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# An improved equipment for continuous measurement of respiration of marine invertebrates*)

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**Abstract**

A respirometer for small benthic marine invertebrates is presented. The advantages of this construction are: compactness and easy transport of the equipment, small respiration chamber, entrance and exit electrodes close to respiration chamber, exact temperature control and equilibration of the experimental medium, exact regulation of the flow-through speed, digital display of consumed $O_2$ values. The equipment is suitable for long-term measurement of $O_2$ consumption by benthos organisms in low oxygen conditions and for a rapid picture of the reaction of experimental animals to abrupt changes in their environment (e.g. temperature, salinity, composition of the medium). Procedure for experiments involving a lowering of the $O_2$ content of the medium is explained.

**Zusammenfassung**

**Ein verbessertes Gerät zur kontinuierlichen Messung der Respiration mariner Invertebraten**

Ein Respirometer für kleine benthische marine Wirbellose wird vorgestellt. Die Vorteile dieser Konstruktion sind: Kompaktheit und leichte Transportmöglichkeit des Gerätes, kleine Respirationskammer, Eingangs- und Ausgangselektroden direkt neben der Respirationskammer, genaue Temperaturkontrolle und Äquilibrierung des Versuchsmediums, genaue Regulation der Durchflußgeschwindigkeit, digitale Anzeige der verbrauchten $O_2$-Werte. Das Gerät ist für langfristige Messungen des $O_2$-Verbrauchs von Benthosorganismen bei herabgesetzten Sauerstoff-Sättigungsbedingungen und zur schnellen Erfassung der Reaktion der Versuchstiere auf plötzliche Veränderungen in ihrer Umwelt (z.B. Temperatur, Salzgehalt, Zusammensetzung des Mediums) geeignet. Das Vorgehen bei Experimenten unter herabgesetzter Sauerstoffsättigung des Mediums wird erläutert.

The measurements of an animal's oxygen consumption in conditions of low oxygen tension requires an apparatus through which water may be passed at a constant, low level of oxygen saturation. Furthermore, it must be possible to determine saturation levels of the water entering and leaving a small respiration chamber. As only slight changes may be expected in the course of an experiment at low oxygen tension, it must be possible to expand the measuring scale. This requires exact control over the experimental temperature as the slightest temperature variation may distort the results.

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Detailed descriptions of open systems for measuring respiration rates of aquatic animals using ''oxygen electrodes'' are given among others by PLATZER (1967), SCHARF (1972) and BULNHEIM (1972). Here a modification of this design offering the mentioned advantages is presented.

For measuring partial pressure of oxygen ''$O_2$-electrodes'' according to the principle of CLARK are used. They were developed by GLEICHMANN and LÜBBERS (1960), modified by LÜBBERS and WINDISCH (1963) and are produced by ESCHWEILER (Kiel). They show a reproducibility of $\pm$ 0,05 % and under favourable conditions a drift of less than 1 % per day.

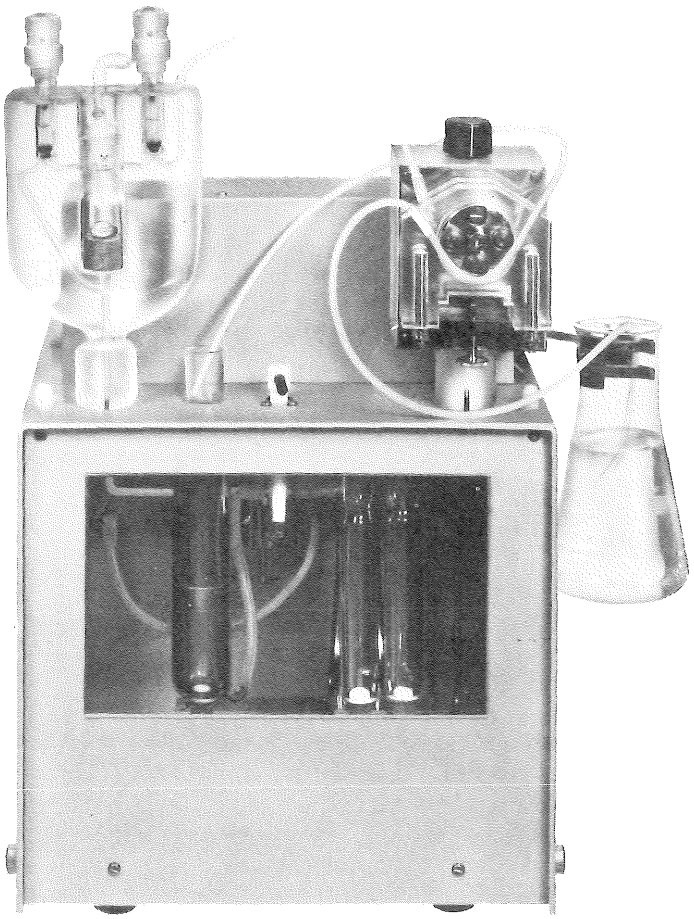

**Figure 1**

Apparatus with temperature control for the measurement of oxygen consumption of small marine invertebrates in a liquid medium.
Lower part: thermostat with equilibration unit. Top left: measuring unit with respiration chamber and 2 $O_2$-electrodes. Top right: peristaltic pump

The experimental temperature is controlled within ± 0,05° C by means of a thermostat (ba 3, made by ESCHWEILER & Co.) and a cryostat (COLORA) (see Fig. 1). The equilibration unit in the thermostat waterbath consists of four fritted glass flasks, two for calibration gas and two for equilibration (Fig. 2) of the experimental medium. These are connected to the capillary system of the respiration chamber unit by means of a multiple tap. The respiration chamber unit (Fig. 3) is situated on top of and cooled by the thermostat. It is composed of the animal chamber and 2 $PO_2$-electrodes placed directly in front of and behind this. A valve enables the experimental medium to be passed through the chamber or to bypass it and flow over the electrodes: withdrawal of this valve provides an opening through which an animal may be introduced into the chamber.

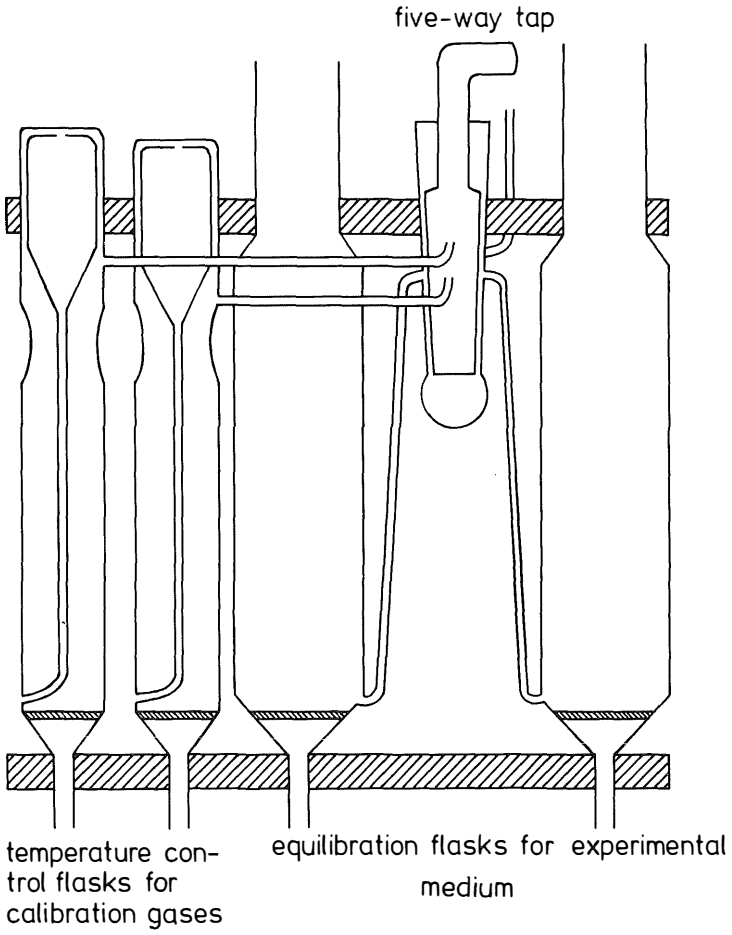


**Figure 2**
Equilibration unit with two temperature control flasks and two equilibration flasks of fritted glass for the experimental medium

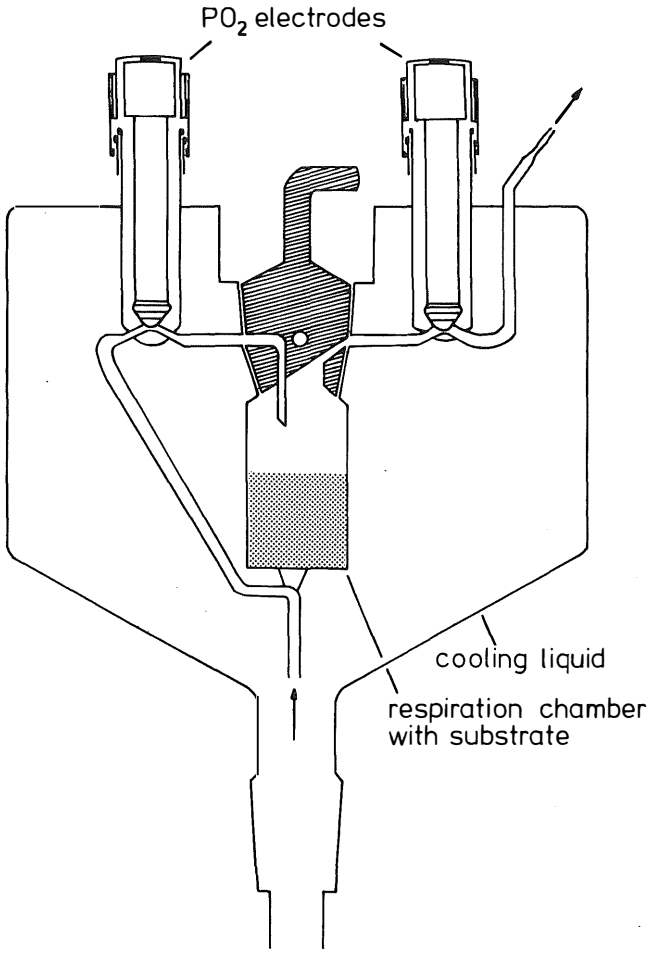


**Figure 3**
Measuring chamber unit with respiration chamber, electrodes and cooling liquid. The respiration chamber is partly filled with burnt-off substrate (800° C) from the specimen's habitat

As PVC and silicon tubes are not impermeable to oxygen, glass, portex or tygen have to be used for the different connetions in order to avoid oxygen saturation of the experimental medium by diffusion from the cooling medium. A capillary tube on top of the equilibration flask prevents the saturation of the experimental medium from the environmental air. The flowthrough speed may be continuously regulated by a peristaltic pump (DESAGA PLG 132100) and be measured by means of a 10 ml measuring cylinder. The peristaltic pump also regulates the level in the equilibration flasks.

The polarisation tension for the two electrodes is provided by a 2 channel $PO_2$-analyser made by ESCHWEILER & Co.; expansion of the measuring scale at low oxygen tensions is achieved with the field magnification switch. A 2-channel compensation line recorder (LINSEIS LS 2244) continually records the values measured. The immediate mathematical evaluation is carried out by an oxygen

consumption evaluator (ESCHWEILER & Co., Kiel) coupled to the analyser, which calculates the difference between channels I and II and gives the results on a digital display. The displayed values are printed by a digital printer (KIENZLE DO 44) at chosen intervals. A schematic reproduction of the circuit is given in Fig. 4. –

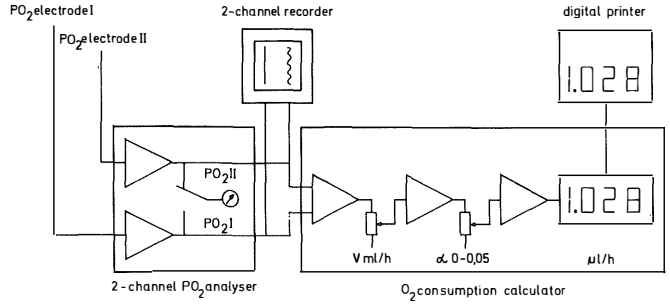


**Figure 4**

Measuring and recording system

The principle of the special procedure of respiration measurements in small marine macrobenthic organisms at reduced external oxygen tensions is presented in Fig. 5.

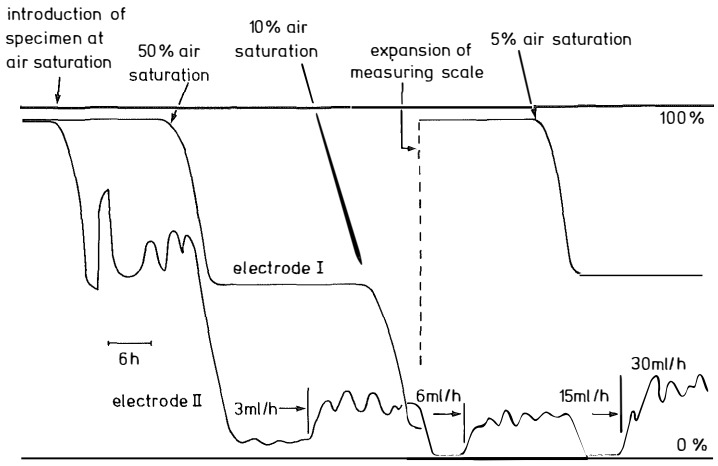


**Figure 5**

Partly schematic reproduction of a recorded measurement of oxygen consumption in relation to oxygen saturation in the described open system (*M.balthica*, 10° C., 19‰ S). Electrode I indicates the oxygen tension before water enters the respiration chamber, electrode II the tension when water leaves it. When oxygen available is lowered to 50 % of air saturation, the value at electrode II sinks almost to zero because of the specimen's consumption. In order to make more oxygen available the flow-through speed is raised from 3 to 6 ml per hour. This raises the value of the partial pressure indicated by electrode II; the oxygen consumption rises owing to the higher flowthrough speed. When the oxygen saturation is further reduced to 10 % of the air saturation, an expansion of the measuring scale becomes necessary. If the oxygen ist still further reduced to 5 % of the air saturation, the flowthrough speed must be increased again (to 30 ml per hour), so that the specimen may obtain sufficient oxygen

For comparsion the oxygen consumption of some marine bivalve species *(Macoma balthica*, *Macoma calcarea and Abra alba)* from the western Baltic at different external oxygen tensions is measured using the open system (Fig. 6). The mean values of 4 registrations, each at 10° C, are compared with those obtained in a closed system (DRIES and THEEDE, 1976). The variations between both results are relatively small.

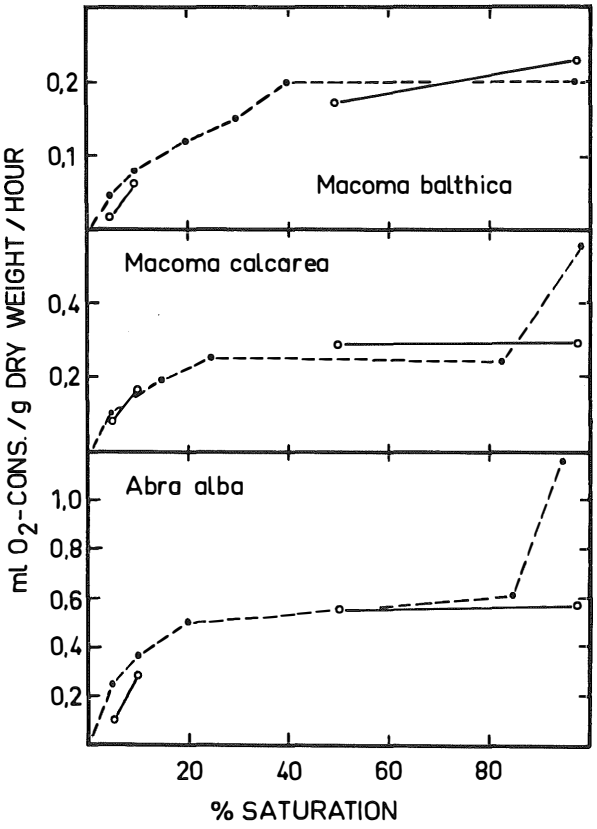


**Figure 6**

Oxygen consumption of *M. balthica*, *M. calcarea* and *A. alba* relative to oxygen tension measured in an open system (open circles). Experimental conditions: 10° C., 19‰ S. Time of the experiments: May – June (1975). Average values from 4 experiments. – For comparsion, the dependence of oxygen consumption on oxygen tension in a closed system is shown (broken line with filled dots)

Contrary to some elevated values for respiration in the closed system at the beginning of the experiments and at high air saturation the respiration values in the open system are not so high. This suggests that the increased metabolic level at the beginning of the experiments in the closed system could be caused by increased activity due to experimental stress. Altogether, all species are able to regulate oxygen consumption within a wide range of oxygen saturations of the medium. However, *Abra alba*, the species from the deeper trenches of the western Baltic, demonstrates the highest metabolic rates. The ecological significance of the metabolic reactions of these bivales is discussed by THEEDE (1978, 1979).

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