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SHORT COMMUNICATION

AN ANNULAR RESPIROMETER FOR MEASURING AEROBIC METABOLIC RATES OF LARGE, SCHOOLING FISHES

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Aerobic metabolic rates of fish at controlled levels of activity are usually measured with individual fish isolated in a flume-type respirometer, in which the fish must swim against a known water velocity (Brett, 1964; Beamish, 1978; Gehrke *et al.* 1990). Their use for large and fast-swimming fish is difficult owing to engineering problems and water turbulence and because swimming in the confined test section of the respirometer affects the performance of the fish. Some of these problems have been partially dealt with by applying corrections to compensate for the altered water flow around the body of the fish (Webb, 1971) and by developing highly sophisticated flume respirometers (Gehrke *et al.* 1990).

Rheotactic stimuli have also been used to control activity in annular rotating vessels such as the ‘fish wheel’ of Bainbridge (1958) and respirometers (Wohlschlag, 1957; Fry, 1971). An alternative way of controlling swimming activity is to utilize the optomotor response of fish (Harden-Jones, 1963). The main advantage of such a system is that the fish swim at a controlled speed, set by a moving background pattern, through still water. Dabrowski (1986) used a small annular respirometer where swimming speed of salmonid fry was controlled by a pattern rotating around the chamber. The apparatus described below has been developed to provide a new solution to the problem of measuring oxygen consumption in larger active fish, such as adult salmon, and in fast-swimming obligate-schooling fish, such as mackerel, which are highly susceptible to stress and for which flume respirometers are unsuitable.

The respirometer tube (Fig. 1) was submerged in sea water in the 10m diameter annular tank at the Marine Laboratory, Aberdeen (He and Wardle, 1988). Size and shape of the tube were a compromise between minimal restriction of fish swimming ability and minimal volume to give reliable measurements of oxygen consumption rates. The respirometer was constructed from twelve, 5mm thick, clear polyvinylchloride (PVC) (Darvic) tubular (0.78m diameter) modules, each containing an angle of 150°, in plan

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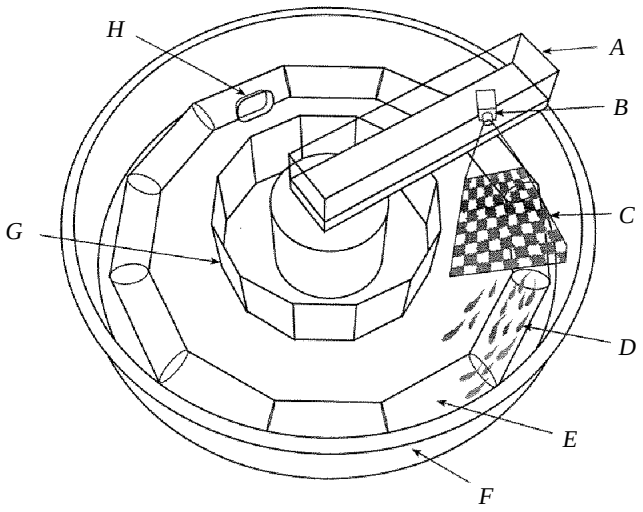

Fig. 1. Schematic diagram of the annular respirometer design in use. Water level is not shown for clarity. The background illumination would normally be low. *A*, gantry; *B*, pattern projector; *C*, pattern; *D*, school of fish swimming with and ahead of pattern; *E*, annular respirometer (submerged below water); *F*, tank wall; *G*, fabric barriers containing fish-holding area; *H*, respirometer door. Note that some fish are shown in the training corridor within the annular tank.

forming a dodecagon (see *E*, Fig. 1) with an external diameter of approximately 9.25m. The 12 modules were butt-jointed and sealed using silicone rubber adhesive. Each respirometer module had a bung hole of 2cm diameter in the upper surface for the removal of trapped air bubbles. A removable access door (0.6 m×0.4m) in one of the modules was attached by quick-release clips. A PVC funnel could be attached under water to the access door, enabling transfer of fish to and from the holding area without handling. Total volume of the respirometer system was 12690l.

Two water sampling points were positioned diametrically opposite one another, each connected to a chamber with an oxygen electrode (Orbisphere 2126). This design was adopted because initially it was feared that the large volume of water and the shape of the respirometer would predispose it to uneven mixing of the water inside, but in initial trials with swimming fish it was found that the two electrodes recorded the same rate of decline of oxygen saturation. Fish swimming around the respirometer mix the water and progressively remove oxygen from it. Thus, in practice, one sampling unit was used, and this was periodically checked for accuracy against the second. Calculations indicated that, in the necessarily large water mass used, predicted oxygen uptake rates would be low. The oxygen electrodes used were therefore chosen for very high stability. The dissolved oxygen level and water temperature within the respirometer were recorded on a two-channel pen recorder (Gould MS272) and data logger (Orion Delta).

Individual experimental runs were not started until oxygen saturation was at least 90%. A submersible pump (flow rate 200 l min^{-1}) with one-way valves was connected to the respirometer to exchange water when required from the surrounding tank, which

contained aerated sea water. Following each experiment, the respirometer was opened and the pump operated to re-establish an oxygen saturation above 90%; this procedure took approximately 3–5h.

Seals were tested by introducing water with a reduced oxygen content into the respirometer, sealing the respirometer and monitoring for 24h for evidence of a rise in oxygen content caused by diffusion. Negligible changes occurred over this period. Dye solutions were injected into the respirometer and allowed to diffuse over several days. No traces of dye were detected in the water surrounding the respirometer.

Prior to introduction to the respirometer, fish were trained to follow a light pattern projected from a rotating gantry (see Fig. 1) for which the speed could be controlled and recorded (He and Wardle, 1988). The fish swam in a corridor adjacent to the respirometer, with a mean diameter of 7.5m. The fish were finally transferred to the respirometer by coaxing them to swim through the guide funnel. The funnel was then replaced by the door and the bung holes were closed. After being given time to acclimate to the respirometer, the group was set swimming at a chosen speed determined by the speed of the rotating gantry, and the metabolic rate was calculated from the rate of decline of oxygen level. Oxygen levels were not allowed to fall below 80% of the air-saturated value at any time. Each measurement was carried out over a period of 60–120min at a controlled speed. In order to remove fish from the respirometer, the portion of the respirometer tube adjacent to the door was blocked with a piece of fabric as a barrier and a pattern was projected from the moving gantry. The fish swam with the pattern towards the open door and swam out of their own accord, or could be caught at the doorway with a dip net.

Separate tests were carried out in sea water with two fish species, adult Atlantic salmon, *Salmo salar* L., obtained from a commercial fish farm, and adult Atlantic mackerel, *Scomber scombrus* L., caught and transported from the west coast of Scotland. The school of salmon consisted of eight fish (mean total length 58.7cm, S.D. 4.4cm; mean mass 1979g, S.D. 366g) and experiments were carried out at 11˚C, S.D. 0.5˚C. The mackerel school consisted of 37 fish (mean total length 34.7cm, S.D. 3.1cm; mean mass 413g, S.D. 147g) and experiments were carried out at 9.9˚C, S.D. 0.6˚C. The mackerel were captured and tank-adapted using the techniques of Glass *et al*. (1986). Water temperature was not observed to vary during any experimental run, although thermometer accuracies were rated to 0.1˚C only.

Fish were not fed while in the respirometer and food was witheld for 48h prior to introduction. Background light levels of 10lx were reduced to 0.1lx during projector operation in the experiments with salmon. For the group of mackerel, the most reliable visual stimulus was obtained using a black plastic sheet (3.0 m×1.5m) trailed on the water surface behind the moving gantry at a background light intensity of 10lx.

Resting rates of oxygen consumption for salmon at zero activity were obtained by reducing ambient light levels to 10^{-4}lx. At this low light level, the fish stopped voluntary swimming, settled on the bottom and remained stationary until light intensity was raised (a period of hours). This was validated by periodic checks with a low-intensity red light which did not disturb the fish, and showed them to be resting, spread out around the respirometer. During these measurements, a small submersible pump was installed

in the respirometer; it created a low flow to aid water distribution and mixing ($<0.01\,m\,s^{-1}$).

The relationship between oxygen consumption and swimming speed for adult Atlantic salmon and Atlantic mackerel is shown in Fig. 2. Semi-logarithmic oxygen consumption ($\dot{M}_{O_2}$, $mmol\,O_2\,kg^{-1}\,h^{-1}$) *versus* swimming speed (U, $m\,s^{-1}$) regressions are shown below and are the lines indicated in Fig. 2. The points obtained for salmon at rest were not included in the regression.

Atlantic salmon:

$$\log\dot{M}_{O_2} = 0.04 + 1.05U \; (r^2 = 0.866);$$

Atlantic mackerel:

$$\log\dot{M}_{O_2} = 0.18 + 0.82U \; (r^2 = 0.847).$$

The data for salmon and mackerel lie along the same approximate line (ANCOVA, slope, F=2.72, P>0.05; intercept, F=0.004, P>0.05), indicating that the costs of swimming per unit mass at the speeds studied are similar for the two species, despite differences in size and morphology. The gradient of the power ($\dot{M}_{O_2}$)–swimming speed relationship can be regarded as an indicator of the efficiency of swimming (Priede, 1985); increasing the drag load of a fish increases the slope of the relationship (Webb, 1971). Slope coefficients for a variety of studies of salmonids (0.27–0.31m in length) in flume respirometers were examined (Brett, 1964; Webb, 1971; Duthie, 1987) and found to vary between 1.2 and 1.6. The relatively low values obtained in the present study appear to indicate that the

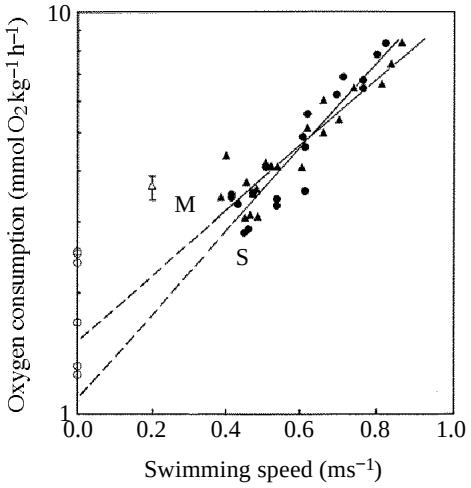

Fig. 2. Relationship between oxygen consumption rate and swimming speed for Atlantic salmon (mean total length, 58.3cm) (●) and Atlantic mackerel (mean total length, 34.7cm) (▲) obtained using the annular respirometer. Data for resting salmon (○) were not included in the regression. The mean value (±S.E.) of data obtained for mackerel at $0.2\,m\,s^{-1}$ ($0.6\,L\,s^{-1}$) at 11°C (△) by Johnstone *et al.* (1992) is included for comparison with the mackerel data. Regression lines for salmon (S: $\log\dot{M}_{O_2}$=0.04+1.05U, r^2=0.866) and mackerel (M: $\log\dot{M}_{O_2}$=0.18+0.82U, r^2=0.847) are shown and are extrapolated to zero activity.

annular respirometer provides a more favourable swimming environment in relation to flume respirometers.

Oxygen consumption measurements for adult Atlantic salmon in sea water have not been made before. Brett (1965) gives the oxygen consumption rate of sockeye salmon, *Oncorhynchus nerka* (mass 1432g), swimming at $0.75\,\mathrm{m\,s^{-1}}$ ($1.3\,L\,\mathrm{s^{-1}}$, where L is body length) in fresh water at 15°C as $6.2\mathrm{mmol\,O_2\,kg^{-1}\,h^{-1}}$, compared to the value of $6.9\mathrm{mmol\,O_2\,kg^{-1}\,h^{-1}}$ found in this study (11°C). In flumes it has not been possible to obtain $\dot{M}_{O_2}$ determinations of salmonids at complete rest and estimates (standard metabolic rate) have been made by extrapolation from the oxygen consumption–swimming speed regression. Our measurements show that true resting oxygen consumption of adult Atlantic salmon is substantially higher than would be predicted by simple extrapolation from the oxygen consumption–swimming speed regression (Fig. 2).

There have been few attempts to measure oxygen consumption in mackerel. Baldwin (1924) sealed seven small mackerel (total mass 138g) in a container over a 60min period and obtained a mean metabolic rate of about $30\mathrm{mmol\,O_2\,kg^{-1}\,h^{-1}}$. Temperature and activity levels were not given but the fish were probably severely stressed. Johnstone *et al*. (1992) have measured oxygen consumption of small groups of tank-adapted mackerel at their lowest swimming speed ($0.2\,\mathrm{m\,s^{-1}}$, $0.6\,L\,\mathrm{s^{-1}}$), and found mean $\dot{M}_{O_2}$ (±S.E.) to be $3.7\pm0.3\mathrm{mmol\,O_2\,kg^{-1}\,h^{-1}}$ (see Fig. 2). This is considerably higher than would be predicted by extrapolating from the swimming speed–$\dot{M}_{O_2}$ relationship (Fig. 2) given here.

Mackerel do not have swimbladders, are heavier than water and gain lift by swimming continuously. At low swimming speeds (below $0.8\,L\,\mathrm{s^{-1}}$, L=0.30–0.39m), mackerel tilt their bodies at positive attack angles of as much as 27° (He and Wardle, 1986). Such changes in swimming behaviour may offset predicted reductions in oxygen consumption at low swimming speeds and may also be linked to changes in gill ventilation mode.

Swimming in a curved path requires greater energy expenditure to accommodate the centripetal force required to maintain the curved course (Weihs, 1981). Theoretical corrections for this can be applied using equations 1–10 of Weihs (1981). Using the 'increased thrust' calculation, which Weihs states is largely independent of speed, theoretical reductions in $\dot{M}_{O_2}$ of 40% for salmon and 15% for mackerel are obtained for swimming in a straight line. The correction for salmon is rather higher, owing to their greater mass. The circle radius makes a large difference; according to Weihs' 'increased thrust' equation, a salmon swimming in a circular path of 1m radius would theoretically exhibit a power requirement 456% greater than if it were swimming in a straight line, largely irrespective of speed. Physiologically, such a large figure at low swimming speeds seems unlikely, but the importance of having as large a radius as possible is clear.

Maximum sustained swimming speeds (for at least 60min) of $0.82\,\mathrm{m\,s^{-1}}$ ($1.4\,L\,\mathrm{s^{-1}}$) were obtained for salmon and $0.90\,\mathrm{m\,s^{-1}}$ ($2.6\,L\,\mathrm{s^{-1}}$) for mackerel in the respirometer. Tang and Wardle (1992) found the maximum sustained swimming speed, U_{ms} (200min), for Atlantic salmon (L, 0.45m) to be $0.91\,\mathrm{m\,s^{-1}}$ ($2.0\,L\,\mathrm{s^{-1}}$), and He and Wardle (1988) measured U_{ms} of mackerel (L approximately 30cm) as $1.16–1.34\,\mathrm{m\,s^{-1}}$ ($3.5–4.1\,L\,\mathrm{s^{-1}}$). Their higher measurements were made in the same Aberdeen annular tank at the same

radius of swimming path and at similar temperatures to this study. However, both the studies of Tang and Wardle (1992) and He and Wardle (1988) relied on selection, from much larger groups, of a few fish which kept up with the optomotor stimulus at even the highest speeds. For respirometry it is essential to maintain all fish at the test speed. At speeds higher than the maximum speeds detailed here, problems were experienced with numbers of fish dropping behind the main school and swimming more slowly, so that control of swimming speed was lost. It is unclear whether this represents a physiological limitation for some individuals or a lack of motivation to keep up with the other members of the school.

The annular respirometer system described has proved effective in measuring rates of oxygen consumption at controlled speeds in schooling fishes, particularly in those that are sensitive to handling. The main disadvantages noticed were the problems in achieving the highest predicted sustainable swimming speeds in a large enough group of fish, and the effect of path curvature on energy expended in swimming, which increases with fish size and decreasing swimming radius.

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