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Some errors in respirometry of aquatic breathers: how to avoid and correct for them

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Abstract

Respirometry in closed and flow-through systems is described with the objective of pointing out problems and sources of errors involved and how to correct for them.

Both closed respirometry applied to resting and active animals and intermittent-flow respirometry is described. In addition, flow-through or open respirometry is discussed, in particular when the system is in non-steady state.

Simulations are used to show how improper analysis can lead to improper conclusions.

Abbreviations used: bw: weight of animal (kg); βwo_2: capacitance coefficient of oxygen in water (mg $O_2.l^{-1}.mmHg^{-1}$); Cwo_2,in: concentration of oxygen in water flowing into the respirometer (mg $O_2.l^{-1}$); Cwo_2,out: concentration of oxygen in water flowing out of the respirometer (mg $O_2.l^{-1}$); $\dot{M}o_2$: total oxygen consumption per unit of body mass (mg $O_2.kg^{-1}.min^{-1}$); Pwo_2,cr: critical partial pressure of oxygen in water (mmHg); Pwo_2,in: partial pressure of oxygen in water flowing into the respirometer (mmHg); Pwo_2,out: partial pressure of oxygen in water flowing out of the respirometer (mmHg); $\dot{V}g$: gill water flow rate ($l.min^{-1}$); Vr: volume of water in the respirometer minus fish volume (L); $\dot{V}w$: water flow rate ($l.min^{-1}$); $\dot{V}w/Vr = \dot{D}$: dilution rate (min^{-1}); $Vr/\dot{V}w = 1/\dot{D}$: average residence time (min); t: time (min); $t_{95\%}$: time for 95% transformation (min).

Introduction

Respirometers of different designs have been important in studying gas exchange in aquatic animals. Three different methods have been used in measuring the respiratory gas exchange. In closed respirometry the time course of gas content of the water in a closed chamber containing the animal is measured (Humboldt and Provencal 1809). In open respirometry the difference in gas content and the rate flow of water through a chamber are measured (Ege and Krogh 1914). The third method is manometric or volumetric (Scholander *et al.* 1943). The first and second methods will be evaluated below with the objective of pointing out sources of errors and possible corrections.

The examples involve teleost fishes, but the problems and recommendations apply to the use of respirometry on invertebrates as well.

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Respirometry in closed systems

The volume of water in the respirometer, Vr, weight of the animal, bw, and the time course of the oxygen concentration of the water, ΔCwo_2, during time period, Δt, permit the calculation of oxygen consumption, $\dot{M}o_2$, according to the formula:

$$\dot{M}o_2 = \frac{Vr \cdot \Delta Cwo_2}{\Delta t \cdot bw} \quad (1)$$

Similarly, the time course of the partial pressure of oxygen in the water, Pwo_2, and the capacitance of oxygen in the water, βwo_2, allow calculation of $\dot{M}o_2$:

$$\dot{M}o_2 = \frac{Vr \cdot \beta wo_2 \cdot \Delta Pwo_2}{\Delta t \cdot bw} \quad (2)$$

This method has been widely used by many investigators since Humbolt and Provencal (1809). Among others, Ege and Krogh (1914) used this technique to investigate the influence of temperature on the gas exchange in small goldfish (*Carassius auratus*) enclosed in a 2.7 l bottle. The oxygen consumption of small fish relative to a large volume of respiration chamber, will result in a slow decline in Cwo_2. In an experiment lasting hours or days, bacterial oxygen consumption may cause a significant error in the calculation of $\dot{M}o_2$ of the fish. As pointed out by Keys (1930), stratification of gas content in the water as well as accumulation of production of carbon dioxide and other metabolites as well as nitrogenous excretory products may also influence the oxygen consumption. The use of an adequate pump for recirculating and mixing the water in a closed respirometer will solve the problem of stratification. $\dot{M}o_2$ of resting postabsorptive animals is defined as standard oxygen consumption (Fry and Hart 1948). If long time periods are used in determining $\dot{M}o_2$, it is likely that some spontaneous activity will occur, causing an increased $\dot{M}o_2$, which was termed routine oxygen consumption (Fry 1957, 1971). Hence, it is important to work with appropriate ratios of fish size to respirometer volume, allowing measurements of oxygen consumption over shorter time intervals.

When $\dot{M}o_2$ is independent of Pwo_2 the animal is considered an oxygen regulator (Prosser 1955). If $\dot{M}o_2$ is dependent on Pwo_2 the animal is an oxygen conformer. The transition Pwo_2 is termed the critical partial pressure of oxygen, Pwo_2, cr.

Fish will be exposed to small changes in oxygen concentration when short periods are used to measure ΔCwo_2, which is desirable, but our ability to measure ΔCwo_2 accurately decreases when ΔCwo_2 is small. With short time spans standard $\dot{M}o_2$ is also more likely to be distinguishable from routine $\dot{M}o_2$.

Flushing of respirometers by automatically controlled valves allows repeated experiments within narrow limits of time and Pwo_2, and problems of accumulation of CO_2 and other excretory products are greatly reduced (Forstner 1983). With the aid of computers in such intermittent-flow respirometers $\dot{M}o_2$ can be determined during 3 to 10 min intervals with a decrease in Pwo_2 of only 2 to 5 mmHg (Bushnell *et al.* 1984; Steffensen *et al.* 1984).

An example of a series of 5 min determinations of oxygen consumption in a rainbow trout, *Salmo gairdneri*, exposed to a natural light regime during an 18 h period is shown in Fig. 1. A modified version of the intermittent-flow respirometer described by Steffensen *et al.* (1984), combining 5 min periods of closed respirometry with 5 min periods of flushing the respirometer, was used. A 385 g trout was introduced into a 4.5 l respirometer at 1400 h. During the following 9 h oxygen consumption varied from 125–350 mg O_2 $kg^{-1}.h^{-1}$. About 2 h after sunset (2039 h) $\dot{M}o_2$ had decreased to a stable value of 100–110 mg O_2 $kg^{-1}.h^{-1}$, maintained for the following 5–6 h. This can be considered standard oxygen consumption. At sunrise (0357 h) the $\dot{M}o_2$ increased to 360 mg O_2 $kg^{-1}.h^{-1}$ and fluctuated during the following 4 h.

If flow-through respirometry had been used, the animal would have been introduced into the respirometer in the afternoon, allowed to acclimate overnight, and the experiment started the following morning. This would have resulted in an erroneously high standard $\dot{M}o_2$, since the animal became exited at sunrise with a concurrent increase in $\dot{M}o_2$.

Determination of active oxygen consumption of swimming fish using closed respirometry was introduced by Fry and Hart (1984). Their respirometer consisted of a circular doughnut shaped

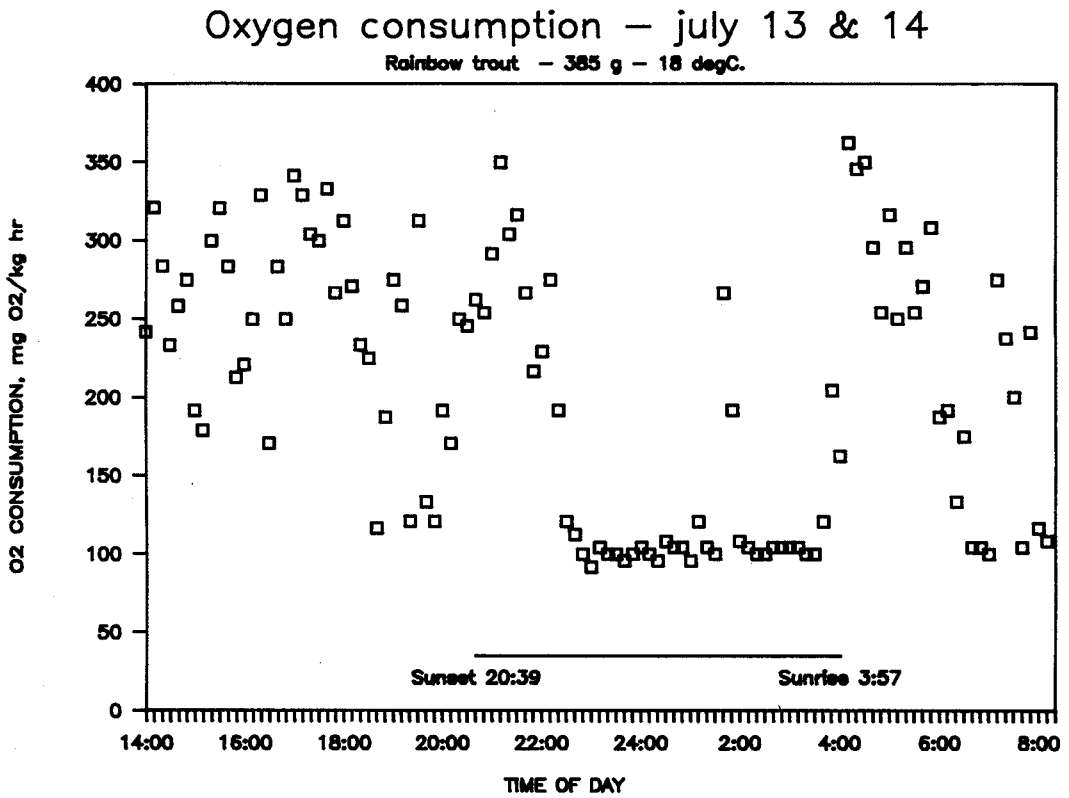

Fig. 1. An example showing the variation in oxygen consumption of a 385g rainbow trout, *Salmo gairdneri*, at 10°C, measured automatically with a computerized respirometer. See text for details. (Steffensen and Lomholt, unpublished data).

chamber, mounted on a turntable. As it rotated, the fish swam against the produced water current. The turntable had to be stopped for sampling water for determination of Cwo_2 at the beginning and termination of an experiment. $\dot{M}o_2$ was calculated according to equation (1) or (2). Since Fry and Hart's studies, several other methods have been developed (Fry 1971).

Active oxygen consumption of fish can be studied accurately using a tunnel respirometer through which the water is recirculated at a constant flow by a pump or propeller (Blazka *et al.* 1960; Brett 1964). The use of closed swimming respirometers for measurement of active oxygen consumption involves the same problems, as discussed above in relation to measuring standard $\dot{M}o_2$ of resting animals. Active $\dot{M}o_2$ increases exponentially with increasing swimming speed as long as the fish is swimming aerobically (Brett 1964). By extrapolating back to zero swimming speed standard $\dot{M}o_2$ can be determined. Brett (1964) reported, however, that fish may show restless behavior, especially at low swimming velocities. This will elevate the $\dot{M}o_2$ at low speeds, causing an over estimation of standard $\dot{M}o_2$ when extrapolating back to zero velocity.

Respirometry in open or flow-through systems

Ege and Krogh (1914) introduced the open respirometry method in experiments with goldfish placed in a small flow-through vessel. From determinations of oxygen content in the water running in and out (Cwo_2,in; Cwo_2,out) and the water flow through the respirometer ($\dot{V}w$), oxygen consumption was calculated according to the Fick principle (Fick 1870) as:

$$\dot{M}o_2 = \dot{V}w.\ (Cwo_2,in - Cwo_2,out) / bw \quad (3)$$

or:

$$\dot{M}o_2 = \dot{V}w.\beta wo_2 \times (Pwo_2,in - Pwo_2,out)/bw \quad (4)$$

Ege and Krogh (1914) reported that after occasional changes in the water flow, $\dot{V}w$, new determinations of water oxygen content were not begun until 20 to 60 min later. Respirometer flow was regulated to cause a 20% reduction of the oxygen content in the water running through the chamber. Application of the Fick principle for the calculation of $\dot{M}o_2$ assumes a steady state of the measured variables. That was the reason for waiting 20 to 60 min after a change in flow.

Hall (1929) used a modified Ege and Krogh type of respirometer. Water flow could be varied, and in this manner the oxygen tension surrounding the fish could be controlled. Hall (1929) reported that 'during experiments several oxygen tensions were taken at intervals of an elapse of an hour, which gave time for adjustment of the fishes to the oxygen tension used' (Hall 1929). However, none of the investigators at that time (Ege and Krogh 1914; Hall 1919; Keys 1930), considered the importance of the ratio of the water flow ($\dot{V}w$) to volume of water in the respirometer (Vr) which is the dilution factor, $\dot{D} = \dot{V}w/Vr$.

Using open respirometry, Spoor (1946) reported a change in oxygen consumption to lag somewhat behind a change in activity. The reason for this lag was the reservoir or wash-out effect of the chamber, which depends on the dilution factor $\dot{D}$.

In an empty flow-through respirometer in steady state, Pwo_2,in will be identical to Pwo_2,out. After a change in Pwo_2,in, Pwo_2,out will approach exponentially a new steady state according to the washout or dilution characteristics of the respirometer, which in turn depends on effective mixing in the respirometer, the volume and the flow. The exponential equation for the transformation from one steady state to another is:

$$\text{transformation } (\%) = 100.(1-\exp(-\dot{D} \times t)) \quad (5)$$

where t = time. Accordingly, for $t \rightarrow \infty$ a new steady state will be attained (100 % transformation).

Equation (5) can be rearranged to give the time required for a certain % transformation from one steady state to another, e.g. t_{95} for 95% transformation:

$$t_{(95\%)} = \frac{-\ln(100-95) \,.\, Vr/\dot{V}w}{100} \quad (6)$$

or,

$$t_{(95\%)} = \frac{-\ln(100-95) \,.\, 1/\dot{D})}{100} \quad (7)$$

where $1/\dot{D} = Vr/\dot{V}w$ = average residence time of water is the time required for 63.2% transformation or wash-out.

Equations (5), (6) and (7) apply to a respirometer without an animal. In the following the transformation from one steady state to another is derived with regards to Cwo_2 when an animal with a constant oxygen consumption is placed in the respirometer having an oxygen consumption of $\dot{M}o_2$ Hence, Cwo_2,in and Cwo_2,out will be different at steady state.

($\rho(o) = Cwo_2,in$; $\rho(t) = Cwo_2,out$; $\dot{M}o_2 \cdot bw = de/dt$; $\rho(t = o) = \rho(o)$ and $\rho(t)$ const. at steady state):

$$d/dt \,.\, \rho(t) = \frac{Vw \,.\, \rho(o)}{Vr} - \frac{Vw \,.\, \rho(t)}{Vr} - \frac{de/dt}{Vr}$$

$$d/dt \,.\, \rho(t) = \frac{Vw \,.\, \rho(o) - de/dt}{Vr} - \frac{Vw \,.\, \rho(t)}{Vr}$$

$$d/dt \,.\, \rho(t) = K - \frac{Vw \,.\, \rho(t)}{Vr}$$

$$\rho(t) = \frac{K \,.\, Vr}{\dot{V}w} + C \,.\, \exp(-\dot{V}w\underline{V}r \,.\, t)$$

for t = o

$$\rho(t = o) = \frac{K \,.\, Vr}{\dot{V}ww} + C$$

$$\rho(t = o) = o(0) = de/dt \,.\, 1/\dot{V}w + C$$

$$C = de/dt \,.\, 1/\dot{V}w$$

for $t = \infty$

$$\rho(t = \infty) = \rho(o) - de/dt \,.\, 1/\dot{V}w$$

solution $\rho(t) = \rho(o) = de/dt \cdot 1/\dot{V}w \cdot (1\text{-}exp(-\dot{V}w/Vr \cdot t))$

or:

$$Cwo_2,out(t) = Cwo_2,in - \dot{M}o_2 \cdot bw \cdot 1/\dot{V}w \cdot (1 - exp(-\dot{D} \cdot t)) \quad (8)$$

or:

$$\beta wo_2 \cdot Pwo_2,out(t) = \beta wo_2 \cdot Pwo_2,in - \dot{M}o_2 \cdot 1/\dot{V}w\ (1\text{-}exp(-\dot{D}\ t)) \quad (9)$$

From equations (8) and (9) it appears that the time-course of the transformation from one steady state to another is independent of the presence of an animal in the respirometer. Hence after a change, equations (6) or (7) allows calculation of the time needed to reach a new steady state when using flow-through respirometry. Changes in $\dot{M}o_2$ of the animal will interfere, however, although the error resulting from this will be small.

The % transformation from one steady state to another *vs* time is shown in Fig. 2 for a number of dilution rates typically used in flow-through respirometry, ranging from 1.0 min^{-1} to .01 min^{-1}. A new steady state is reached faster with increasing dilution rates. From Fig. 3, t_{99}, t_{95}, t_{90}, $t_{63.2}$ and t_{50} can be read for dilution rates commonly used in respirometry. Increasing the flow 10 times or decreasing the volume of the respirometer 10 times results in a new steady state 10 times faster.

In recapitulating Hall's (1929) experiments on the effect of hypoxia on resting toadfish, *Opsanus tau*, knowing that the volume of the respirometer was 10 l (Vr), and assuming the flow ($\dot{V}w$) was 0.5 l min^{-1} and that the toadfish is an oxygen regulator, it appears from Fig. 3 that t_{95} and t_{99} for a dilution rate of 0.05 min^{-1} was 60 min and 92 min respectively. Hall reported that the Cwo_2 determinations were taken after an hour had elapsed. Thus at least 95% transformation had occurred, and the use of equation (3) or (4) can be justified. If 99% transformation had been the criterion for a new steady state, no samples should have been taken for the first 92 min. However, in order to expose the toadfish to hypoxic water (20–25% air-saturated) assuming a unchanged $\dot{M}o_2$, Hall had to decrease the water flow, $\dot{V}w$, 4 times since Pwo_2, out and $\dot{V}w$ are inversely proportional. Hence t_{95} and t_{99} would be 240 min and 368 min respectively, and

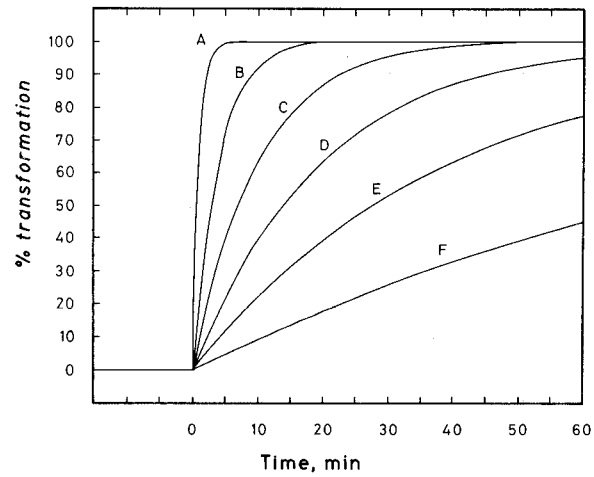

Fig. 2. Time versus % transformation from one steady state to another at different dilution ratios. A = 1.0 min^{-1}, B = 0.25 min^{-1}, C = 0.1 min^{-1}, D = 0.05 min^{-1}, E = 0.025 min^{-1}, F = 0.01 min^{-1}.

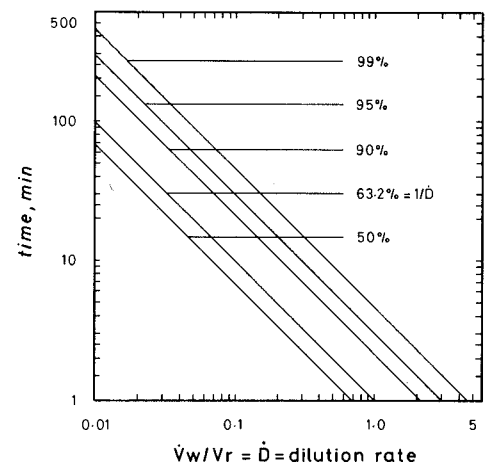

Fig. 3. Approximate times required for wash-out of a respirometer at various dilution rates (= flow through the respirometer/volume of the respirometer). Example applies to a respirometer with a volume of 1.0 liter and a flow of 0.1 liter.min^{-1}. The dilution rate is 0.1 min^{-1}, and 99% wash-out time is reached in about 46 minutes.

equation (3) or (4) cannot be used until at least 240 min after the change in flow through the respirometer. Equation (5) indicates that after 60 min only 53% of the transformation has occurred. If, in this case, equation (3) or (4) had been used to calculate $\dot{M}o_2$, the result would be that $\dot{M}o_2$ after 1 h was 47% lower than 3 h later. This erroneous

use of open respirometry may be the reason why Hall (1929) reported the toadfish to be an oxygen conformer. This classic study by Hall has often been used as an example of an oxygen conformer (Schmidt-Nielsen 1979, Randall and Daxboeck 1984). In contrast to Hall's results, Ultsch *et al.* (1981) recently showed that the toadfish is an O_2 regulator.

In most open respirometry studies on fish, the dilution ratio varies from 0.5 min^{-1} to 0.05 min^{-1} with a corresponding t_{99} of 9.2 min to 92 min respectively. However, in experiments on invertebrates $\dot{V}w/Vr$ ratios as low as 0.05 to 0.01 min^{-1} have been used with t_{99} lasting up to 460 min. Obviously it is essential to correct for wash-out in studies of the effect of acute changes in ambient conditions or behavior.

Fry (1971) focused attention on this problem and discussed how to account for the lag or wash-out by the use of a factor to correct for the changes in oxygen content in the water in the respirometer during the period of measurement. Unfortunately this correction factor has been overlooked by most investigators. In 1978, Niimi described the following formula for approximation of $\dot{M}o_2$ in non-steady systems:

$$\dot{M}o_2 = \beta wo_2.\dot{V}w. \left(\frac{Pwo_2,out.t - o.\exp(-\dot{V}w.\Delta t/Vr) - Pwo_2,out,t_1}{+ (1 - \exp(-\dot{V}w.\Delta t/Vr)} + Pwo_2,in \right) .1/bw \qquad (10)$$

where Pwo_2,out,t_0 and Pwo_2,out,t_1 is the partial pressure of oxygen in water leaving the respirometer at t_0 and t_1. $\Delta t = t_1 - t_0$.

From this equation $\dot{M}o_2$ can be approximated for any time interval, even during the transformation from one steady state to another.

Fig. 4 gives an hypothetical example of the effect of acute hypoxia. An animal with a constant $\dot{M}o_2$ of 30 mg O_2 min^{-1} independent of Pwo_2 (an oxygen regulator) is assumed. At time 0 the respirometer with a $\dot{V}w/Vr$ ratio of 0.1 min^{-1} is in steady state and Pwo_2,in and Pwo_2,out are 150 mmHg and 120 mmHg, respectively. At time 0, Pwo_2,in is changed in a stepwise fashion to 110 mmHg, and Pwo_2,out approaches the new steady state of 80 mmHg according to equation (5) (--- in Fig. 4). By incorrectly using equation (4) when the system is in non-steady state it appears that $\dot{M}o_2$ immediately after the change will come out negative (+ + + in Fig. 4), and only after 46 min will approach the correct value. Conversely by using equation (10) $\dot{M}o_2$ will immediately approach the assumed $\dot{M}o_2$ of 30 mg O_2 min^{-1} (xxx). This example illustrates how the use of equation (10) allows an approximation of $\dot{M}o_2$ in a non-steady state system immediately after a stepwise change in Pwo_2.

After supplying the respirometer with hypoxic water for 50 min, it was changed back to normoxic water with a Pwo_2, in of 150 mmHg. As stated above, the use of equation (4) would now overestimate $\dot{M}o_2$ by more than 100% shortly after the acute change in Pwo_2,in (+ + +). After 46 min the correct $\dot{M}o_2$ would again be approached.

Hence the use of open respirometry, without consideration of the dilution properties of the respirometer and the corresponding corrections, may result in misinterpretations of the effects of hypoxia. The application of equation (4) in Fig. 4 will, for example result in the incorrect conclusion that $\dot{M}o_2$ was depressed, after acute exposure to hypoxia, and that the animal slowly adjusted to the hypoxic condition as $\dot{M}o_2$ approached the normoxic value. Upon return to normoxia the false increase in $\dot{M}o_2$ might erroneously be attributed to a recovery process where an oxygen debt, caused by the acute hypoxia was paid back.

A modification of a flow-through respirometer was introduced by van Dam (1938). The van Dam-method involved a 2 compartment system separating the head and mouth from the opercular opening by a rubber membrane. Ventilation volume as well as inspired and expired Pwo_2 were measured directly. From this information, $\dot{M}o_2$ was calculated as in equation (4) using the Fick principle. This method allowed van Dam to investigate the influence of a progressive fall in water oxygen content on ventilation volume, oxygen extraction and by the Fick principle derive $\dot{M}o_2$ of rainbow trout, *Salmo gairdneri*, and other species. Rainbow trout showed a four-fold increase in ventilation volume

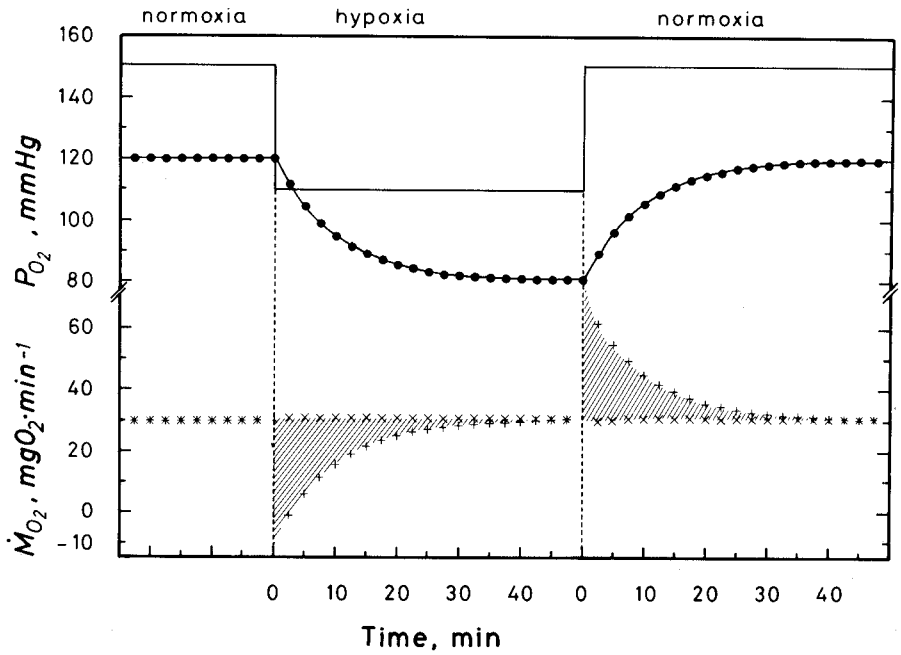

Fig. 4. Theoretical example of a flow-through respirometer experiment when exposing a fish to acute hypoxia, and return to normoxia. Oxygen tension in the respirometer was changed in a stepwise fashion (——), and combined with a selected dilution factor of 0.1, the oxygen tension in the water leaving the respirometer will exponentially approach a new steady state (— • — • —). Oxygen consumption was calculated from the Fick principle (+ + +) as well as the formula given by Niimi (1978) (xxx).

in hypoxic water and oxygen consumption increased by 79%, 'owing to the greatly increased respiratory movements'. This study is frequently cited by investigators of the energetic cost of branchial ventilation (Jones 1971; Cameron and Cech 1970; Randall 1970). Van Dam (1938) decreased inspired water oxygen content progressively from 100% of air saturation to 25% during an experiment lasting approximately 1.5 h. Unfortunately, the volume of the expiration side of the 2 compartment chamber is not given. Hence, it is not possible to calculate the dilution factor, and for that reason impossible to correct for the wash-out and calculate the correct $\dot{M}o_2$. Most likely the system was in steady state only for the initial measurement of $\dot{M}o_2$ in 100% saturated water.

In contrast to Van Dam's study (1938), the energetic cost of ventilation in the rainbow trout has recently been reported to be only 8 to 13% of the total oxygen consumption (Steffensen 1985).

van Dam's method has been used extensively to study the effect of acute hypoxia, hypercapnia, pH changes in the water or respiratory gas exchange. Few investigators appear to have been aware of the dilution factor. Among them are Burggren and Cameron (1980), who studied the effect of acute hypoxia on the channel catfish, *Ictalurus punctatus*. They suggested that the expiration compartment, with an effective volume of 1.7 l (2.7 l – 1.0 l fish vol), would be washed out in 8–12 min and 5–7 min with flow rates of 200 $ml.min^{-1}$ and 300 $ml.min^{-1}$ respectively. They reasoned that this would match the time required to fill the empty expiration chamber. After waiting this initial period $\dot{M}o_2$ was calculated by the Fick principle. This is, however, not a reasonable estimate of the displacement time of water in the chamber. From equation (5) it can be calculated that t_{99} at a flow of 200 $ml.min^{-1}$ is 39 min and t_{99} is 26 min at 300 $ml.min^{-1}$, and therefore equation (10) should have been used instead.

Direct and continuous measurements of ventilation volume and oxygen extraction are possible on fish equipped with snugly fitted rubber masks. This allows the application on an electromagnetic flow probe at the outlet of the mask for direct measure-

ments of ventilation (Piiper *et al.* 1977; Lomholt and Johansen 1979). A similar method has been used to measure ventilation in flatfish naturally buried in the sediment, by placing a 30 ml plastic funnel in the sand surrounding the upper opercular outlet. A flow probe mounted in the exit channel allows continuous recording of ventilation (Kerstens *et al.* 1979). Expired water was sampled through polyethylene tubing from the lumen of the mask or funnel. The volume of the mask or funnel (Vr) to gill water flow ($\dot{V}g$) ratio with this design is typically ≥ 2 at normoxic conditions. This results in a rapid dilution, with a t_{95} of ≤ 1.5 min and a t_{99} of ≤ 2.3 min. When the animals are exposed to hypoxia, $\dot{V}g$ increases, resulting in a $\dot{V}g/Vr$ ratio ≥ 5 and a t_{99} ≤ 1 min. This method makes continous and direct measurement of gill ventilation ($\dot{V}g$) as well as oxygen extraction possible. Since dilution is so rapid, it also allows calculation of $\dot{M}o_2$ across the gills from the Fick principle, even when acute changes in ambient O_2 tension are applied.

Flow-through respirometry with active animals

The first experiments measuring $\dot{M}o_2$ in fish during activity were performed by Spoor (1946), using an open respirometer. He reported a lag between increase in activity and the attendant increase in $\dot{M}o_2$. This phenomenon has since been discussed by several investigators, e.g. Kausch (1968). The lag was due to the wash-out of the respirometer.

In Fig. 5 another hypothetical example is shown for a respirometer with a realistic dilution factor $\dot{D}$ = .1 min^{-1}. The fish has a constant $\dot{M}o_2$ of 20 and 80 mg O_2 min^{-1} at exercise levels I and II, respectively. Initially at exercise level I the system is in steady state, and $\dot{M}o_2$ is 20 mg O_2 min^{-1}. After a step change to exercise level II, $\dot{M}o_2$ calculated by the Fick principle (4) exponentially approached 80 mg O_2 min^{-1} according to the transformation equation (+ + + in Fig. 5). $\dot{M}o_2$ should have been 80 mg O_2 min^{-1} immediately after the change in activity. If $\dot{M}o_2$ is calculated from equation (10), a value nearly identical to the given oxygen consumption is obtained (xxx) and when returning to exercise level I the oxygen consumption returns to 20 mg O_2 min^{-1} immediately. However, $\dot{M}o_2$ exponentially approached 20 mg O_2 min^{-1} when calculated using the Fick principle (+ + +). With the use of equation (10) $\dot{M}o_2$ (xxx) is found to be nearly identical to the theoretical $\dot{M}o_2$. An incorrect conclusion could be drawn from the curve in Fig. 5 based on equation (4) namely that the adjustment of $\dot{M}o_2$ to the sudden increase in activity to level II, is gradual. The area A would represent the error (underestimation) in the quantity of oxygen consumed during the period at exercise level II. Upon returning to activity level I a positive error results, represented by area B. Again $\dot{M}o_2$ only gradually approached the steady state value of 20 mg O_2 min^{-1}.

If open respirometry is performed on animals showing spontaneous activity additional misinterpretations can occur. For determination of standard oxygen consumption, it is common to measure $\dot{M}o_2$ at several different activity levels and extrapolate back to zero activity. This is shown in Fig. 6 as points A,B,C and Y-intercept, S. In an experiment in which an animal is allowed to perform spontaneous activity, most time would be spent at a preferred activity level, with a $\dot{M}o_2$ corresponding to point B. The system would likely be in steady state at this activity level. If the animal increased its activity for a short period, apparent $\dot{M}o_2$ would increase, but only to C* when calculated from equation (4) if the system was in non-steady state. In fact, apparent $\dot{M}o_2$ would have increased to C if calculated from equation (10) or if the system was in steady state.

If the animal decreased its activity level, the use of equation (4), in the case of non-steady state, would result in an erroneously high apparent $\dot{M}o_2$ corresponding to A* in Fig. 6, compared to a $\dot{M}o_2$ of A if calculated from equation (11) or at steady state. When using equation (4) the slope of the activity vs log $\dot{M}o_2$ line will be rotated clockwise (relative to the 'correct' line) around $\dot{M}o_2$ at the preferred activity level. This would result in overestimation of standard $\dot{M}o_2$. Similar problems in flow-through respirometry where fish are subjected to forced swimming for short periods were ad-

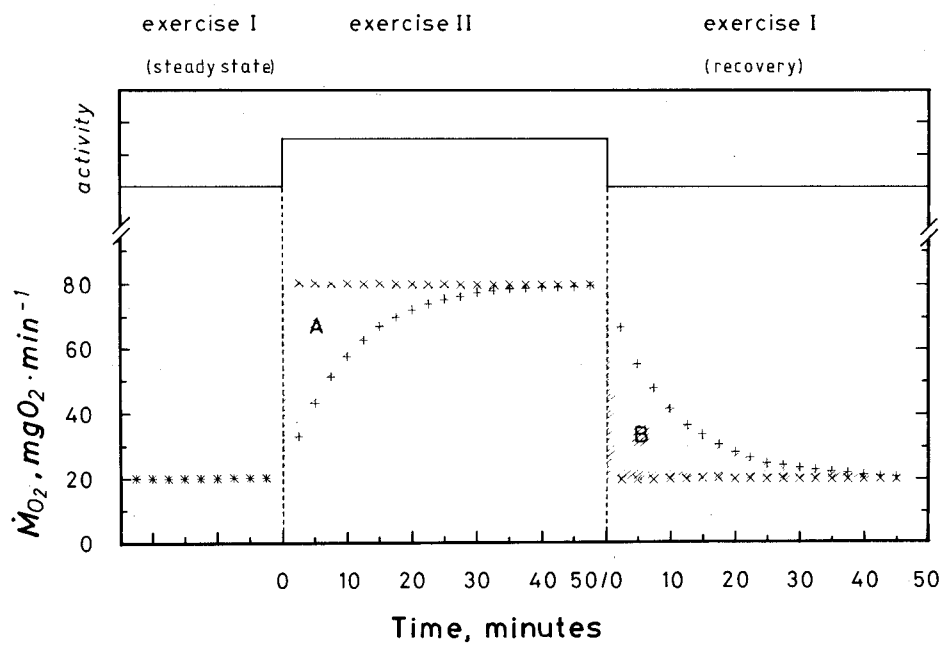

Fig. 5. Theoretical example showing oxygen consumption *vs* swimming speed based on flow-through swimming respirometry with a dilution factor of 0.1 min^{-1}. The fish was forced to swim at 2 different constant speeds. Oxygen consumption was calculated from the Fick principle (+ + +) as well as according to Niimi (1978) (xxx). The shaded areas A and B express the difference obtained when using the two formulas. Theoretically $\dot{M}o_2$ should be constant at the 2 exercise levels. Using the Fick principle will underestimate the quantity of oxygen consumed during the period at exercise level II by the area A.

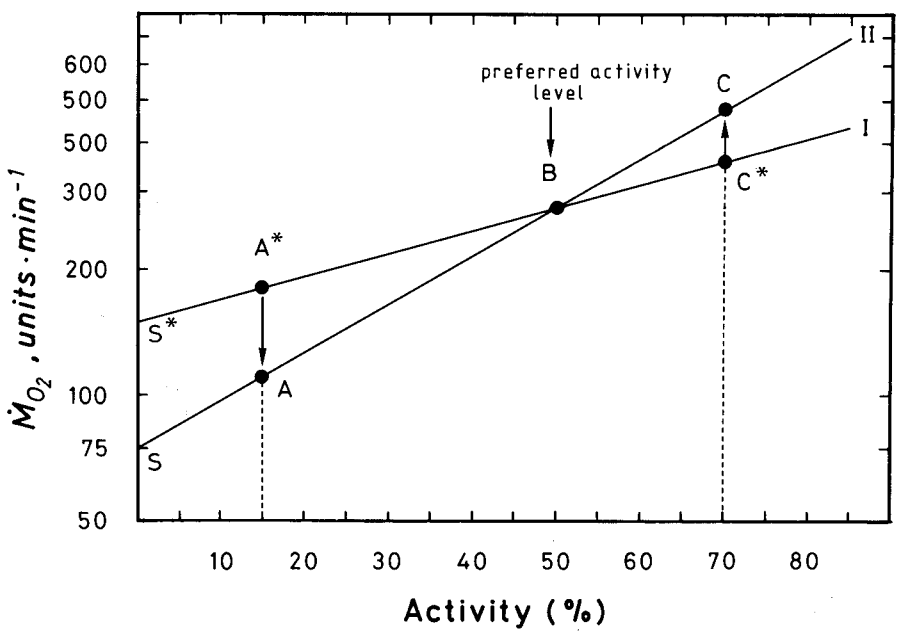

Fig. 6. A theoretical example of oxygen consumption *vs* swimming speed using a flow-through respirometer with a dilution factor of 0.1 min^{-1}. The fish can choose the swimming speed or activity level. At the preferred activity level the system will most likely be in steady state. At higher and lower activity levels oxygen consumption will be A and B, if the system is in steady state at these activity levels. The system might be in a transformation state, however, which will rotate the log $\dot{M}o_2$ *vs* activity line clockwise around B, and through A′ and C′. If the system is in non-steady state it wil result in an overestimation of the standard oxygen consumption according to the Y-intercept S′.

dressed by Blazka *et al.* (1960), but his analysis has been largely ignored despite its consequences.

Conclusion

Based on the discussion above, measurement of oxygen consumption of aquatic breathers is recommended as follows:

1. Avoid using closed respirometry which cause accumulation of CO_2 and other excretory products as well as large changes in ambient oxygen concentration.
2. Avoid using flow-through respirometry since it is difficult and troublesome to correct for washout.
3. Use intermittent-flow respirometry, which involves none of the problems described above.

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