotation of the impeller wheel through a 2 mm thick Perspex wall. The accuracy of the measurements was tested after background correction was made for the oxygen consumption rate caused by microbial populations. It was found by experiment that 1,30 (± 0,11) ml; 3,55 (± 0,20) ml and 4,65 (± 0,21) ml of air dissolved in 900 ml of water at 90%, 73,6% and 62% air saturation respectively. In comparison, these values agree closely with the calculated values. This indicates the effectiveness of agitation by the impeller and an efficient air/water equilibrium. The impeller wheel was designed to reduce excessive splatter, but not to impede the air/water mixing ability. Furthermore, the respirometer was tested for its ability to measure the oxygen consumption rate ($\dot{V}O_2$) of the same crab, both when in air and when in water. The results indicate that the mean difference between aerial and aquatic respiration is less than 6%.

'n Goedkoop tuisvervaardigde respirometer, geskoei op die meetbeginsels van die Scholander konstante drukrespirometer, word beskryf. 'n Ontwerpaspek van hierdie respirometer is dat gaslekkasies tydens die metinge vir beide lug- en waterlewende diere heeltemal uitgeskakel word. Hiervoor is gebruik gemaak van 'n magneet geïnduseerde aandrywing van die skyfskroef deur 'n 2 mm dik Perspex-afskorting. Die betroubaarheid van die metinge is getoets nadat agtergrondkorreksies gemaak is van die suurstofverbruikskoers wat deur mikrobiese populasies veroorsaak is. Dit is eksperimenteel vasgestel dat 1,30 (± 0,11) ml; 3,55 (± 0,20) ml en 4,65 (± 0,21) ml van die opgeloste lug in 900 ml water oplos, by respektiewelik 90%, 73,6% en 62% lugversadiging. Hierdie gevonde waardes kom baie goed ooreen met die waardes wat bereken is vir dieselfde persentasies lugversadigde water. Dit is 'n aanduiding dat die klitsing van die water deur die skyfskroef genoegsaam plaasvind vir 'n goeie lug/waterewewig. Die skyfskroef is ook so ontwerp dat 'n minimum van spatwater gevorm word maar dat die lug/watervermenging nogtans effektief is. Die respirometer is verder getoets vir sy vermoë om die suurstofverbruikskoers by die krap te meet tydens lug- en waterasemhaling. Die resultate toon aan dat die gemiddelde verskil tussen lug- en waterasemhaling minder as 6% is.

The development of the constant pressure respirometer by Winterstein (1912, 1913), Scholander (1942, 1949, 1950) and Scholander & Iverson (1958) led to the design of respirometers that can be used for both terrestrial animals (Davies 1966) and for aquatic animals (Gilson 1963). With the exception of some ultramicrorespirometers (Tyler & Berg 1941; Tuft 1950) aquatic respirometers should be equipped with a water agitation device. This ensures that the respiring animal is adequately supplied with oxygen-saturated water so that the rate of oxygen uptake by the animal is not greater than can be replaced by the diffusion of oxygen from the atmosphere to the fluid (Umbreit, Burris & Stauffer 1972). Because the rate of diffusion of oxygen in air is approximately ten thousand times more rapid than in water (Schmidt-Nielsen 1983) it is not necessary to agitate the air in the respiration chamber for the terrestrial type of respirometer (Davies 1966; Scholander 1942, 1950).

Two methods have been employed to agitate the water in the respiration chamber so as to enlarge the surface layer between air and water for diffusion. In the designs of Scholander (1949) and Wennesland (1951) the entire respirometer is shaken. In the Gilson respirometer the manometers are stationary and easily read while the respiration and compensation chambers are agitated (Gilson 1963). Scholander (1949), however, employed a steel wire drive shaft that turns an impeller in the respiration chamber for his aquatic respirometer. A disadvantage of this stirring method is the real possibility of leakages where the drive shaft enters the respiration chamber.

In this study, a Scholander-type respirometer was designed where the impeller, sealed off from outside atmosphere by the Perspex respiration chamber wall, is magnetically rotated by a magnet bar. This arrangement permits the design a respiration chamber which is both water and air tight. This, in turn, means that it should be possible to use the respirometer not only for aquatic respiration but also for animals employing aerial respiration.

Apparatus, reagents and operation

The respirometer consists of the following main parts made out of Perspex: respiration chamber (A), compensation chamber (B), manometer block (C), water-tight compartment for the electric motor (D), (Dixie type, 200 rpm), a brass block that serves as a ballast (E), and a chassis (F) onto which the parts are glued or screwed (Figure 1). On one side of the chassis, facing the respiration chamber, the impeller (F_1) is separated from the respiring animal by a perforated Perspex disc (G). The impeller is made from polyvinyl chloride. It is designed so that, at a speed of 200 rpm, an

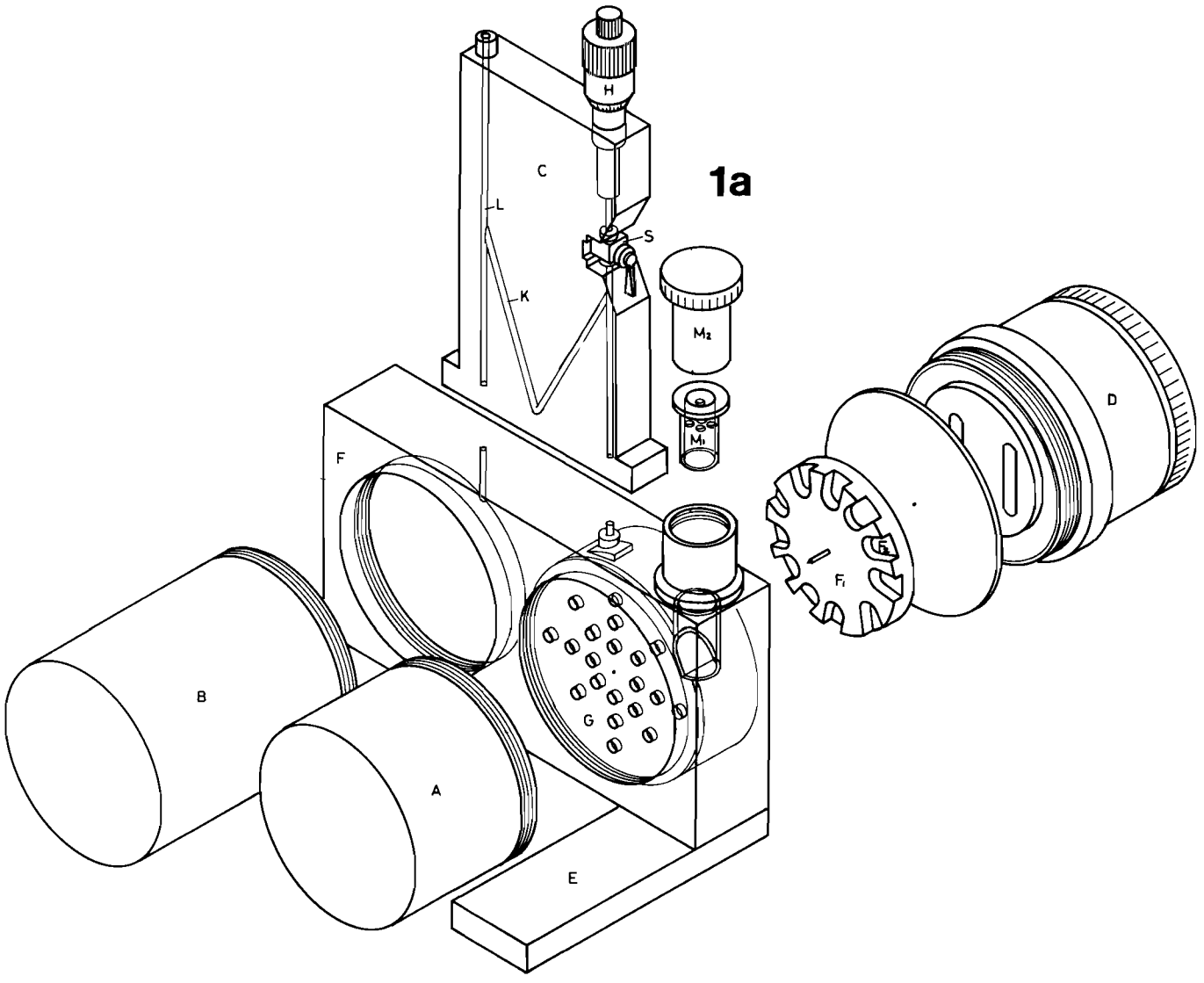

Figure 1 A Scholander-type constant pressure respirometer. The dimensions indicated on the figures are in millimetres. **1a:** A three-dimensional view of the respirometer without the oxygen-filled neoprene bag.

air bubble of 0,82 ml could be submerged under the water level by each of the eleven notched holes (F_2). During operation, with the impeller exactly halfway submerged in the water, an air-to-water exchange of 1782 ml air per min could be achieved at 200 rpm. A hole (16 mm by 50 mm) is drilled through the top part of the chassis block to perforate the impeller chamber at its side. In this cylindrical space, a removable Perspex vial (M_1) is suspended to hold potassium hydroxide. The KOH vial is perforated on its upper part and is filled with 2,0 ml of a 20% KOH solution. A strip of Whatman no. 1 filter paper is placed in the KOH solution so that the KOH saturated paper surface can be seen through the perforations. The position of the KOH vial, and its CO_2 absorption surfaces are such that they face the highest air and water convection created by the impeller. A Perspex plug (M_2) screws on top of the KOH vial to make an air-tight seal. The manometer block was made in two halves to make possible the construction of the V-shaped manometer (K) (1,2 mm inner diameter) and the connecting tubes (L) in the manometer block (C). Grooves were machined out on these halves. A V-shaped glass tube (1,8 mm in outer diameter) was fitted in the grooves of the two blocks. The blocks were then glued together with Tensol no. 7 cement. A water-based manometric fluid described by Krebs (1951) was used.

To adjust the manometer level for determining the amount of oxygen consumed, a 2,0 ml capacity micrometer syringe (H) (Cole Parmer, USA) was used, with a reading accuracy of 0,2 μl. The piston was made of teflon and sealed off by a Viton O ring. Pure oxygen, used to correct the manometer level, was kept in a neoprene rubber bag which was fitted with a three-way gas-tight stopcock (S).

Measurement procedure

The respirometer is placed in a thermostatted waterbath with glass-sided walls and completely covered with water except for the manometer block. To measure oxygen consumption rate ($\dot{V}O_2$) of an aquatic animal, the respiration chamber is filled to 80% of its volume with

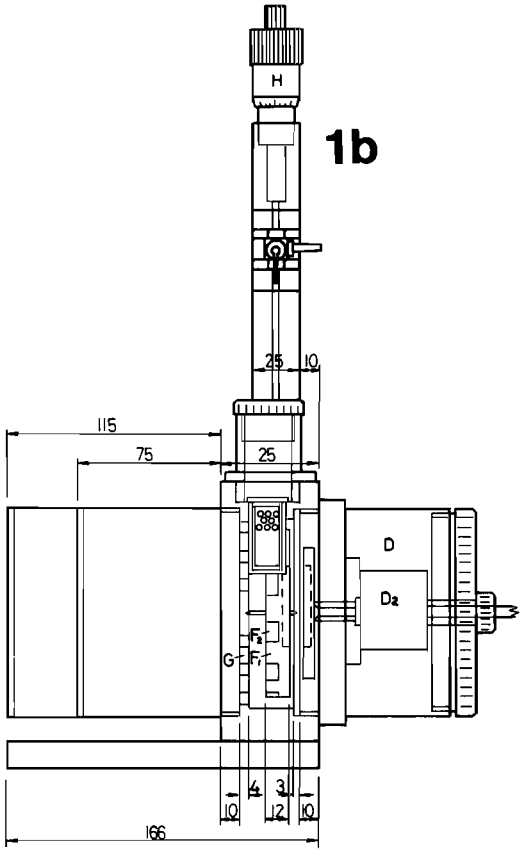

Figure 1b Side view of respirometer to show the position of the electric motor (D), impeller (F) and KOH absorption vial (M).

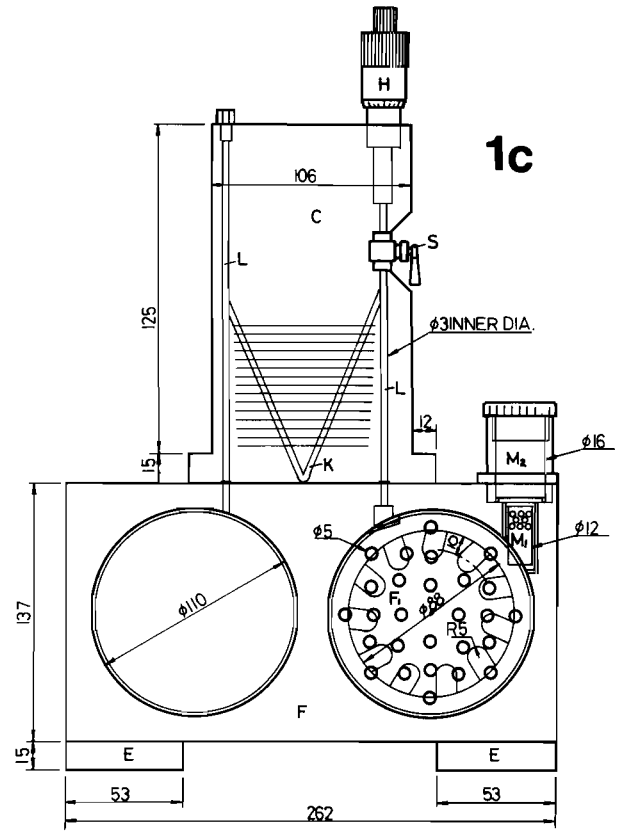

Figure 1c Front view of respirometer without respiration chamber (A) and compensation chamber (B) to show the pattern of the notched holes (F) on the impeller.

water. The animal is placed in this chamber. For purposes of temperature equilibration, and for possible handling stress inflicted on the animal, the compensation and respiration chambers are kept open and in contact with the atmosphere for at least 2 h. Five millilitres of water are pipetted into the compensation chamber (Umbreit *et al.* 1972).

During the equilibration period the impeller is operative to ensure 100% oxygen saturation of the water by air. For measurement of $\dot{V}O_2$ of a terrestrial animal, the same procedures are followed except that no water is used in the respiration chamber. After temperature equilibration, and before the readings are made, outside openings leading to the chambers are closed. At appropriate time intervals the oxygen from the microsyringe is used to level the manometer. The amount of oxygen added to the respiration chamber from the rubber bag is equal to the amount of oxygen used by the animal at a given time interval, uncorrected for standard temperature and pressure (Umbreit *et al.* 1972).

Accuracy of the measurements

The accuracy of the respirometer was determined by means of blank runs at 25°C.

An important accuracy test is to measure how much air, in millilitres, is needed for equilibration between the gas phase and a volume of unsaturated water phase in the respiration chamber. If the measured values agree closely with the calculated values, it is a good indication of the efficiency of the system, particularly in the design of the notched impeller wheel. This test was done by measuring the quantity of air in millilitres that dissolved into a body of 900 ml of tap water at three levels of saturation (Table 1). For 90% saturated water, 810 ml water was equilibrated in the respiration chamber open to the atmosphere. Tap water, kept at the same temperature as the equilibrated water, was degassed with an Edwards vacuum pump. Ninety millilitres of the degassed water was placed under the water surface in the respiration chamber by means of a syringe fitted with a piece of tygon tubing. In the same manner, 237 ml and 341 ml degassed water were carefully placed into 663 ml and 559 ml equilibrated water to give a percentage saturation of 73,6% and 62% respectively. The manometer was closed off from the atmosphere, and the electric motor was switched on. Readings were taken from the manometer at 30-s intervals for 6 min. The results are depicted in Table 1 and Figure 2. In this table, the calculated values of the exact quantities of air in millilitres, that can dissolve in 62%, 73,6% and 90% saturated water are also presented. For these calculations particular care was taken to compensate for barometric pressure, water vapour pressure and the solubility of air in water (Hale 1958). According to Weiss (1971) the solubility of nitrogen, oxygen and argon from

Table 1 The amount of air in millilitres that dissolves in tap water when the magnetically driven impeller wheel is in operation in 900 ml water. The respiration chamber has a total volume of 1,24 l. The air is dissolved in the water at 25'C, at a barometric pressure of 656 mmHg. The results are the means of five experiments done on each of the chosen saturation levels of the water; ± standard deviation from the mean

% Saturation of water	Calculated air dissolved (ml)	Measured air dissolved (ml)	Calculated O_2 dissolved (ml)	Equilibration time (min)
90,0	1,278	1,30 (± 0,11)	0,432	2,5
73,6	3,554	3,4 (± 0,20)	1,201	3,6
62,0	4,858	4,65 (± 0,21)	1,642	5,5

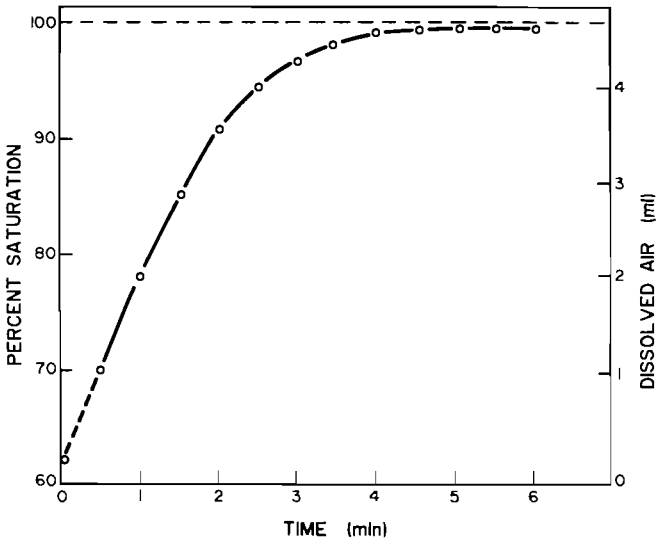

Figure 2 A graphical representation of the rate of gas absorption into 62% saturated water at 25°C. The impeller wheel was rotated at a speed of 200 rpm to mix the degassed water (341 ml) with the gassed water (559 ml).

moist air at one atmosphere total pressure, expressed in millilitres per litre is 10,910 ml, 5,775 ml and 0,2830 ml, respectively, at 25°C. For a barometric pressure of 656 mmHg (Potchefstroom) the solubility of nitrogen, oxygen and argon from moist air at 25°C with a P_{H_2O} of 23,8 mmHg could be 14,114 ml per litre. If moist air is allowed to dissolve in 62% air saturated water with a volume of 900 ml, it can be calculated that 4,858 ml air at 25°C will be dissolved (Table 1, Figure 2). When the calculated values are compared with the experimental values (Table 1), it can be deduced that the amount of air that dissolves into 90% saturated water, is practically the same as the calculated value. However, for the 73,6% and 62% saturated water the experimental data show that less air is dissolved in the water when compared with the calculated data. An explanation for this discrepancy could be that air, because of a time lapse, is already dissolved in the 73,6% and 62,0% saturated water before the manometer ports are closed. This occurs during the time needed to empty the syringe and to remove it. Furthermore, it takes time to insert the KOH well and to screw on the lid to close the KOH well before the manometer ports are closed.

The lower experimental values found for the 73,6% and 62% saturated water tested will not influence the manometer readings during the actual measurements because, in practice, the manometer is always closed when in operation. Furthermore, as can be deduced from data in Table 1, the saturation of the air should always be very high. This could be ascribed to the effectiveness of the oxygen dissolution into the water. Data from Table 1 show that only 2,5 min are neccessary for 0,432 ml O_2 to dissolve into 90% saturated water. If the $\dot{V}O_2$ of a 100 g crab is 3,5 ml per hour, it can be calculated that a sudden shortage of 3,5 ml O_2 in the water can be replenished by the respirometer's impeller wheel within 20 min of the available 60 min. This indicates that the agitation provided by the newly designed impeller is adequate.

The main background component to be subtracted from the oxygen uptake by the animal consists of oxygen consumed by microbial populations in the respiration chamber (Scholander 1949; Wightman 1977). The mean

Table 2 The specific oxygen consumption rate of ten crabs at 25°C with a mass variation between 41 and 150 g. The rates were first determined in water and, on the same day, in 98% relative humidity air; ± standard deviation from the mean

In water (A) ($\mu l\ g^{-1}h^{-1}$)	In air (B) ($\mu l\ g^{-1}h^{-1}$)	B/A.100
55,0	52,0	95,0
33,2	34,7	104,5
37,7	43,9	116,4
37,4	40,2	104,4
50,3	57,6	114,5
36,0	41,6	115,5
57,5	59,0	102,6
36,6	35,4	96,7
57,5	59,0	102,6
36,6	35,4	96,7
		105,2 ± 8,0

oxygen consumption rate in 900 ml of respiratory water was determined for 3 h at 25°C. This was done with the same water in which the individual crabs were placed prior to the measurement of their $\dot{V}O_2$. The data collected from four experiments lasting 6 h showed that the mean oxygen demand by microbial organisms was 48 μl / h, with a standard deviation of 36 μl. The mean oxygen consumption rate of a 100-g crab, calculated from the data in Table 2, was 3,50 ml / h. From this it can be readily calculated that the $\dot{V}O_2$ by bacteria may account for approximately 1,37% of the standard metabolic rate of *Potamon warreni* at 25°C, measured in 900 ml tap water for a 3-h period. A possible further increase of the $\dot{V}O_2$ by bacteria derived from the crabs, after removal of the animals from the chamber, was not investigated. To keep the $\dot{V}O_2$ caused by bacteria in the water medium below 1% of the standard metabolic rate of mature crabs, fresh tap water was used for the $\dot{V}O_2$ determination of each crab.

The ability of the respirometer to measure the oxygen consumption of animals that respire both in water and in air was tested on the river crab *Potamon warreni* Calman. The individual crabs, when placed inside the respiration chamber, were prevented from emerging above the water level during aquatic measurements. This was done with the aid of a rectangular stainless steel mesh, firmly pushed into grooves made on the inner sides of the respiration chamber. The results of the specific oxygen consumption rate from 10 crabs is presented in Table 2. The largest difference between two data sets from each crab, expressed as a ratio (B/A) percentage, is 16,4%, and, the mean 5,2%, with a standard deviation of 8,0.

The accuracy of the respirometer can be improved upon if the volume of the respiration chamber is kept as small as practically possible relative to the compensation chamber. A large respiration chamber volume necessitates a bigger microsyringe volume to compensate for the volume decrease augmented in both the respiration chamber and manometer by the animal.

The respirometer described is at least ten times less expensive than respirometers using electrometric methods (e.g. polarography) to analyse oxygen or carbon dioxide. This does not include standard registration equipment. Maintenance and service are kept to a minimum. Furthermore, as Scholander (1949) pointed out, a whole battery of respirometers can be constructed and mounted on the same frame, each containing an experimental animal. The impellers could be driven with a single electric motor by a belt and pully arrangement, enhancing the versatility of the instrument.

Acknowledgements

The financial support of the Council for Scientific and Industrial Research and the University of Potchefstroom for CHE, are gratefully acknowledged. We thank Mr J.P. van der Walt, B.Sc. of the Instrument-making Workshop, Potchefstroom University, for the design of the impeller and construction of the respirometer.

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