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## Technical Paper - 22

#### SUMMER INSTITUTE IN

#### RECENT ADVANCES IN FINFISH AND SHELLFISH NUTRITION

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#### DETERMINATION OF METABOLIC RATES AND QUOTIENTS IN FISH

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Before any growth can be achieved by an organism there must be sufficient energy in the food to provide for the metabolic demands of maintenance and any activity associated with food intake. In culture practices it is possible to reduce these energy costs to a minimum and to optimize food conversion. This can be done through an understanding of the environmental and biological factors affecting metabolic rate with a view to reduce the demands on the system and hence increase the production. It is therefore desirable to examine the broad picture of energy exchange within an animal, then proceed to more specific considerations in fish. Since it is the principles and prospects which are of major interest here, no attempt is made to make this a major review of the subject.

A general balance sheet of the biological uses (profits) and losses associated with the conversion and utilization of energy by fish can be prepared by using the available sources of information. Starting with a given intake of 100 calories, an average of 80 calories remaining from assimilation as net energy available for biological use. If the metabolic demand is low, nearly 50 calories may be converted into growth. If the metabolic demand from activity is high, all 80 calories may be involved in total metabolism. The sources of energy loss (20 calories) are feces, urine+NH, and heat increment.

Metabolic rate/power, or the rate of expending energy, has usually been measured by determining the rate of oxygen consumption of fish at various levels of activity. It should be stressed that the assumption implicit in all such measurements is that a state of equilibrium exists between oxygen demand and supply.

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In practice, basal metabolism is defined as the minimum energy cost when the animal is at rest in a temperature acclimated environment in the post-absorptive condition. The possibility of obtaining absolute rest has been questioned frequently for animals subject to excitement from handling. This has led to the use of standard metabolism which is usually measured after 24 hours of fasting, during the slump of any diurnal metabolic cycle.

Active metabolism is the maximum rate consistent with the highest continued level of activity.

Routine metabolism has been used to express the average oxygen consumption of fish which are moderately active. It forms a closer approximation of normal demand because fish are neither continuously resting nor continuously moving.

Many factors influence the metabolic rate. The abiotic factors are temperature, salinity, oxygen, carbon dioxide, ammonia, pH, photoperiod, season and pressure, and the biotic factors are activity, weight, sex, age, group (schooling),  $O_2$  debt, condition, starvation and diet. To illustrate the multiplicity of involvement between metabolic rate and environment it is apparent that within the bounds of knowledge the factors of temperature, oxygen and activity exert the greatest effect on metabolism--in unpolluted waters.

Even oxygen at 100% air saturation may act as a limiting factor to sustained performance when the combined factors of temperature and activity are high. It is perhaps surprising that salinity does not appear to have more effect on reducing metabolic rate since it might be expected that the lack of ions in freshwater would impose a fair energy demand to maintain osmotic balance. On this basis it may be predicted that except in the estuary any advantage which the marine environment might confer on growth, through the energy-saving mechanism of reduced osmoregulation, would be small.

Activity has a tremendous effect on metabolism, frequently elevating the oxygen uptake by a factor of 4 times at optimum temperatures and reaching a maximum of 8 times in some fish species. In the case of active metabolism, undernourished, unexercised, disease-inhibited, sluggish or lazy fish could reduce the potential level. However, excitation and oxygen debt replacement (unless depressed by excessive waste products) make large demands on metabolism. Since the ventilation of the gill chamber could be the limiting factor, and free swimming rather than just opercular movement can facilitate ventilation, the circumstances within the respirometer must not be overlooked.

The gill system of fish is remarkably efficient, working on the counter-current principle and capable of removing 80% of the dissolved oxygen. When there is excess oxygen (respiratory independence) no limitation to activity is imposed. Below this level of excess, active metabolism is dependent on oxygen availability (respiratory dependence). Where standard metabolism becomes dependent, any continued activity will result in death from irreplaceable oxygen debt (level of no excess activity).

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More recent manifestations of interest in fish metabolism studies are apparent in the various tests on stamina, using water tunnels, flumes, or rotating circular troughs - the nearest equivalent of the step-test or bicycle ergometer for man. The step that was necessary to relate metabolism and performance was to conduct experiments where swimming speed could be accurately documented while determining the accompanying the stable respiratory demand for oxygen. In recent years, the apparatus commonly used for metabolism studies are Fry's respirometer and Blazka's respirometer.

The Fry's respirometer is a modification of the annular respirometer of Fry and Hart (1948) and of Smit (1965). The earlier respirometer (Smit, 1965) had a concave transparent plastic lid covering the annulus of the chamber and did not provide an effective seal against diffusion of gases. The present one is modified in that its top is covered by a transparent plastic sheet with only two wells opening above. Fitting these wells are two plastic cups with a hole at the bottom in each. These wells alone offer open surface to the exterior. The design of the respirometer is such that the diffusion of gases into and out of the respirometer is minimized. The activity of the fish is recorded by the electronic counter which is a part of the respirometer assembly. When the fish moves in the annular chamber it interrupts the light beams focussed on the photocells, and these events are recorded in a counter. An interlocking system is provided so that, when the fish obstructs the same light consecutively, the second and the following events will not be recorded for counting, unless otherwise the other light is interrupted in between the two events to actuate the counter. Thus mal-operation due to minor events such as the flick of tail or head, is avoided. More details of the apparatus are given in Kutty et al. (1971).

Blazka's respirometer (Blazka et al., 1960) consists mainly of two concentric transparent (Perspex) cylinders, one fitted inside the other. A propeller is fixed on one side and a shocker grid on the other side. The propeller is fixed to rotate just inside the inner cylinder. The rotation of the propeller induces a water current inside the inner cylinder. The water circulates back to the propeller-end through the clearance between the outer and inner cylinders. Water currents in required velocities could be obtained with the help of a voltage regulator connected to the motor. The apparatus is so designed that water can flow in and out of the respirometer (open system) or can be left to stagnate (closed system) when the fish is being exercised depending on the experimental needs. The shocker grid serves two purposes, viz., one of closing the inner cylinder without letting the fish escape to the outer cylinder and the other of preventing the fish from resting on it. When a fish rests on the grid the exposed wires become connected and a circuit is established resulting in a shock to the fish. Unless a fish is unable to swim due to low ambient oxygen or is really fatigued it will not rest on the grid.

Metabolic rates can be estimated by simultaneous measurements of rate of  $O_2$  consumption,  $CO_2$  output and  $NH_3$ -N excretion. Anaerobic metabolism and its link with protein utilization can also be estimated from concurrent measurements of R.Q. and A.Q.

R.Q.	(Respiratory	Quotient)	=	volume	OI	<u> </u>	, produce
				Volume	of	°2	consumed

A.Q. (Ammonia Quotient)

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Under high ambient oxygen concentrations R.Q. near unity will be maintained by the fish, which indicates that the fish was drawing energy aerobically or maintaining aerobic metabolism. If the R.Q. goes above '1' which indicates the anaerobic energy utilization. There are instances (during hypoxia) where the increased carbon dioxide production is combined with increased ammonia excretion. This may be of edvantage to the fish in acid-base regulation (preventing acidosis) and iono-osmotic regulation (conservation of Na<sup>+</sup>). It is possible that non-ionic ammonia is excreted by passive diffusion down a concentration gradient across the gill surface from blood to water, and the activities of glutaminase and glutamic acid dehydrogenase are high in fish gills. However, the ammonia source could be peripheral or otherwise.

The discussions made at present although provides some insight into the importance of physiological aspects especially metabolic rates and quotients in fish, more information is necessary to elucidate the metabolic and behavioural responses of more species in view of their importance as cultivable warm-water fishes. However, the involvement of environmental interaction is not a deterrent to progress but a measure of the complexity to be met. .. 7 ..

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