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AN AUTOMATED SWIMMING RESPIROMETER

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Abstract—An automated respirometer is described that can be used for computerized respirometry of trout and sharks.

INTRODUCTION

There has been an increasing interest in studying physiological characteristics of swimming fish under minimal constraints. This interest has led to improved techniques for semichronic cannulations of blood vessels (Smith and Bell, 1964; Soivio and Nyholm, 1975) and to the development of improved respirometers allowing studies at controlled water velocities and ambient water qualities.

This report concerns a tunnel respirometer designed for studies of swimming performance in fish or other aquatic animals performing horizontal movements.

The system is designed for computerized monitoring of water velocity, temperature and level of oxygenation. The system can sample and compute O_2 consumption at programmed conditions for longer periods, i.e. 24 hr.

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DESCRIPTION OF THE SWIMMING RESPIROMETER

The use of respirometry in studies of oxygen uptake in swimming fish was pioneered by Fry and Hart (1948), and further developed by Blazka *et al.* (1960) and by Brett (1964).

The swimming respirometer to be described below is of the Brett type (Brett, 1964) and a modification of one previously described by Christensen *et al.* (1982). It is built from stainless steel tubing, except for the expansion and contraction cones and the interchangeable transparent swimming section, which were made from PVC. The swimming section (Fig. 1.) (a), with an i.d. from 10 to 20 cm, was fitted with an electrified screen at the downstream end (e) to secure

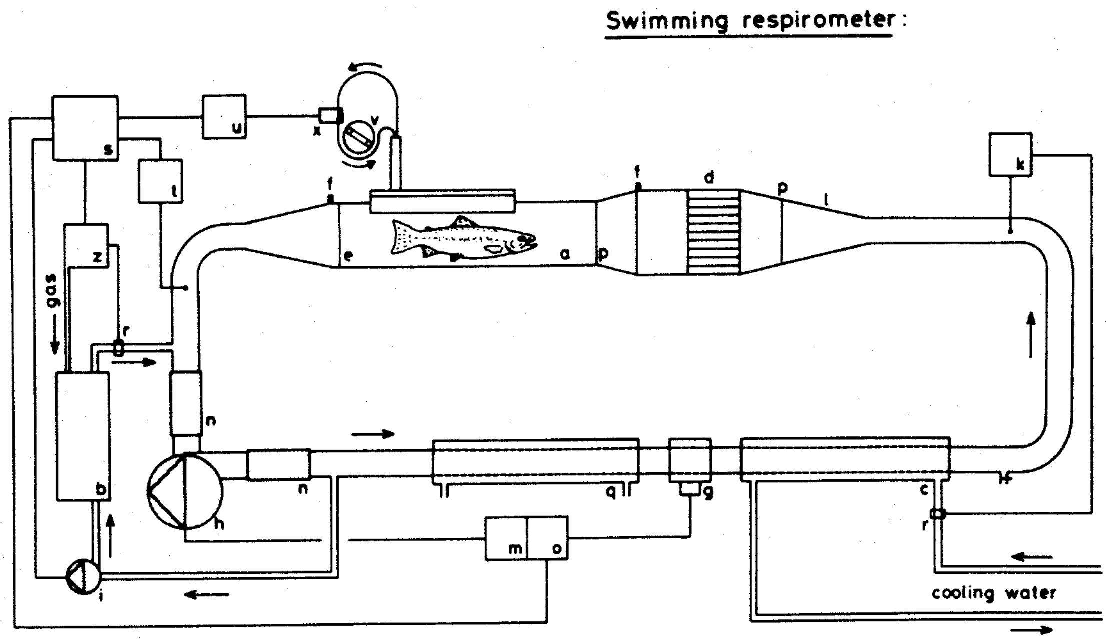

Fig. 1. Swimming respirometer: (a) swimming chamber, (b) artificial lung, (c) cooling shell, (d) turbulence tubes, (e) electrified screen, (f) bubble trap, (g) turbine flowmeter, (h) main pump, (i) lung circuit pump, (j) thermistor, (k) temperature control unit, (l) expansion cone, (m) motor control, (n) rubber joints, (o) flow gauge, (p) turbulence screens, (q) heating shell, (r) magnet valve, (s) computer with interface, (t) thermometer, (u) oxygen meter, (v) roller pump, (x) oxygen electrode, (z) oxygen regulator.

continuous swimming. The swimming section is made to be easily interchangeable, allowing studies on fish of variable size. Vertical strips of tape on the outer surface of the upstream end of the swimming section were used to provide visual cues for orientation. An expansion cone (i) with a turbulence screen (p), small turbulence tubes (d) and a contraction cone, also with a turbulence screen, reduced the magnitude of axial velocity perturbations and turbulence, and produced an almost uniform velocity profile in the swimming section. When working with f. ex. rainbow trout (*Salmo gairdneri*), a swimming section with an i.d. of 15 cm was used, and the constant volume of water (in this case 40.1 l) was circulated in a closed circuit by means of a variable 1.5 hp centrifugal pump (h) with a motor control unit (m). Velocities in the swimming section could be regulated up to 60 cm·sec^{-1}, and were measured by a turbine flowmeter (g and o). In order to assure sufficient mixing, water velocities less than 5 cm·sec^{-1} were never used. The centrifugal pump was connected to the respirometer through rubber joints (n) to reduce vibrations.

A thermostat (k) controlled experimental temperature to a preset value ±0.2°C by means of a magnet valve (r) opening or closing for supply of cooling water to the cooling unit (c).

Water oxygen tension (PwO_2) in the respirometer was measured continuously by recirculating a small fraction of the water through an O_2 electrode cuvette (x) (Radiometer, D-616, E-5046) by a roller pump (Istmatic Mini-S, 840) fitted with gas tight tygon tubing. The O_2 electrode was connected to a Radiometer PHM-71 acid-base analyser (v) and via an analog-digital interface fed to a Hewlett-Packard A-9825 computer (s). Water oxygen tension in the respirometer was controlled to a preset value by the computer and an oxygen regulator (z) by opening or closing a magnet valve (r) and turning on or off a magnetic-centrifugal aquarium pump (i) controlling the amount of water shunted through a membrane gas exchanger (artificial lung) (b), made of a silicone membrane with a surface of 3.5 m^2 (Sci-Med Kolobow). The computer was also controlling two magnet valves in the oxygen regulator, directing either nitrogen or an air/O_2 mixture through the lung countercurrent to the water flow. Nitrogen was only used when the water oxygen tension was far above the preset PwO_2 (> 25 mm Hg). Otherwise PwO_2 was allowed to decrease to the set value by the oxygen consumption of the fish.

The computer was also used to sample water velocity and correct for solid blocking effect of the fish as described by Bell and Terhune (1970), and it continuously displayed the corrected actual swimming speed. This was corrected as given in Bell and Terhune (1970):

$$V_F = V_T(I + \varepsilon_s)$$

where V_F are corrected velocities with the fish present, and V_T the value in the absence of the fish. The fractional error due to solid blocking can be calculated as:

$$\varepsilon_s = \tau \cdot \lambda \left(\frac{A_o}{A_T}\right) 3/2$$

where $\tau = 0.8$ and $\lambda = 0.5 \times$ (length of fish/body thickness). A_o was the maximum cross-sectional area of the fish and A_T was the swimming section area. The correction factor was usually 10–20% for rainbow trout weighing 250–900 g.

APPLICATION OF THE RESPIROMETER

A normal experimental procedure could be as follows: the fish was usually transferred to the swimming respirometer the day before an experiment to recover from the effect of handling and for acclimation to a low swimming speed—the pre-experiment period (PE on Fig. 2). The duration of this period was preset in hr and min on the computer, and a preset PwO_2 (PS_{zero}) was controlled to within ±2 mm Hg. The regulation of PwO_2 to a PS_{zero} value (in Fig. 2 at 155 mm Hg) is in progress in the pre-experiment period (PE). The open squares at the top

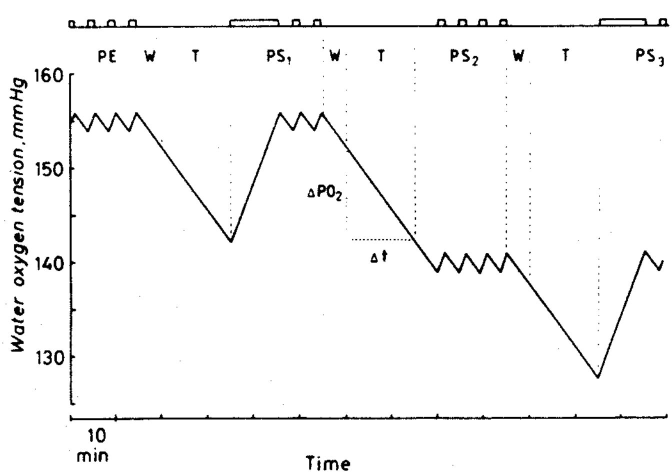

Fig. 2. Schematic diagram which illustrates the application of the respirometer. PE = pre-experiment period (hr, min). PS_n = preset water oxygen tension (mm Hg), W = wait period (min), T = test period (min). The open squares at the top indicate when a supply of air/O_2 mixture was directed through the lung.

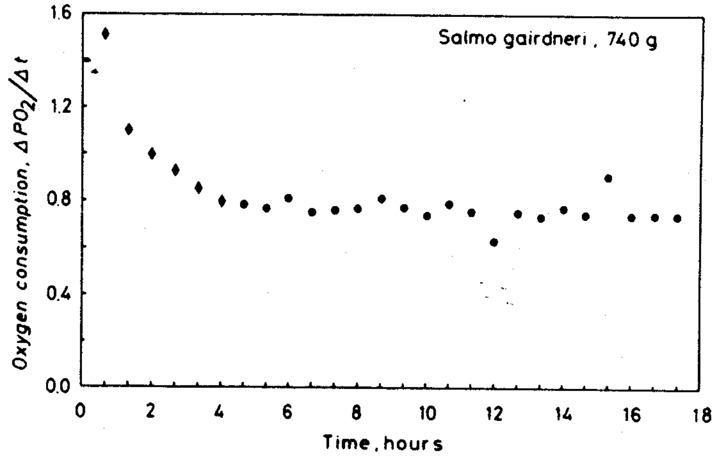

Fig. 3. Time course of oxygen consumption ($\Delta PO_2/\Delta t$) in a rainbow trout swimming $25\,cm \cdot sec^{-1}$. There was an adjustment period of 5 hr after the fish was transferred to the respirometer, during which oxygen consumption declined (◆), before stable readings were obtained (●).

indicate when a supply of air/O_2 mixture was directed to the lung. At the termination of the PE period, the wait period (W) was started, the lung circuit was shut off, and PwO_2 started to decrease. A 5 min wait period was introduced because the decrease of PwO_2 was not linear over the first 1–2 min due to a combination of lag time in the gas exchanger and the O_2 electrode. After the wait period, the test period (T) started, and a pre-determined number of data point pairs consisting of oxygen tension and time were collected by the computer. The test period lasted from 15 to 40 min, in which PwO_2 was measured once per sec and averaged every 30 sec, i.e. $30 \times 30 = 900$ data points for a 15 min test period. At the termination of the test period a linear regression analysis was conducted and the following information immediately printed out: "measurement number"—all data from test periods were stored in files on tape in the computer, and numbered 0–160; "No. of samples"—in this case 30 samples, consisting each of 30 measurements, i.e. 900 data points: "PO_2 mean value" was the mean PwO_2 during a test period; "slope"—$\Delta PwO_2/\Delta t$ calculated from linear regression is expressed by:

$$\Delta PwO_2/\Delta t = \frac{n \cdot \sum x \cdot y - \sum x \cdot \sum y}{n \cdot \sum x^2 - \left(\sum x\right)^2}$$

n = number of samples

and the "correlation coefficient" (r) as

$$r = \frac{n \cdot \sum x \cdot y - \sum x \cdot \sum y}{\sqrt{\left[n \cdot \sum x^2 - \left(\sum x\right)^2\right]\left[n \sum y^2 - \left(\sum y\right)^2\right]}}.$$

Oxygen consumption, VO_2, was calculated as

$$VO_2 = \frac{\Delta PwO_2/\Delta t \times V_{s.r.} \times \alpha}{B.W.}$$

where $V_{s.r.}$ = volume of swimming respirometer, B.W. = body wt and α = oxygen solubility coefficient. Furthermore maximum and minimum temperature and swimming velocity and PwO_2 at the beginning and at the end of the test period were printed out.

Simultaneously the computer was engaged in regulating PwO_2 at the next preset value (PS_1), and this period usually lasted 20–40 min. Up to 10 different preset PwO_2 values (PS_{zero}–PS_{10}) could be stored and the computer would run the above described procedure automatically and continue from PS_{zero} after a test in PS_{10}.

The computer could also be programmed to disconnect and terminate a test period earlier than the preset time and continue to the next PS_n if the relative decrease in PwO_2 was more than a certain value (usually 20 mm Hg). It was also programmed not to go from PS_n to a wait period unless PwO_2 was within a certain range of the preset value (usually ± 5 mm Hg), and if that happened, the PS_n period would of course last longer than the preset time.

Figure 3 shows the time course of oxygen consumption when a rainbow trout had been transferred to the respirometer. Swimming speed: $25\,cm \cdot sec^{-1}$, temperature: 15°C. There is clearly an initial adjustment period during which oxygen consumption declines before stable readings were obtained (◆). The oxygen consumption during the following 12 hr (●) are seen to be very stable (mean $\pm$ SD; $0.765\,mm\,Hg \cdot min^{-1} \pm 0.051\,mm\,Hg \cdot min^{-1}$).

The use of computerized respirometry as described above was used by Bushnell *et al.* (1984) and Steffensen (1984) in studies of oxygen consumption in swimming rainbow trout and ram ventilating sharksucker, *Echeneis naucrates*. It allows acquisition of data points at a rate several orders of magnitude greater than would be possible with manual operation of instruments, and should prove to be a valuable tool also for open and closed respirometry of resting animals.

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