J. exp. Biol. ***185****, 357–365 (1993)*
Printed in Great Britain © The Company of Biologists Limited 1993

SHORT COMMUNICATION

A METHOD FOR LONG-TERM MULTIPLE-CHANNEL RECORDING OF OXYGEN CONSUMPTION IN AQUATIC ANIMALS

TROND NORDTUG AND ANDERS JOHNY OLSEN
Department of Ecotoxicology, ALLFORSK-AVH, N-7055 Dragvoll, Norway

Accepted 12 July 1993

The objective of the present work has been to develop a method for measuring the rate of oxygen consumption on a routine basis during toxicity testing and environmental monitoring. This requires a method that is reliable over long periods and can be used with a minimum of maintenance work in a variable environment. The system should consist of a compact unit easily transported and available at a reasonable cost. Visual updated records of the oxygen consumption should be available at all times.

A compact unit for continuous long-term multiple-channel recording of oxygen consumption in an open flow-through system has been developed. The system has primarily been designed for use in routine ecotoxicological testing and monitoring during suboptimal recording conditions. The unit is built around a single standard oxygen electrode inside a multiple-port system with 12 input channels, which successively collect water from the respiration chambers. By using frequent calibrations, the effects of chemicals on the electrode and variations in recording conditions, such as temperature changes, are compensated for. Emphasis has been placed on the use of simple mechanical components and a modular principle that allows a large degree of freedom for modifications through exchange of components. The system was tested with blue mussels (*Mytilus edulis*), during exposure to a number of organic and inorganic chemicals.

Computer control of the system is based on a minimum of peripheral lines (two analogue inputs and two digital lines). The software is entirely screen-based and has few input variables with a number of continuously updated graphical and numerical displays that convey information about the status of the experiment.

The driving force for flow through the system is the pressure difference between the inlet and the outlet created by the difference between the surface levels of the two water columns (Fig. 1). The flow into each chamber is regulated by reduction valves made from Teflon capillary tubes. The flow rate through the tube is described by the Poiseuille–Hagen equation:

$$\dot{Q} = \Delta P \times \left(\frac{\pi r^4}{8\eta \times L} \right), \quad (1)$$

Key words: aquatic respirometry, rate of oxygen consumption, long-term multiple-channel respirometry, automated-flow respirometry.

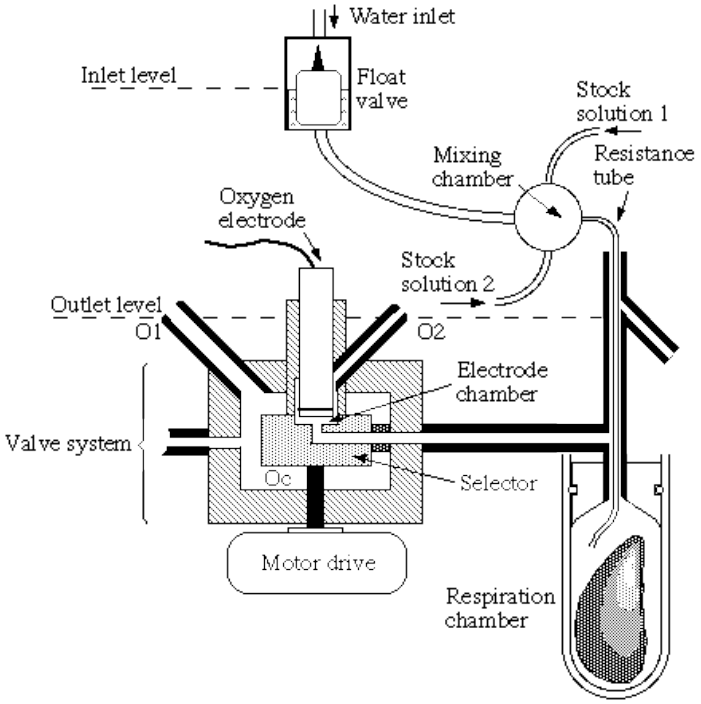

Fig. 1. Transected view of the 12-channel experimental set-up for measuring the rate of oxygen consumption. The set-up consists of 12 identical respiration chambers with inner diameter 32mm (of which one is shown) connected to a multiple-port valve system. The flow through each respiration chamber is defined by the resistance of Teflon capillary tubes (resistance tubes) and the height difference between the inlet and outlet levels. The surface level of the inlet water column is defined by a vertically adjustable float valve. For each respiration chamber, two different stock solutions may be mixed into the main stream through a mixing chamber. The outlets from the respiration chambers are guided through a valve system that successively connects them to the oxygen electrode by means of a selector driven by a motor drive. Water from the eleven chambers that are not connected to the electrode chamber circulates the outer chamber (Oc) and helps to stabilise the temperature of the electrode. The surface level of the outlet water column is defined by overflow from the multiple-port valve (O1 and O2).

where $\dot{Q}$ is the flow rate, ΔP is the pressure difference, L is the length of the tube, r is the radius of the tube and η is the viscosity of the fluid. Thus, for a given tube, the only variables affecting the flow are the pressure difference and the viscosity of the fluid.

Identical high-resistance tubes are placed upstream of each respiration chamber, whereas the resistance of the rest of the flow system is kept at a minimum by using tubes with a large inner diameter. In addition to the main flow, two different solutions may be mixed into each individual chamber by adding them into the mixing chamber placed in front of the resistance tube (Fig. 1). Addition of stock solutions displaces a similar amount of water from the main inlet and does not affect the flow rate unless the viscosity changes.

The respiratory chambers consist of a glass centrifuge tube mounted on a piston-like

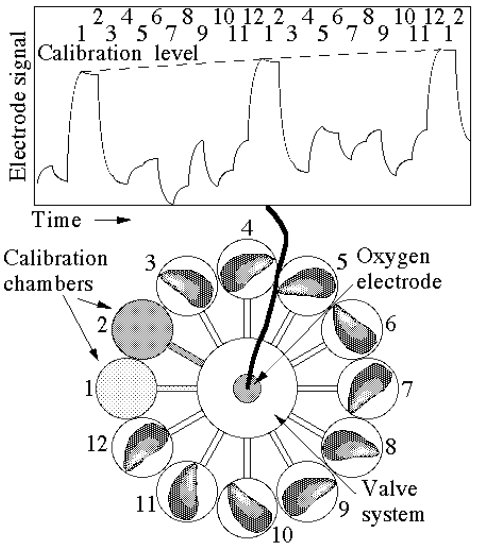

Fig. 2. Schematic view of the multiple-port system seen from above (lower section) and a constructed chart record of the electrode signal obtained during two revolutions of the system (upper section) with successive records from all twelve chambers (as indicated above the record). Channels 1 and 2 are used to calibrate the recordings relative to pure aerated sea water (C1) and exposure solution (C2). Extrapolation between successive calibration measurements in one solution is used to obtain a continuous reference level (broken line) that is used to determine the relative oxygen tension in chambers where animals are exposed to the same solution. The flow was adjusted according to the rate of oxygen consumption of the animals so that a maximum of 15% of the total oxygen content of the water was extracted by the animals. At each input, the electrode signal was allowed to stabilise before the selector proceeded to the next port. The time between successive calibration measurements was about 30min.

top which fits inside the tube (Fig. 1). The inner surface of the top is conical to allow air bubbles to escape from the chamber. The volume of the chamber may be adjusted by sliding the tube vertically along the piston. The chamber is opened by pulling the tube off the piston.

The valve system consists of a circular stainless-steel chamber with a rotating channel selector (Fig. 1). The input channels are selected one at a time, and the water is led into the electrode chamber at the top of the selector by rotating the selector by a step motor. Water from the selected channel is led from the electrode chamber along the electrode to an outlet, which can be connected to a fraction collector or used for automatic recording of, for instance, the content of algae in the water.

All measurements should be carried out at a room temperature varying by no more than ±3˚C and the apparatus should be stabilised by immersion in a water reservoir at room temperature. The inlet water should also be at room temperature, guided in a thin layer over an air diffuser to equilibrate it to ambient air pressure.

In Fig. 2 a schematic chart recording of two rotational sequences of the valve system is shown. The figure demonstrates the principle of a moving calibration level obtained by linear interpolation between successive calibration measurements.

Software for control of the valve system and the data assembly as well as analysis of the data was developed by the icon-based LabVIEW programming system (National Instruments). During recording, the electrode signal is continuously displayed in a separate chart window (time resolution 10s) for visual inspection of response characteristics and noise. Updated curves of the oxygen tension of individual respiratory chambers relative to the calibration input are displayed on the computer screen. The software also includes modules for position control and automatic resetting of the multiple-port system. For each position of the valve, the electrode is allowed to equilibrate for a period defined by the user. The electrode signal is recorded at the end of this period, before the valve proceeds to the next port. Each electrode reading is stored together with the channel number, a time tag and a temperature reading.

Calculation of the rate of oxygen consumption is based on the recorded value from each individual chamber relative to the constructed calibration value. The relative deviation between the control and the actual measurement is then expressed as rate of consumption (X_{i}; expressed as $\mathrm{mg\,O_2\,g^{-1}\,h^{-1}}$) by using the content of oxygen in the aerated inlet water (C in $\mathrm{mg\,l^{-1}}$), the flow through the chambers (f in $\mathrm{ml\,h^{-1}}$) and the dry mass of the animals (M in g) and is calculated according to the equation:

$$X_{\mathrm{i}} = 1 - \left\{ \frac{R_{\mathrm{i}}}{C_{\mathrm{n}} + [(C_{\mathrm{n+1}} - C_{\mathrm{n}}) \times (T_{\mathrm{R_i}} - T_{\mathrm{C_n}})/(T_{\mathrm{C_{n+1}}} - T_{\mathrm{C_n}})]} \right\} \times C_{\mathrm{O_2}} \times f \times M^{-1}, \qquad (2)$$

where R_{i} is the electrode signal from a particular channel at time $T_{\mathrm{R_i}}$ and C_{n} and $\mathrm{C_{n+1}}$ are the two nearest calibration readings recorded at time $T_{\mathrm{C_n}}$ and $T_{\mathrm{C_{n+1}}}$, respectively.

Changes in air pressure and temperature affect the solubility of oxygen in water. When using a fixed value for the oxygen content of the aerated water, as in the previous example, changes in these variables during the experiments will introduce an error. The oxygen contents of the exposure chambers are obtained as a fraction of the values for aerated water. Thus, the error added to the calculated oxygen consumption rate by these variables is directly proportional to the relative change in the pressure- and temperature-dependent solubility of oxygen of the inlet water. The magnitude of this error is about $2\%\ ^{\circ}\mathrm{C^{-1}}$ for a temperature change, and $1\%\,\mathrm{kPa^{-1}}$ change in air pressure. Exchanging the fixed value of the oxygen content of the inlet water with a function involving continuous records of temperature and air pressure removes this error.

Changes in temperature will also affect the sensitivity of the electrode. Since the water in the control and the experimental channels has been treated in exactly the same way, a slow drift in temperature is compensated for by the calibrations. Faster changes in temperature at the site of the electrode require corrections according to the temperature/response function of the electrode.

The electrode requires a standard oxygen analyser unit with a stable cathode current. Sequencing of the current input to the step motor is performed by a standard circuit. The system requires access to a small computer with one analogue input for the electrode signal and another for temperature recording. In addition, one digital output to control the step motor and another from a photo-interrupter that conveys information on the position of the multiple-port system are required. The system used was a Macintosh SE computer

with a MacAdios peripheral board and a Radiometer P_{O_2} electrode (E5046) connected to a Chemiware MKS 2 unit with an analogue output.

Natural sea water without micro-filtration (collected from 90m depth in Trondheimsfjorden) was used for the experiments, and after some days there was some growth of algae and fungi in the system. However, in experiments lasting up to 1 week no fouling was observed within the capillary tubes, probably because of the high velocity of the water through the tubes. In experiments lasting 1–2 weeks, flow reductions of a maximum of 5% were sometimes observed. Hence, during long-term experiments, micro-filtration of the water should be carried out. Accumulated faeces have to be removed from the exposure chambers at intervals, otherwise the system needs very little maintenance.

The size of the respiration chambers used can be adjusted from 80 to 25ml. In the experiments with *M. edulis*, a chamber size of 75ml and a flow rate of 10mlmin^{-1} were used. The optimal flow rate depends on the oxygen consumption of the animals and is a compromise between the accuracy of the measurements, which will decrease with increasing flow rate, and the possible effect of chronically lowered oxygen tension on the metabolic rate at low flow rates. In the present experiments, the oxygen tension in the respiration chambers was kept above 85% of that of the aerated sea water. The size of the chamber should be determined in accordance with the frequency of recordings relative to the half-time of the exchange of water in the chambers. The equilibration period at each input channel was 2.5min. Thus, the interval between successive recordings for each channel was 30min.

The response time of the system when moving from one chamber to the next is the result of the combined effects of dead space within the valve system and the time constant of the response from the electrode in a particular situation. This was tested by filling one chamber with nitrogen-bubbled water and observing the response while shifting the valve position between the two channels using a flow rate of 10mlmin^{-1}. Owing to the minimum of dead space within the valve system, the lag time is short (about 10s). After the initial lag time, the response is almost exponential and corresponds to the characteristics of the electrode, reaching 90% of full response within 90s. The results show that the response of an electrode used for about 3 weeks without changing the membrane is only slightly slower than that of an electrode with a new membrane.

The wash-out properties of a 75ml chamber with a flow rate of 10mlmin^{-1} were examined (Fig. 3). The data show that the high velocity of the inflowing water results in good mixing in an empty chamber. Mixing is further increased by the activity of an animal within the chamber, and is very close to the theoretical time course.

The problems of wash-out properties and instantaneous changes in oxygen consumption have been dealt with in detail by Niimi (1978) and Steffensen (1989). Using the equations of Steffensen (1989), the theoretical time for exchanging 95% of the water (95% transformation time) in the situation shown in Fig. 3 is 22.5min. In the present tests, about 98% of the water is exchanged between consecutive recordings from each chamber. Using recording intervals as large as 30min prevents the detection of momentary changes in the rate of oxygen consumption, and for this purpose other recording methods have to be used. In the present application, however, the main purpose

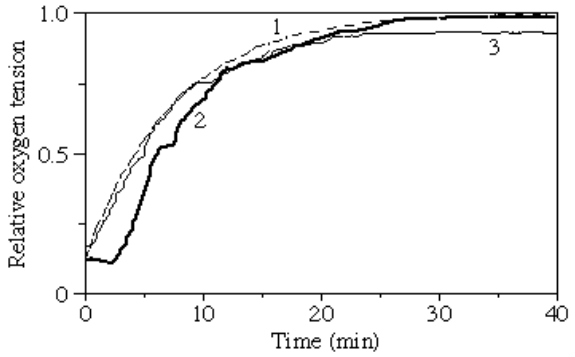

Fig. 3. The wash-out properties of the respiration chambers. The flow was 10mlmin^{-1} and the size of the respiration chamber was 75ml. The chamber was initially filled with nitrogen-bubbled water and the curve illustrates the time course of the dilution of this water by air-equilibrated sea water when the chamber was empty (2) and for a chamber containing a blue mussel (3). For comparison the theoretical time course for the dilution in a chamber with complete mixing is included (1). No stirring was applied.

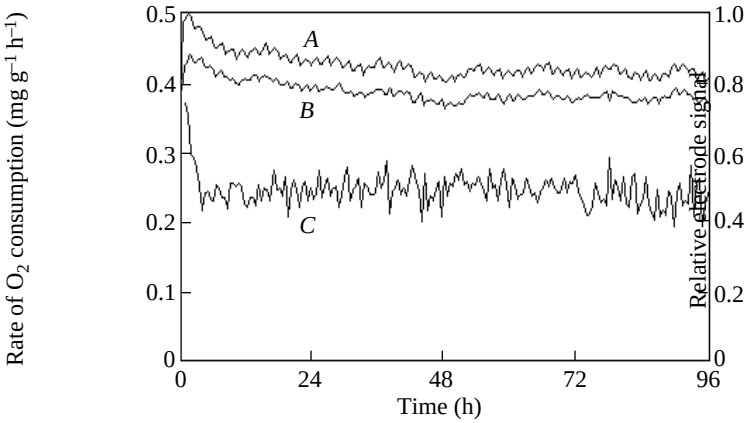

Fig. 4. Plot showing the electrode signals (arbitrary units) recorded from the control chamber (*A*) and a respiratory chamber (*B*) during a period of 96h. Curve *C* shows the rate of oxygen consumption for the animal in the respiratory chamber calculated according to the present method. The record was obtained during a period with relatively large drift in the electrode signal. The temperature of the laboratory was 10±2˚C.

is to record changes occurring over hours or even days. In this case, the lag time of the recordings due to the wash-out time of the respiration chambers acts as a low-pass filter to smooth recordings containing sudden changes in the rate of consumption. The characteristics of this filter effect may be altered by changing the ratio between flow rate and chamber size.

The effect of slow temperature changes of a few degrees is corrected for by the calibration procedures. This is demonstrated in Fig. 4. The effects of both the temperature changes and the changes in electrode sensitivity are almost completely eliminated by the calibration procedure.

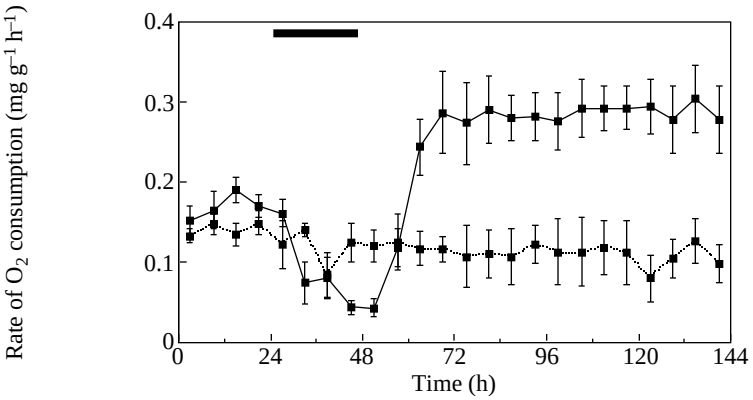

Fig. 5. Results obtained during an exposure experiment with *Mytilus edulis.* Six animals were exposed to 350mgl^{-1} of phenol for 24h during the second day (bar), and four animals served as the control group. The plot shows the mean rate of oxygen consumption during successive 6h periods for exposed (open squares) and control (filled squares) animals, with standard errors indicated.

To test the effects of chemical exposure on experimental animals, two chambers were used for calibration measurements, one being circulated with aerated sea water containing the exposure solution and the other with clean sea water only. Of the remaining 10 chambers, six contained animals exposed to the test chemical and four contained control animals. Fig. 5 shows an example of exposure for 24h to 100mgl^{-1} phenol. Phenol was added by replacing 10% of the inlet water with 1000mgl^{-1} phenol in seawater stock solution. The rate of oxygen consumption was recorded 24h before and 96h after the exposure period. By comparing the two control values, the effect of the chemical on the electrode signal can be detected; but no such effect was observed for phenol.

The amount of food available influences the rate of oxygen consumption in the blue mussel (Bayne *et al.* 1973). Thus, in experiments extending over several days, the animals should be supplied with sufficient food to cover at least energy maintenance. In our present apparatus, algae may be added through one of the inlets available for stock solution. The effect of feeding in *M. edulis* is illustrated in Fig. 6, which shows that admission of algae clearly stabilises the rate of oxygen consumption compared with that of the starved animal.

This method does not record the rate of activity, so changes in metabolic rate due to changes in the rate of activity cannot be separated from changes in resting or basal metabolic rate. However, the inclusion of intrumentation for measuring activity levels in this system is currently being tested in our laboratory. The measurements are not continuous, but the rate of consumption is tested every 30min, sufficient for observing changes in rates of oxygen consumption that occur during periods of an hour or more.

When the method is used according to the present description, some measuring accuracy is lost in order to rationalise the data assembly and to make the system as easy as possible to operate under different conditions. In order to obtain very accurate measurements, the equilibration time of the electrode should be extended and correction

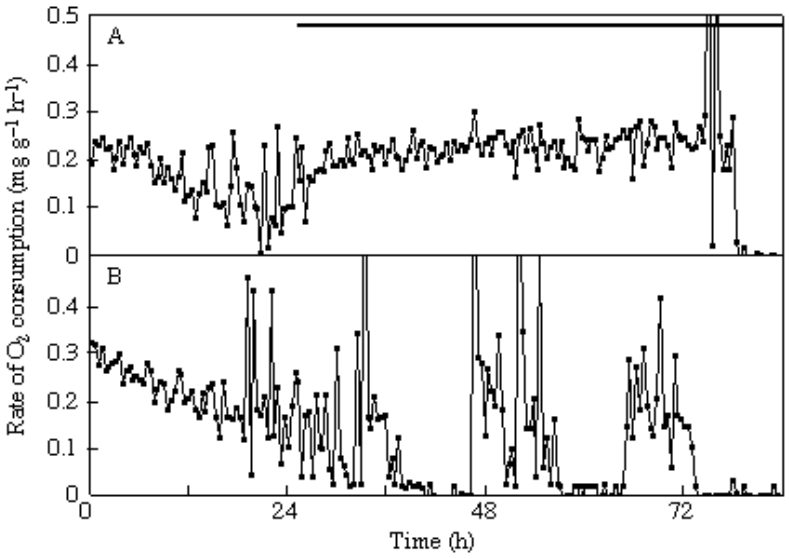

Fig. 6. The effect of feeding in *Mytilus edulis* shortly after spawning. (A) The rate of oxygen consumption in an individual supplied with water containing algae (*Tetraselmis* sp., 10^6–10^7 cells l^{-1}) after 24h (horizontal bar). (B) Corresponding data from an individual from the same experiment that was starved throughout the experimental period. The animals were removed from the respiration chamber after 78h while the recordings continued in order to test the possible effect of the algae and bacteria in the chambers. This effect was insignificant as seen by the very low rate observed after 78h. The experiment was performed in darkness at 10±2˚C.

procedures, such as compensation for air pressure and temperature changes, should be used. When using electrodes with a significant zero current, a channel for zero calibration should be included in the recording sequence. However, the use of only one electrode to record from multiple channels by a single-valve system reduces the cost and economises on the use of computer capacity. The entire system contains only one active mechanical component (the input selector), and the danger of mechanical failure is greatly reduced compared with more complicated mechanical solutions. Three different solutions can be mixed into each respiration chamber, and the system is well suited for maintaining specified water qualities with respect to both abiotic factors and xenobiotics over long periods. The system has proved reliable during long-term monitoring of the rate of oxygen consumption in *M. edulis*, and the method is very robust against slow changes in the recording conditions. The entire unit for recording the rate of oxygen consumption can be built into very compact, easily transported units. The system is fully automated and requires little maintenance work and, as a result of automated calibration and monitoring procedures, it is very easy to operate.

The present work is part of the BECTOS research programme financed by FINA Exploration Norway u.a.s. The authors are indebted to Hege Myhren for supplying data from feeding experiments and to Dag Altin for supplying the algae used in the same experiments.

References

BAYNE, B. L., THOMPSON, R. S. AND WIDDOWS, J. (1973). Some effects of temperature and food on the rate of oxygen consumption by *Mytilus edulis* L. In *Effects of Temperature on Ectothermic Organisms* (ed. W. Wieser), pp. 181–193. Berlin, New York: Springer-Verlag.

NIIMI, A. J.(1978). Lag adjustments between estimated and actual physiological responses conducted in flow-through systems. *J. Fish. Res. Bd Can.* **35**, 1265–1269.

STEFFENSEN, J. F. (1989). Some errors in respirometry of aquatic breathers: how to avoid and correct them. *Fish Physiol. Biochem.* **6**, 49–59.