SOME ASPECTS OF THE METABOLISM OF BROWN TROUT AND PERCH

by

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The work presented in this thesis is the result of my own investigations and has neither been accepted nor is being submitted for any other degree.

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..... Date

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SOME ASPECTS OF THE METABOLISM OF BROWN TROUT AND PERCH

A Thesis submitted for the degree of Ph.D.

by R. I. G. MORGAN

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A B S T R A C T

Using recirculating water fish tunnel respirometer, the metabolism of brown trout, Salmo trutta (L) and perch, Perca fluviatilis (L), was investigated. The fish were subjected to increasing velocity swimming tests at 5°C, 10°C and 15°C. After swimming failure, following the final speed increase, the recovery of the fish was monitored for a period of three hours.

Whilst undergoing the routine and active metabolic conditions of the experiments, analyses were carried out on the uptake of oxygen and excretion of carbon dioxide and ammonium nitrogen. A new method for the analysis of carbon dioxide in fresh water was described, based on the so called carbon dioxide electrode. The measurements made enabled the respiratory quotient to be estimated.

It was found that brown trout, undergoing routine activity, catabolised the same percentage (28%) of protein at both 5°C and 15°C, in relation to the total metabolism at each temperature. The lipid and carbohydrate contributions to the total metabolic rate, however, demonstrated a marked temperature effect. At 5°C

lipid provided 49% of the total energy utilised and carbohydrate 22%. At 15°C the situation was reversed, lipid providing 27% and carbohydrate providing 46% of the total energy utilised. At 5°C and 10°C brown trout maximum sustained swimming speeds were accompanied by a catabolic metabolism comprised of 70% lipid oxidation. At 15°C the swimming metabolism of brown trout was suggested as being characterised by a hetogenic condition coupled with a gluconeogenesis. Perch active metabolism at all three temperatures indicated a strongly anaerobic energy supplying mechanism was operating. The perch routine metabolism, measured at only two temperatures, 10°C and 15°C also showed a temperature effect. In contrast, however, to brown trout; as the temperature decreased lipid oxidation also decreased.

The recovery metabolism for both brown trout and perch enabled an hypothesis to be propounded relating to acid base balance. It was suggested that brown trout, which appeared to resist overt anaerobic metabolism, possess a system of NH_4^+ excretion which can utilise metabolically produced H^+ ions to maintain blood pH stability. This hypothesis was discussed in relation to the findings of the study and previously reported observations.

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PLATE, FIGURE AND TABLE CONTENTS

I. GENERAL METABOLISM

The two main objectives of the study were to measure respiratory quotients (R.Q's) in active fish and to use the R.Q's obtained to describe the metabolism taking place.

"The ratic (moles CO_2 produced/moles O_2 consumed) is called the respiratory quotient, usually referred to by students of metabolism as the R.Q." (Kleiber 1961)

Metabolism includes all anabolic and catabolic processes occuring within an organism. The speed or intensity of these processes is referred to as the metabolic rate. In an organism without net synthesis of body substances (endogenous tissue, milk, eggs, etc.) metabolic rate and catabolic rate are equal because there is no anabolism. (Kleiber 1965). The energy and gaseous equivalents of energy substrates can be established in vitro by use of the bomb calorimeter. Care has to be taken, however, in applying oxidation data directly to organisms, because complete oxidation takes place in a calorimeter. Lipids and carbohydrates are metabolised to complete oxidation products within organisms. Proteins are not always completely oxidised in metabolic processes and different end products of protein catabolism can be observed. (Prosser and Brown 1965). However, typical oxidation values are shown in Table I. From these values the relationships between protein, carbohydrate and lipid can be defined in terms of oxygen, carbon dioxide and nitrogen.

TABLE 1.

CHEMICAL OXIDATION OF SOME SUBSTRATES

GLUCOSE (CARBOHYDRATE)

$C_6H_{12}O_6 + 6\ O_2 = 6\ CO_2 + 6\ H_2O + 686$ Kcal.

180 g. 134.4 L 134.4 L 108 ml.

TRIBUTYRIN (LIPID)

$C_3H_5O_3(OC_4H_7)_3 + 18.5\ O_2 = 15\ CO_2 + 13\ H_2O + 1941$ Kcal.

302 g. 414.4 L 336.0 L 234 ml.

LEUCINE (AMINO ACID)

$C_6H_{13}O_2N + 7.5\ O_2 = 5.5\ CO_2 + 5.5\ H_2O + 0.5\ CO(NH_2)_2 + 780$ Kcal.

131 g. 168.0 L 123.2 L 99 ml. 0.5 mol.

UREA

$CO(NH_2)_2 + 1.5O_2 = CO_2 + 2\ H_2O + N_2 + 151$ Kcal.

60 g. 33.6 L 22.4 L 36 ml. 22.4L

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TABLE 2

OXIDATION PRODUCTS AND RELATIONSHIPS BETWEEN TYPICAL ENERGY SUBSTRATES. (after Brown & Breugelmann 1965)

SUBSTRATE	ENERGY (Kcal/g.)			RESPIRATORY EQUIVALENT			VOLUME	
	Bomb Calorimeter	Human Oxidation	Physiologic Value	Oxygen (Kcal./L)	Carbon Di. (Kcal./L)	R.Q. $\frac{\text{Vol. } CO_2}{\text{Vol. } O_2}$	Oxygen (L/g)	CO_2 (L/g)
CARBO.	4.1	4.1	4	5.05	5.05	1.00	0.81	0.81
PROTEIN	5.4	4.2	4	4.46	5.57	0.80	0.94	0.75
LIPID	9.3	9.3	9	4.74	6.67	0.71	1.96	1.39

The R.Q. of an organism can therefore be used to estimate the metabolism that is taking place. However, it is a very sensitive measure in that the organism may not be displaying complete oxidative metabolism. Synthesis may be taking place, substances other than lipids, proteins and carbohydrates may be involved in the oxidations; and there may be a difference in the CO_2 produced and that appearing outside the organism. (Krogh 1919). Further, if the measurement of gaseous exchange is only made over a short time period, there may be a serious error present due to the problems of washout or CO_2 storage. (Kleiber 1961). Not only lipids, carbohydrates and proteins are metabolised in an organism. Richardson (1929) notes that the catabolism of pyruvic acid and ethanol produces R.Q. values of 1.2 and 0.67 respectively. Figure 1 shows some R.Q. values of commonly encountered substrates.

The question of synthesis taking place is an interesting one and worth pursuing from the effects on R.Q. measurements. The two main synthetic situations that can give rise to markedly different R.Q's (i.e. outside the general range 0.7 - 1.0, see Table 2) are the synthesis of lipid from carbohydrate and the synthesis of carbohydrate from lipid. In the former case, a high R.Q. has been noted in geese (Bleibtreu 1901 , Benedict cited by Wierzuchowski et al 1925) adipose cells (Mirski 1942) Lepidoptera (Zebe cited by Niemerko 1959) and young hogs (Wierzuchowski et al 1925). These high R.Q's were explained

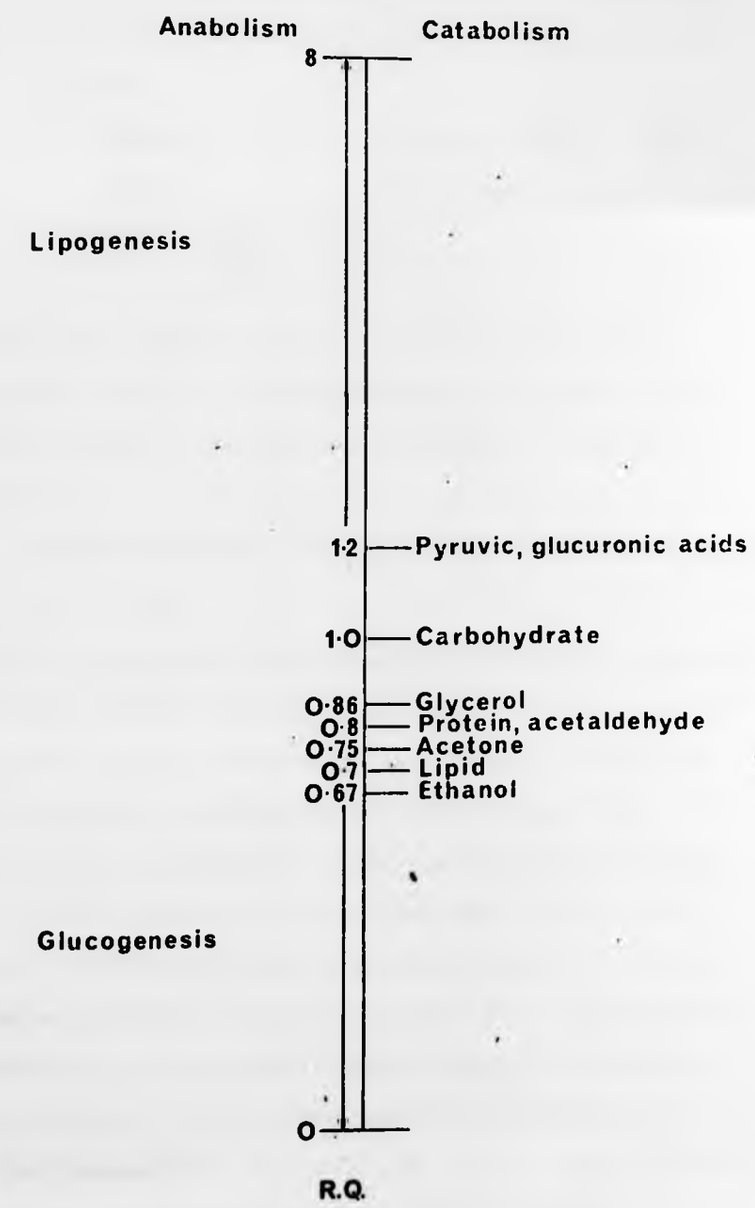

Fig. 1. Some examples of R.Q. values.

on the ground of lipogenesis from both theoretical and analytical observations. Lusk (1917) explained the theoretical aspects for the observations and demonstrated that an R.Q. of 8.0 is possible.

$$\underset{\text{glucose}}{4C_6H_{12}O_6} + O_2 = \underset{\text{palmitic acid}}{C_{16}H_{32}O_2} + 8CO_2 + 8H_2O$$

$$\text{R.Q.} = \frac{CO_2}{O_2} = 8.0$$

An alternative system for an R.Q. of greater than 1.0 was suggested by Cohen (1954) who demonstrated than an R.Q. of 2.0 could be obtained by oxidative decarboxylation of 6-phospho-gluconate.

$$\text{6-phospho gluconate} + 0.5O_2 = \text{pentose-5-phosphate} + CO_2$$

$$\text{R.Q.} = 2.0$$

Low R.Q's as a measure of carbohydrate synthesis are not commonly encountered, probably the lowest persistent R.Q's being observed inhibernating animals. (Kayser 1940 a, b, 1961). Here the R.Q. drops to about 0.7 (cf. Table 18) for long periods of time. However, despite the fact that lipid is probably the main energy source in this situation some glucogenesis does occur but at a low rate. It is interesting to note that on arousal the R.Q. of an hibernating animal does increase to about 0.9. The explanation for this being a switch to carbohydrate oxidation combined with hyper ventilation. Soskin (1941) noted that the theoretical range for gluconeogenesis from lipid was 0.65 to 0, depending on the number of betahydroxybutyric acid molecules formed.

It would appear, however, that the incidence of low synthetically derived R.Q.'s is not as great as that of high synthetically derived R.Q's.

The normal range of R.Q. values encountered, in the absence of any obscuring anabolic processes, is about 0.75 - 0.95. Probably the most intensively studied animal with regard to R.Q. values and their implications is man. The effect of a high carbohydrate diet has been shown to produce an R.Q. of about 0.95 (Benade et al 1971, Christensen and Hansen 1939), indicating a predominant carbohydrate metabolic energy source. The "normal balanced diet" has been shown to produce an R.Q. of 0.8 - 0.85 (Christensen and Hansen 1939, Krogh and Lindhard 1920, Roughton et al 1942). A lipid diet gives rise to R.Q's of 0.79 - 0.75 (Bortz 1969, Christensen and Hansen 1939, Krogh and Lindhard 1920). A normal value of R.Q. of 0.94 was reported by Benade et al (1971) which, on first inspection appears too high. However, this value was obtained from Bantu mineworkers prior to work. Examination of the diet reveals that it is high in carbohydrate and low in protein and fat (Seftel and Walker 1966) and so for this group a high R.Q. is normal. The commonest deviation from the normal R.Q. in man, normal here being used to indicate an R.Q. between 0.8 and 0.9, is that observed after exercise. Roughton et al (1942) have described a series of experiments using the bicycle ergometer - mild exercise and the treadmill - severe exercise. The R.Q. values obtained just before the end of the exercise were 0.92 and 1.11. respectively. These deviations from the resting R.Q. of 0.80 being correlated with the appearance of blood lactic acid and the presence of an alactacid debt (Margaria 1969). The addition of lactic acid, produced by the exercising muscle as a result of anaerobic contraction,

to the blood causing a transitory metabolic acidosis. This being compensated for or causing an increase in carbon dioxide output. The relationship between lactic acid and exercise will be described later.

So far the R.Q. ratio of oxygen consumed to carbon dioxide produced has been used to indicate whether carbohydrate or lipid or a mixture of both is the predominant oxident. However, a third component of energy substrate is also present, protein. The oxidation of protein produces a variety of nitrogenous end products which appear in the urine. Proteins can be oxidised to give rise to uric acid, urea or ammonia or combinations of all three. In birds uric acid predominates, in mammals urea predominates and in fresh water teleosts ammonia predominates. The derivation of the different nitrogenous end products in relation to the environment will not be discussed here, pertinent reviews and chapters on the subject being available. (Campbell 1970, Prosser and Brown 1965, West et al 1967).

Proteins are composed of amino acids, and amino acids in the body can be used in three major ways. They can be used to build up endogenous protein i.e. tissue, they can be converted into a component of the Tricarboxylic Acid Cycle (TCA) and they can be converted into either carbohydrate or lipid or both. The conversion of an amino acid into a ketuglutaric acid (for TCA utilisation) or carbohydrate or lipid involves the removal of an amino or non-amino nitrogen group. In the mammals, the nitrogen is excreted in the urine chiefly as urea with a small amount as ammonia (Berg and

Kolenbrander 1970). Bollman et al (1924) established that in the mammal, ammonia formation occurs mainly in the kidneys and urea formation in the liver. Therefore, by measuring the nitrogen content of urine, an estimate may be made of the protein turnover occuring in the organism. Average proteins contain about 16% nitrogen so by measuring the nitrogen output and applying the appropriate correction factor (either multiply by 6.25 or divide by 0.16) the protein turnover may be established. An example of this type of calculation, applied to a cow, is given in Kleiber (1961).

II. FISH METABOLISM

Early work on fish R.Q. and the methodology employed has been reviewed by Gardner (1926) and Kutty (1966). Accordingly it will be of little value to cover this area again. However, some measurements of fish R.Q. over the last forty years can be seen in Table 19. Prior to the work of Kutty (1966), no measurements of active fish R.Q's were described, all values reported probably being routine values. The terms standard, routine and active metabolism are used here as defined by Fry (1971). Also, the effects of stress and asphyxia were not always absent from the experimental regimes. Remarkably, Kutty (1972) in the most recently published values of R.Q., did not remove the possible causes of stress from his experiments - despite the large body of literature available on the subject. It would appear, however, that the range for fish R.Q's is approximately the same as for man and other animals. In contrast to man, but apparently in common with piglets (Curtis et al 1970), Indian meal moth larvae (Hunter and Hartsell 1971) and Paramecium sp.(Pace and Kimura 1944), some investigators have described changes in fish R.Q's in relation to temperature. (Baudin 1931, Gardner 1926, Stroganov 1956). The data of Stroganov (1956) is obtained from fish subjected to rapid temperature change and suffering consequent death and so it is doubtful whether the observed R.Q's represent true physiological adaption. The work of Gardner and associates (1914, 1922, 1923, 1926) is suspect owing to the

undoubted stress present during his investigations. The work of Baudin (1931) was only carried out on one fish and remains to be substantiated.

At low temperatures, the presence of a high R.Q. (above 1.0) has been said to be indicative of lipid synthesis from carbohydrate (Blazka 1958). His method of analysis of fatty acids and experimental techniques have recently been carefully re-evaluated. (Burton and Spehar 1971). These authors could find no increase in fatty acids in four species of fish subjected to hypoxic conditions. They came to the conclusion that the Declux method for the anlysis of fatty acids had been incorrectly interpreted by Blazka (1958). These findings would appear to remove the foundation for a lipid synthesis from carbohydrate. However, the possibility still exists that the synthesis might take place slowly over a period of time under cold or hypoxic conditions. In this respect, the work of Moroz (1971) on overwintering carp is relevant in that a slow interconversion rate is indicated. The reverse of this situation, synthesis of carbohydrate from lipid, has not yet been demonstrated in fish. Similarly, the effect of diet composition has not yet been described as having an effect on the R.Q. Also, although sex, size and age have been cited as modifiers of R.Q. (Curtis et al 1970, Prosser and Brown 1965) these effects on fish, if present, have not yet been demonstrated.

The effect of exercise on the R.Q. of fish has only been studied by Kutty (1966, 1968 b, 1972) using Carassis auratus, Salmo gairdnerii and Tilapia mosambica. Kutty has also examined

the R.Q. in relation to ambient oxygen concentration in those three species. Regarding the effects of ambient oxygen concentrations, he demonstrated an increase in R.Q. in all species with decreasing oxygen concentration below a critical level. However, it is probable that apart from the goldfish which appears to be capable of withstanding hypoxic conditions, neither the rainbow trout or Tilapia are capable of doing so for extended periods of time. The work of Mahdi (1973) on Tilapia nilotica showed a 52% mortality in 24 hours at 36°C and a 40% mortality in 24 hours at 37°C and an initial oxygen concentration of 3.0 and 2.8 mg/L. respectively. Kutty (1971, 1972) working at 30°C and below 2.0 mg. O_2/L. showed an increase in the R.Q. of Tilapia mosambica. He concluded "... a high intensity of anaerobiosis in Tilapia mosambica at oxygen concentration below 2mg/L". He also stated (1972) "... in a few experiments at low oxygen levels the fish began losing their equilibrium.". A similar situation obtained with rainbow trout at 15°C (Kutty 1968 b). It would appear likely that the apparent anaerobiosis exhibited by Tilapia sp and rainbow trout is a moribund phenomenon and not a common metabolic state. The exercise metabolism described by Kutty (1968 b) on the evidence of R.Q. measurements, however, is supported by acceptable physiological responses. Gold fish subjected to increasing velocity tests demonstrate (3-12 hours of an imposed speed) R.Q. values of over 1.0. However, at about 50%v.crit. (50% of critical swimming speed,

Webb 1971) the R.Q. starts to drop and falls below 1.0 (Table IV p. 1715). Rainbow trout at 15°C exhibit the reverse situation, the R.Q. initially being 0.85 - 0.93 rising to 1.0 at the maximum sustained speed. Three rainbow trout and three goldfish were used under retest conditions for these experiments. The general conclusions from the work of Kutty (1968 b) were that initially the fish derived energy anaerobically at the onset of a speed increment and that goldfish have a greater anaerobic capacity than rainbow trout. No measurement of excreted nitrogen was made and so partition of the energy substrates was not possible. However, in a recent work Kutty(1972) measured the ammonia production of an active fish. Using Tilapia mosambica at 30°C, a routine rate of 21 mg NH_3-N/kg.hr. and an active rate of 75 mg. NH_3-N/kg.hr. were recorded. Unfortunately, the fish was placed in the respirometer just prior to the experiments and this has undoubtedly influenced the excreted compounds.

Before concluding this brief discussion of measured respiratory quotients in fish, it will be of value to outline some of the mechanisms and physiology of carbon dioxide production.

FACTORS AFFECTING THE OUTPUT OF CARBON DIOXIDE

Carbon dioxide production, as observed by measures of excreted carbon dioxide in fish is susceptible to a number of phenomena.

Four major groupings can be described:

a) Metabolism - catabolic and anabolic processes.

b) Blood buffering - includes metabolic acidosis and possible washout effects.

c) Ionic excretion of HCO_3^-

d) Swimbladder effects.

a) Metabolism:

Although the metabolism underlying production of CO_2 will be fully discussed in the sections on the validity and interpretation of R.Q., brief mention will be made here of its role in differential CO_2 production. The oxidation of energy substrates to provide high energy phosphate compounds produces CO_2 as one of the end products. Depending upon the type of substrate oxidised and the degree of oxidation involved different quantities of CO_2 are produced per unit weight of substrate. The quantity of CO_2 produced is a characteristic of the substrate being oxidised.

Examples are:

Carbohydrate glucose

$$C_6H_{12}O_6 + 6\,O_2 = 6CO_2 + 6H_2O$$

180 g. 134.4L 134.4L 108 ml.

(0.77 L/g)

Lipid tributyrin

$C_3H_5O_3(OC_4H_7)_3 + 18.5\ O_2 = 15CO_2 + 13\ H_2O$

302 g. 414.4L 336.0L 234 ml.

(1.11 L/g)

Amino acid leucine

$C_6H_{13}O_2N + 7.5\ O_2 = 5.5CO_2 + 5.5H_2O + 0.5CO(NH_2)_2$

131 g. 168.0L 123.2L 99 ml. 0.5 mol.

(0.94 L/g)

It can be seen that different types of substrate give rise to different quantities of CO_2 per unit weight. It is possible that because of the slightly different composition of fish energy that the type of example cited above is not entirely accurate. However, to be able to distinguish between a respiring homeotherm and a respiring poikilotherm tissue on the basis of CO_2 production is somewhat of an experimental nicety. The degree of difference between the two tissue types is unlikely to affect the interpretation of CO_2 production.

Regarding possible fixation processes, the blood retention of metabolic CO_2 will be discussed later. If the assumption that CO_2 once formed, leaves the tissues and ultimately the organism is made, only active anabolic, anapleurotic or fixation processes are likely to interfere. Regarding fixation per se evidence has been produced for fixation of CO_2 by testicular tissue of cod (Mounibard Eisen 1972). However, the quantities involved are very small (nMolar) and are unlikely to have any significant bearing on the total production.

b) Blood buffering:

To a certain degree this section is very similar to the following section on ionic exchange and so certain comments will occur in both sections. Basically this section will deal with the possible effects due to increased ventilation causing "washout" and to metabolic acidosis. The buffering capacity of fish blood is low in relation to mammals, and elasmobranchs possess an even lower blood buffering capacity than teleosts. (Piiper and Baumgarten 1969, Piiper et al 1972, Albers 1970, Robin and Murdaugh 1967). Blood buffering in mammals is achieved both by the lungs and the kidney acting as regulating mechanisms (West et al 1967, Masoro and Siegel 1971). The lung acts as a control for the PCO_2 HCO_3^- system and the kidney controls the release of H^+ ions. Metabolic acidosis caused by increased CO_2 content of blood can be relieved by an increase in ventilation rate which causes an increase in blowoff of CO_2 which lowers the blood PCO_2 and hence HCO_3^- content. Metabolic alkalosis is controlled by a reverse process i.e. depressed ventilation rate. The kidney acts by controlling the levels of H^+, Na^+, CC^-, HCO_3^- and HPO_4^- secretion. Briefly, CO_2 combines with water under the influence of carbonic anhydrate to form H_2CO_3. This dissociates and appears in the tubular fluid, where H^+ exchanges with Na^+, Na^+ passing back into the tubular cells and eventually into the plasma. This preserves the Na^+ content of filtered plasma. The H^+ in the tubular fluid can then exist either with HCO_3^- or with HPO_4^- to act as the anion buffer preserving the pH of the rubular fluid. Any HCO_3^- present in tubular fluid will be reabsorbed by the tubular cells until the plasma HCO_3^- content is at a "normal" level.

Above this level, HCO_3^- content tends to be excreted giving rise to an alkaline urine. Simultaneously NH_3 is produced by tubule cells (Pitts 1971) which passes into the tubular fluid and combines with H^+ from H_2CO_3 dissociation, to form the ionised form of ammonia, NH_4^+. This weak cation then combines either with Cl^- or an organic anion and is excreted. In cases of metabolic acidosis the output of NH_3 is increased to keep up with the increased production of H^+ ions. The lower the pH of tubular fluid the more NH_3 will be trapped in to pH sink created by high levels of H^+ ions (Pitts 1971).

Apparently this type of blood buffering situation does not apply in fish to the same extent as in mammals. Hyperventilation does not give rise to a control of blood PCO_2 and HCO_3^-. Blood pH appears to be controlled by an increase in blood HCO_3^- (Randall and Cameron cited in Cameron and Randall 1972). The exchange of CO_2 and hence control of blood HCO_3^- in fish is suggested as a function of ion exchange rather than a passive diffusion. (This is discussed in the following section) The role of the kidney in the excretion of H^+ also appears to have a lesser bearing on acid base regulation in fish. Hunn (1969) analysing the urine of hypoxia stressed rainbow trout demonstrated a urine pH decrease of about 0.5 units pH over a period of four hours after hypoxia. There was an increase of HCO_3^- one hour after hypoxia and an increase of PO_4^{--} up to four hours after hypoxia. However, the urine flow had returned to normal after four hours. Working with the dogfish Scyliorhinus stellaris, Piiper et al (1972) demonstrated an H^+ deficiency, as compared with measured lactate, in the blood after stimulation to exhaustion. This H^+ could not be demonstrated in either the urine or the aquarium water.

They came to the conclusion (Piiper and Baumgarten 1969, Piiper et al 1972) that the H^+ ions were retained within the fish and buffered in the tissues. However, Dejours et al (1968) working with goldfish noted that in fishes confined with asphyxia the increase in NH_4^+ output was much greater than those confined without asphyxia. It would appear from the results with dogfish and goldfish that the two types differ metabolically, goldfish perhaps behaving more like mammals under conditions of metabolic acidosis in that they utilise NH_3^o as a method of removing excess H^+ ions. However the question of production of NH_3^o or NH_4^+ by ammonotelic fish remains in doubt, Kerstetter et al (1970) maintain that the ammonia excreted is all in the molecular form NH_3^o, whilst Maetz (1972) is of the opinion that ammonia excreted is in the ionised NH_4^+ form.

c) Ionic exchange of HCO_3^-

It would appear that CO_2 at gill surfaces is not like O_2, a simple diffusion phenomena; but an ionically regulated function. Further, the exchanged ion is HCO_3^- which is accompanied by an influx of CC^- ions. This is the situation in freshwater teleosts, the exchange phenomena is marine teleosts being as yet undescribed.

Lloyd and Jordan (1964), Garcia Romeu (1964) Dejours (1966), Lloyd and White (1967), Dejours et al (1968), Dejours (1969) and Cameron and Randall (1972), all describe experiments showing the influence of ambient PCO_2 and CC^- upon HCO_3^- excretion. Perhaps the most dramatic are the experiments of Dejours (1969) working with goldfish and different concentration of CC^- ions in the ambient water. Goldfish acclimated to water containing high levels of CC^- (10 m M/L) and then abruptly transferred to water containing negligible levels of CC^- ions demonstrated a marked reduction of CO_2 output. This

This reduction became a negative CO_2 balance in some cases. The effect lasted for up to three days, after which the CO_2 excretion rate had returned to its reference level. The reverse effect was observed in fish going from low to high CC^- containing water. Lloyd and White (1967) following changes in plasma HCO_3^- and CC^- of rainbow trout, whilst acclimating to high levels of ambient PCO_2 showed an increase in HCO_3^- and a decrease in Cl^-. This response took about 24 hours before stabilising. The increase in HCO_3^- was also noted by Cameron and Randall (1972) in a study of blood PCO_2 in relation to ambient PCO_2 in rainbow trout. They noted a constant PCO_2, blood/ambient water, of about 2 mm Hg; blood HCO_3^- increasing with increase of ambient PCO_2. The changes in blood HCO_3^- and CC^- are interpreted as being due to the HCO_3^-/Cl^- exchange at the gills, HCO_3^- efflux being correlated with Cl^- influx. In this way the fish can compensate for osmotic Cl^- loss. When fish are faced with high ambient PCO_2 they adjust the blood HCO_3^- so as to give rise to a blood PCO_2 about 2 mm Hg above ambient PCO_2 (Cameron and Randall 1972). This is achieved by retaining HCO_3^- thus preventing CC^- influx, explaining the findings of Lloyd and White (1967). The time course of 24 hours for adjustment of HCO_3^- levels is borne out by the findings of Eddy and Morgan (1969) who demonstrated that blood O_2 capacity is unaffected by 24 hours acclimatisation to high PCO_2. The blood buffers itself against a fall in pH due to increased PCO_2 by an increase in blood HCO_3^-, thus preventing a reduced blood O_2 capacity.

The Root effect in this case is probably a transitory phenomena. Maetz and Garcia Romeu (1964) actually altered CC^- movements across the gills by altering the CO_2 gradient across the gills. It is interesting to note in this respect that carbonic anhydrase which facilitates the reaction

$$H_2CO_3 \rightleftharpoons H^+ + HCO_3^-$$

is found in gill tissue of both marine elasmobranchs and teleosts and also freshwater teleosts (Maren 1967). However the concentration of the enzyme appears to be much higher in freshwater teleosts, and the pseudobranch of Perca sp. demonstrates the highest activity of all fish organs or tissues. (Maetz 1956).

Although exchange of HCO_3^- with CC^- has been demonstrated, no data has been published relating total CO_2 production to CO_2 production from HCO_3^-. However, the inference would appear to be that CO_2 production from HCO_3^- exchange represents a significant proportion of total CO_2 production (c.f. Dejours 1969). With this in mind recent values of CO_2 production (Kutty 1966, 1968 b, and 1972, Kutty et al 1971, Morris 1967, Bosworth et al 1936) must be interpreted with caution, as in all cases the experimental fish were held under different water conditions to the holding tank water conditions.

d) Swimbladder effects

The amount of CO_2 in the swimbladder normally is negligible (Fange 1966 Table 2) although there is a difference in the rate of gas secretion between perch and trout. According to Fange (1966) trout are characterised by a slow gas secreting ability and perch are capable of fast gas secretion. Regarding the actual rate of carbon dioxide secretion into the swimbladder, Wittenberg et al (1964)

demonstrated a CO_2 secretion of 4 ml/kg. hr. into the swimbladder of the bluefish (Pomatomus). Even at the very high rate of secretion which is believed to be of blood bicarbonate in origin - the gas gland itself being incapable of this rate of secretion - it would not have a significant effect on the total CO_2 measured outside the fish. High percentages of carbon dioxide usually occur exclusively during the first phase of gas secretion when the secretory mucosa is relaxed. The carbon dioxide being extremely rapidly reabsorbed as soon as the secretory mucosa contracts. (Fange 1953). This has also been demonstrated by MacNabb and Mecham (1971) working with blue gill sunfish. They evaluated the effects of temperature on the rate of gas secretion and gas composition at various temperatures. The rate of gas secretion into 50% deflated swimbladder for the first eight hours was independent of temperature and was of the order 3 ml. gas /kg.hr. For CO_2 the maximum rate was at 12°C where secretion amounted to about 0.4 ml/kg.hr.

The possibility of CO_2 entering the swimbladder and being retained although possible, would not appear to have any serious significant bearing on the appearance of CO_2 outside the fish. Taking the secretion figures of either 4 ml/kg.hr. (Wittenberg et al 1964) and 0.4 ml/kg.hr. (MacNabb and Mecham 1971) the maximum possible errors of CO_2 production at 5°C would be less than 10% and at 15°C less than 4%. However, as these CO_2 secretion rates represent maximum values, it is felt that the acclimation procedure would cause these effects to be minimised and the % error of CO_2

smaller. This being the case it was not taken into account and no correction factor for swimbladder uptate of metabolic CO_2 applied.

THE VALIDITY OF THE R.Q. MEASUREMENT IN FISH

Some of the physico chemical and metabolic factors affecting the R.Q. of fish have been discussed in the previous section. The derivation of R.Q. and its interpretation in the normal animal have been discussed in the Introduction. It is the purpose of this section to place the measurement of the fish R.Q's on a firm basis by describing some of the conditions that are essential to reliable measures of R.Q.

Severe criticisms of R.Q. measures and their interpretation are mentioned in Winberg (1956). He is sceptical about R.Q. measures outside a range of 0.7 1.0, referring to one R.Q. measure of 1.63 in bream at 20°C (Bogdanov and Streltsova 1953)as ".. an absurd value .... cannot continue for any length of time". According to Blazka (1958) carp are capable of sustaining an R.Q. of 2.0 by utilising a lipogenic pathway from carbchydrate. He demonstrated an increase in short chain fatty acids after hypoxia in carp coupled with an absence of oxygen debt. His analytical methodology has however been criticised by Burton and Spencer (1971) who could demonstrate no increase in short chain fatty acids after hypoxia in four species of freshwater teleosts. They also describe how an error in the analysis of short chain fatty acids using the method of Friedemann (1938) as used by Blazka could arise. This criticism would appear to remove the

metabolic foundation of lipogenesis giving rise to an R.Q. above 1.0. No temperature effect giving rise to high R.Q's were detected during the present study.

However, that an R.Q. above 1.0 can be measured under certain circumstances cannot be rejected. High R.Q's, although transient in nature can arise in exercising mammals due to the presence of lactic acid and washout effects due to hyperventilation. (Asmussen 1961, Kleiber 1961) The fish situation is somewhat different owing to the nature of lactic acid diffusion at low temperatures (Johnson et al 1945, Black 1958). Further, the ability of fish to cope with blood lactate in terms of synthesis to carbohydrate is a much slower process (Bilinsky and Jones 1972). High R.Q's have been measured by Kutty (1966, 1968b) in rainbow trout and goldfish both under declining oxygen tensions and activity conditions. Further work by Kutty (1972) on Tilapia also demonstrates high R.Q's under activity conditions, however in the latter work the fish were undoubtedly stressed prior to measures being taken of O_2 and CO_2. The main objection to his work however, is on the grounds that the fish were tested in water other than that to which they were acclimatised. Thus the effect of ionic exchange (Dejours 1969) could have had severe effects on the measured CO_2 production. This comment is especially relevant to his Tilapia work. Mather (1967) working with Rasbora daniconius in a sealed system desribed some remarkable O_2 consumption and CO_2 excretion figures obtained from his hypoxic experiment. His measure of CO_2 by titration with

phenolpthalein as an indicator is hard to understand, as the initial water pH was 8.5 and phenolpthalein changes colour at pH 8.2. (B.D.H. 1969). This particular instance is unfortunate and recent authors (Doudoroff and Shumway 1970) use it as an example of why R.Q's are suspect.

Providing that the experimental system and analytical regimes are sound, there is no reason why steady state fish R.Q's cannot be measured and interpreted to describe the metabolism giving rise to them. What appears to be lacking in published works is a sound description of factors affecting R.Q. - especially CO_2 production and also the metabolism giving rise to the observed R.Q. It is not sufficient to give the explanation that some anaerobic metabolism has occurred to explain high R.Q. values.

The utilisation of energy substrates in fish has been examined from various viewpoints other than by the R.Q. Brief mention will now be made of these.

b) STARVATION

The analysis of fish metabolism by means of starvation experiments can yield information relating to mobilisation of storage materials, rate of utilisation of storage or structural compounds and patterns of excretion. It must be borne in mind throughout this type of experiment though, that a fish starving over a long period of time is possibly atypical, and interpretation of the results be made cautiously. An interesting series of observations were made by Moroz (1971) on the body composition of overwintering carp in hibernation ponds. He noted a large increase in the lipid content of the muscles of these fish whilst protein levels stayed constant.

He suggested that the increase in lipid was due to transformation from other compounds. In the Spring, when the fish became active, the lipid content of the muscle dropped rapidly and was followed by a drop in liver and muscle glycogen. Unfortunately, no records of lipid around the gut were reported and it is possible that the muscle lipid increase merely reflected a mobilisation phenomenon. In contrast, in herring and plaice larvae, Ehrlich (1972) demonstrated that starvation brought about a large decrease in protein content; attributed to a modification for a planktonic way of life. Loss of protein making the larvae lighter and therefore able to remain in the upper layers of water for feeding. Loss of protein during fasting was also described by Lovern (1939) for immature Anguilla sp. Here protein appeared to be the only component lost, lipid levels staying constant. Inui and Ohshima (1966) also working with eels, demonstrated that glycogen decreased most rapidly, an observation opposite to that of Lovern.

In the carp starved at 20°C, Nagai and Ikeda (1971) showed an hepatopancreas lipid drop after 22 days whilst glycogen stayed more or less constant. After 100 days the lipid had completely gone and only 1.6% of the original glycogen was left. Interestingly, blood glucose slowly dropped over the 100 days, reaching a value of 50% of the original at the end. Working with pirahna, Rooseveltiella natteri. Bellamy (1968) showed a rapid drop in liver glycogen, 10.3% to 0.5% on a wet weight basis, after only 8 days starvation. Likewise Hochachka and Sinclair (1962) showed a drop in the liver glycogen of Salmo gairdueri after 14 days starvation, but no change in blood glucose concentration. Finally, regarding protein utilisation,

Drilhon (1954) showed that the effect of starvation on body protein is seen sooner in active than in sluggish species. Electrophoresis of blood proteins of Salmo trutta showed a change 30 days into starvation whereas Cyprinus carpio showed the same change only after 100 days of starvation. Further, Love (1970) states, ".... fish would tend to draw upon their protein reserves more readily when building up gonads than during simple depletion." This comment implies an hormonal control of protein mobilisation, a phenomenon now coming under investigation as a method of promoting the growth of farmed fish (Cowie and Sargeant 1972).

From this brief survey it can be seen that no hard and fast rule for substrate preference in starvation can be established. Depletion of energy sources would appear to be a function of species, ambient temperature and state of maturation.

c) FEEDING

In a sense, feeding is the opposite of starvation, in that an excess food intake is present in relation to the maintenance requirements. This excess is normally laid down as either lipid carbohydrate or protein. Analyses of tissues after feeding can yield information relating to the fates of the various dietary components. Possible interrelations may be established and maximum thresholds for particular component tolerance may be noted. Phillips et al (1948) working with trout stated that the maximum digestible carbohydrate in the diet amounted to some 12% net weight.

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Excess over this level causing high liver glycogen deposition and mortality. This was disputed by Buhler and Halver (1961) who fed chinook salmon up to 20% dry weight dietary carbohydrate without adverse effects. Phillips (1969) made the point in relation to the work of Buhler and Halver that, when expressed on a wet weight basis, their levels of digestible dietary carbohydrate and hence confirmed earlier findings (Phillips et al 1948). The addition of extra carbohydrate in the diet leading to the deposition of glycogen in the liver and muscle of salmon was also reported by Wendt(1964). He, however, believed it to be of advantage to fish about to be transported. The rationale being that the extra glycogen acting as an energy store reduced the effects of stress. The presence of carbohydrate in the diet demonstrates the "diabetic physiology" of fish in relation to mammals. Phillips et al (1948) and Palmer and Ryman (1972) have demonstrated a prolonged hyperglycaemic effect in trout fed carbohydrate rich diets. The latter authors showed a postcibal hyperglycaemic effect in rainbow trout of over five times the resting level of blood glucose seven hours after the administration of oral glucose (1 ml. 1 mg/ml. D(+) glucose). Liley (1970) has shown that the insulin producing islets of Langerhans are sparse in fish. The ability of fish to cope with carbohydrate rich diets therefore appears to be a function of hormone action. This inability to reduce blood glucose levels is an interesting phenomenon when it has been demonstrated that stress causes an increase in blood glucose (Menten 1927, Scott 1921, Wardle 1972)

The inclusion of high levels of lipid in dietary formulations gives rise to increased body fat, fatty infiltrated livers and mortalities. (Davis 1953, Phillips et al 1951) However, Ono et al (1960) have fed lipid levels up to 15% wet weight of diet without mortalities, providing that the dietary lipid was not oxidised. A curious example of lipid deposition was noted by Roberts (1970) in intensively farmed plaice. Lateral line lipidosis was diagnosed in which the lymphatic system was blocked with a creamy white substance. Starvation alleviated the phenomenon which occurred when the plaice were fed a diet of fresh white fish. Cowie and Sargeant (1972) suggested that the symptoms were possibly caused by an imbalance between W3 and W6 dietary fatty acids.

Finally, protein, which appears to be the only major dietary component that has not yet been demonstrated as harmful when fed in excess. In fact protein, when taken in quantities above the net protein utilisation (NPU) level, is simply not stored as such. Ogino and Saito (1970) working with carp and casein as the dietary protein, showed that NPU in terms of increase in body protein did not increase above a level of 38% net weight dietary protein. This phenomenon appears to be common to all fish studied and Cowie and Sargeant (1972) review the literature on this subject. Gerking (1952) showed that in green eared sunfish NPU decreased with age. A similar observation was made by Savitz (1969, 1971) who also demonstrated decreased endogenous nitrogen excretion with increase in size. A point to be noted however, in respect of the work of Gerking and Savitz and nitrogen balance experiments in general, is the source of the measured excreted nitrogen in relation to the protein reserve or store.

This stems from the work of Martin and Robison (1972) who measured the nitrogen excretion of subjects on low and high protein diets. When a subject in equilibrium on a high protein diet was switched to a low protein diet, a time lag occurred before a low constant level of nitrogen excretion was attained. Similarly, the reverse effect occurred in going from a low protein diet to a high protein diet. This protein store has now been established as being the cytoplasmic proteins and especially the cytoplasmic proteins of soft tissues. (Schoenheimer 1942). Despite the fact that proteins are structural components they do exist in a state of constant turnover (Sinclair and Hollingsworth 1969) and will be used as energy sources if insufficient energy is available from other sources.

d) ACTIVITY

The effect of activity upon body energy reserves is an interesting study area and is one that has not been extensively studied. Oddly enough all the fish that have been studied have been in a starving condition. This applies to both laboratory and wild fish i.e. migrating salmonids which are reputed not to feed. However, the use of migrating salmon to determine body depletion is complicated by the fact that gonads are being formed and this will constitute an energy drain on the fish. According to Idler and Bitners (1959, 1960) in Oncorhynchus nerka 8% of the total lipid of the female and 0.5% of the total lipid of the male is laid down in the gonads. The total loss of lipid during the

spawning migration of O. nerka has been shown by Thurston and Newman (1962) to range from 9.7 to 1.8% in white muscle and from 27.4% to 6.8% in red muscle. Regarding levels of carbohydrate, Fontaine and Hatey (1953) measured the amount of glycogen present in the muscles and liver of salmon during maturation. They found a low glycogen level,0.1 0.3 mg/g. The liver of these salmon, Salmo salar, showed a marked reduction in glycogen content; the females going from 18.4 mg/g to 0.5 mg/g and the males from 27.1 mg/g. to 24.9 mg/g. This severe loss in female salmon is further born out by the studies of Idler and Clemens (1959) who sampled a run of salmon traversing a distance of 750 miles. From the sea to the spawning grounds, the female sockeye salmon lost 96%of body lipid and 53% of body protein. Macleod et al (1960) studied maturing coho salmon in a laboratory setting and showed that the lipid levels of tissues stayed fairly constant confirming that the activity metabolism required in migration requires large energy stores. A point noted by Idler and Bitners (1960) was that during the first 250 miles of the 750 miles migration, most of the lipid and protein disappeared from the alimentary canal. This indicates a sparing action on muscle storage materials. Attempts to simulate these long migrations have been carried out by Brett (1965) and Kreuger et al (1968). Brett (1965) used an exercise cage to force sockeye salmon to swim for known distances at known speeds. He correlated oxygen consumption (Brett 1964) against loss of body materials and found good agreement between the two types of experiment. Kreuger et al (1968) using small coho salmon (about 8 cms.) subjected them to swimming against

water speeds of either 52 cms/sec. or 59 cms/second. The results from this work are quite remarkable and indicate that continuous swimming at 52 cms/sec. the calorific losses come 55% from protein and 45% from lipid. At 59 cms/sec. however, 84% of the calorific losses come from protein and only 16% from lipid. The mean swimming times for the two velocities differed greatly, being 1141 minutes at 52 cms/sec. and only 398 minutes at 59 cms/sec. Kreuger et al (1968) concluded that their data must be augmented to determine the content of protein in exercised salmon to evaluate the validity of this deduction.

It can therefore be seen from this brief survey, that various dietary and activity conditions, can, and do, give rise to shifts of metabolic emphasis. The type of shift appears to depend upon temperature and species and no clear cut conclusions emerge.

This study represents an attempt to elucidate the type of substrate being oxidised to provide energy under routine and active conditions in two fish species. The fish used were the brown trout Salmo trutta (L) and the perch Perca fluviatilis (L). Both of these are freshwater teleosts and indigenous to the British Isles (Wheeler 1969). The choice of species was for two main reasons. The first was that both species are indigenous in Loch Leven and part of the reported work formed a contribution to the Loch Leven I.B.P. Project (Morgan 1974 and Proceedings of the Royal Society of Edinburgh, Series B, 1974 in press). The second being that both are carnivores occupying separate ecological niches and, presumably, demonstrating different metabolism and physiology.

MATERIALS AND METHODS

I. Respirometers

II. Analyses
- a) Oxygen
- b) Carbon Dioxide
- c) Ammonium nitrogen

III. Fish stocks.

IV. Experimental regimes.
- a) Active
- b) Routine
- c) Nutritional state

I Respirometers.

a) Active,

The recirculating water fish tunnel respirometer was designed after that described by Brett (1964). This particular type of respirometer was chosen in preference to that of Blazka et al (1960) for a variety of reasons. The Brett system allows for a more efficient temperature control than the Blazka system, which either has to be immersed in a water bath (Dickson and Kramer 1971) or run in a controlled ambient temperature to obtain low or high temperature control (Tytler, pers. comm.) Water flow characteristics can be more controlled in the Brett system and wall effects are much reduced. In a recent paper by Muir and Niimi (1972) the Blazka system employed was reported to have a water velocity reduction of 20% at the walls, compared with the flow down the centre of the tube.Introduction and removal of a test fish from a Blazka respirometer can be difficult due to the double skin of tubes present.Loading of a Blazka respirometer has been achieved by use of a pivot at the centre point of the respirometer, (Smith and Newcomb 1970) but this does not appear to offer any operational advantage. Of other types of swimming activity respirometers those described by Fry and Hart (1948), Farmer and Beamish (1969) and Johnson (1960) are available. The respirometer of Johnson (1960) is essentially a Brett system that employs a propeller as the water moving agent. The doughnut respirometer of Fry and Hart (1948) suffers from the disadvantage of rotational slippage of the fish which has to be allowed for in swimming speed estimations, the

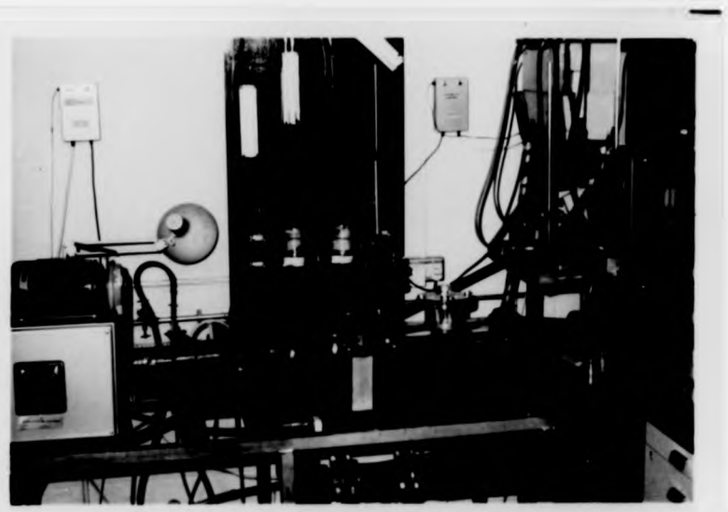


Plate I. Layout of Respirometer

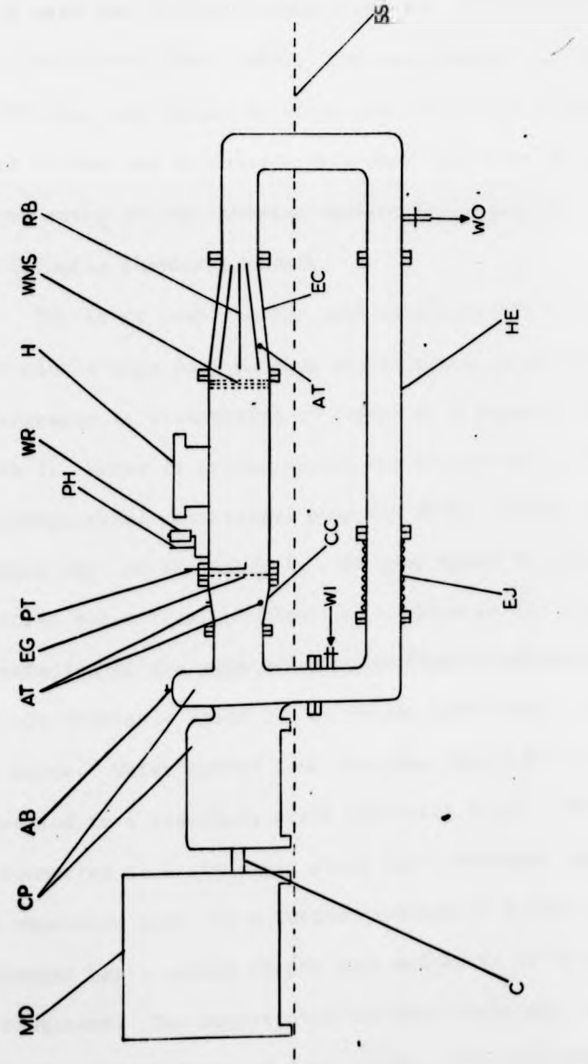
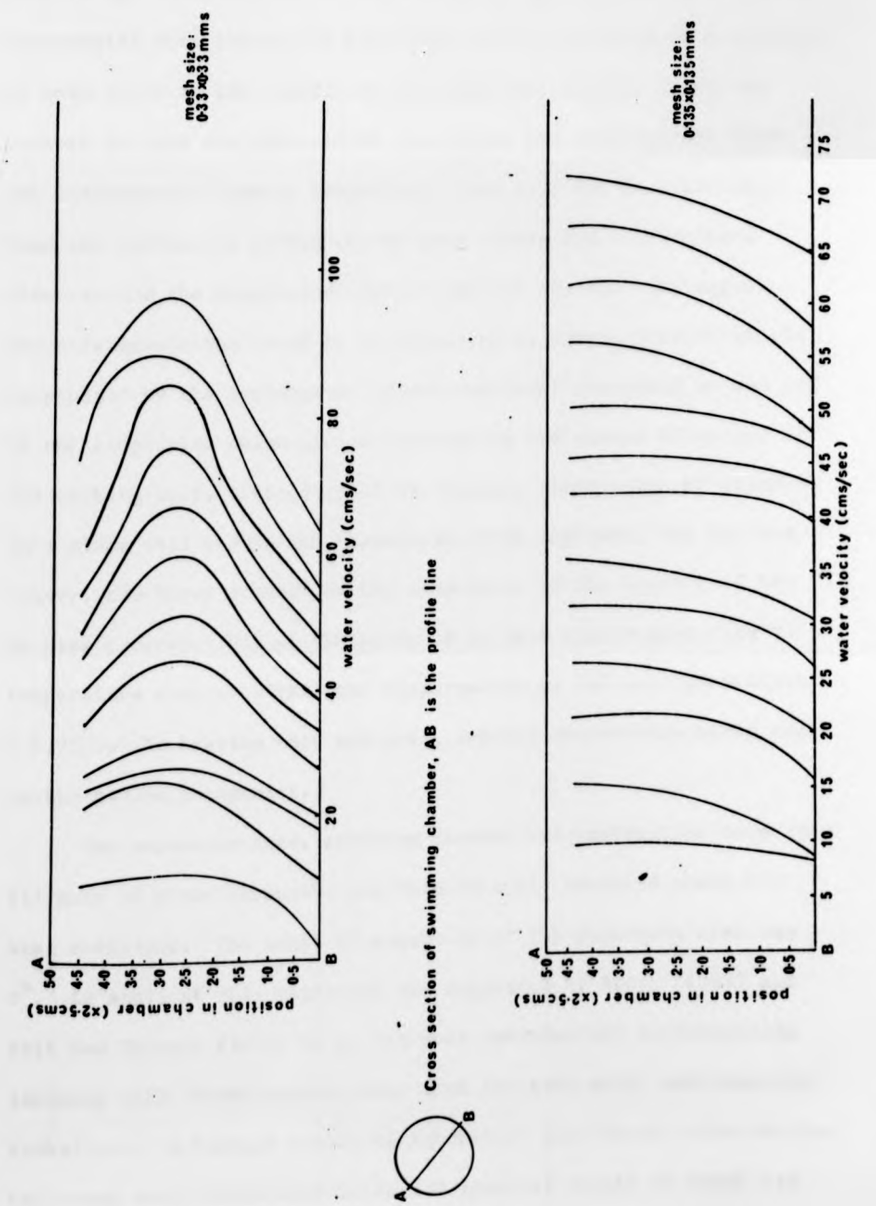


Figure 2. The tunnel respirometer. scale approximately 1/

the different swimming radius available to the fish; and a need for external temperature control. It has however, the advantage of being used for routine metabolic rate studies by being fitted with photo detectors (Smit 1965). The respirometer of Farmer and Beamish (1969) does not appear to offer any particular advantages over the Brett system and is considerably more difficult to reduce the water volume owing to the swimming chamber being smaller than the pre swimming chamber pipework.

The Brett respirometer used in this study consists in essence of a closed pipe loop made up of different sections. The sectional arrangement is illustrated in Figure 2, a general view is shown in Plate 1. Water is driven around the circuit by a closed vane stainless steel centrifugal pump (3" JTVS Holden and Brooke Ltd.) forming part of the circuit. The pump speed is achieved by a 3 H.P. electric motor (English Electric) coupled to the pump. The speed of rotation of the pump being controlled by an external electronic circuit (Variable Speed Drive, Heenan and Froude Ltd.) acting through the motor. Water passes from the pump into a P.V.C. (Durapipe) pipe connected to a stainless steel expansion joint. The expansion joint is connected to a stainless steel heat exchanger pipe which leads to the expansion cone via a further section of P.V.C. pipe. The heat exchanger has a spiral copper tube welded to it which contains refridgerant. The copper pipe and heat exchanger are surrounded by an insulating layer of foam rubber. The refridgerant is driven in the opposite direction to the water flow to achieve counter current heat exchange. A small compressor unit (Sterne & Co. Ltd.) is mounted under the respirometer to control the flow of refridgerant.

Figure 3. Water flow profiles in the swimming chamber The profiles were obtained at 10°C using one pitot static tube.



Cooling is achieved by a mercury contact thermometer mounted downstream of the heat exchanger in such a way that the thermometer bulb is approximately in the centre of the water flow. The contact thermometer acts through an electronic relay connected to a solenoid by pass valve in the compressor refridgerant circuit. When the contact is made the solenoid by pass opens and refridgerant from the continuously running compressor flows over the heat exchanger. When the contact is broken the by pass closes and refridgerant flows around the compressor circuit and not the heat exchanger. The arrangement was found to be necessary as direct control of the compressor by the thermometer caused continual switching on and off of the compressor which caused overheating and severe vibration of the working unit. Shielding of the mercury thermometer by glycerol in a glass vial around the thermometer bulb improved, but did not remove, the heavy demands on the compressor in the absence of the by pass control. Use of the solenoid by pass system gave rise to a temperature control within the respirometer of the set temperature $\pm$ 0.05°C. No heating unit was used, ambient temperature being used as the cooler antagonist.

The expansion cone, swimming chamber and contraction cone were all made of clear"perspex", and were directly amounted above the heat exchanger. The angle of expansion of the expansion cone was 6°. An angle of this magnitude was suggested by Brett (1964) and Bell and Terhune (1970) to be the most satisfactory in preventing incoming water from breaking away from the tube walls and creating turbulence. A limited series of laboratory experiments were carried out using entry cones with different internal shapes to check the

validity of the 6° cone. It was found that the 6° cone was the most satisfactory. The expansion cone was fitted with a radially spread baffle system to remove water swirl due to the right angled pipe leading into the expansion cone. Between the expansion cone and the swimming chamber was fitted a flange "slice" containing three stainless steel mesh grids. The dimensions of the mesh were 0.135 mms. x 0.135 mms, the mesh wire being 0.07 mms. in diameter. An earlier "slice" holding grids of mesh size 0.33 mms. x 0.33 mms. and the same wire diameter was also tested and Figure 3 shows the velocity profiles created by the two mesh sizes. The swimming chamber was 39 cms. long, mesh to electric grid and 12.5 cms. internal diameter. The combined entry and exit hatch was sited approximately half way along the swimming chamber. The removable hatch component was made of solid "perspex" machined on the inside to the curvature of the swimming chamber. The hatch was sealed by means of an "O" ring compressed against the hatch flange by four bolts.

Downstream from the hatch and also mounted in the top of the swimming chamber was a combination inlet connected via a three way stop tap to a small water resevoir (about 500 mls.) and a stand pipe. The stop tap was used either to equalise the pressure in the system after filling from the header tank by exposing the respirometer to atmospheric pressure through the standpipe; or to allow water from the resevoir to enter the respirometer whilst samples were being removed. Just upstream from the electric grid, and mounted at right angles to each other, are two stainless steel pitot static tubes.

The pitot static tubes are attached to a manometer system of the type described by Bell and Terhune (1970). The manometers are available containing either kerosene and sudan red (S.C. 0.78) distilled water (S.G. 1.0) or mercury (S.C. 13.6). The kerosene manometer was mounted at 45^{o} to improve the accuracy of low water speed readings. A 5% accuracy was placed on the measurement of water velocity, dictated mainly by the readibility of the manometer scales. This level of accuracy allowed use of the kerosene manometer up to a water speed of 30 cms/sec., the water manometer up to 75 cms/sec. and the mercury manometer at speeds greater than 75 cms/sec. The pitot tubes were mounted such that they could be traversed across the swimming chamber to estimate the velocity profiles. The velocity profiles obtained are illustrated in Figure 3. The profiles obtained with the smaller mesh size (0.135 mms. x 0.135 mms.) were the ones present in all the experiments. The pitot static tubes were both drawn up to the side walls of the swimming chamber whilst experiments were being conducted.

The downstream end of the swimming chamber is delineated by horizontal stainless steel electrodes, 1 cm. apart. These are connected to a power supply of 5 V. 3 amps in such a way that when two adjacent electrodes are connected they become live. This prevented the fish from resting on the grid and gave rise to continuous swimming. The contraction cone between the swimming chamber and the pump is fitted with two small access tubes. By connecting the oxygen electrode and sampling curvette (Figure 4) between these two tubes, a peristaltic pump can be used to draw

water from the respirometer through the curvette and pump it back into the respirometer.

The volume of the system was determined by direct drainage of the entire respirometer through the water outlet tap. The respirometer was filled with water, the hatch bolted down and the oxygen electrode mounted in the sampling curvette. Water was circulated around the respirometer for 20 minutes at 10°C. The water was then drained into a bucket and measured in a 5L measuring cylinder. Any water remaining in the swimming chamber was siphoned out and the inside of the swimming chamber wiped with absorbant paper. The inside of the collecting bucket was also wiped with absorbant paper. The increase in weight of the absorbant paper was taken as the volume of water taken up (assuming S.G. = 1.0) This procedure was carried out five times to give a respirometer volume of 21.5L ± 0.01 (1 S.E.)

All materials in contact with the respirometer water were made of stainless steel, plastic or "perspex" and therefore there was no corrosion problem. All joints were flanged and held by bolts. All flanges are separated by rubber gaskets coated with glycerol to form a water tight seal and to prevent molecular adhesion between the rubber gasket and the "perspex" flange. Isolation of the pump from the respirometer circuit by means of rubber joints to reduce vibration was not carried out and excessive vibration was not in evidence. The use of rubber joints does not appear to confer any advantage (Webb 1970). The respirometer was cleaned by monthly soaking in "Decon 75" a non ionic rinsable detergent. The respirometer was filled with water and detergent to give a 5% solution. The pump was switched on

and the hatch left open. The mixture was allowed to circulate for about 10 hours with periodic injections of air through a ceramic aerator block. This was followed by extensive rinsing with water. This technique was found to be adequate to keep the system clean without undergoing major dismantling.

Water can be exchanged through the respirometer from the header tank, which is maintained at approximately 0.3°C higher than the respirometer to avoid problems of supersaturation. Water enters the respirometer through a tap upstream of the expansion joint and drains out through the tap in the lowest part of the circuit (downstream of the heat exchanger). The drain is fitted with a flowmeter mounted in such a way that if the water supply fails whilst the respirometer is in open circuit, i.e.whilst flushing, the respirometer would not run dry. The header tank supplying water to the respirometer is mounted above the respirometer. It is fitted with an immersion heater and a cooling coil. The surface of the water is insulated with polystyrene balls and the outside walls of the tank are covered with expanded polystyrene sheets. The water is thoroughly stirred and aerated. Water continuously flows into the header tank and into the respirometer or down to drain through a standpipe overflow.. The curvettes holding the carbon dioxide electrode and calibrating system for the oxygen electrode are temperature controlled using header tank water.

b) Routine.

The routine respirometer chamber is made up from a length of "perspex" tube. One end is permanently closed by a place of "dural" metal, the inside face of which is coated with "Araldite" (C.I.B.A.) to prevent corrosion. The metal plate sets up as a heat exchanger. The removable lid of the respirometer is also made of "perspex". The internal dimensions of the chamber are 27 cms. by an internal diameter of 16.5 cms. The lid is pierced by two glass tubes, one protruding into the chamber about 2 cms., the other by about 25 cms. After introduction of a fish, the lid if bolted down and the chamber placed on its long axis in a temperature controlled water bath. The short inlet tube is located at the bottom of the chamber and the long outlet tube at the top. This arrangement ensured mixing of the respirometer chamber and caused the fish to orientate towards the inlet whilst resting on the bottom. The water bath was covered by a piece of wood which gave rise to darkened conditions inside the respirometer. Activity of the fish was not monitored but it was found by visual observation that spontaneous activity was almost totally absent. The volume of the system was determined by direct drainage and was 6.0L.

II Water Analysis

a) Water quality

The water used for maintaining fish stocks and in the respirometers came from the same source. A chemical analysis is presented in Table 3.

b) Oxygen

The oxygen concentration in the respirometer water was measured using an oxygen electrode (Radiometer E5046). The electrode was made up to manufacturer's specifications with a polypropylene membrane. The principle of the oxygen electrode is that oxygen diffuses through the membrane until equilibrium is reached between the sample and the film of electrolyte in contact with the platinum cathode. If a polarizing voltage of the order 650mV is applied to the platinum cathode a current proportional to the partial pressure of oxygen, PO_2, will be generated through reduction of the oxygen diffusing towards the cathode.

Calibration of the electrode was achieved by placing the electrode such that two thirds of its length was immersed in water in a glass vial. The glass vial was mounted in a "perspex" water jacket connected to the respirometer header tank. Using a peristaltic pump water was drawn from the header tank into the water jacket and then back into the header tank. The electrode calibration temperature was therefore approximately 0.3°C higher than the respirometer temperature. Air or nitrogen was passed

TABLE 3

CHEMICAL ANALYSIS OF STOCK AND EXPERIMENTAL WATER

	Parts per Million
Total dissolved solids	40.0
Total Alkalinity as $CaCO_3$	14.0
Chlorine (Cl)	7.0
Free Ammonia	0.007
Albuminoid Ammonia (N)	0.063
Nitrous Nitrogen (N)	none
Nitric Nitrogen (N)	trace
Sodium (Na)	2.6
Potassium (K)	0.3
Iron (Fe)	0.12
Copper (Cu)	0.04
Lead (Pb)	0.01
Free Chlorine (Cl)	0.04
pH Value	7.0

(Analysis carried out by R.R. Tatlock and Thomson, Analytical Chemists, Glasgow)

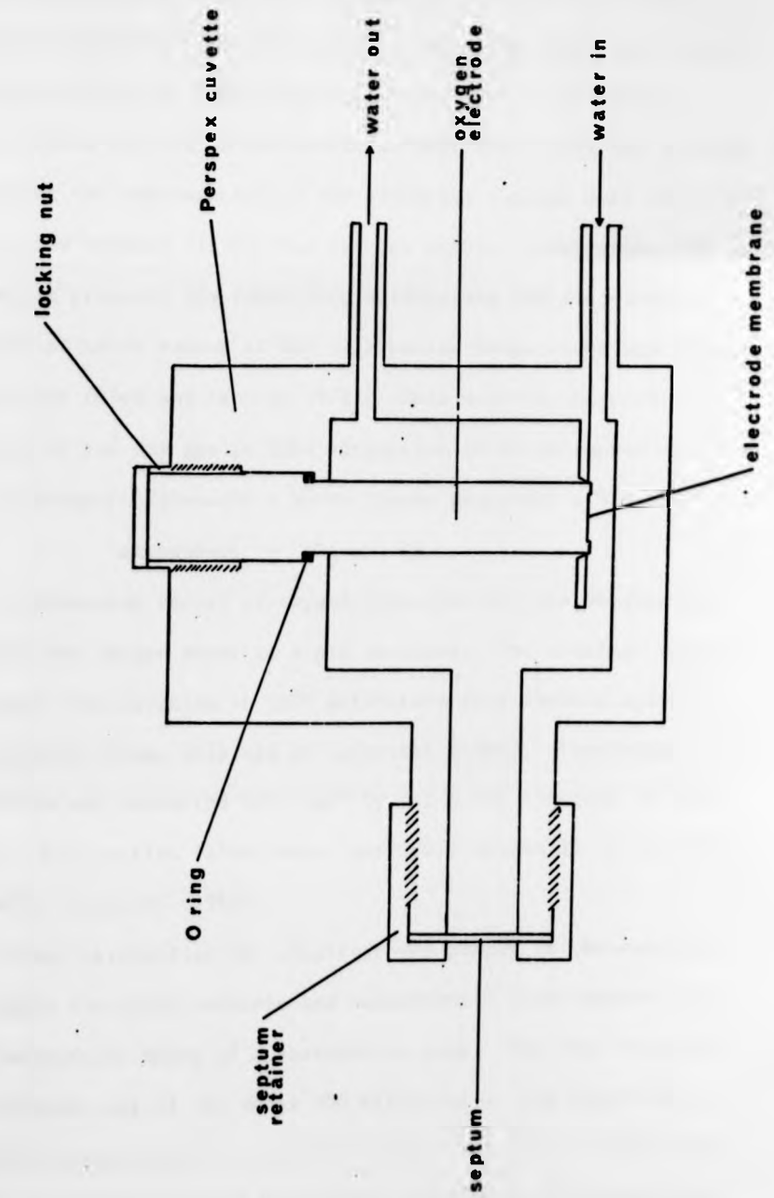


Figure 4: Oxygen electrode and sampling cuvette.

Scale: approximately full size.

through gas tubing coils in the header tank and bubbled into the water surrounding the electrode. The electrode was calibrated at 0% with nitrogen and 100% with air. Calibrating gases were bubbled into the calibration cuvette in such a way that non of the gas bubbles could collect on the electrode membrane. This was achieved by placing the membrane end of the electrode further into the calibration cuvette liquid than the gas bubbles penetrated. The barometric pressure was taken when calibrating and the partial pressure of water vapour at the calibration temperature was taken from tables (Diem and Lentner 1972). This enabled the partial pressure of the dry gas at 100% saturation to be estimated as:-

(Barometric pressure - Water vapour pressure) x % O_2 in atmosphere = PO_2 mm. Hg.

A permanent record of oxygen concentration was obtained by coupling the oxygen meter to a pen recorder. The obtained values of oxygen concentration at 100% saturation were checked against experimental values obtained by Carpenter (1966). Percentage saturation was converted into mg/L by using the nomogram of Hart (1967). Calibration values were invariably within 2% of the values quoted by Carpenter (1966).

After calibration the electrode was placed in the sampling and oxygen electrode curvette and respirometer water passed over the electrode by means of a peristaltic pump. The flow rate over the electrode was of the order 900 mls/minute. The electrode was relatively insensitive to pressure effects but it was found that using the peristaltic pump to produce flow rates of greater than 1500 mls/minute gave rise to fluctuating readings. It is of interest to note at this point that in the initial stages of the work, the

the electrode was mounted directly in the contraction cone. This, however, gave rise to readings that were water velocity dependent, unlike the findings of Beamish (1970). After insertion into the cuvette water was continually flushed over the electrode whilst the respirometer was in open circuit i.e. fresh water was running from the header tank through the respirometer and down to drain. When the electrode had stabilized, as demonstrated by a steady line, on the pen recorder chart, the concentration of oxygen in the respirometer water was estimated.

Approximately once a week a dummy run was carried out on the respirometer for inherent oxygen consumption. Any respirometer uptake was allowed for in the calibration of fish oxygen consumption. Due to the quality of the water used however, little algal growth took place within the respirometer and inherent oxygen consumption rarely occurred.

Although the electrode was effectively pressure insensitive, it was temperature sensitive to the extent of 1.5% change per ^{o}C (% of full scale deflection on recorder chart). This was noted experimentally and indicated by the manufacturer. This phenomenon was virtually eliminated by the close temperature control ($\pm$ 0.05^{o}C) of the respirometer water. The effect of calibrating at 0.3^{o}C higher than the respirometer temperature was, in terms of mgO_2/L, experimentally negligable.

About once every five to six months the electrode would display erratic behaviour not due to membrane or polarizing voltage defects. When this occurred the electrode was treated by removing the accumulated silver coat from the reference electrode (anode) with 0.1M NH_4OH (LeFevre et al 1970). Rechloriding of the anode was achieved by

applying 1.5V to the anode against a piece of silver wire in 0.1M HCl for thirty seconds. The cathode was cleared of the silver contamination at the same time by just applying the electrode tip into 0.1M HNO_3 for sixty seconds.

c) Carbon Dioxide

The method used for the anlysis of carbon dioxide was based on the use of the so called carbon dioxide electrode. The basic design for this electrode was independently described by Stow et al (1957) and Gertz and Loeschcke (1958). The electrode consists of a glass pH electrode covered with a "teflon" membrane. A thin layer of water containing chloride and bicarbonate ions is held between the glass and "teflon" by a spacer-cellophane, Josef paper, nylon stocking or glass wool. Carbon dioxide gas molecules diffuse through the "teflon" from the sample and react with the bicarbonate solution. The pH of this solution will vary with changes in carbonic acid concentration. The carbonic acid concentrations of the bicarbonate solution will vary with changes in the partial pressures of the carbon dioxide (PCO_2). The PCO_2 of the bicarbonate solution will vary directly with the PCO_2 of the sample measured because the "teflon" membrane is permeable to carbon dioxide gas molecules. The pH of the bicarbonate solution is measured by the pH electrode and transformed into a carbon dioxide gas measurement by either the use of a calibration line relating log CO_2 to pH or displayed on the measuring meter as PCO_2.

In order to convert the partial pressure of the gas (PCO_2) into a concentration, the relationship between partial pressure, concentration and solubility has to be defined. This relationship from Henry's Law is:

$$PCO_2 = \frac{C}{\alpha}$$

Where

PCO_2 = partial pressure exerted by CO_2 in mm. Hg.

C = concentration of CO_2 in mM/L.

α = solubility coefficient of CO_2 in the sample at the experimental temperature.

The value for α, the Bunsen coeifficient, depends upon the temperature and ionic composition of the sample.

i) Estimation of α, the Bunsen coeifficient.

The values of α were calculated from that data given in Randall (1970).

Temp. °C.	Solubility of CO_2 in pure water (ml/L/mm.Hg)
5	1,874
10	1.571
15	1.341

Taking the density of CO_2 as 1.977 g/L (Petrusewicz and Macfadyen 1970), the values for α become:

Temp. °C	α in pure water (mM/L/mm.Hg)
5	0.0841
10	0.0705
15	0.0602

In order to check the validity of this estimation of the value of α at 25°C was calculated. This gave a value of 0.0448 which agrees well with the α value at 25°C of 0.04392 as determined by Bartels and Urbitzsky (1960). As the CO_2 was present in an HCl solution (see later) a correction had to be made for the effect on the solubility due to the presence of the HCl. Van Slyke et al (1928) demonstrated from their work, that the effect of H^+ ions was negligable and the "salting out" effect was due only to the Cl^- ions.

A correction factor, fCO_2 (Siggard-Anderson 1962), was therefore calculated for a 10 mM HCl solution. The value of fCO_2 was obtained from data in Van Slyke et al (1928) by the relationship:

$$fCO_2 = \frac{\alpha}{\alpha_o} \qquad \text{(Siggard-Anderson 1962)}$$

where

α_o = solubility factor for pure water = 0.5468

α = solubility factor for 10 mM HCl = 0.5452

This gives a value for fCO_2 of 1.003.

Therefore the values of α_o for the temperatures of 5°, 10° and 15°C were obtained by dividing the values of α by fCO_2.

Temp. °C.	α_o (mM/L/mm.Hg)
5	0.0838
10	0.0702
15	0.0600

Again, these values were checked by comparison with the literature. Rispens and Hoek (1968) derived a value at 37°C for α o of 0.03286 which agrees well with the estimated value of α o at 37°C of 0.0305 from the calculated data of this study. The difference can be explained by the different concentrations of HCl used in the two acidification processes.

ii) Standardisation of the electrode and the determination of CO_2.

When gaseous CO_2 dissolves in water it hydrates to form carbonic acid. Depending upon the ambient pH the carbonic acid dissociates. The resulting bicarbonate union can then react with a free cation if available. The overall reaction may be represented as:

$$CO_2 + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons H^+ + HCO_3^-$$

$$\Big\Downarrow + \text{Cation}^+$$

$$\text{Cat. } HCO_3$$

Addition of H^+ ions to the mix in the form of acid forces the reaction to the left to give only gaseous CO_2. This can exert a partial pressure proportional to the concentration of total CO_2 in the system. This partial pressure in related to the concentration (C) and solubility, the relationship defined by Henry's Law.

$$\text{i.e.} \quad PCO_2 = \frac{C}{\alpha}$$

As the electrode was to be used at low temperatures to detect and measure small increases of CO_2 against a background concentration of CO_2 in the sample water, modifications to both the electrolyte and gas permeable membrane was made. The "teflon" membrane was replaced by a silicone rubber membrane (Rispens and Hoek 1968) which permits faster gas diffusion. The Josef paper was omitted and the electrolyte composition changed to 0.001 M $NaHCO_3$ and 0.1M Na Cl made up in a 2% high substitution methyl cellulose medium (Severinghaus 1965). These modifications permit faster response time - important at the low temperatures, and ability to register small changes.

Carbon dioxide standards were made up using deionised (Permutit Mk. II) boiled water (= B.D.W.). The water was allowed to cool connected to the atmosphere through a soda lime tube. Anhydrous sodium carbonate (B.D.H. microanalytical reagent) heated overnight at approximately 100°C was used to make up the standards. An 0.25 mM CO_2/L standard was prepared by making up 13.3 mg. Na_2CO_3 to 500 mls. with B.D.W. Addition of the water was made quickly without splashing. The volumetric flask was sealed with a vaccination stopper ("Suba Seal"). A second flask was filled using only B.D.W. and sealed in a similar manner. To remove liquid from either the standard or B.D.W. flask a procedure was adopted to prevent any contact with air. Nitrogen is injected through the stopper into the flask from a glass syringe. A second glass syringe is filled with 0.1 M HCl which is ejected from the barrel, leaving the needle dead space filled with acid. This "rinsing" also creates a liquid seal between the barrel and the plunger. The needle of the acid prepared syringe is then gently inserted into the inverted volumetric flask and the gas pressure within the flask forces liquid

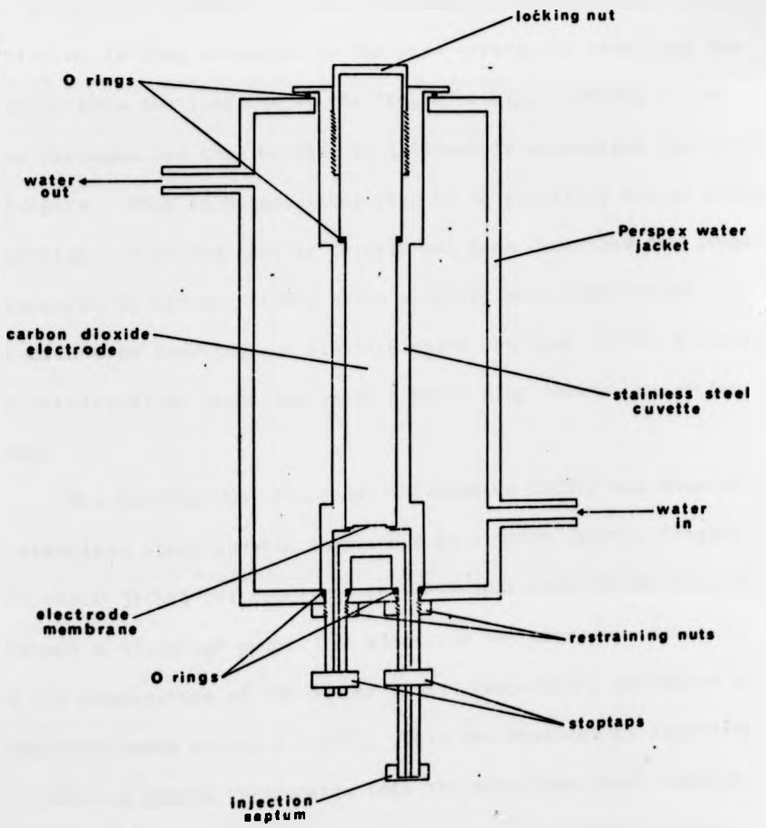


Figure 5: Carbon dioxide electrode cuvette.

Scale: approximately Half size.

into the syringe. By only partially filling the syringe with standard solution and then filling with B.D.W., any dilution of the standard required can be made up within the syringe. A second acid syringe, the needle fitted with a short length of "tygon" tubing, is then again filled with 0.1 M HCl. and acid expelled to a residual volume of 1 ml. The syringe containing the standard solution is then connected to the acid syringe by inserting the needle into the free end of the "tygon" tubing. Mixing of the two syringes can then be made by alternately depressing the syringe plungers. This technique gives rise to an acidified mix of standard solution. A method similar to this has been described for oxygen standards by LeFevre (1969) and a slightly more complicated procedure by Severinghaus (1962).Rispens and Hoek (1968) described an acidification technique using a metal ring inside one syringe only.

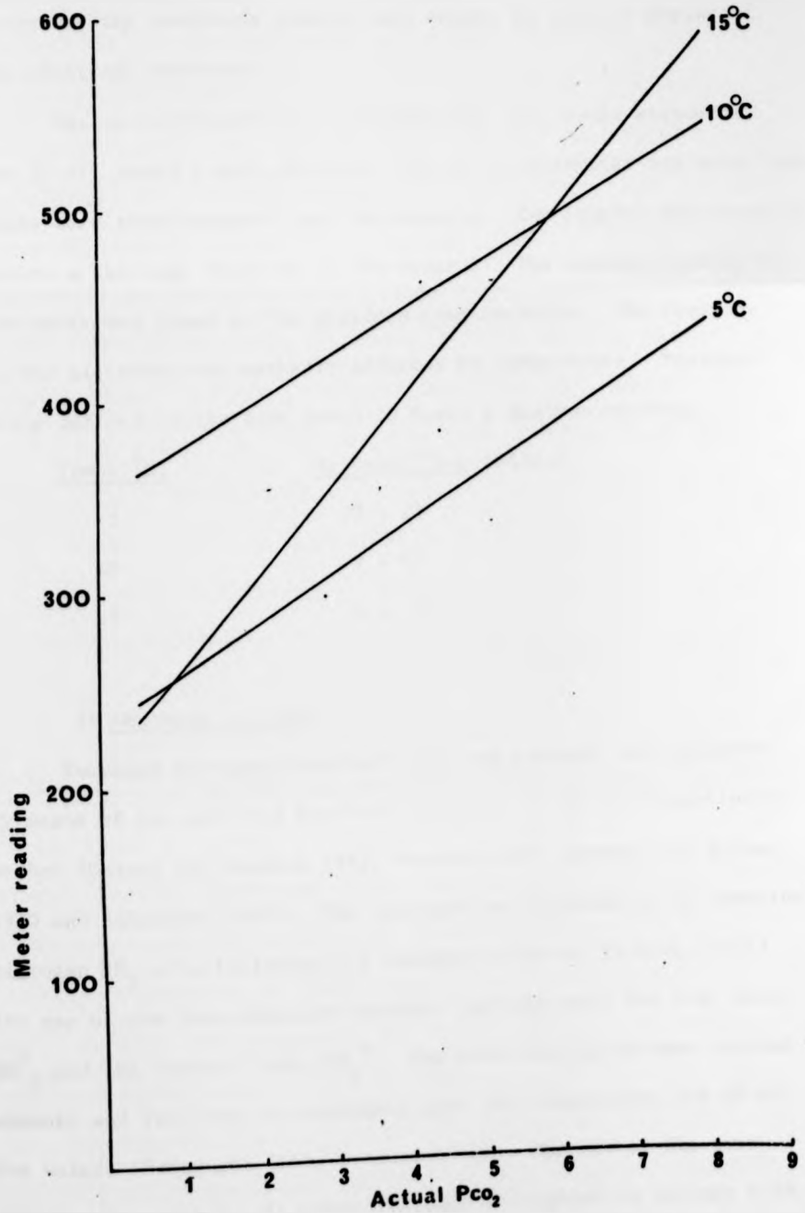
The modified CO_2 electrode (Radiometer E5036) was mounted in a stainless steel cuvette surrounded by a water jacket. (Figure 5) The water jacket was connected to the header tank of the respirometer through a diaphragm pump. The electrode was therefore maintained at the temperature of the header tank. Temperature variation of the electrode never exceed $\pm$ 0.08°C. This was measured by inserting an oscillating quartz thermometer into the stainless steel cuvette (Hewlett Packard 2801A). In use the electrode was always mounted at above 45° with the sensing head downwards, to avoid faulty recordings caused by the air bubble within the pH electrode. Samples and standards were injected through the lower part while the cuvette was placed horizontally. This ensured that air bubbles did not get trapped within the cuvette. The volume of the cuvette was approximately 2.5 mls.

In order to calibrate the electrode, a one-standard method was adopted. An arbitary dilution of the standard was made, acidified and injected. The meter was then adjusted to give a very high, in relation to the actual PCO_2 of the standard, reading. This reading was 512 mm.Hg. for 7 mls. of standard diluted to 10 mls. This technique was adopted in order to achieve a scale expansion. The standard reading of 512 mm.Hg. was taken as the reference or standard calibration. After calibration with the reference, a second standard of different concentration was injected, and the reading noted. This was followed by another reference injection. If the reading obtained from the second reference differed from that of the first reference, the differential was added to the reading obtained from the test sample. In practice the error or drift of the second reference was always within 2% of the expected reading. In this way a series of known standards was used to obtain a calibration line for the electrode at each experimental temperature. A graph of meter reading against actual PCO_2 was drawn for each temperature (Figure 6). It is interesting to note that although the regression coeifficient of the lines at $5^{\circ}C$ and $10^{\circ}C$ are similar, the regression constants vary. This is due to the fact that the $5^{\circ}C$ electrode was calibrated to a reference point of 366 mm Hg. and not 512 mm Hg. as in the $10^{\circ}C$ electrode. The line for the $15^{\circ}C$ electrode shows a markedly different regression coeifficient to both the $5^{\circ}C$ and the $10^{\circ}C$ lines. This is due to the fact that a fresh batch of electrolyte was used for the $15^{\circ}C$ electrode and probably differed slightly in composition. The constant slope is a feature remarked upon by Gambino (1961) who noted that with the same

Figure 6: Carbon dioxide electrode calibration line.

Regression equations:

5°C	Y = 224.15 + 90.29 X	r	= 1.00	n = 13
10°C	Y = 291.77 + 85.04 X	r	= 1.00	n= 25
15°C	Y = 219.35 + 99.14 X	r	= 1.00	n = 19



electrolyte the slope was invariate between 10°C and 40°C. At no stage was the electrode cuvette left empty, to prevent drying of the electrode membrane.

Having calibrated the electrode with two 10 ml. standards, the 10 ml. samples were obtained from the respirometer and acidified. These were then injected into the cuvette. Two samples were used to ensure a thorough flushing of the cuvette. The maximum reading of the meter was taken as the measured concentration. The response of the electrode was markedly affected by temperature, response being defined as the time taken to reach a maximum reading.

Temp. °C.	Response Time (mins.)
5	20 - 23
10	9 - 12
15	4 - 6

d) Ammonium nitrogen

Excreted nitrogen from both urea and ammonia, was measured by means of the modified Berthelot reaction or phenol hypochlorite method (Chaney and Marbach 1962, Cocking 1967, Harwood and Huyser 1970 and Solorzano 1969). The nitrogen was referred to as ammonium nitrogen NH_3 - N, following the recommendation of Winberg (1971). The use of the term ammonium nitrogen includes both the free base NH°_3 and the ionised form, NH_4^+. The relationship between ionised ammonia and free base is dependent upon the temperature and pH of the water. (Burroughs 1964, Trussel 1972). The use of the term ammonium nitrogen is therefore followed throughout to include both forms of the molecule.

Two 20 ml. samples of respirometer water were obtained at each sampling via the sampling cuvette by means of a 20 ml. all glass syringe. These samples were placed in "Pyrex" boiling tubes (15 cms. x 2 cms. I.D.), sealed with "Parafilm" and stored overnight at 4°C. It was not possible to analyse the samples when obtained due to the length of time involved in each experimental fish run. Preliminary analyses showed that no appreciable change took place in the overnight samples. The water quality was such that no pretreatment of the samples prior to analysis was required.

The reagents were made up as follows. (Chaney and Marbach 1962 - concentrated reagents):

Phenol	25 g.	made up to 500 mls.
Sodium nitroprusside	0.125 g.	
Sodium hydroxide	12.5 g.	made up to 500 mls.
Sodium hypochlorite	52.5 mls	

All reagents Analar (B.D.H.)

The sodium hypochlorite contained 10-14% available chlorine. The solutions were made up using boiled deionised water. (= B.D.W. Permutit Mk.II). Solutions of the reagents were made up in 500 ml. volumetric flasks and sealed with "Parafilm" and a screw top. The flasks were covered with black polythene and when not required were stored at 4°C. Fresh reagents were made up monthly, no measurable deterioration taking place between batches.

An ammonium nitrogen standard was made up by dissolving 0.9433 g. ammonium sulphate (Analar, B.D.H.) in B.D.W. and making up to 1 L. The ammonium sulphate was oven dried at 100°C for 24 hours before being used. The resulting standard containing 200/mg NH_3 - N/ml. was further diluted to give a working standard of

2/mg NH_3 - N/ml. To analyse the samples the following routine was adopted.

Three urease tablets (Sigma Type 111-20) were crushed and mixed with B.D.W. to provide a urease solution containing 60 units of urease activity. One unit of activity being defined as producing 1 mg. NH_3 - N from urea in five minutes at pH 7.0 and 30°C. The crushed tablets in B.D.W. were allowed to stand for 30 minutes at room temperature before being centrifuged at 3,000 r.p.m. for 20 minutes. This yielded a clear supernatant for use.

To each of the total NH_3 - N samples was added 1 ml. of urease solution. The tubes were resealed with "Parafilm" and incubated at 37°C for 30 minutes with occasional gently shaking. Ammonium nitrogen standards were made up from the working standard solution to contain 5,2,1 and 0/ng NH_3-N/20 ml. Again B.D.W. was used as the diluent. To the standards and the remaining 20 ml. samples was then added 1 ml. B.D.W. to make all volumes up to 21 mls. All samples and standards then receive 1 ml. of phenol/ nitroprusside reagent followed by 1 ml: of hydroxide/hypochlorite reagent. The order of addition was most critical (Harwood and Huyser 1970). Each tube was then resealed with "Parafilm", immediately inverted to mix the contents, and incubated at 37°C for 30 minutes. Each rack of incubating tubes being periodically gently agitated. After incubation the tubes were allowed to stand and cool to room temperature for about 20 minutes. At the end of this cooling period the contents of the tubes were again mixed by inversion.

The developed colours were then analysed spectroscopically in a Zeiss PM 2 II spectrophotometer using 1 cm. quartz cells, slitwidth 0.05 cms. and at a wavelength of 625 mm. A standard curve was drawn and the concentration of ammonium nitrogen in the urease treated and the untreated samples was determined.

III Fish Stocks

The fish used were brown trout, Salmo trutta (L) and perch, Perca fluviatilis (L). The trout were obtained from the Howietoun and Northern Hatcheries, Stirling; and the perch were seine netted from the wild population indigenous in Loch Leven, Kinross. All fish on entry to the aquarium were bathed for 30 minutes in an aerated bath containing potassium permanganate (1g/100L. Reichenbach-Klinke and Elkan 1965). The fish were then sized, and those in a 17-23 cm. size range were retained, the others being discarded. Apart from rejection for reasons of size, external damage, infection or injury, no further distinction was made in the fish stocks.

The fish were maintained in opaque, creamy yellow circular fibre glass tanks containing approximately 1000 L of water. After an outbreak of light induced Saprolegnia sp. infection in the trout stocks, the intensity of the artificial illumination at the surface of the trout tanks, was reduced by shading. (For details of trout infection see Appendix V) The trout were maintained on Beta floating pellets (Coopers Nutritional Products Ltd.) with once weekly supplements of beef liver to combat any possible dietary deficiencies. Perch were fed on a diet of beefliver and maggots, (larvae of bluebottles). The tanks had a continual flow-through of fresh water and were aerated. No routine dosing with antibiotics or chemicals was undertaken as the fish ordinarily remained healthy. To ensure a relatively stable population no fish were used for experimental acclimatization until a residence of at least one month was completed. Once the laboratory population was established no new fish were introduced to avoid problems associated with new stock (c.f. Brett 1964)

The effect of exercise on fish used in the study of metabolism has been described by Brett (1964), Hochachka (1962) Jones (1971), and in a review of tank fish problems, Love (1970). All experimental trout were therefore subjected to continuous exercise conditions similar to those described by Alderdice et al (1962) and Jones (1971) Vertically mounted pressure pipes provided a running water circulation against which the trout maintained station. Water speeds imposed in this manner were of the order 0.7-1.0 bl/sec. The perch were not subjected to such vigorous conditions, but were maintained under water velocities sufficient to cause them to orientate to the water current and hold station with gentle movements of the pectoral and caudal fins.

Temperature acclimatization was carried out for both species either by using an immersion heater or by holding the fish in constant temperature rooms. Experimental temperatures used corresponded as closely as possible to the temperatures prevailing in near by natural water bodies. Photoperiods were maintained between 10-12 hours per day, again depending on the prevailing natural photoperiod. Complete paralleling of the natural photoperiod was impossible as this would have entailed light and dark periods during experiments which might have introduced complicating metabolic and activity factors (Chaston 1968, Roberts 1964). Temperature acclimatization was judged to be complete when a fish stock had been held at a described temperature, $\pm$ 0.5^{o}C, for a period of at least three weeks. (Brett 1964)

IV Experimental regimes

a) Active

An experimental fish was placed in a bucket containing anaesthetic (MS 222 Sandoz, 0.1g/L = 1/10,000). If during capture and anaesthesia a fish struggled excessively it was rejected and not used for that experiment. When the fish was sufficiently narcotised to be handled, it was quickly blotted on absorbant paper, weighed to the nearest 0.1g. measured to the nearest 0.1 cm. (fork to snout) and returned to the anaesthetic. Whilst being handled it was inspected for disease, injury or scale loss, and if any was present it was rejected. The bucket was then covered and fresh water run into it to recover the fish. After 5 - 10 minutes the fish recovered equilibrium and responded to visual and tactile stimuli. At this stage the fish was placed into the respirometer at a water speed corresponding to 0.7 bl/sec. with the electric grid switched off. A piece of black polythene sheeting about 3" broad was placed around the upstream end of the swimming chamber. The black polythene acted both as a visual cue and light "shelter". A lamp with a 40 watt bulb was placed against the wall adjacent to the respirometer and switched on. It was found that recovering the fish in the manner described gave rise to minimal excitement and activity after introduction to the respirometer. In the case of trout it was necessary to leave the hatch open for a period of 2-3 minutes after introduction of the fish. This enabled the gas blown off from the swimbladder to be collected with a polythene tube and removed from the system. The hatch was then closed and water flushed through the respirometer, exchanging at a rate of 1-1.5 L/minute. A piece of brown cloth was wrapped around

the entire swimming chamber and hatch. Using a "fish eye" lens mounted in a piece of plywood, the fish could be observed in the swimming chamber, through a hole cut in the cloth cover. This arrangement allowed inspection of the fish and operator movement in the vicinity of the respirometer without any apparent visual stimulus to the experimental fish. The lamp was fitted with a time switch that was synchronised to the holding conditions photoperiod. This allowed the switching on of the main laboratory lights the following day to elicit minimum excitement. The electric grid was switched on 30-40 minutes after introduction of the fish. The fish was left in the respirometer to acclimatise to the system overnight - a period of 15-18 hours.

The following day the oxygen and carbon dioxide systems were calibrated and the experiment started.

Each fish was held at a desired swimming speed for a period of two hours, measures being made of O_2 consumed and CO_2 and NH_3-N excreted every hour. This regime was adopted in order to obtain estimates of the "steady state" - the second hour, and the effect of an increase in swimming speed - the first hour. At the end of the second hour the swimming speed was increased by approximately 0.3 bl/sec. at a rate of 2 cms/minute. The speed increases therefore, took place over a period of 4-5 minutes. This procedure was carried out to avoid undue stress and excitement of the test fish. After a speed increase a period of unsteady swimming (5-10 minutes) existed, after which the fish settled down. The velocity increases were carried out until the test fish collapsed. Collapse was seen as the fish falling back, hitting the grid and being unable to lift itself off. At this point the grid was switched off and the water

water velocity lowered to a swimming speed of 0.7 bl/second. The recovery metabolism was then monitored for a further period of three hours. The recovery was timed from the end of the second hour of the last successful swimming speed. Collapse invariably took place 10-15 minutes after the speed increment, similar times to those noted by Webb (1971b).

The respirometer was flushed with fresh water for 30 minutes every 90 minutes at 15°C. Flushing taking place during the first 30 minutes of the second hour of a particular speed. At 10°C and 5°C this was not deemed necessary, as the metabolism displayed was such that the concentrations of O_2, CO_2 and NH_3-N never approached levels that could be limiting. In practice, the O_2 concentration was used as the main monitor, the concentration never being allowed to fall below 120 mmHg. (this is about 75% saturation and at 10°C is equivalent to 8.5 mg O_2/L) It has been demonstrated with rainbow trout at 8-15°C that a decrease in O_2 concentration below 8.0 O_2mg/L (110 mm Hg.) causes a decrease in heart rate (Randall and Smith 1967). Likewise, Gibson and Fry (1954) showed that up to 14°C, using lake trout (Salvelimus namaycush), the active metabolic rate is not reduced until oxygen concentration falls below a PO_2 of 120 mm.Hg. Kutty (1968a)using rainbow trout demonstrated that at 15°C the swimming speed was not reduced until the oxygen concentration fell below 2.5 mg/L. The effect of CO_2 concentration appears to be variable, but Dahlberg et al (1969) showed that the swimming speed of coho salmon decreased only when the CO_2 concentration increased by 18 mg/L over the acclimatisation level. However, this effect at 20°C only took place when the oxygen concentration dropped below 9 mg/L. It is of interest to note that this effect decreased rapidly with decrease in temperature.

When an experiment was finished, the test fish was placed in a small tank overnight. This was done in order to determine if any post experimental effects were present.

b) Routine

The respirometer used here is described in Section I b). It was used for perch only at 15°C and 10°C. The fish were prepared as before, except that all recovery from anaesthetic and handling took place in the respirometer. The respirometer was sealed, placed in the water bath and water flushed through it at a rate of 1-1.5L/minute. Acclimatisation again took place overnight, a period of 15-18 hours.

After calibration of instruments the experiments consisted of closing the respirometer for 60 minutes, measurements being made at the onset of closure and 60 minutes after closure. The system was then flushed for 30 minutes. This procedure was followed so as to obtain three sets of readings for each experimental fish. Activity was not monitored but it was found by observations that the fish rested quietly on the bottom of the darkened respirometer throughout the experimental period.

c) Nutritional state of the experimental fish.

Before an experiment, the experimental fish was not fed for a period of time depending upon the temperature. It has been shown (Beamish 1964a, Edwards 1971, Elliot 1972, Glass 1968, Sethi - cited by Beamish and Dickie 1967, Molnar and Tölg 1962 and Windell and Norris 1969) that the presence of food in the gut gives rise to an elevated metabolic rate and that the rate of gastric evacuation is temperature dependent. Accordingly, food had been withheld from

the experimental fish at the start of respiratory experiments for the following periods of time:

Temp $^{o}C.$	Time (Hrs.)
5	72
10	48
15	36

This time period includes the overnight acclimatisation in the respirometer. Preliminary experiments using trout in the routine respirometer at $5^{o}C$ agreed, on the basis of respiratory data, with the conclusions of Elliot (1972).

d) Nature of the metabolic rates described.

Without anticipating too much of the discussion that follows, some points relating to the metabolic rates used in this study will be usefully described at this stage. Two major metabolic rates are examined in this study, the routine metabolic rate and the sustained metabolic rate. A third, and minor rate, the standard metabolic rate (for oxygen consumption alone) is mentioned in the R.Q. Discussion section. Commonly quoted metabolic rates are those of standard, routine and active. The standard metabolic rate is defined as the metabolic rate (in terms of oxygen consumption) obtained by extrapolation of the regression line of the log. of oxygen consumption against swimming speed to zero activity. (Brett 1964, Fry 1971). The routine metabolic rate is variously described at that metabolic area lying between standard and active metabolism (Doudoroff and Shumway 1970) or the metabolic rate displayed when an unstressed fish is only undergoing spontaneous activity (Brett 1972, Fry 1971).

In this study, the routine rate for perch is described only at 10°C and 15°C, when measures were taken from fish being respired in the routine respiration chamber. At all three temperatures in the tunnel respirometer the perch swam, even at the lowest water velocities. This latter condition, in the case of perch, cannot be considered to be spontaneous activity. The brown trout, on the other hand when placed in the active (tunnel) respirometer at water speeds corresponding to swimming velocities of 0.7 bl/sec., did not swim. Under these conditions they rested on the bottom of the swimming chamber. Sutterlin (1968) demonstrated that the heart rate of brown trout in this situation did not increase above the resting level. Likewise careful observation of the brown trout in this study showed that no visually detectable stress was present. Accordingly, the routine metabolic rate of brown trout in this study was taken from measures obtained under these conditions. A sustained metabolic rate in this study was taken to be the measured metabolism during the second hour of a swimming speed. The trout routine rates were also taken from the second hour of the routine condition.

Another term or physiological condition that will be referred to is that of the steady state condition. This is used in this study to refer to the metabolism displayed during the second hour of a set swimming speed. In the second hour, the fish under experiment is deemed to be displaying the metabolic characteristic resulting from the energy requirements only. In other words, such factors as excitement, burst of anaerobic metabolism and exchanges between fish and environment are taken as being absent or reduced to a low

low level, and the fish is in a metabolic equilibrium. Studies are available describing the short term effects of excitement and anaerobic metabolism during stress (Black et al 1961, Hunn 1969, Kutty 1968b, Smit 1965). However, Kutty (1968b) also showed that the R.Q. of a rainbow trout swimming at 1.6 bl/sec. at 15°C had essentially stabilised by the second hour of the speed increase. Similar stability in rainbow trout was also noted by Wood and Randall (1973 a,b,c) in the measurements of sodium and water exchange during swimming.

RESULTS AND DISCUSSION

I AMMONIUM NITROGEN OUTPUT

a) During swimming activity

An examination of Appendix Tables 1-6 showing total data collected from both trout and perch shows that ammonium nitrogen (NH_3-N) and total ammonium nitrogen levels differ. However, there is no consistent difference, the total NH_3-N measurement not always being greater than the NH_3-N measurement. The determination of the ammonia content of urea must therefore be considered unsatisfactory. In view of the low urea content produced by fresh water teleosts, of urine and gill excretions (Smith H.W. 1929, Solomon and Brafield 1972) it was perhaps optimistic to attempt to measure the urea NH_3-N in such a large volume of water. All discussion of nitrogen excretion will therefore relate to the measures of NH_3-N and not total NH_3-N. Solomon and Brafield (1972) analysed the nitrogenous output of perch and came to the conclusion that the use of ammonia as the sole nitrogen excrement would only give rise to a maximum error of 0.5%. Brett (1962) suggested that 98% of amino derived nitrogen appeared as ammonia and so the use of NH_3-N in this study to indicate protein catabolism would appear to be reasonable.

The actual quantities of excreted NH_3-N in the second hour of a swimming speed are shown in Tables 6 and 7. The routine rates obtained for perch in the routine respirometer are also included in the table. Comparison of the routine rates obtained with some reported values may be made by reference to Table 8 which includes

TABLE 4

NH_3-N PRODUCED BY BROWN TROUT DURING FIRST HOUR OF A SPEED INCREMENT

	ACTIVITY (bl/sec.)			
Temp. °C.	0.7	1.0	1.4	1.7
5	2.7 ± 0.4 (5)	3.0 ± 0.4 (5)	4.8 ± 0.3 (2)	
10	3.8 ± 0.5 (7)	3.3 ± 0.4 (8)	5.3 ± 0.5 (8)	5.2 ± 0.4 (3)
15	5.3 ± 0.9 (7)	6.3 ± 0.6 (7)	7.6^{M} ± 0.7 (7)	7.1^{M} ± 0.5 (7)

M denotes significance ($P < 0.01$)

TABLE 5

NH_3-N PRODUCEDD BY PERCH DURING FIRST HOUR OF A SPEED INCREMENT

	ACTIVITY (bl/sec.)			
Temp. °C.	0.7	1.0	1.4	1.7
5	2.6 ±0.5 (4)	3.2 ±0.2 (4)		
10	2.7 ±0.5 (7)	2.9 ±0.2 (7)	2.7 ±1.0 (3)	
15	4.4 ±1.5 (6)	4.1 ±1.0 (6)	8.9 ±0.9 (5)	

Tables show the mean, in mg. NH_3-N/kg.hr. ± 1 standard error, n being the number of samples.

data of this study. The Q_{10} (5-15°C) for NH_3-N for both trout and perch is 2.1 which is similar to that reported by Maetz (1972b) for ammonia loaded Carassius auratus. His normal fish gave an NH_3-N Q_{10} (6-16°C) of 3.9 from which he concluded that ammonia excretion was very temperature sensitive. As the temperature change took place over 30 minutes only, this latter Q_{10} can hardly be indicative of the fully acclimatised fish as used in this study. Pequin and Serferty (1963) also using Carassius auratus show a NH_3-N Q_{10} (7-20°C) of 2.1, the same as observed with trout and perch in the present study.

Two main points arise from the NH_3-N production data, the first is that within each temperature regime there is no statistically significant increase in NH_3-N production with increasing swimming speed. The second is that there is no statistically significant difference between the NH_3-N production during the first hour of swimming and that during the second hour of swimming at the same speed. These observations are therefore in disagreement with those of Smith et al (1971) and Kutty (1972). Smith and coworkers using migrating Pacific salmon stated that ammonia excreted increased with increasing activity. Unfortunately, no temperatures, swimming speeds or experimental regimes were described. Kutty (1972) using Tilapia mossambica at 30°C swimming at 2.4 bl/sec. showed an increase in NH_3-N production from about 30 mg/Kg. hour to 75 mg/Kg. hour (Figure 3) after 5½ hours. The conclusions stated were that protein was being used as an energy source or that ammonia was being used to maintain acid base balance. Either possibility would require a protein catabolism. The relationship between acid base balance and ammonia production is discussed fully in the following section. The

(Tables 4 and 5)

TABLE 6

NH_3-N PRODUCED BY BROWN TROUT DURING SECOND HOUR OF A SPEED INCREMENT

	ACTIVITY (bl/sec.)			
Temp. °C.	0.7	1.0	1.4	1.7
5	2.1 ±0.5 (5)	3.4 ±0.5 (5)	5.1 ±1.0 (2)	
10	3.2 ±0.7 (8)	3.2 ±0.8 (8)	5.3 ±0.8 (7)	5.7 ±1.5 (3)
15	4.5 ±0.5 (7)	5.2 ±1.0 (7)	5.0 ±0.5 (7)	4.3 ±0.6 (7)

TABLE 7

NH_3-N PRODUCED BY PERCH DURING SECOND HOUR OF A SPEED INCREMENT

	ACTIVITY (bl/sec.)			
Temp. °C.	R	0.7	1.0	1.4
5		2.4 ±0.8 (4)	2.3 ±0.7 (4)	
10	2.8 ±0.2 (12)	3.2 ±1.0 (7)	3.6 ±0.3 (7)	4.1 ±0.7 (3)
15	3.3 ±0.3 (12)	5.0 ±1.6 (6)	3.6 ±0.6 (6)	

R denotes routine rate.

TABLE 8

SOME REPORTED VALUES OF NH_3-N OUTPUT IN FRESHWATER TELEOSTS

SPECIES	TEMP. °C.	NH_3-N mg/kg.hr.	AUTHOR
Carassius Auratus	6 16	1.5 4.5	Maetz 1972
Cyprinus Carpio	14.5-15.5	2 - 9	De Vouys 1968
Carassius Auratus	7 20	2.3 6.2	Pequin and Serfaty 1963
Ameirus Nebulosus	not stated	3.4 - 11.9	Wøhlbach et al 1957
Tilapia Mossambica	30	21 R 75 A	Kutty 1972
Salmo Gairdneri	12 - 14	15.8	Fromm 1963
Salmo Gairdneri	13	1.9	Olsen and Fromm 1971
Salmo Gairdneri	12 - 13	5.2	Fromm and Gillette 1968
Perca Fluviatilis	14	16.7	Solomon and Brafield 1972
Perca Fluviatilis	17	3.0	Birkett 1969
Lepomis Macrochirus	7.2 15.6 23.9 29.4-32.2	2.3 2.0 4.8 11.9	Savitz 1969
Salmo Trutta Perca Fluviatilis	5 10 15 5 10 15	2.1 3.2 4.5 2.4 2.8 3.3	This study

R - routine A - active

The possibility of increased protein utilisation during swimming activity is supported by the work of Kreuger et al (1968) on juvenile coho salmon. Again in this last case, if the protein/amino acid energy source hypothesis is correct, one would expect an increase in NH_3-N output.

b) <u>During Recovery</u>

The production of NH_3-N in the recovery phase following swimming failure, is markedly different from that observed during swimming activity. (Tables 9 and 10) It can be seen that not only is there an increase in output, but this increase appears to be temperature dependent. At 5^oC for both trout and perch the recovery phase production does not differ significantly from the production during the last successful swimming speed. However, for both species at 10^oC and 15^oC there is a significant increase output. The explanation for the recovery phase increases could either be of shock or stress or metabolic origin.

The suggestion of shock or stress mediated NH_3-N output increases after swimming failure is not supported by reported observations. In the experiments utilising hypoxia or loading of the blood with ammonia (cf. Hunn 1969, Maetz 1972b, Wolbach et al 1957) there is no marked or significant increase in either urine production or ammonia content of urine. Using rainbow trout at 7.5^oC Wood and Randall (1973 c) showed an increase in urine production from 3.9 ml/Kg. hour to 7.5 ml/Kg. hour. These figures were obtained in swimming experiments going from a water velocity of 42.8cms/sec to one of 107 cms/sec. Even if this diuretic effect

Tables 9 and 10 show the mean, in mg. NH_3-N/kg.hr $\pm$ 1 standard error, n being the number of samples.

$^{xx}_{x}$ $P < 0.001$

xx $P < 0.01$

x $P < 0.05$

Highest ——— Mean ——— Reading before swimming failure

Lower temperature equivalent

TABLE 9

NH_3-N PRODUCED BY BROWN TROUT DURING RECOVERY FOLLOWING SWIMMING FAILURE

	RECOVERY (hours)		
TEMP. °C.	1	2	3
5	5.9 $\pm$ 1.3 (4)	5.8 $\pm$ 1.1 (4)	4.7 $\pm$ 0.8 (4)
10	^{x} 10.0 ^{x} $\pm$ 1.2 (6)	$^{xx}_{x}$ 11.8 $\pm$ 1.0 (5) $^{xx}_{x}$	^{x} 11.6 $\pm$ 2.0 (3) ^{x}
15	^{xx} 11.1 ^{xx} $\pm$ 2.0 (7)	$^{xx}_{x}$ 15.8 ^{x} $\pm$ 0.8 (7) $^{xx}_{x}$	$^{xx}_{x}$ 15.4 $\pm$ 1.0 (7)

TABLE 10

NH_3-N PRODUCED BY PERCH DURING RECOVERY FOLLOWING SWIMMING FAILURE

	RECOVERY (hours)		
TEMP. °C.	1	2	3
5	3.8 $\pm$ 0.4 (5)	3.4 $\pm$ 0.6 (5)	2.3 $\pm$ 0.7 (4)
10	xx 6.2 xx $\pm$ 0.8 (7)	^{x} 6.2 $\pm$ 1.1 (7)	4.5 $\pm$ 0.8 (6)
15	$^{xx}_{x}$ 9.3 $^{xx}_{x}$ $\pm$ 0.8 (6) ^{x}	$^{xx}_{x}$ 9.4 $\pm$ 1.0 (5) ^{xx}	$^{xx}_{x}$ 11.1 $\pm$ 0.4 (3)

were maintained into recovery, which it is not as demonstrated by Wood and Randall (1973c) it would only yield an increase in NH_3-N production of 4% (Maetz 1972). The possible diuretic effect of swimming failure would not therefore be a satisfactory explanation for the large increases observed. As the only other known site of ammonia production is the gill, which produces the majority of excreted ammonia in the fresh water teleost (Maetz 1972 , Smith, H.W., 1929, Solomon and Brafield 1972); the recovery phase ammonia must be secreted from the gill. At this point the debate becomes complicated as it is not known whether the gill itself or the liver which actually produces the ammonia (Pequin and Serfaty 1963). It has been demonstrated that ammonia exchanges across the gill with sodium (Na^+) ions (Garcia Romeu and Maetz 1965, Maetz and Garcia Romeu 1964, Maetz 1972, 1973) but the exact source of the ammonia is not known. Goldstein and Forster (1961) working with a marine teleost (Myxocephalus sp.) showed a partition between ammonia excretion from the gills and elsewhere (undefined) whilst Maetz (1972) was of the opinion that the gill only produced ammonia when an excess to the metabolic production of ammonia by the liver was present i.e. in a loading situation. This latter suggestion points to a possible explanation for the large increases in NH_3-N production observed during recovery after swimming failure.

In the mammal, NH_3-N content of the urine increases with metabolic acidosis, as does the venous blood (McCullough 1968, Pitts 1971, Rector et al 1954, Schwartz et al 1958). The increase in blood ammonia is believed to be due to muscle metabolism and the breakdown of components of the purine nucleotide cycle (Lowenstein 1972) and has been suggested as having a buffering capacity additive

to that of whole blood (Prosser and Brown 1965). The increase in the urine content of NH_3-N following exercise of metabolic acidosis has been ascribed to a combination of acid conditions increasing the rate of deamination and deamidation in the kidney tubule cells and an acid urine acting as a pH "sink" (Pitts 1971). The production of ammonia in the free base (NH_3°) form enables a combination with H^+ ions to take place to reduce the drop in pH of the urine. The presence of the resulting NH_4^+ ions also acting as a preservation mechanism for Na^+ ions which are reabsorbed by the tubule cells. Rector et al (1954) however, were careful to point out that the evidence does not prove an increased concentration of deaminases or deamidases or preclude an increased rate of enzyme activity. In the mammal, therefore, an increase in urine ammonia content is associated with a kidney response to physiologically acidic conditions.

In the fresh water teleost this situation apparently does not occur, although Hunn (1969) has shown that the urine of the rainbow trout after severe hypoxia demonstrates classic indications of metabolic acidosis viz, decreased pH, increased lactate, sodium and phosphate ions. Maetz (1972) has indicated no increase in urine ammonia content after blood ammonia loading. The large increases in NH_3-N production after swimming failure must therefore be a gill mediated phenomenon. The question arises as to what is the source of the NH_3-N observed and what, if any, is its metabolic function?

It is the contention of this study that the large increase in NH_3-N production after collapse constitutes a system for the removal of the large quantities of H^+ ions as a result of lactic acid production and respiratory acidosis. In doing so the sodium balance

is restored having undergone a depletion due to the failure of kidney tubule absorption under the prevailing acidotic conditions. The rationale for this hypothesis includes both a free base and an ionised form of the ammonium molecule and a temperature effect. At neutrality the ammonium is about 99% dissociated and in most biological fluids the ration of NH_4^+ to NH_3^o is about 100:1 (Goldstein and Forster 1970, Pitts 1971). Weak acids and bases pass through biological membranes mainly in their unionised form and Maetz (1972 and 1973) has demonstrated with goldfish at 16°C that a variable fraction of the ammonia can be excreted in the free base form. Alteration of the external sodium concentration has been interpreted as causing ammonia excretion to be all in the free base form possibly as a result of a conformational change in the apical carrier for Na^+ in the epithelial cell (Garcia Romeu 1971, Garcia Romeu et al 1969, Maetz 1973). However, Kerstetter et al (1970) using rainbow trout, demonstrated a drop in the pH of the medium surrounding the respiring fish and came to the conclusion that ammonia was excreted in the free base form (NH_3^o) and that H^+ alone was responsible for an Na^+ exchange system. All the experiments described depended upon either exogenous loading of the fish with ammonium sulphate or altering the composition of the external medium. No direct work has been carried out on the whole fish producing endogenous ammonia.

The metabolic situation at swimming failure appears to be as follows. Lactic acid is produced as a result of intense muscular activity combined with a failure of the circulatory system to provide sufficient oxygen and the predominant muscle type - the

white fibres - receiving only about 15% of the circulation system (Beamish 1968, Black et al 1962, 1966, Bone 1966, Johnston and Goldspink 1973a, b, Stevens 1968). The rate of appearance of lactic acid in the blood being a function of temperature affecting diffusion rate, (Black 1958, Johnson et al 1945) adrenaline concentration (Wardle pers. comm.), disappearance of adrenaline due to low oxygen concentration (Demael-Suard 1972) and glucogneogenesis from lactate in the liver and other tissues (Bilinsky and Jonas 1972). Furthermore, once in the blood, lactic acid dissociates to give rise to H^+ cations and lactic anions - the rate of dissociation depending upon temperature (Lockwood et al 1965).

Using Scyliorhynus stellaris stimulated to exhaustion Piiper et al (1972) and Piiper and Baumgarten (1969) demonstrated a curious deficit of H^+ ions in the exhausted fish blood. This deficit could be quantified by using values for blood buffering capacity (Van Slyke et al 1928, Albers 1970), blood lactate concentration, blood pH and total carbon dioxide content of blood. Piiper et al (1972) noted that Murdaugh and Robin (1967) after loading Squalus acanthias with either H_2CO_3 or HCl, had concluded that H^+ and HCO_3^- excretion took place through the gills. Piiper et al (1972) came to the conclusion that the deficiency of H^+ ions in the blood was compensated for by either an increase in Na^+ or a decrease in Cl^- ions. Using goldfish and sculpins in rebreathing experiments, Dejours et al (1968) showed that the goldfish going into hypoxic conditions produced more ammonia than the goldfish

in well aerated water. This final observation suggests the mechanism underlying the recovery phase output of NH_3-N demonstrated in this study.

Swimming failure at high speeds, as noted earlier, is associated with the appearance in the blood of anaerobically produced lactic acid. This lactic acid dissociates and if the blood buffering capacity is insuffiçient the blood pH will drop. If the blood pH drops below a critical level mortality occurs. Jonas et al (1962) showed a critical pH for rainbow trout at 11°-14°C of about 6.85. If a mechanism existed for the removal of metabolic H^+ ions it would be an addition to the buffering capability of the blood. Also, if this mechanism were coupled with an uptake of Na^+ ions which are lost via the kidney and urine under conditions of metabolic acidosis; further benefit would result. It has been shown that resting stressed fish in partly saline water significantly reduces mortality (Collins and Hulsey 1963) indicating a possible beneficial effect of high ambient Na^+ ions. The hypothesis that may be advanced to explain the output of NH_3-N after swimming failure is as follows.

Swimming failure results in, or is a result of, anaerobic muscular metabolism giving rise to increased blood lactate. This lactate dissociates and produces H^+ ions which, if not buffered or removed, cause a drop in blood pH. If this drop in blood pH goes below a physiologically critical level, mortality occurs. The presence of H^+ ions causes both a drop in blood pH and intraepethelial gill cell pH. The decrease in intraepithelial gill cell pH causes

both an increase in rate of deaminase and deamidase activity and percentage of free base, NH_3^o, present. This NH_3^o combines with the excess H^+ ions to form NH_4^+ which diffuses out of the epithelial cells and into the surrounding water. Electrical neutrality being maintained by exchange of NH_4^+ with Na^+ ions. If this is the case, it should be possible to predict the increase in excreted NH_3-N from data for blood lactate, blood pH and decrease in blood HCO_3^- concentration. Using the H^+ deficit equation of Piiper et al (1972) i.e.

$$H^+ \text{ deficit} = \Delta[\text{Lactate}] + \Delta[HCO_3^-] + \Delta[B^-]$$

where

$\Delta[\text{Lactate}]$ = increase in lactate concentration.

$\Delta[HCO_3^-]$ = decrease in blood bicarbonate concentration.

$\Delta[B^-]$ = non bicarbonate buffer capacity.

all concentrations being in meq/L.

The increase in lactate concentration, $\Delta[\text{Lactate}]$, may be obtained from Black et al (1966) for a 12°C rainbow trout. The same source also provides data on the decrease in blood bicarbonate concentration, $\Delta[HCO_3^-]$. To obtain a value for $\Delta[B^-]$ the work of Cameron (1971) and Eddy (1971) may be combined. Using the 15°C data of Eddy and the 13°C data of Cameron a graph may be drawn of blood pH against total CO_2 content. This gives a slope of 10.8 i.e. the non bicarbonate buffer capacity if 10.8 meq/L/pH unit. This agrees well with values

values of 9.0 meq/L/pH unit (Squelus suckleyi Lenfant and Johansen 1966), 10.0 meq/L/pH (Scyliorhinus stellaris Albers and Pleschka 1967), 8.0 meq/L/pH (Scyliorhinus stellaris Piiper et al 1972) obtained by other workers. Taking the data of Black et al (1966) and tabulating the blood component concentrations during recovery gives:

Recovery (Hrs)	Blood Component Concentration			
	Δ[Lactate]	$\Delta[HCO_3^-]$	$\Delta[B^-]$	Δ pH
1	12.39	4.99		can be ignored
2	16.67	4.94		Black et al (1959)
4	10.41	3.06		showed no change of pH in recovery.

Combining these and using the H^+ deficit equation, the H^+ deficit during the recovery may be calculated.

Recovery (Hrs)	Deficit (meq H^+/L)
1	7.40
2	11.73
4	7.35

As one mole of H^+ ion is equivalent to one mole of measured NH_3-N (i.e. $H^+ + NH_3^o = NH_4^+$), the quantity of excreted NH_3-N resulting from this mechanism may be calculated. Table 11 shows values of observed NH_3-N production and predicted values for brown trout at 15°C.

Taking salmonid blood volume to be 5 mls/100g. (Conte et al 1963, Houston and De Wilde 1968 , Smith 1966, Smith and Bell 1964, Wood and Randall 1973a) and calculating for a kilogram fish.

Recovery (Hrs)	Predicted	Observed
1	10.9	11.1
2	14.9	15.8
3		15.4
-4	10.9	

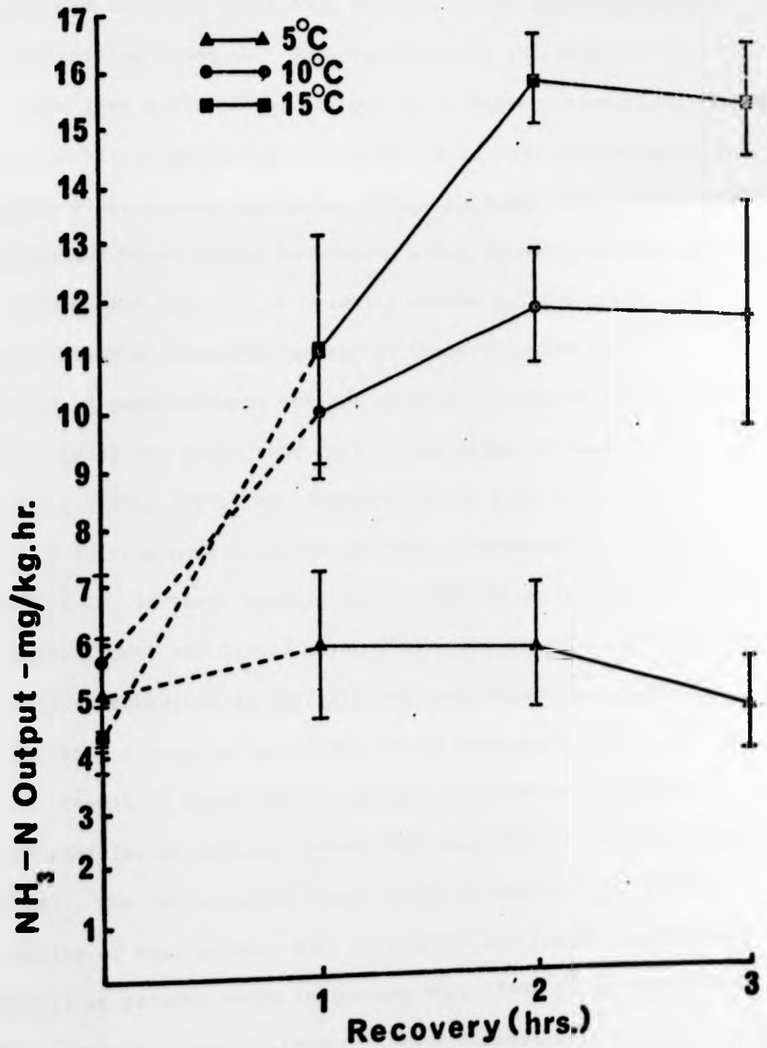
Table 11. Showing predicted and observed values for NH_3-N production, in mg/Kg. hour, for brown trout at 15°C recovering from swimming failure.

An interesting confirmatory finding for the observed level of excreted NH_3-N in the first hour of recovery of brown trout at 15°C, comes from the work of Wood and Randall (1973a). These workers followed the branchial exchange of Na^+ in rainbow trout at 15°C and showed a net Na^+ influx in the first hour of recovering amounting to some 13.8 mg. Na^+/Kg. hour. This finding demonstrates the two possible exchange mechanisms for Na^+ suggested. The first (Shaw 1960 , Maetz 1971) is that an Na^+/NH_4^+ exchange exists under "normal" conditions. The second is that an Na^+/H^+ exchange occurs to explain the high Na^+ uptake when the NH_4^+ output is not stoichiometric (Maetz 1971) The higher Na^+ influx noted by Wood and Randall (1973a) over the observed NH_3-N output, could well be possible on the basis of an Na^+/H^+ exchange. These correlations would appear to be compatible with the suggested schematic exchange of Maetz (1971).

In the absence of any data relevant to either salmonids or perch at lower temperatures, the equivalent lower temperatures calculation cannot be made. However, from the observed NH_3-N production and R.Q. data, certain predictions may be made on the basis of the calculated 15°C condition for the brown trout. It has been shown by Burton and Spehar (1971) that brown trout subjected to hypoxic conditions can produce the same quantity of muscle lactate at both 5°C and 15°C. Further, Bilinsky and Jonas (1972) have demonstrated, on the basis of tissue slice incubation, that at 15°C rainbow trout only oxidise about one fifth of the lactate available. The remaining four fifths of the lactate load being used for anabolic purposes. It is interesting to note that gill tissue possesses the highest oxidative ability with regard to lactate. This observation coupled with that of Conte (1969) of mitrochondial concentration in gill filaments suggests that perhaps the energy required for increased deaminase and deamidase activity is self generated. The lower rates of NH_3-N production in the brown trout at 10°C and 5°C could well be due to a temperature mediated reduction in enzyme diffusion of the relatively large NH_4^+ molecule across the epithelial cell walls could explain the apparent temperature effect. (Fig. 7)

The occurence of mortalities in the 5°C brown trout test (40%) group, characterised by a cramping and stiffening of the body, is indicative of a high blood lactate condition. This high blood lactate appearing about 2 hours into recovery as evidenced by the large increase in R.Q. at this time. Perch, on the other hand, do not display such high NH_3-N outputs during recovery. They

Fig. 7 NH_3-N produced by brown trout at 5°C, 10°C and 15°C during recovery following swimming failure. Each point represents the mean ± 1 standard error.



also display an R.Q. of greater than 1.0 throughout except in the 15°C condition. Two possibilities exist, either the lactic acid is produced in lower quantities or a different mechanism exists for controlling blood pH. Both appear to be relevant.

The less active fish, for want of a better definition, appear to be capable of producing less lactic acid than the active fish (Beamish 1968, Burton and Spehar 1971, Caillouet 1964, 1967, 1968). Ferguson and Black (1941) have shown a low blood buffering capacity per gram Hb for the carp in relation to the rainbow trout. It is possible that anaerobic energy in these sluggish fish is supplied through pathways other than those ending in lactic acid. Blazka (1958) has shown that carp do not repay an oxygen debt and Kutty (1966, 1968b) has demonstrated an R.Q. of 2.0 for a goldfish lasting over a period of days. Alternately, the large output of CO_2 in these species could indicate an increased HCO_3^-/Cl^- exchange. (Maetz and Garcia Romeu 1964) The presence of high levels of carbonic anhydrase in the gill and pseudobranch of perch (Maren 1967) aiding in the production of excretable HCO_3^- ions. The net result of this type of exchange would be an increased Donnan equilibrium effect (Albers 1970) and hence blood CO_2 carrying capacity. The influx of Cl^- ions aiding in maintaining electroneutrality of the plasma. Work of Cameron and Randall (1972 and in prep.) on rainbow trout indicating that blood pH is controlled by HCO_3^- levels substantiating the above suggestion.

Before leaving this area related to high levels of NH_3-N output, the possibility of ammonia production from the purine mucleotide cycle (Lowenstein 1972) must be mentioned. Work on the preservation

preservation of fish for the food industry has indicated a large degeneration of the phosphate nucleotide compounds in dead fish (e.g. Burt and Stroud 1966, Fraser et al 1966, 1967, 1968a, b, Jones and Murray 1961, 1966, Kassemsarn et al 1963, Saito et al 1959, Tarr 1966). This type of situation could lead to a large output of "non metabolic" ammonia and the observation of Johnston and Goldspink (1973 b) of increased free creatine in the muscles of exhausted carp indicates a sudden energy demand. However, the work of Khalyapin et al (1972) showing that fatigue is not accompanied by a decrease in A.T.P. content of fish muscle discounts a purine nucleotide cycle ammonia production.

II RESPIRATORY QUOTIENTS

Although carbon dioxide excretion has been little studies fishes, attention has been paid to oxygen consumption. Measures of oxygen consumption and hence metabolic rates under a variety of conditions have been described (Doudoroff and Shumway 1970, Brett 1964, 1970a, Brett and Glass 1973, Fry 1971, Winberg 1956). Accordingly no attempt will be made to review the literature on this subject but comparison between the results obtained in this study (Tables 14-17) and some reported values (Tables 15 and 16, Appendix III) may be made.

The values obtained for standard metabolic rate (S.M.R.) for both brown trout and perch at the three experimental temperatures are indicated in Tables 12 and 13. The method used to obtain these values is that of extrapolation of the regression line of the logarithm of the oxygen consumption against swimming speed to zero activity (Brett 1964). Other values for S.M.R. obtained by other workers using this method are shown in Table 15 (Appendix III). Table 16 (Appendix III) on the other hand, shows some values for S.M.R. obtained by extrapolation of the regression line of oxygen consumption against spontaneous activity to zero activity (Beamish and Mookjeri 1964). Although conditions prevailing in the experimental methods differ, it would appear that two types of S.M.R. may be measured. If an increasing velocity test regime is employed, a lower S.M.R. is generally obtained. This is a result of applying a linear extrapolation to what is probably a non linear situation. In other words, below a certain swimming

TABLE 12

PARAMETERS OF THE OXYGEN CONSUMPTION/SWIMMING SPEED REGRESSION LINE FOR BROWN TROUT

TEMP. °C.	REGRESSION EQUATION	CORRELATION (r)	P (of r)	ESTIMATE OF REGRESSION S.E.	S.M.R.	C.S.S.
5	LOG.Y = 1.4785 + 0.5183 X	0.07474	< 0.01	Log. S.E. = 1.5092	30.1	1.29 ±0.09
10	LOG.Y = 1.9175 + 0.2471 X	0.6247	< 0.001	Log. S.E. = 1.6721	82.7	1.70 ±0.11
15	LOG.Y = 2.0233 + 0.2511 X	0.7873	< 0.001	Log. S.E. = 1.5623	105.5	1.78 ±0.0

Y - oxygen consumption in mg/kg. hr.

X - swimming speed in bl/sec.

Estimate of the standard error (S.E.) of the regression line - in mg.O_2/kg.hr.

S.M.R. - standard metabolic rate in mg.O_2/kg.hr.

C.S.S. - critical swimming speed (calculated after Brett 1964) i.e. C.S.S. = $A + (a \times \frac{T^1}{T^o})$ bl./sec.

where:

A = last successful sustained speed (bl/sec.)

a = speed increment (bl/sec.)

T^1 = time to swimming failure (min.)

T^o = imposed velocity test time (min.)

TABLE 13

PARAMETERS OF THE OXYGEN CONSUMPTION/SWIMMING SPEED REGRESSION LINES FOR PERCH

TEMP. °C.	REGRESSION EQUATION	CORRELATION (r)	P (of r)	ESTIMATE OF REGRESSION S.E.	S.M.R.	C.S.S.
5	LOG.Y = 1.1072 + 0.9989 X	0.8827	< 0.01	LOG. S.E. = 1.0820	12.8	1.05 ±0.0
10	LOG.Y = 1.8029 + 0.2929 X	0.7422	< 0.01	LOG. S.E. = 1.2662	63.5	1.24 ±0.14
15	LOG.Y = 1.7362 + 0.5741 X	0.7789	< 0.01	LOG. S.E. = 1.7339	54.5	1.12 ±0.0

swimming speed it is unrealistic to expect exponential decreasing oxygen consumption. It is for this reason that S.M.R. measurements are more useful and meaningful measures. The use of, and the possible errors arising from, extrapolated swimming speed S.M.R's in estimating natural fish metabolic rates have been discussed elsewhere (Morgan 1974, In Press)

Despite these comments it can be seen that the S.M.R. values obtained for both trout and perch at the temperatures employed, agree well with published values for S.M.R. It will be noted from Tables 12 and 13 that no indication of deviation or error about the extrapolated values for S.M.R. is given. Instead, an estimate of the standard error of the regression line of oxygen consumption and swimming speed is given. As all experimental data obtained was included in the calculation of the regression line this method of presentation of the S.M.R. and related regression line parameters describes fully the derived situation. Although not indicated in the Tables, analysis of variance tests were carried out on both the slopes and elevations of the regression lines. For trout it was found that there was no significant difference between the regression line slopes at $5^{o}C$, $10^{o}C$ or $15^{o}C$ (at 5% level). The regression line elevations however were significantly different ($P < 0.001$) between $5^{o}C$ and $10^{o}C$, and $10^{o}C$ and $15^{o}C$. The regression line slopes for perch between $5^{o}C$ and $10^{o}C$ were significantly different ($P < 0.01$) but not between $10^{o}C$ and $15^{o}C$. The regression elevations between $5^{o}C$ and $10^{o}C$ were not significantly different, but between $10^{o}C$ and $15^{o}C$ a significant difference ($P < 0.001$) was found. This last point illustrates

the dangers inherent in extrapolated values. The S.M.R. values for perch at 10°C and 15°C were found to be 63.5 mg O_2/kg.hr. and 54.5 mg O_2/kg.hr. respectively. If these values are compared with the routine metabolic rates obtained for perch at 10°C and 15°C (see Appendix Tables 5 and 6) they are found to be reversed. The error probably being caused by the extrapolation of a relatively short regression line.

The critical swimming speeds obtained for both species at the three experimental temperatures employed can also be seen in Tables 12 and 13. Each critical speed indicated with its associated standard error. Perch only demonstrated a significant difference between critical swimming speeds at 5°C and 10°C ($P < 0.01$) Likewise trout critical swimming speeds only differed significantly between 5°C and 10°C ($P < 0.001$). This plateau response to increasing temperature parallels that seen in the measurements of the standard metabolic rates. These apparently low critical swimming speeds obtained are interesting. The use of electric grids has been shown to give rise to a higher critical swimming speed than that obtainable in their absence (Jones 1971, Smit et al 1971). Likewise, the use of exercised fish promotes performance (Brett 1964, Hochachka 1961, Hammond and Hickman 1966). The imposition of a longer test period decreases the critical swimming speed (Doudoroff et al 1968). Taking these factors into account the critical swimming speeds for salmonids (rainbow trout - Bainbridge 1962, Hudson 1973, Webb 1971b, Priede pers. comm.; brown trout - this study) in the United Kingdom appear to be consistently lower than those of America and Canada (Brett 1964,

Dickson and Kramer 1971, Horak 1972, Kutty 1968 a,b.) Webb (1970) reviews some further swimming speeds described in the literature. Further, this difference appears to be a function of the differing hatchery management techniques practices in the countries concerned. Brett and Glass (1973) make the point in their comprehensive review of the metabolism and swimming performance of Oncorynchus nerka that all fish used were obtained from fresh run wild parent stock. Whereas Webb (1971b) stated that the rainbow trout that he used (also Hudson 1973) were obtain from hatchery stock that had been inbred for about 50 years. The brown trout of this study were obtained from a hatchery where possible brood stock replacement with wild fish did not take place. Bams (1967) demonstrated that the rearing techniques for the eggs and juvenile stages also affect swimming performance again indicating a management response. Another possible source of differing performance lies in the use of different rainbow trout varieties. Commonly encountered types include Kamloops, Steelhead and Shasta (Mills 1971). On a subjective basis Kamloops possess more musculature and are capable of muscular contraction of a more intense and prolonged nature (Sperber pers. comm.)

There is very little data available for perch comparison, but what there is agrees fairly well with the findings of this study. Ferguson (cited by Fry 1964) obtained a maximum swimming speed for Perca Flavescens at 15^{o}C of about 1.5 bls/sec. Ohlmer and Schwartzkopff (1959) showed that perch burst speeds did not exceed 5 bls/sec. and comparing this result with the burst speeds reviewed by Blaxter (1967) would demonstrate sustained and critical swimming speeds of the order found in this study.

R. Q. Measurements

a) During Activity

The R.Q's described are obtained from the volume ratio CO_2/O_2, both gases being calculated as dry and at S.T.P. (760 mm.Hg. 273°K). It will be noted that no standard errors are indicated on an R.Q. measurement. An indication of the error in an R.Q. measure can be obtained from the standard errors computed for the gas volumes of oxygen and carbon dioxide and also from the standard error on a measurement of NH_3-N excretion. The R.Q's described here are obtained from the means of the measures for oxygen consumption and carbon dioxide and NH_3-N excretion. As such they can be used to determine overall metabolic trends and regimes.

The R.Q's obtained during the first hour and the second hour (steady state) are shown in Tables 14-17 respectively. The steady state routine values for perch are included in Table 17. The measured values for recovery of R.Q's can be seen in the following section (Tables 20 and 21). Both trout and perch demonstrate similar patterns of R.Q. (Figures 8 and 9) during activity at the different temperatures employed. At 5°C the trout R.Q. is relatively constant until a swimming speed of greater than 1.0 bls/sec. is imposed, at which point the R.Q. rises to 1.28. Perch demonstrate the same change except that an R.Q. of 1.28 is realised at a swimming speed of 1.0 bls/sec. At 10°C an R.Q. of greater than 1.0 is only seen in trout at a swimming speed of 1.0 bls/sec. The 10°C trout routine R.Q. of 0.66 is probably low. This is borne out by examination of the data of Appendix I, Table 2

TABLE 14

THE R.Q. DURING THE FIRST HOUR OF A SWIMMING SPEED, DISPLAYED BY BROWN TROUT

SPEED (bl/sec.)	5°C.	10°C.	15°C
0.7	0.35	0.92	0.93
1.0	0.70	1.00	0.81
1.4	1.37	0.93	0.74
1.7		0.86	0.77

TABLE 15

THE R.Q. DURING THE FIRST HOUR OF A SWIMMING SPEED, DISPLAYED BY PERCH

SPEED (bl/sec.)	5°C	10°C	15°C
0.7	0.74	1.59	0.75
1.0	1.72	1.39	0.83
1.4		1.48	0.84
1.7			

TABLE 16

THE OBSERVED AND NITROGEN FREE R.Q's OF BROWN TROUT DURING THE SECOND HOUR OF A SWIMMING SPEED

	5°C		10°C		15°C	
Speed (bl/sec.)	OBSERVED	NON-P.	OBSERVED	NON-P.	OBSERVED	NON-P.
0.7	0.80	0.79	0.66	0.61	0.87	0.88
1.0	0.73	0.69	1.02	1.07	0.62	0.55
1.4	1.28	1.45	0.86	0.86	0.65	0.60
1.7			0.70	0.66	0.64	0.61

TABLE 17

THE OBSERVED AND NITROGEN FREE R.Q's OF PERCH DURING THE SECOND HOUR OF A SWIMMING SPEED

	5°C		10°C		15°C	
Speed (bl/sec.)	OBSERVED	NON-P.	OBSERVED	NON-P.	OBSERVED	NON-P.
ROUTINE			0.93	0.98	0.89	0.91
0.7	0.88	0.90	1.30	1.46	0.62	0.53
1.0	1.28	1.37	1.68	1.99	0.60	0.55
1.4			1.53	1.71		
1.7						

A non protein R.Q. is one that is calculated by removing the contribution of gases involved in protein oxidation from the overall gaseous exchange.

TABLE 18 SHOWING SOME VALUES OF R.Q. IN ANIMALS OTHER THAN FISH

SPECIES	AUTHOR	TEMP.°C	CONDITIONS	R.Q.	COMMENTS
MAN	Benade et al 1971		Normal Work	0.94 0.80	Return to 0.94 with sucrose feed.
SQUIRREL	Bintz et al 1971		Water deprived Starved	0.80 0.70	
GEESE	Benedict		Stuffed with grain	1.33	
	Boell	25	Embryonic development	0.95 0.71	0.95 for about one day. Over 45 declined to 0.71
MAN	Bortz 1969		Maintenance diet	0.74	Grossly obese hospitalised patient on hospital diet. Fat metabolism
LOBSTER	Bosworth et al 1936	15	Normal Collodian coating	1.30 0.92	Respiratory CO_2 + carbonate of crustacean chitin produce HCO_3^-. This effect removed by collodion coating.

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TABLE 18 (contd.)

SPECIES	AUTHOR	TEMP. °C	CONDITIONS	R.Q.	COMMENTS
MAN	Christensen 1939		Basal	0.96 0.83 0.74	Carbohydrate) "Normal") Diet types. Fat)
	Crescitelli 1935	20-39	Pupal development	0.69	Not affected by temperature.
PIGLETS	Curtis et al 1970	5 32 5 32	12 hours old " " " 84 hours old " " "	0.90 0.95 0.85	Older pigs have lower R.Q. at both temperatures. Fat metabolism is higher in the cold.
MAN	Haldi and Bachmann 1935		Basal Recovery	0.75 1.06	Bicycle ergometer for exercise.
	Hedman 1957		Basal Recovery	0.70	Skiers. Recovery R.Q. about 2 hours after race. Breath smelt of acetone..
BLOWFLY	Hitchcock 1935 and Haub		End of pupation Adult	0.7 0.7	Fat disappeared, glucose increased, concluded gluconeogenis present.

TABLE 18 (contd.)

SPECIES	AUTHOR	TEMP. °C	CONDITIONS	R.Q.	COMMENTS
LEECH	Jolyet, Regnault 1877	13.5	Starved Fed	0.69 0.9	104 tested, same group for both measurements.
COMMON DOORMOUSE	Kayser 1940 (c)	9	Hibernating	0.64	Used fats in hibernation, On arousal R.Q. increases due to hyperventilation and carbohydrate utilisation.
GROUND SQUIRREL	1940 (b) 1961	7.8	Hibernating	0.71	
HEDGEHOG		6	Hibernating	0.72	
MAN	Krogh and Lindhard 1920		Basal 0.74	0.95 0.74	Carbohydrate) Diet types Lipid)
ADIPOSE CELLS	Mirski 1942	37	Hunger Recovery	0.64 1.05	Described two types as hunger fat and glycogen fat.
SEA URCHIN SPERMATOZOA	Mohri 1961 and Horiuchi	2[illegible]		0.7	Correlated R.Q. with utilisation of endogenous phospho lipids.

TABLE 18 (contd.)

SPECIES	AUTHOR	TEMP. °C	CONDITIONS	R.Q.	COMMENTS
LEPIDOPTERA	Niemerko 1959		Glucose fed	1.5 2.0	Cites Zebe. Carbohydrate to lipid on basis of
			Forced to fly	0.7 0.9	R.Q. and confirmed by analysis. Concludes that carbohydrate has to
			Recovery	1.5+	be converted into lipid for use.
PARAMECUM AURELIA PARAMECUM CAUDATUM	Pace and Kimura 1944	15 - 35		0.7 - 0.94	Increase of R.Q. with increase of temperature.
MAN	Roughton et al 1942		Basal Bicycle Treadmill	0.80 0.92 1.11	Calculated from Table 1. Severity of exercise increased R.Q.
YOUNG HOG	Wierzuchowski et al 1925		Basal Fed starch and glucose	0.86 1.58	This author calculated that at the maximum R.Q., 2 mg.fat/second were being synthesised.

TABLE 19 SHOWING SOME VALUES OF THE FISH R.Q's

SPECIES	AUTHOR	TEMP.°C	CONDITION	R.Q.	COMMENTS
CARASSIUS	Baudin	3 - 5	1 fish 100 g.	1.07	C. auratus died at 35°C.
AURATUS	1931	6		0.98	Temperature acclimation only
		7	2 fish 86 g.	0.88	for 24 hours.
		8	1 fish 100 g.	0.75	All routine values.
		10	2 fish 86 g.	0.83	
		15	1 fish 72 g.	0.75	
		20		0.91	
		25		0.97	
		30		1.2	
COTTUS		18	1 fish 15 g.	0.74	
BUBALIS		20		0.87	
		22		0.94	
STENOTOMUS	Bosworth	22	Routine	0.92	Removed HCO_3^- by addition of HCl.
VERSICOLOR	et al		Sea Water		Titrated to pH 8.2 with NaOH
PLATICHTHYS	1936	11-22		0.89	
FLESUS					

TABLE 19 (contd.)

SPECIES	AUTHOR	TEMP. °C	CONDITION	R.Q.	COMMENTS
CYPRINUS CARPIO	Blazka 1958	5	Routine (anoxia)	1.0	Respiration medium, tap plus distilled water.
		20		0.56	
CARASSIUS AURATUS	Dejours 1968	25	Anaesthetised	0.87	Calculated from Table 1.
SALMO TRUTTA	Gardner 1926	0.75-5.4	Routine	0.63	Summary of
		5.9 -7.4	to	0.40	Gardner +
		10 -16.8	Stressed	0.74	Gardner +
		20.1-22.7		0.76	Gardner +
TINCA TINCA	Jolyet and Regnault 1877	14	Two fish	0.66	Probably routine
CYPRINUS AURATUS		11.5	Ten fish	0.8	
ANGUILLA ANGUILLA		14	Eight fish	0.79	
CARASSIUS AURATUS	Kutty 1968	20	Routine	0.93	Phosphate buffer as experimental medium
SALMO GAIRDNERI		15	Routine	0.85	

TABLE 19 (contd.)

SPECIES	AUTHOR	TEMP. °C	CONDITION	R.Q.	COMMENTS
TILAPIA MOSSAMBICA	Kutty 1971	30	Routine	0.92	Decarbonated tap water for experiment
TILAPIA MOSSAMBICA	Kutty 1972	30	Routine	1.03	Measured after introduction
TILAPIA NILOTICA	Mahdi 1973 (b)	35	Routine	0.85	Calculated from Table 1
AEQUIDENS	Morriss	20	Routine	1.14	Used G.L.C. for gas analysis.
PORTALEGRENIS	1967	30		1.06	
ICTALURUS		15		1.0	These are calculated means.
NATALIS		25		1.13	
LAGODON RHOMBOIDES	McFarland	not given		0.84	In Trizma buffer
LAGODON RHOMBOIDES	Smith et al 1935	25	Fasting Fed	0.68 0.83	

which shows that the carbon dioxide output under routine conditions is low. The reasons for this are not known. At speeds greater than 1.0 bls/sec. the trout 10°C R.Q. shows a steady decline to a final reading of 0.70. Perch, on the other hand, start from a routine R.Q. of 0.93 which is then maintained at over 1.0 for all 10°C swimming speeds. The 15°C condition for both trout and perch is remarkably similar. Both species have routine rates of 0.87 and 0.89, trout and perch respectively. Likewise, when swimming at all speeds, they both exhibit R.Q. values of between 0.60 and 0.65.

To put the results into perspective, Tables 18 and 19 show some reported R.Q. values for both fish and other animals under a variety of conditions. From these tables two main points emerge, the first is that the reported values for fish R.Q's are extremely variable; and the second is that the R.Q. values obtained in this study would appear to agree well with normal and other values reported to occur in animals other than fish. From this study there is no evidence to suggest that synthesis of the type observed by Benedict (cited by Wierzuchowski et al 1925), Bleibtreu (1901) Wierzuchowski (1925) and Zebe (cited by Niemerko 1959) are taking place. That is not to say that measurements of fish R.Q. after feeding would show that lipid synthesis from carbohydrate does not take place, but that fish starved for the periods used in this study (see Materials and Methods) do not exhibit this phenomenon. All R.Q's greater than 1.0 would suggest that some metabolic disturbance has taken place. The decrease of R.Q. in trout at 10°C and 15°C and perch at 15°C can be seen in animals other than fish.

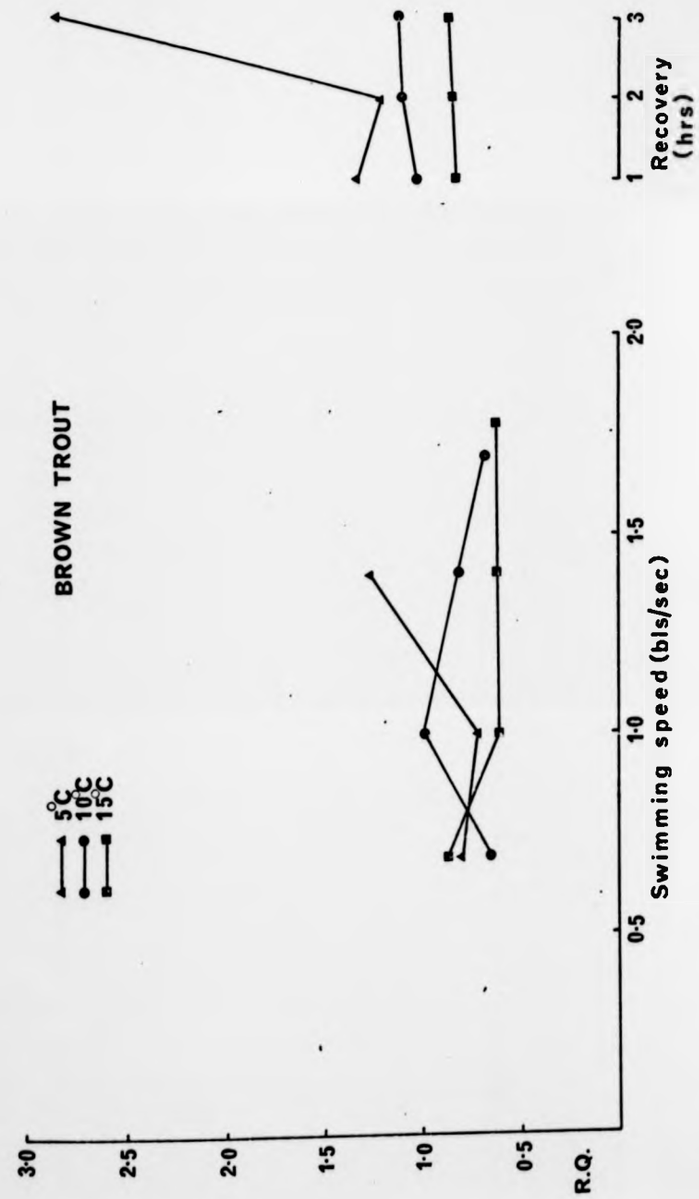


Fig. 8. Measured R.Q's during swimming (steady state) and recovery in brown trout.

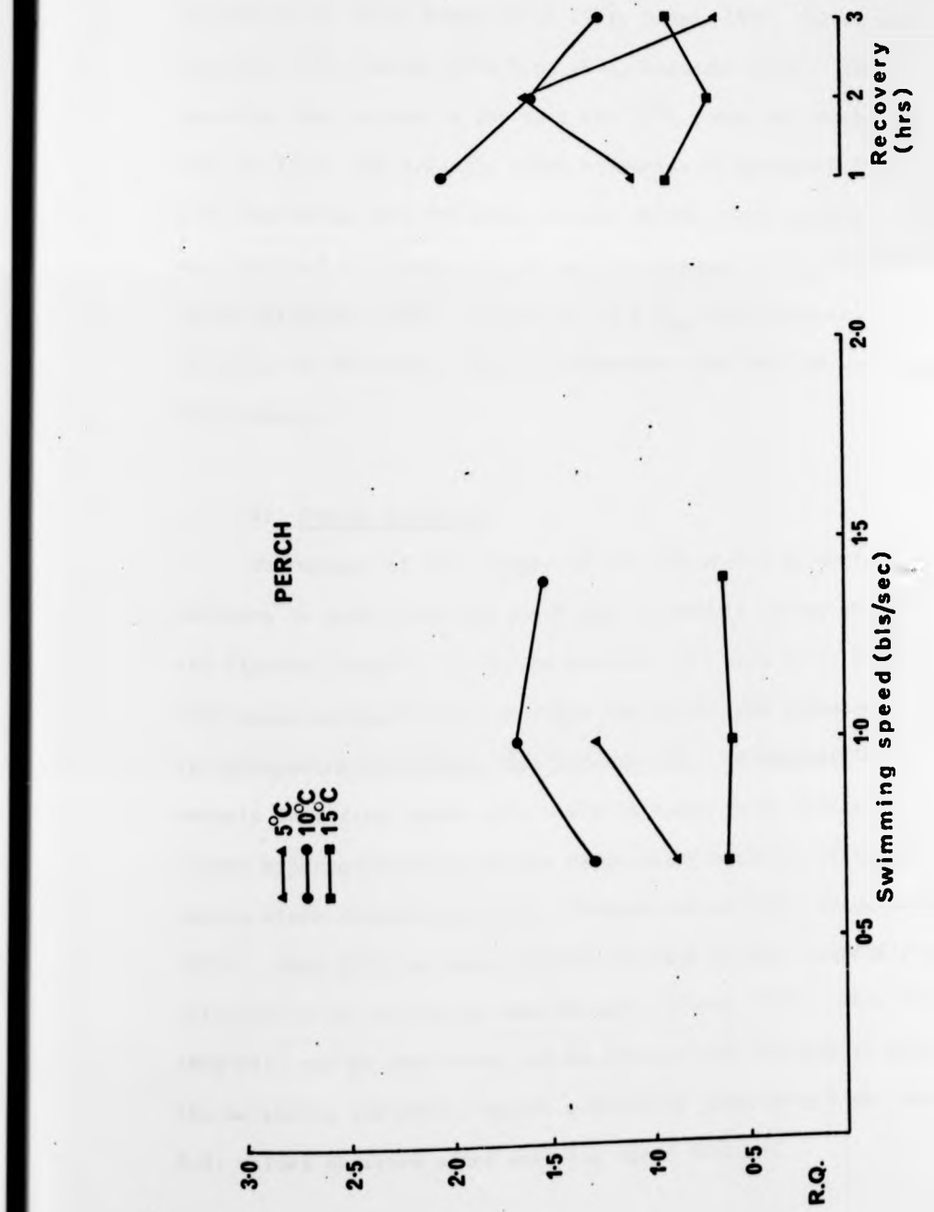


Fig. 9. Measured R.Q's during swimming (steady state) and recovery in perch.

Under exercise or starvation a decrease in R.Q. has been reported. (Benade et al 1971, Bintz et al 1971, Hedman 1957, Jolyet and Regnault 1877, Kayser 1940 b,c, 1961, Niemerko 1959). The question that arises is why does the 15°C trout and perch R.Q. fall so low? The R.Q. for lipid oxidation is between 0.70 and 0.73 depending upon the exact nature of the lipid oxidised. Fish are reported to possess lipids mainly composed of C_{18}, C_{20} and C_{22} types (Sinhuber 1969). Oxidation of a C_{18} lipid produces an R.Q. of 0.73, so the answer must lie elsewhere (see section on Energy Substrates).

b) <u>During Recovery</u>

The extent of the changes in the measured R.Q. during recovery in both trout and perch may be seen in Tables 20 and 21 and Figures 8 and 9. It may be quickly seen that apart from the 15°C experiments, recovery of trout and perch from swimming failure is accompanied by an R.Q. that exceeds 1.0. In terrestrial animals including humans this would be taken to be indicative of either hyperventilation and non respiratory acidosis which commonly occurs after violent exercise. (DeLanne et al 1959, Siggard-Anderson 1971). When this has been recorded in fish it has frequently been referred to as evidencing anaerobiosis. (Kutty 1972). This blanket term will not be used here, but an attempt will be made to describe the metabolic and physiological conditions underlying high (over 1.0) R.Q. values observed after swimming speed failure.

TABLE 20

RESPIRATORY QUOTIENTS OF BROWN TROUT DURING RECOVERY FOLLOWING SWIMMING FAILURE

TIME (hrs.)	5°C.	10°C.	15°C.
1	1.34	1.05	0.84
2	1.22	1.12	0.86
3	2.86	1.14	0.88

TABLE 21

RESPIRATORY QUOTIENTS OF PERCH DURING RECOVERY FOLLOWING SWIMMING FAILURE

TIME (hrs.)	5°C.	10°C.	15°C.
1	1.10	2.04	0.91
2	1.63	1.60	0.70
3	0.65	1.25	0.92

In the air breathing animal which carries out gaseous exchange across a moist, permeable, vascularised barrier, carbon dioxide exchange occurs by diffusion. The basic chemical equation describing the carriage and exchange of CO_2 is:

$$CO_2 + H_2O = H^+ + HCO_3^-$$

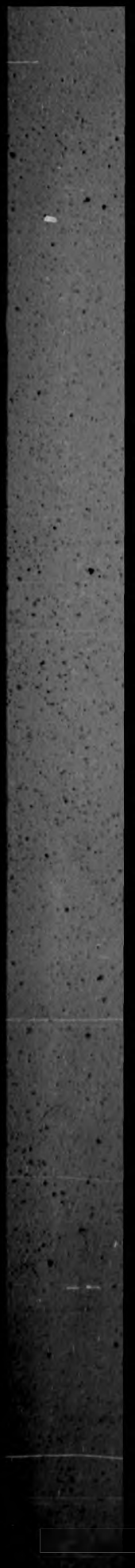
Thus carbon dioxide is hydrated and under the influence of carbonic anhydrate dissociates to form hydrogen and bicarbonate ions. At the gaseous exchange membrane the reaction is reversed and gaseous CO_2 is blown off. This does not occur in fish, the bicarbonate ion being the chemical combination in which CO_2 is excreted. Further the process is one of ionic exchange, the reciprocal ion being Cl^- (Dejours 1969, Garcia-Romeu and Maetz 1964, Maetz and Garcia-Romeu 1964). This aspect has been fully described and discussed in a previous section.

Although the appearance of CO_2 outside a freshwater teleost is the product of ionically exchanged HCO_3^-, the rate of HCO_3^- excretion does not appear to be a limiting factor. The change in CO_2 output as measured by this study would indicate that providing the exchange anion is present in sufficient quantities the actual method of transfer from fish to environment is not rate limiting. These introductory comments are necessary in order to show that a large production of carbon dioxide under conditions of low or non existent swimming activities is a result of the metabolic changes that have occurred prior to the measurements being made. As the brown trout used in this study present the apparently conventional

physiological condition, i.e. a predominantly aerobic metabolism, most of the following remarks will apply to brown trout. That is not to say that the perch do not exhibit similar metabolic and physiological homeostasis, but that the evidence relating to perch metabolism from this and other studies (on non salmonid teleosts) indicates the presence of non aerobic metabolic processes. That the recovery R.Q. values of brown trout following swimming failure, are a function of both oxygen and carbon dioxide exchange is amply illustrated in Figure 10. It can immediately be seen that the high R.Q. measured in the 5°C trout is a function of an abnormally high carbon dioxide production and not an abnormally low oxygen consumption. Although Figure 10 shows the dramatic increase in CO_2 output at 5°C, it also shows that at 10°C and 15°C the CO_2 output drops from the first to the second hour of recovery, but then remains more or less constant between hours two and three. The oxygen consumption exhibits similar changes. In order to attribute an R.Q. of over 1.0 to the presence of anaerobic metabolites in a predominantly aerobic organism, it must be demonstrated that anaerobic metabolites are present under the experimental conditions and the manner of their presence in terms of acid base equilibria .

Albers (1970) has summarised the transport of CO_2 in fish blood as follows:

1) Carbon dioxide formed by metabolic processes in the tissues diffuses into the blood and reacts with water to form carbonic acid. The reaction is shown in the plasma. Within the red cells it is speeded up by the enzyme carbonic anhydrase.



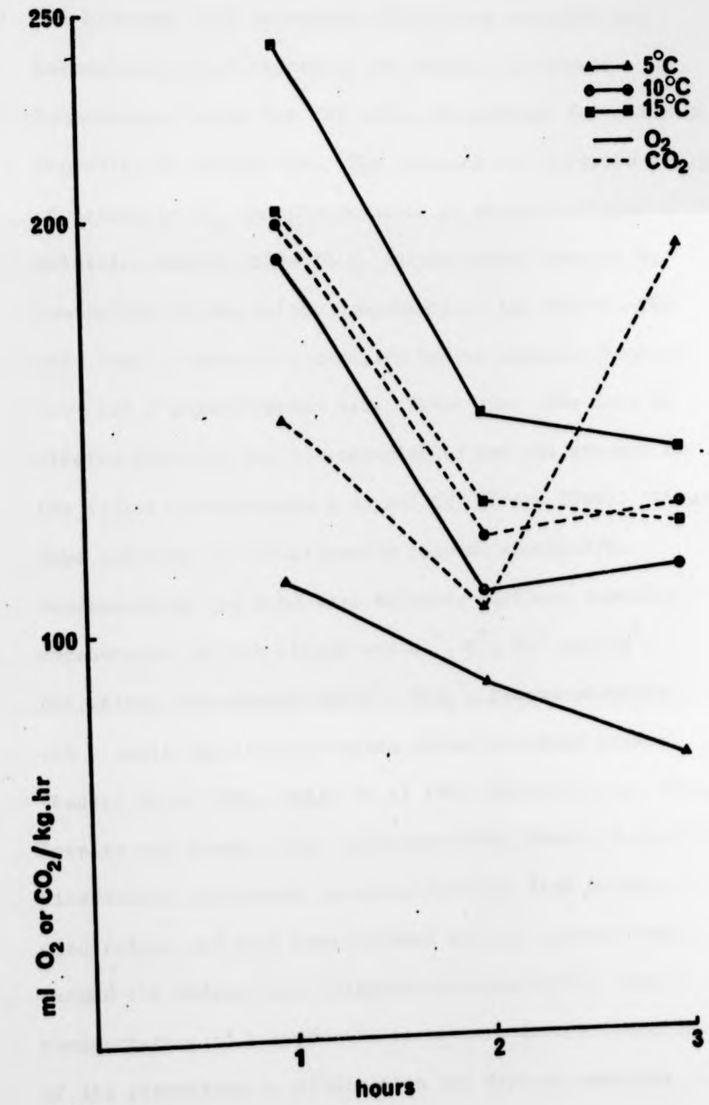


Fig. 10. The oxygen consumption and carbon dioxide output (mean values only) of brown trout during recovery following swimming failure.

2) Carbonic acid dissociates into hydrogen ions, bicarbonate ions and to a small extent into carbonate ions.

3) The hydrogen ions recombine with plasma proteins and haemoglobin which represent the buffer sustances.

4) Bicarbonate leaves the red cells in exchange for chloride according to Donnans Law. The presence of increased levels of metabolic CO_2 and the presence or absence of non volatile organic acids (i.e. lactic acids) have to be controlled by the buffer components of the blood. The acid base balance is controlled by two physico chemical laws and a physiological law. These are: the laws of electronentality and iso-osunolarity and the attempt of the tissues to maintain a normal pH (Rooth 1969). These laws indicate the relationship between electrolyte concentration and acid base balance. Cations commonly encountered in fish plasma are Na^+, K^+, Ca^+ and Mg^+. The anions encountered are Cl^-, HCO_3^-, plasma proteins and a small quantity of anions termed residual anions. (Becker et al 1958, Field et al 1943, Hartman et al 1941, Pereira and Sawaya 1957, Robertson 1954, Smith, H.W. 1929). Bicarbonate and plasma proteins form the link between electrolyte and acid base balance and are collectively termed the buffer base (Siggard-Anderson 1971). The concentration of bicarbonate is never a direct function of its production or elimination but depends upon the surplus of cations over anions, e.g.

$$Cations^+ - (Cl^- + Prot^- + Residual\ anions^-) = Bicarbonate^-$$

Bicarbonate is thus the non fixed ion, the concentration of which can be altered by respiration and by buffer reactions.

Returning to the recovery of fish from swimming failure, there is a considerable literature indicating the production of lactic acid following severe exercise conditions (e.g. Secondat and Diąz 1942, Black 1955 1957a.b.c. Black et al 1959, 1960, 1962 1966, Beamish 1966, 1968, Heath and Pritchard 1962, Pritchard et al 1971, Johnston and Goldspink 1973 a,b). It is not unreasonable to suppose that fish collapsing under the experimental conditions of this study would also exhibit high levels of muscle and blood lactate.

The time course of the appearance of blood lactate is interesting and has been studies by some workers (Beamish 1968, Black 1957 a,b,c). It has been shown that blood and muscle lactic acid concentrations are not equal and that the increase in blood lactate takes place over a period of hours. The permability coefficient of lactic acid being temperature dependant (Johnston et al 1945) has often been quoted to explain this phenomenon (Black 1958). Further, some studies have shown that a relationship exists between blood lactic acid concentration and either blood bicarbonate or total CO_2 (Black et al 1959, 1966, von Buddenbrock 1938, Secondat and Diaz 1942, Piiper et al 1972). The relationship being an increase in blood lactic acid and a decrease in total blood CO_2. The relationship is thus established, the problem remaining being to describe the physiology of an excretion of bicarbonate ion as opposed to CO_2 gas.

Although freshwater teleost blood pH is controlled by HCO_3^- levels (Cameron and Randall 1972, Randall and Cameron 1973 a,b quoted in Cameron and Randall 1972) the rate of the adjustment of blood HCO_3^- levels is slow taking up to 24 hours (Dejours 1969, Eddy and Morgan 1969, Lloyd and Jordan 1964, Lloyd and White 1967). A challenge of lactic acid to the blood buffering capacity has to be accommodated by the existing blood buffering components or else a drop in blood pH will occur. It has been shown by Jonas et al (1962) that if blood pH is lowered to 6.8-6.9 in rainbow trout and sockeye salmon at 11-14°C mortalities will occur. The initial reduced rate of CO_2 output in brown trout during recovery could be interpreted as being due to the buffering capacity of the blood coping with the appearance of lactic acid in the circulation. The plateau of oxygen consumption and carbon dioxide output seen at 10°C and 15°C could well indicate a maximum blood lactate that is not only buffered but subject to tissue oxidation. This would explain the maintained oxygen consumption and carbon dioxide output at these temperatures. The excretion of NH_4^+ as described in the relevant section of this study would assist the acid base components to maintain a pH equilibrium. The 5°C situation however, indicates an inability to deal with an increased blood lactate. The appearance of large quantities of lactic acid causing an increase in the anionic component as the blood and bicarbonate being excreted as a function of electroneutrality. The inability to deal with the lactic acid load could be due to a combination of the low temperature permeability coeifficient permitting a sudden appearance of lactic acid, and the reduced response of tissues to oxidise the lactate.

This type of acid base response to the presence of lactic acid can therefore be used to explain the measured R.Q's of 1.0. However, the perch situation differs from that of the trout and although acid base disturbance can be implicated to explain high R.Q. values the possibility of fundamental metabolic differences exists.

III ENERGY SUBSTRATES

The energy supplying substrates involved in routine and active swimming metabolism may be evaluated from the values obtained for oxygen consumption and carbon dioxide and ammonium nitrogen excretion. Using the gaseous equivalents of Table 2 simultaneous equations may be derived that allow calculation of the substrates involved.

These equations are:

$N(NH_3\text{-}N) = 0.16P$

$VO_2 = 0.81C + 0.94P + 1.96L$

$VCO_2 = 0.81C + 0.75P + 1.39L$

Where

N = ammonium nitrogen (NH_3-N) (mgs)

VO_2 = volume of oxygen consumed (mls)

VCO_2 = volume of carbon dioxide excreted (mls)

P = protein (mgs)

C = carbohydrate (mgs)

L = lipid (mgs)

These equations are derived from measures obtained in human physiology, and as such may not be accurate for fish work. However, apart from protein which is suggested to have a nitrogen -protein conversion factor of 6.025 (Bailey 1937, Nottingham 1952), no other fish equivalents are available.

Before describing and tabulating the changes in energy substrates occurring, mention must be made of data that is unsuitable for interpretation. Although the R.Q. itself can be used to estimate the percentage contribution of carbohydrate and lipid to the displayed

metabolism, it will not be used here. The nitrogen free R.Q. (Tables 16 and 17) will however be used to indicate those measurements unsuitable for analysis. A nitrogen free R.Q. is simply an R.Q. measurement that has been calculated after the oxygen and carbon dioxide volumes associated with the excreted nitrogen have been subtracted from the observed exchanges. Where a nitrogen free R.Q. exceeds 1.0 - the maximum value for 100% carbohydrate oxidation, no attempt will be made to determine the substrate. The rationale for this being the possibility of blood acid base disturbances caused by lactic acid production giving rise to artificially high or low gaseous exchanges. Lactic acid has been demonstrated to be produced by fish under active conditions and has been shown to elevate the R.Q. in humans. (Åstrand and Rodahl 1970, Krogh and Lindhard 1920, Visser et al 1964). Likewise, a nitrogen free R.Q. of less than 0.7 will be taken to indicate the presence of metabolism other than that giving rise to 100% lipid oxidation. With these provisos the data of Tables 22 and 23 has been calculated taking the equivalents of 1 mg. NH_3-N to be 5.9 mls. oxygen and 4.9 mls. carbon dioxide (Kleiber 1961) to estimate the nitrogen free R.Q. The gaps in Tables 22 and 23 indicate the occurrence of metabolism giving rise to nitrogen free R.Q's of greater than 1.0 or less than 0.7

Apart from indicating the type and quantity of substrate utilised these Tables should be interpreted with caution. To obtain a true comparison of energy production from the utilisation of substrates, the calorific relations should be discussed. Before this certain interesting points arise from the actual substrate turnover observed.

TABLE 22

BROWN TROUT ENERGY SUBSTRATE TURNOVER

A

	5°C			10°C			15°C		
SPEED (bl/sec)	PROTEIN	LIPID	CARBO.	PROTEIN	LIPID	CARBO.	PROTEIN	LIPID	CARBO.
0.7	12.6	12.8	13.5	19.2	45.9	25.5	27.0	15.7	62.5
1.0	20.4	27.8	0.2	19.2	NC	NC	31.2	NC	NC
1.4	30.6	NC	NC	31.8	25.4	69.4	30.0	NC	NC
1.7	-	-	-	34.2	56.3	16.5	25.8	NC	NC

B

	5°C			10°C			15°C		
SPEED (bl/sec)	PROTEIN	LIPID	CARBO.	PROTEIN	LIPID	CARBO.	PROTEIN	LIPID	CARBO.
0.7	69.3	121.6	55.4	105.6	436.1	104.6	148.5	149.2	256.3
1.0	112.2	264.1	0.8	105.6	NC	NC	171.6	NC	NC
1.4	168.3	NC	NC	174.9	241.3	325.5	165.0	NC	NC
1.7	-	-	-	188.1	534.9	67.7	141.9	NC	NC

TABLE 22 A Energy substrate turnover expressed in mg/kg.hr.

TABLE 22 B Energy substrate turnover expresses as cal/kg.hr.

TABLE 22 C Energy substrate turnover expresses as percentages of individual components, the total calorific turnover being taken from Table 22 B.

NC - not calculated (non protein R.Q. being greater than 1.0 or less than 0.7)

— - speed not achieved

TABLE 22 (contd.)

	5°C			10°C			15°C		
SPEED (bl/sec.)	PROTEIN	LIPID	CARBO.	PROTEIN	LIPID	CARBO.	PROTEIN	LIPID	CARBO.
0.7	28	49	23	16	67	16	27	27	46
1.0	30	70	0	NC	NC	NC	NC	NC	NC
1.4	NC	NC	NC	23	33	44	NC	NC	NC
1.7	-	-	-	23	68	9	NC	NC	NC

TABLE 23

PERCH ENERGY SUBSTRATE TURNOVER

	5°C			10°C			15°C		
SPEED (bl/sec)	PROTEIN	LIPID	CARBO.	PROTEIN	LIPID	CARBO.	PROTEIN	LIPID	CARBO.
R	NM	NM	NM	16.8	0.7	44.6	18.0	8.4	52.7
0.7	14.4	5.0	26.6	19.2	NC	NC	30.0	NC	NC
1.0	13.8	NC	NC	21.6	NC	NC	21.6	NC	NC
1.4	-	-	-	24.6	NC	NC	-	-	-

A

	5°C			10°C			15°C		
SPEED (bl/sec)	PROTEIN	LIPID	CARBO.	PROTEIN	LIPID	CARBO.	PROTEIN	LIPID	CARBO.
R	NM	NM	NM	92.4	6.7	182.9	99.0	79.8	216.1
0.7	79.2	47.5	109.1	105.6	NC	NC	165.0	NC	NC
1.0	75.9	NC	NC	118.8	NC	NC	118.8	NC	NC
1.4	-	-	-	135.3	NC	NC	-	-	-

B

TABLE 23 A — Energy substrate turnover expressed in mg/kg.hr

TABLE 23 B — Energy substrate turnover expressed in cal/kg.hr.

TABLE 23 C — Energy substrate turnover expressed as percentages of individual components, the total calorific turnover being taken from Table 23 B.

NC - not calculated (non protein R.Q. being greater than 1.0 or less than 0.7, or data incomplete)

— - speed not achieved

NM - not measured

R - routine metabolic rate.

TABLE 23 (contd.)

	5°C			10°C			15°C		
SPEED (bl/sec)	PROTEIN	LIPID	CARBO.	PROTEIN	LIPID	CARBO.	PROTEIN	LIPID	CARBO.
R	NM	NM	NM	33	2	65	25	20	55
0.7	34	20	46	NC	NC	NC	NC	NC	NC
1.0	NC	NC	NC	NC	NC	NC	NC	NC	NC
1.4	-	-	-	NC	NC	NC	-	-	-

As the temperature decreases from 15°C to 10°C, perch under routine conditions show a reduction in lipid utilisation. Unfortunately the 5°C condition was not measured, but it would appear that the observations of Blazka (1958) and Moroz (1971) are substantiated. That is, as the temperature decreases, lipid metabolism changes from one of utilisation to one of storage. This effect is not as marked in trout going from 15°C to 5°C. Regarding the trout at 10°C, these figures are possibly in error as a low R.Q. (0.66) was measured. Why this should be is not known but inspection of Appendix I Table 2 indicates that it is caused by what appears to be a low CO_2 production. Despite the low 10°C trout R.Q. comparison of predicted weight loss with that reported by Phillips et al (1960) for Salvelinus fontinalis at 8.5°C over a 12 week period is remarkably similar. Assuming that weight is lost at the same rate as that indicated under routine conditions, after 12 weeks the weight loss would amount to some 18% of starting body weight. Phillips et al (1960) obtained a figure of 20% weight loss. Apart from this indirect evidence the inevitable conclusion is that the 10°C routine R.Q. for trout is suspect. The low R.Q. and resultant inability to calculate the 15°C condition for both trout and perch is however, explainable.

It can be seen from Tables 16 and 17 that trout at 15°C subjected to a water velocity of 0.7 bl/sec and perch respiring in the routine respirometer display R.Q's that are similar, i.e. 0.87 and 0.89 respectively. When the perch are subjected to a water velocity corresponding to a swimming speed of 0.7 bl/sec. they swim continually. Likewise, the trout at 1.0 bl/sec. swim continually. Once both species are under constant swimming conditions at 15°C the observed R.Q. drops to 0.62 in both cases. The R.Q. observed during swimming

is therefore a real phenomenon and not one that is caused by, for example, an analytical error. This effect has never been reported before and the only other work on active fish R.Q's (Kutty 1968b, 1972) does not mention this situation. Further, as both species are subjected to increasing swimming speeds, this low R.Q. is maintained. It is interesting to note (Tables 14 and 15) for both species at all temperatures, that the first four of a set swimming speed gives rise to a higher R.Q. than the second steady state hour. The excitement and stress of the speed increment apparently giving rise to an element of anaerobic metabolism. Kutty (1968 b) in Figure 12 shows a similar situation for rainbow trout at 15°C and maintained at a swimming effort of 1.6 bl/sec. The text however, carries no mention of the low R.Q. In the absence of confirming chemical analysés no definitive statement regarding low R.Q's may be made, but from the circumstantial evidence obtained suggestions may be put forward relating to the metabolism occurring.

Oxidation of lipid by the β oxidation cycle produced molecules of acetyl Co.A (coenzyme A). Acetyl Co.A condenses with oxaloacetate and enters the tricarboxylic acid cycle (West et al 1967). If the rate of production of acetyl Co.A exceeds the rate at which condensation can take place an accummulation of ketone bodies occurs. Ketone bodies have been chemically identified as acetoacetate, beta hydroxy butyrate and acetone. (Masoro 1968). In humans suffering from this condition the breath smells strongly of acetone (e.g. Hedman 1957). Ketosis is the result of a high rate of fatty acid catabolism and a low rate of carbohydrate catabolism (Masoro 1968).

Blood glucose suppresses fatty acid catabolism primarily by inhibiting the rate of fatty acid metabolism. If, therefore, the availability of glucose or carbohydrate for aerobic catabolism is low, ketosis will occur. Fish are reputed to have a poorly defined blood glucose control system. When challenged by hyperglycaemic conditions they demonstrate a low ability to reduce blood glucose (Palmer and Ryman 1972, Phillips et al 1948a). Fish characteristically show increased blood glucose as a result of stress (Menten 1927, Scott 1921, Wardle 1972). Further, the presence of insulin producing cells in the islets of Langerhans have been demonstrated (Epple 1969, Khanna and Gill 1972, Khanna and Rekhari 1972, Umminger 1971). These observations coupled with those of Gray and Hall (1930) and Leibson (1972) who demonstrated higher blood glucose concentrations in active teleosts as opposed to sluggish teleosts, suggest the explanation for the observed low R.Q's.

At a physiologically favourable temperature fish, when exercised, utilise blood glucose to a point at which hypoglaecemic conditions prevail. Lipid then becomes the major energy source, the oxidation of which leads to an accummulation of ketone bodies. The R.Q. drops to between 0.5 and 0.7 indicating a formation of hydroxbutyrate molecules (Soskin 1941). It is possible that gluconeogenesis is taking place to restore blood glucose (Bloch 1947, Buchanan and Hastings 1946, Lorber et al 1945, Rittenberg and Bloch 1945). The presence of an increased blood β hydroxybutyrate concentration (0.9 - 2.47 mg/100 ml) after exercise has been demonstrated by Jones and Bilinski (1965) to occur in rainbow trout. Also short term exercise

has been shown to have no effect on liver glycogen whilst long term (24 hours) exercise can lower liver glycogen concentration (Stevens and Black 1966). Low temperature exercise probably has the same effect, but here the rate of lipid oxidation is low. This could be a result of the increasing proportion of unsaturated fatty acid present (Kanungo and Prosser 1959) or of a primarily anabolic function of lipid. Leibson (1972) suggests that active fish are less resistant to insulin hypoglycaemia but if they survive, restoration of blood glucose is rapid. Gray and Hall (1930) tested the effect of insulin injections and found that active fish demonstrated insulin shock convulsions about 2-4 hours after injection. The sluggish fish that they tested although showing a decrease in blood glucose after insulin, did not demonstrate any convulsions. The lack of perch mortalities in this study could be due to the resistance of perch to hypoglycaemic conditions. It is interesting that trout mortalities occurred at 5°C in this study but not in the work of Brett (1964) with coho salmon. Brett demonstrated mortality at 24°C, perhaps illustrating two very different types of stress mortality.

The data of Tables 22 and 23 with regard to protein utilisation can now be considered. It can be seen that for trout going from 5°C to 15°C there is an approximately 5 times increase in carbohydrate utilisation, virtually no change in lipid utilisation and a doubling in protein utilisation. Although the routine protein utilisation approximately doubles, at both temperature extremes, there is no statistical increase in protein turnover with increasing activity for either trout or perch. This observation is in agreement with

the findings of Astrand and Rodahl (1967), Cathcart and Burnett (1926), Chauveau (1896), Crittenden (1904), Hedman (1959) and Pettenkofer and Voit (1866) that protein does not act as an energy source during activity. As such it disagrees with the findings of Kreuger et al (1968), Kutty (1972) and Mercy Bai (1970 cited by Kutty 1972), that protein utilisation increases with activity. Kreuger et al (1968) were perhaps demonstrating a phenomenon relating to the physiological age of their experimental juvenile salmon. Erhlich (1972) has shown that larvel herring and plaice during starvation obtain 60-70% of their calorific requirements from protein. The proteolytic enzyme systems associated with yolk sac utilisation could perhaps still be present and active. The findings of Kutty (1972) on Tilapia mossambica showing high ammonium nitrogen excretion (see Table 8) indicate routine protein turnover of 129 mg/kg.hour and an active turnover of 450 mg/kg.hour. Taking fish to be about 15% net weight of protein (McLeod pers.comm. Swift 1955), this latter figure implies a body protein loss of about 0.3%/hour. This remarkable figure is inexplicable on protein as an energy source grounds. Perhaps the relevant comment is that of West et al (1967) who state "...found in animal experiments that a sharp premortal rise in nitrogen excretion occurs shortly before death."

When the substrates are converted into energy equivalents (calories, Winberg 1971), direct comparison between protein, lipid and carbohydrate may be made. Tables 22 and 23 show the calories arising from each of the three substrates and each substrate as a percentage of the total energy output. Here the relationships

between protein, lipid and carbohydrate utilisation are clearly illustrated. Although trout at 5°C show an apparent increase in actual quantity of protein utilised, on a calorific basis the lipid utilisation increases with increasing swimming speed. At the same time the carbohydrate utilisation drops to zero and the R.Q. for the following swimming speed (1.4 bl/sec) rises to above 1.0. Likewise at 10°C the highest sustained swimming speed for trout is accompanied by a high lipid utilisation rate. Although the 15°C trout condition cannot be analysed the evidence suggests that again lipid is the predominant energy source. Because of the inability to determine active perch energy sources, the same observations cannot be made. It is interesting though that the 15°C perch R.Q. follows the same pattern as the 15°C trout R.Q., implying a similar metabolism, i.e. predominant lipid. This being the case, the two fish species used exhibit exactly the same utilisation of energy substrates as man. With increasing work intensity human metabolism shifts towards a predominantly lipid metabolism.

IV GENERAL DISCUSSION

From the observed R.Q's during swimming activity and recovery, the energy substrates used during swimming, an overall assessment of fish energy requirements may be made. It is proposed to relate in the following discussion, such factors as muscle types and function, aerobic and anaerobic metabolism, temperature, degree of activity and energy reserves.

It has long been recognised that muscle is of two basic types, red and white (review, Needham 1971) and several reviews of their functions and composition in fish exist (Bodekke et al 1959, Bone 1966, Braekkan 1959, Love 1970, Webb 1970). It is not proposed to sum the contents of these and other papers but rather to bring out the relevant points where required. The proportions of the red and white or ordinary muscle vary with the habit of the fish concerned (visual illustration Braekkan 1959, Bodekke et al 1959, Love 1970, composition by weight Webb 1970, Greer Walker 1970). Active fish such as tunny possess both lateral line and deep sited red fibres (Carey and Teal 1969) whilst sluggish fish possess only a thin strip of red muscle in the region of the lateral line. It has been suggested that the red fibres are aerobic and capable of slow contractions over a long period of time whilst the white fibres are capable of fast contraction of a short duration. (Bodekke et al 1959, Braekkan 1959, Bone 1966, Love 1970). The division into red and white muscle types is not absolute, intermediate fibres designated as mosaic are found in varying proportions in the predominantly white muscle blocks (Webb 1970).

Chemical analyses indicate a higher lipid and lower glycogen content of red fibres as opposed to white fibres. (Braekkan 1959, Bligh and Scott 1966, Pritchard et al 1971, Nagayama 1961, Thurston 1962) although the latter point is open to question (Love 1970). Bilinski (1963) demonstrated that red or dark muscle was capable of much greater oxidation of fatty acids than the white muscle as did George (1962) and George and Bokdawala (1964). Stevens (1968) calculated that the blood content per unit weight of red to white muscle types was about 2.5:1, although the ratio of total blood content of red to white was about 1:26. Dark or red muscle types also contain higher concentration of nitro mitrochondria (Buttkus 1963, George 1962, Nishihema 1967) and a higher concentration of myoglobin (Hamoir 1953, Matsure and Hashimoto 1954). From these and may other observations has developed the concept that routine activity is carried out using red muscle and fast demand activity is provided for by the white fibres (the "coiled spring" Love 1970). Analysis of the A.T.P. ase activities of the two muscle types has indicated a higher activity in the white muscle confirming the fast muscle properties of white muscle (Johnston et al 1972, Nag 1972). In carrying out these functions the fibres act aerobically and anaerobically respectively.

This type of conclusion is supported by the numerous studies on blood and muscle lactate (lactic acid) concentrations. The variety of experimental techniques employed tend to preclude a definitive statement on the formation of lactic acid and its relation to metabolic demand. Commonly quoted experimental

techniques are those of chasing a fish in a container of water for between 5 and 15 minutes or until it collapses (Black 1955, 1957, Black et al 1959, 1966, Jones and Murray 1960, Miiler et al 1959, Tomlinson et al 1965, Wendt 1965). The invariable result from this type of work is an increase in white muscle lactate and blood lactate and a decrease in white muscle glycogen. From this comes the conclusion that severe exercise causes high activity of white muscle which operates anaerobically by converting glycogen into lactic acid. The lactic acid enters the blood slowly either as a result of slow diffusion rates due to temperature (Johnston et al 1945) or impaired or poor circulation (Black 1959, Redfield and Medearis 1926). Associated with this type of change are elevations in the levels of blood epinephrine and norepinephrine (catecholamines) phosphates and pyruvic acid. (Black et al 1966, Nakano and Tomlinson 1967, Nakatani 1957). Sustained swimming experiments where the fish is allowed to metabolically equilibrate with its activity level tend to show slightly different results. Liver glycogen can be shown to decrease if the fish are made to swim for 24 hours instead of 15 minutes (Miller et al 1959). Blood and muscle lactate rises only at about 70% of the critical swimming speed whilst muscle glycogen drops throughout activity (Beamish 1968).

Analysis of the high energy phospjate compounds viz: A.T.P., A.D.P., A.M.P. and C.P. (adenosine tri, di and mono phosphate and creatine phosphate) also yields information aposite to the activity of the muscles. It has been shown that A.T.P. is the source of energy for muscular contraction (Cain and Davis 1962, Needham 1971)

and that A.T.P. may be replenished by resynthesis from A.D.P. by the transphosphorylation of creatine phosphate by creatine phosphotransferase. If a muscle is allowed to recover from a tetanic contraction, the resulting levels of A.T.P., A.D.P. and A.M.P. are restored. If, however, a series of tetanic twitches are carried out, the level of freee creatine rises due to the transfer of the "phosphate bond energy" from the creatine to the adenosine moiety (Sproŋck 1965, Wilkie 1968). If the capacity of the muscle to restore either the levels of C.P. or adenosine phosphates is impaired by anaerobic conditions or an excessive energy demand, glycolysis occurs. Once the glycolytic pathway is induced, lactic acid is formed which may lower the intracellular pH and cause pH changes in the blood by diffusion through the fibre membrane. At this point the work of Karlsson (1971) is relevant. In the review by Karlsson, covering a number of exercise physiology experiments on human subjects, some interesting points emerged. It was shown that muscle lactate only started to rise at about 60% V max O_2 (60% of the maximum recorded oxygen consumption) and that the muscle lactate obtained from a brief exhaustive exercise was the highest attainable. Further, at exhaustion, the levels of C.P. and A.T.P. were 30% and 70% of the resting levels respectively. Karlsson came to the conclusion that lactate glycolysis was relatively unimportant provided that the C.P. depletion was not at a maximum, i.e. 30% of the resting level. These relationships demonstrate the three sources of energy for muscular contraction, oxidative or the "normal" combustion of lipid or carbohydrate, lactacid or glycolysis leading to lactic acid formation and alactacid or the depletion of A.T.P. and C.P. stores without lactic acid formation (Margaria 1969).

In fish these relationships have not been well explored, although limited data is available for phosphate compound concentration in muoribund or dead fish. As this work is mainly related to commercial fisheries the results are of the "rested and during storage" nature (Burt and Stroud 1966, Fraser et al 1966, 1967, 1968, Jones and Murray 1957, 1966, Kassemsarn et al 1963, Tarr 1966). As a lot of the samples used were trawl caught the degree of exhaustion is not known. The overall conclusions of the work of these authors is that the severe exhaustion of trawl netted fish leads to a reduction in the muscle concentrations of A.T.P., A.D.P., and A.M.P. whilst the concentrations of I.M.P. (inosine monophosphates) and hypoxanthine increase. It therefore appears that the purine nucleotide cycle (Lowenstein 1972) is operative in these fish, the levels of adenosine phosphate compounds being pushed so low as to initiate removal of A.M.P. through pathways leading to I.M.P. and hypoxanthine. The time course of these changes in dead rainbow trout are illustrated in Saito et al (1959 Table 2). It is doubtful though whether these severe changes would take place in a fish subjected to conditions that are removed when swimming failure occurs. Felton (1956) demonstrated an increase in the free creatine content of the dorsal muscle of rainbow trout (Steelhead variety) following strenuous exercise. Recently, Johnston and Goldspink (1973 b) showed a significant increase in the free creatine concentration over the resting levels in the white muscle of crucian carp subjected to 15 minutes of vigourous swimming activity. At about 50% of the critical swimming

speed these authors also showed an increase in the free creatine content of the white muscle (P 0.05) but after 200 minutes of sustained swimming. Because of the time scales involved the two results are not strictly comparable and it is possible that the 50% critical swimming speed condition, if sampled after 15 minutes, would show the same result as obtained after 15 minutes of vigorous swimming activity. It is interesting to note that Khalypen et al (1972) reported that fatigue in fish was not accompanied by a significant reduction in A.T.P. concentrations.

It can be argued that swimming failure and the factors either causing it or accompanying it, can be described in terms of post swimming failure metabolism i.e. recovery. From the results obtained for the nature of the energy substrate utilised and the R.Q. associated with recovery as measured in this study, at attempt will be made to relate the factors governing energy supply in the active fish. Undoubtedly high speed swimming failure is accompanied by a large increase in muscle lactate, a proportion of which will appear in the blood. However, the questions that arise are: at what stage does anaerobic glycolysis occur, what causes the onset of anaerobic glycolysis, and at what stage in steady swimming does white muscle become operative? The concept of the coiled spring of Love (1970) and Bone (1966) presents too much of an either or reaction - as do the rigid ideas relating to aerobic and anaerobic capacities of the red and white muscle systems. The work of Braekkan (1959) showed that pantothenic acid, the precursor of CoA-SH (coenzyme A) is present in high quantities in the dorsal (white) muscle of the salmon (Salmo salar). Further it is present

in higher concentration than in the red muscle. Coenzyme A is readily acylated in the metabolism of substrate molecules i.e. pyruate and free fatty acids. The acetyl CoA resulting from these reactions condenses with oxaloacetic acid to enter the tricarboxylic acid cycle as citric acid (Ochoa 1954). Although Bilinski (1963) has demonstrated the superior ability of red muscle to oxidise fatty acids comparative to white muscle, the fact remains that white muscle does contain lipid or interfibre adipose tissue (Greer Walker 1970) and also possesses the precursor of CoA - SH. The analyses of this study indicate that at 5°C and 10°C lipid oxidation increases to provide 70% of the calorific output in the brown trout at the maximum sustained swimming speeds. The balance of the energy output being derived from protein sources. As protein expenditure does not increase and carbohydrate utilisation decreases the increased energy requirement is provided by lipid - up to a point. This decrease in carbohydrate utilisation either reflects an inability to mobilise glycogen depots to provide aerobically useable carbohydrate, or a preferential demand for lipid. The latter possibility raises the interesting prospect of possible competition between lipid and carbohydrate, for available CoA-SH to enable product entry into the tricarboxylic acid cycle. However, it would appear that the situation is not one of competition but temperature modified enzymic induction. Hochacka (1967) demonstrated that cold adapted trout muscle favoured anaerobic glycolysis as opposed to aerobic glycolysis as a result of the induction of allostearic forms of lactic dehydrogenase (L.D.H.). The L.D.H. present in cold

adapted muscle was such that the pyruvate/lactate equilibrium favoured the formation of lactic acid. That a certain amount of carbohydrate is utilised is indicated by a decrease in muscle glycogen without an increase in muscle lactate (Beamish 1968, Hunter et al 1971, Johnston and Goldspink 1973 a,b). This implies either an aerobic function of white muscle at low to medium swimming speeds or the utilisation of glycogen to form blood glucose. This is further supported by the work of Hudson (1973) who demonstrated that white muscle activity could be detected electromyographically at speeds as low as 50% of the critical swimming speed in rainbow trout. Increasing swimming speeds causing an increase in the electrical activity of the white muscle fibres. The work of Greer Walker (1970, 1971 and quoted by Hudson 1973) on the growth of red and white muscle fibres of cod and the hypertrophy of muscle fibres in exercised coalfish, also indicates a low swimming speed activity of white muscles. Greer Walker (1970) noted a dominance of white muscle in cod under 15 cms. and suggested that this could be an adaptation to darting behaviour for predator avoidance. He also stated "... surprising if 83% of the musculature were reserved for escape reactions and only underwent passive contractions during sustained activity", a remark followed by the suggestion that fatigue could possibly be reduced by rotational activity of white fibres. The stress associated with an increase in swimming speed leading to an anaerobic contribution from the musculature, evidenced by an increase in oxygen consumption and in R.Q. (Kutty 1968b, Smit 1965, this study), is still a valid condition preceding an aerobic phase of white muscle activity.

These type of findings would bear out the 15°C condition as indicated in this study. Here the R.Q. evidence points to a high lipid utilisation that results in incomplete oxidation, i.e. the end products of the oxidation of lipids being produced in excess to the capacity of the tricarboxylic acid cycle to utilise them. This does not preclude the possibility that some gluconeogenesis from lipid is taking place. The higher requirement for carbohydrate at 15°C as opposed to 5°C is supported in feeding experiments (Withnall pers. comm.) This is not a new situation, Soskin (1941) stated "...the diabetic R.Q. of 0.7 can best be interpreted as the resultant of at least two factors, namely, a low component due to gluconeogenesis (especially from fat) and ketogenesis in the liver." Since gluconeogenesis and ketgenesis occur in the liver one would expect the liver to give low R.Q's under these conditions and this has been shown to be correct for rats and cats (Blixenkroner Müller 1938, Gemmill and Holmes 1935, Stadie et al 1940 b). Further, Jonas and Bilinski (1965) using rainbow trout, showed that chasing the fish for five minutes followed by leaving them in air for five minutes caused a three fold increase in the concentration of hydroxybutyric acid in the blood. This is a similar result to those of Grollman and Phillips (1954) who observed a rise in the concentration of blood ketone bodies of exhaustively exercised laboratory animals. This was interpreted as resulting from an insufficient carbohydrate supply, more fatty acids being utilised, but being incompletely oxidised. However, it would appear that the capacity of a fish to carry out the utilisation of lipid to this extent is temperature controlled. The evidence

available suggests that a decrease in temperature is accompanied by an increase in unsaturation of fish lipids (Hilditch and Williams 1964, Johnston and Roots 1964, Kemp and Smith 1970, Knipprath and Meud 1968, Roots 1968, Smith and Kemp 1968). It is possible that unsaturated lipids are not capable of being mobilised and oxidised at such a rate that ketosis takes place. In this respect it is interesting that homeotherms subjected to cold acclimation are resistant to fasting ketosis explained on the grounds that the tissues of cold acclimated animals have an increased capacity to oxidise free fatty acids to CO_2 and water (Masoro 1968). This shift of metabolic emphasis also appears to occur in teleosts (Hochachka 1967, Hochachka and Hayes 1962, Dean 1969). The capacity of the muscles of laboratory animals to oxidise free fatty acids has also been shown to increase with training (Mole and Holloszy 1970). For brown trout at 15°C lipid provides 27% of the calorific expenditure under routine conditions and at 5°C lipid provides 49% of the calorific requirements. Perch, on the other hand, under routine conditons show a decrease in lipid utilisation going from 15°C to 10°C (20% and 2% respectively). In doing so perch display the characteristics noted by Blazka (1958, 1960) in carp of liponeogenesis under cold conditions. Similar observations of lipid sparing were made by Moroz (1971) on overwintering carp. Brown trout and perch would therefore appear to differ in their energy metabolism with respect to decreasing temperature. Ekberg (1962) found that the output of organic CO_2 in relation to metabolic CO_2 was increased by cold acclimation in goldfish gills.

The metabolism accompanying recovery, as measured in this study, indicated that the 15°C fish was more competent to undergo vigorous swimming activity than the 5°C fish. The 5°C fish demonstrated large increases in R.Q. during recovery from swimming failure indicating large acid base disturbances. This was especially true of the brown trout who also suffered mortalities during recovery (40%). If the low temperature brown trout metabolism was such that aerobic oxidation of lipid was such that aerobic oxidation of lipid was the main energy source up to a certain swimming speed, beyond which anaerobic glycolysis became an important energy source, it must be considered to be inefficient. The consequence of high activity levels being the presence of large quantities of lactic acid. The 15°C condition did not demonstrate any large acid base disturbances as indicated by R.Q. changes. The trout appear to be capable of controlling 15°C blood lactate both by excretion of NH_3-N and by tissue utilisation. Bilinsky and Jonas (1972) have shown that at 15°C rainbow trout tissues are capable of utilising lactic acid, only about 1/5 of a lactate load being oxidised to CO_2 and water. However, again a species difference is evident. It seems that active fish have a greater capacity for lactic acid production than sluggish fish (Beamish 1966, Burton and Spehar 1971, Pritchard and Heath 1964, Prosser et al 1957). This is reflected in the lower output of NH_3-N during recovery in the perch. This again enphasises the presence of different energy supplying pathways in sluggish fish in comparison with active fish. Confirmation of this is found in the work of Morriss (quoted by Hochachka and Somero 1971) who demonstrated that the heat production

of cichlid fish to be 1½ to 2 times greater than that predictable on the basis of oxygen comsumption. (Kutty 1968b) showed that a goldfish could maintain an R.Q. of 2.0 at 20°C over a period of weeks, again implying a metabolism that could not be adequately described on the basis of oxygen consumption alone.

Finally, consideration must be made of the possible hormonal control of the availability of energy substrates. Probably, the main hormones involved are those arising from the adrenal medulla, the catecholamines, epinephrine and norepinephrine, and those arising from the pancreatic tissue, insulin and glucagon. In fish the responses of tissues and organs to these hormones are not clearly understood although a certain amount of information is now available. The occurrence of catecholamines in various tissues of fish has been demonstrated by Ostlund (1954) and von Ehler and Fänge (1961). Further, at rest, Nakano and Tomlinson (1967) have shown that in the blood of rainbow trout the concentrations of epinephrine and norepinephrine are approximately equal. These authors also showed that disturbance elevated the levels of epinephrine and norepinephrine by factors of 26 and 8 respectively. This agrees with the findings of Fontaine et al (1963) that epinephrine is the predominant catecholamine in Atlantic salmon, but disagrees with Mazeaud (1964) who found that norepinephrine to be the predominant catecholamine in carp. It does appear, however, that epinephrine is on a weight basis, more potent than norepinephrine (Larsson 1973, Minick and Chavin 1973). This agrees with the findings for mammals where the hyperglycaemic activity of norepinephrine is about an eighth of that of epinephrine (West et al 1967). The metabolic

effect of catecholamine administration to teleost fish is invariably a blood hyperglycaemia and either an increase or a decrease in blood free fatty acids (F.F.A., Larsson 1973, Farkes 1967, Minick and Chavin 1972 , 1973, Nakano and Tomlinson 1967, Perrier et al 1973, Young and Chavin 1965). The latter effect still requires clarification but the differing responses could be due in part to the concentrations of the catecholamines administered by the various authors. Larsson (1973) using eels and epinephrine doses of 0.5 mg/kg. and 5.0 mg/kg showed with the smaller dose a slight and transient hyperlactaemia. The larger dose produced a more vigorous and persistent hyperlactaemia. Farkes (1967, 1969) using a norepinephrine does of 2 mg/kg showed decreased serum F.F.A. levels lasting for 6 - 24 hours in pike perch, bream and carp. In regard to the response to varying dose concentrations of epinephrine, Young and Chavin (1965) demonstrated that in the goldfish epinephrine hyperglycaemia did not increase with doses greater than 0.1 mg/kg. This is in contrast with the greater hyperglycaemia effect noted by Larsson (1973) in eels responding to epinephrine concentrations of 5 mg/kg as opposed to 0.5 mg/kg. However, it is possible that the hyperglycaemic effect of catecholamines is controlled both by the glycogen stores available and the augmentation of insulin production in the presence of increased levels of catecholamines (Minick and Chavin 1973). If the action of teleost fish catecholamines is to increase the rate of liver glycogen breakdown to blood glucose, then the experimental dietary and maintenance conditions will dictate the extent of the observed hyperglycaemia. If a hyperglycaemic

condition arises the decrease in serum F.F.A. can be explained in conventional mammalian physiology terms.

Insulin production is controlled by blood glucose levels. As blood glucose rises, insulin is released from the pancreatic tissues to enable facilitated transfer of glucose across cell membranes (Ashmore and Carr 1964). Inside the cells and particularly in adipose cells, glucose is converted into L-α-glycerophosphate which reacts with F.F.A. to form triglycerides, thus limiting the release of F.F.A. to the extracellular fluid. Thus as the F.A.A's present in the circulation are oxidised the concentration drops (Masoro 1968). Rises in blood glucose in the rainbow trout have been shown to cause a decrease in serum F.F.A. (Palmer and Ryman 1972). The insulin enhanced glucose uptake has been shown to occur in isolated muscles of the lamprey, sea urchin and fog by Plisetskaya (1971). The true relationship between catecholamine administration and blood F.F.A. concentration is therefore experimentally difficult to ascertain especially if non physiological doses of the catecholamine are used. This would explain the sometimes conflicting results for changes in F.F.A. levels after catecholamine administration that are to be found in the literature. If insulin production is augmented by catecholamines, hyperglycaemic conditions would be under the control of two antagonistic effectors, viz. insulin and the catecholamines. Insulin adminstration experiments all show a hyperglycaemic response in teleost fish, although Gray and Hall (1930) found that sluggish fish did not demonstrate insulin induced convulsions. Stress has been shown to produce β cell degranulation

in the pancreatic tissue of goldfish (Chavin 1964, Chavin and Young 1970 b). Wardle (1972) using plaice, demonstrated a triphasic response of blood glucose during capture and subsequent recovery in a tank environment. The response of the tank adapted fish to stimuli was only a slight elevation of blood glucose indicating an improved blood glucose control attributed by the author to a combination of epinephrine induced insulin production and an increased glucose diffusion space. It is interesting to note the results obtained by glucagon administration to fish and the production of glucogen by fish. Khanna and Rekhari (1972) using mammalian glucogen demonstrated hyperglycaemia and glycogenolysis in the freshwater teleost Heteropneustes fossilis (Black) after administration. They also noted degranulation of the pancreatic β cells and some of the α cells after the glucagon dose. In a study on the seasonal production of insulin and glucogen in Tilapia and Mugil sp. Said and Al Hussaini (1966/67) showed that glucogen was a constant factor of pancreatic tissue extracts. Insulin could not be detected in pancreatic tissue extracts obtained in the autumn and winter. Autumn and winter extracts injected into a rabbit produced an increase in blood glucose lasting for 3-4 hours. Spring and summer extracts produced a transient blood hyperglycaemia lasting about ½ hour followed by a hypoglycaemia lasting for about 5 hours. This seasonal variation in insulin production and hence control of carbohydrate utilisation and oxidation clarifies and confirms the results obtained in this study.

To conclude, therefore, at low environmental temperatures insulin production is absent or at a very low level (Said and Al Hussaini 1966/67) and fatty acid oxidation rates are increased

(Dean 1969, Hochachka and Hayes 1962). This is reflected in the routine activity level substrates utilised by brown trout and in the substrate composition at the highest successful swimming speeds at 5°C and 10°C. (Tables 22 and 23). In going from 15°C to 5°C there is an approximate doubling of the lipid oxidation and a halving of the carbohydrate oxidation under routine activity conditions. The highest successful swimming speeds for brown trout at 5°C and 10°C both show that lipid oxidation increases to provide about 70% of the utilised energy. At 10°C there is also a small (9%) contribution from carbohydrate oxidation. At 15°C for both brown trout and perch the evidence indicates the presence of ketogenic conditions derived from high rates of incomplete lipid oxidation coupled with a gluconeogenesis of lipid origin, during swimming activity. It is remarkable that the R.Q. of both brown trout and perch undergoing swimming activity at 15°C is constant around 0.6. This indicates that incomplete lipid oxidation does not constitute the major metabolic pathway, and that a gluconeogenesis is present. If a gluconeogenesis is present under these conditions, the evidence for a seasonal insulin production supports a carbohydrate contribution to the overall energy production at all swimming speeds. The perch metabolism differs from that of the brown trout for two reasons. The first being the apparent reduction in lipid utilisation with decrease in temperature and secondly the increased contribution of an anaerobic metabolism giving rise to active R.Q's of greater than 1.0 This further emphasises the difference between the conventionally designated active and sluggish fishes.

An important region of metabolic similarity, however, between perch and brown trout, lies in the utilisation of protein as an energy

source. Both metabolic types do not utilise protein above a level of 30% of the total energy production between 5°C and 15°C. In this respect they are similar to the human condition where protein never supplies more than 18% of the energy requirements. This is in contrast to some zooplankton which can utilise protein to provide up to 80% of the total energy requirements (Blazka 1966).

SUMMARY

(1) A recirculating water fish tunnel respirometer was built and tested. The physical characteristics of the system were determined.

(2) The methods used for the determination of O_2, CO_2 and NH_3-N in the respirometer water are described. The analysis of CO_2 necessitated the development of a new system, based on the use of the carbon dioxide electrode.

(3) The excretion of CO_2 in freshwater teleosts was discussed in detail and critiques of the analysis of excreted CO_2 were described.

(4) Brown Trout and Perch were subjected to increasing velocity swimming tests at 5°C, 10°C and 15°C. Following swimming failure, the metabolism of the first three hours of recovery was monitored.

(5) The metabolism of the fish undergoing swimming and post swimming failure recovery was described in terms of the R.Q. displayed. From the R.Q. ratios obtained and the simultaneous measures of excreted NH_3-N, the aerobic metabolism was described in relation to the energy substrates oxidised.

(6) Under the diet and experimental regimes of this study it was found that brown trout undergoing routine activity at 5°C derived 49% of their energy requirements from lipid, and 23% from carbohydrate. At 15°C under the same conditions, 27% of the energy requirements came from lipid and 46% from carbohydrate. At 5°C and 10°C, the maximum sustained swimming speeds were supported by a predominantly lipid oxidation (70% of

of energy output). At 15°C, however, swimming activity was characterised by a metabolism that indicated ketosis (incomplete lipid oxidation) and gluconeogenesis. Perch undergoing routine activity at 15°C also utilised carbohydrate as the main energy substrate, 55%, whilst lipid only contributed 20%. At 10°C lipid oxidation was almost absent (2%) and carbohydrate oxidation increased to 65% of total energy output. The perch 5°C routine condition was not determined. At 15°C perch swimming activity was supported by a metabolism similar to that of brown trout viz. ketosis and gluconeogenesis. At 5°C and 10°C perch swimming metabolism was almost entirely anaerobic. Protein utilisation varied little both between species and at the different temperatures, contributing between 25% and 34% to the total energy utilised.

(7) The recovery phase following swimming failure for both species at 5°C and 10°C indicated the presence of either an anaerobic metabolism or the products of an anaerobic metabolism. At 15°C, however, the respiratory exchanges were such as to indicate an ability to deal with the products of an anaerobic metabolism. The brown trout exhibited increases in the output of NH_3-N, particularly at 15°C, during recovery. The phenomenon was suggested as representing a means of acid base maintenance in the presence of high levels of lactic acid. The increase in NH_3-N in recovery being a reflection of increased production of the free base, NH_3°, which combined with the H^+ from dissociated lactate to provide a method for the excretion of the acidic ion. This effect was not as marked in perch.

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APPENDIX I

Tables showing the raw data obtained from the respiration experiments

TABLE I OXYGEN CONSUMPTION, CARBON DIOXIDE AND NH_3-N

BROWN TROUT 5°C.

		O_2		CO_2		NH_3-N	
SPEED	DURATION	mg/kg.hr.	ml/kg.hr.	mg/kg.hr.	ml/kg.hr.	NH_3	TOTAL
0.7	60	93.3 ± 13.1 (6)	63.7 ± 8.8 (6)	45.1 ± 8.9 (3)	22.1 ± 4.0 (3)	2.7 ± 0.4 (5)	1.9 ± 1.1 (3)
	120	69.8 ± 7.4 (6)	47.8 ± 5.0 (6)	77.6 ± 20.9 (4)	38.1 ± 10.3 (4)	2.1 ± 0.5 (5)	2.8 ± 0.9 (3)
1.0	60	128.1 ± 17.4 (6)	87.8 ± 12.0 (6)	125.4 ± 41.9 (5)	61.8 ± 20.5 (5)	3.0 ± 0.4 (5)	4.4 ± 1.6 (3)
	120	107.2 ± 11.9 (6)	73.4 ± 8.0 (6)	109.3 ± 24.1 (6)	53.7 ± 11.8 (6)	3.4 ± 0.5 (5)	5.0 ± 1.0 (3)
1.4	60	178.5 ± 12.9 (3)	121.5 ± 9.7 (3)	339.9 ± 98.7 (3)	166.5 ± 49.3 (3)	4.8 ± 0.3 (2)	4.4 (1)
	120	162.6 ± 39.6 (3)	110.8 ± 27.6 (3)	290.8 ± 142.5 (3)	142.3 ± 70.5 (3)	5.1 ± 1.0 (2)	9.3 (1)

Speed - bl/sec.

Duration - minutes

NH_3 - ammonia only

Total - ammonia + urea ammonia

Numbers given are mean ± 1. Standard Error.

Numbers in brackets are numbers of fish tested.

TABLE 2 OXYGEN CONSUMPTION, CARBON DIOXIDE AND NH_3-N

BROWN TROUT 10°C

		O_2		CO_2		NH_3-N	
SPEED	DURATION	mg/kg.hr.	ml/kg.hr.	mg/kg.hr.	ml/kg.hr.	NH_3	TOTAL
0.7	60	154.6 ±13.2 (8)	103.2 ± 8.7 (8)	192.1 ±44.4 (8)	94.9 ±21.8 (8)	3.8 ±0.5 (7)	2.2 ±0.7 (6)
	120	130.8 ± 7.5 (8)	87.5 ± 5.2 (8)	116.8 ±26.9 (8)	57.7 ±13.1 (8)	3.2 ±0.7 (8)	3.7 ±0.7 (7)
1.0	60	151.4 ±6.8 (8)	101.1 ± 4.5 (8)	205.2 ±39.6 (8)	100.6 ±19.5 (8)	3.3 ±0.8 (8)	4.0 ±1.0 (7)
	120	132.6 ± 6.5 (8)	88.6 ± 4.5 (8)	181.1 ±39.6 (6)	90.3 ±19.5 (6)	3.2 ±0.8 (8)	4.4 ±1.0 (7)
1.4	60	221.1 ±25.5 (8)	147.8 ±17.2 (8)	283.3 ±63.3 (7)	128.0 ±29.9 (7)	5.3 ±0.5 (8)	4.8 ±1.2 (7)
	120	214.4 ±30.8 (7)	143.5 ±20.8 (7)	248.5 ±60.8 (5)	123.0 ±28.6 (5)	5.3 ±0.8 (7)	6.4 ±0.9 (6)
1.7	60	246.2 ±40.8 (2)	163.3 ±26.8 (2)	276.5 ±99.7 (2)	129.9 ±50.4 (2)	5.2 ±0.4 (3)	6.9 ±0.5 (3)
	120	195.0 ± 7.9 (2)	129.3 ± 5.4 (2)	179.2 ±67.2 (2)	90.7 ±24.0 (2)	5.7 ±1.5 (3)	5.5 ±1.3 (3)

TABLE 3 OXYGEN CONSUMPTION, CARBON DIOXIDE AND NH_3-N

BROWN TROUT 15°C

		O_2		CO_2		NH_3-N	
SPEED	DURATION	mg/kg.hr	ml/kg.hr.	mg/kg.hr.	ml/kg.hr.	NH_3	TOTAL
0.7	60	186.0 ±22.9 (7)	123.7 ±15.1 (7)	235.8 ±22.1 (7)	115.4 ± 9.6 (7)	5.3 ±0.9 (7)	5.8 ±1.4 (7)
	120	160.2 ± 6.5 (7)	106.5 ± 4.5 (7)	182.6 ±39.5 (3)	92.4 ±20.0 (3)	4.5 ±0.5 (7)	4.3 ±1.0 (7)
1.0	60	214.5 ±27.7 (7)	142.8 ±18.9 (7)	236.0 ±32.0 (7)	116.3 ±16.5 (7)	6.3 ±0.6 (7)	7.5 ±1.1 (7)
	120	200.6 ±20.0 (7)	133.4 ±13.5 (7)	162.9 ±53.4 (4)	82.0 ±27.3 (4)	5.2 ±1.0 (7)	3.7 ±1.2 (5)
1.4	60	263.4 ±21.0 (7)	175.4 ±14.7 (7)	262.8 ±15.9 (7)	129.5 ±8.5 (7)	7.6 ±0.7 (7)	8.7 ±2.4 (6)
	120	215.8 ± 8.4 (7)	143.4 ± 5.5 (7)	188.0 ±28.8 (7)	92.8 ±14.8 (7)	5.0 ±0.5 (7)	7.4 ±3.0 (7)
1.7	60	333.1 ±10.4 (7)	221.4 ± 6.7 (7)	345.5 ± 9.8 (7)	170.0 ± 5.1 (7)	7.1 ±0.6 (7)	7.9 ±1.8 (7)
	120	315.7 ±15.5 (7)	200.5 ± 9.9 (7)	260.3 ±28.9 (7)	128.3 ±14.9 (7)	4.3 ±0.6 (7)	6.9 ±1.5 (7)

TABLE 4 OXYGEN CONSUMPTION, CARBON DIOXIDE AND NH_3-N

PERCH 5°C

		O_2		CO_2		NH_3-N	
SPEED	DURATION	mg/kg.hr.	ml/kg.hr.	mg/kg.hr.	ml/kg.hr.	NH_3	TOTAL
0.7	60	87.8 ±16.5 (4)	59.8 ±11.7 (4)	86.9 ±42.4 (3)	44.0 ±21.4 (3)	2.6 ±0.5 (4)	3.4 ±0.9 (4)
	120	66.1 ± 9.0 (4)	44.8 ± 6.1 (4)	77.5 ± 6.9 (3)	39.2 ± 3.5 (3)	2.4 ±0.8 (4)	2.1 ±0.4 (4)
1.0	60	123.0 ±15.0 (4)	83.4 ±10.2 (4)	287.4 ±132.9 (4)	143.2 ±62.2 (4)	3.2 ±0.2 (4)	4.1 ±1.1 (4)
	120	127.9 ± 3.9 (4)	86.6 ± 1.9 (4)	219.4 ±57.5 (4)	111.0 ±29.6 (4)	2.3 ±0.7 (4)	2.5 ±0.3 (4)

TABLE 5 OXYGEN CONSUMPTION, CARBON DIOXIDE AND NH_3-N

PERCH 10°C

SPEED	DURATION	O_2		CO_2		NH_3-N	
		mg/kg.hr.	ml/kg.hr.	mg/kg.hr.	ml/kg.hr.	NH_3	TOTAL
0.7	60	118.8 ± 4.6 (7)	79.4 ± 1.8 (7)	249.9 ±66.4 (7)	126.4 ±33.6 (7)	2.7 ±0.7 (7)	2.4 ±0.7 (6)
	120	107.8 ± 4.5 (7)	71.9 ± 3.0 (7)	184.4 ±49.8 (6)	93.3 ±25.2 (6)	3.2 ±1.0 (7)	3.1 ±0.7 (7)
1.0	60	129.8 ±10.3 (7)	86.6 ± 6.8 (7)	237.5 ±48.5 (6)	120.2 ±24.5 (6)	2.9 ±0.5 (7)	3.4 ±0.7 (7)
	120	115.8 ± 7.9 (7)	77.3 ± 5.8 (7)	256.0 ±46.1 (6)	129.5 ±23.3 (6)	3.6 ±0.3 (7)	2.7 ±0.5 (7)
1.4	60	194.0 ±17.6 (3)	129.5 ±11.3 (3)	379.2 ±46.3 (3)	191.8 ±23.4 (3)	2.7 ±1.0 (3)	3.0 ±1.8 (3)
	120	179.0 ±10.5 (3)	119.5 ± 7.0 (3)	362.6 ±51.7 (3)	183.4 ±26.1 (3)	4.1 ±0.7 (3)	4.2 ±2.4 (3)

	O_2		CO_2		NH_3-N	
	mg/kg.hr.	ml/kg.hr.	mg/kg.hr.	ml/kg.hr.	NH_3	TOTAL
ROUTINE	78.2 ± 5.6 (15)	53.1 ± 3.8 (15)	102.2 ± 8.2 (12)	49.5 ± 4.0 (12)	2.8 ±0.2 (12)	2.6 ±0.3 (12)

TABLE 6 OXYGEN CONSUMPTION, CARBON DIOXIDE AND NH_3-N

PERCH 15°C

		O_2		CO_2		NH_3-N	
SPEED	DURATION	mg/kg.hr.	ml/kg.hr.	mg/kg.hr.	ml/kg.hr.	NH_3	TOTAL
0.7	60	131.2 ± 5.8 (6)	87.1 ± 4.0 (6)	134.5 ±18.9 (6)	65.4 ± 9.5 (6)	4.4 ±1.5 (6)	4.3 ±1.4 (6)
	120	138.1 ± 6.5 (6)	91.7 ± 4.3 (6)	118.6 ±12.9 (6)	57.3 ± 6.0 (6)	5.0 ±1.6 (6)	5.4 ±2.0 (6)
1.0	60	175.1 ±11.3 (6)	116.1 ± 7.4 (6)	198.5 ± 6.4 (6)	96.1 ± 2.7 (6)	4.1 ±1.0 (6)	4.0 ±0.8 (5)
	120	208.7 ±19.8 (6)	138.6 ±13.3 (6)	166.7 ±32.8 (4)	82.5 ±17.2 (4)	3.6 ±0.6 (6)	2.8 ±0.6 (5)
1.4	60	270.5 ± 9.0 (2)	180.6 ± 5.8 (2)	318.4 ±45.9 (2)	151.1 ±21.6 (2)	4.7 ±1.1 (2)	8.1 (1)
	120	198.2 (1)	132.5 (1)	178.4 (1)	84.8 (1)	1.7 (1)	3.0 (1)

	O_2		CO_2		NH_3-N	
	mg/kg.hr.	ml/kg.hr.	mg/kg.hr.	ml/kg.hr.	NH_3	TOTAL
ROUTINE	131.7 ±12.4 (12)	87.5 ± 8.1 (12)	153.5 ±13.0 (12)	77.6 ± 6.6 (12)	3.3 ±0.3 (12)	3.4 ±0.3 (12)

TABLE 7 OXYGEN CONSUMPTION, CARBON DIOXIDE AND NH_3-N

BROWN TROUT 5°C RECOVERY

		O_2		CO_2		NH_3-N	
SPEED	DURATION	mg/kg.hr.	ml/kg.hr.	mg/kg.hr.	ml/kg.hr.	NH_3	TOTAL
0.7	60	164.2 ±34.8 (5)	112.0 ±23.9 (5)	307.9 ±58.0 (5)	150.5 ±28.3 (5)	5.9 ±1.3 (4)	6.6 ±0.8 (3)
	120	125.8 ±17.3 (5)	85.9 ±12.0 (5)	213.3 ±56.0 (5)	104.4 ±27.5 (5)	5.8 ±1.1 (4)	6.6 ±1.3 (3)
	180	98.0 ±10.5 (5)	66.8 ± 7.2 (5)	393.1 ±97.8 (3)	191.2 ±47.9 (3)	4.7 ±0.8 (4)	5.2 ±0.4 (3)
	240	99.3 (1)	68.0 (1)	198.6 (1)	97.4 (1)		

TABLE 8 OXYGEN CONSUMPTION, CARBON DIOXIDE AND NH_3-N

BROWN TROUT 10°C RECOVERY

		O_2		CO_2		NH_3-N	
SPEED	DURATION	mg/kg.hr.	ml/kg.hr.	mg/kg.hr.	ml/kg.hr.	NH_3	TOTAL
0.7	60	284.1 ±44.9 (6)	189.6 ±30.0 (6)	407.6 ±104.5 (6)	199.0 ±51.1 (6)	10.0 ±1.2 (6)	9.4 ±2.1 (5)
	120	162.3 ± 7.0 (6)	108.2 ± 4.4 (6)	244.2 ±58.4 (3)	121.5 ±29.4 (3)	11.8 ±1.0 (5)	11.5 ±1.3 (4)
	180	169.2 ±10.7 (4)	113.1 ± 7.0 (4)	270.9 ±31.7 (3)	129.4 ±14.4 (3)	11.6 ±2.0 (3)	7.9 ±1.2 (2)
	240	130.8 ±33.2 (3)	87.5 ±22.1 (3)				

TABLE 9 OXYGEN CONSUMPTION, CARBON DIOXIDE AND NH_3-N

BROWN TROUT 15°C RECOVERY

		O_2		CO_2		NH_3-N	
SPEED	DURATION	mg/kg.hr.	ml/kg.hr.	mg/kg.hr.	ml/kg.hr.	NH_3	TOTAL
0.7	60	362.4 ±29.9 (7)	240.8 ±19.8 (7)	406.6 ±40.8 (7)	201.7 ±20.2 (7)	11.1 ±2.0 (7)	11.7 ±2.1 (7)
	120	227.4 ± 7.6 (7)	151.1 ± 4.9 (7)	262.2 ±27.1 (7)	129.6 ±14.5 (7)	15.8 ±0.8 (7)	13.5 ±2.0 (7)
	180	212.6 ± 9.9 (7)	141.5 ± 7.0 (7)	251.5 ±10.3 (7)	123.9 ± 6.0 (7)	15.4 ±1.0 (7)	12.4 ±1.2 (7)
	240	198.2 ±17.3 (6)	131.8 ±12.2 (6)				

TABLE 10 OXYGEN CONSUMPTION, CARBON DIOXIDE AND NH_3-N

PERCH 5°C RECOVERY

		O_2		CO_2		NH_3-N	
SPEED	DURATION	mg/kg.hr.	ml/kg.hr.	mg/kg.hr.	ml/kg.hr.	NH_3	TOTAL
0.7	60	139.3 ±11.0 (5)	94.2 ± 7.6 (5)	205.4 ±41.4 (5)	103.9 ±21.0 (5)	3.8 ±0.4 (5)	4.2 ±0.5 (5)
	120	97.1 ± 8.8 (5)	65.7 ± 6.9 (5)	211.5 ±36.2 (4)	107.0 ±18.3 (4)	3.4 ±0.6 (5)	3.4 ±0.7 (5)
	180	81.6 ± 6.7 (5)	55.2 ± 4.8 (5)	70.4 ±20.8 (3)	35.6 ±10.5 (3)	2.3 ±0.7 (4)	2.1 ±0.7 (5)
	240	68.4 ±7.4 (4)	46.1 ± 5.0 (4)				

TABLE 11 OXYGEN CONSUMPTION. CARBON DIOXIDE AND NH_3-N

PERCH 10°C RECOVERY

		O_2		CO_2		NH_3-N	
SPEED	DURATION	mg/kg.hr.	ml/kg.hr.	mg/kg.hr.	ml/kg.hr.	NH_3	TOTAL
0.7	60	174.3 ± 4.9 (7)	116.4 ± 3.8 (7)	469.1 ±57.5 (6)	237.3 ±29.1 (6)	6.2 ±0.8 (7)	7.5 ±1.3 (7)
	120	136.3 ±13.0 (6)	90.8 ± 8.6 (6)	287.2 ±64.4 (6)	145.3 ±32.6 (6)	6.2 ±1.1 (7)	6.1 ±1.4 (7)
	180	117.8 ± 8.9 (7)	78.6 ± 5.9 (7)	194.3 ±94.8 (5)	98.3 ±47.9 (5)	4.5 ±0.8 (6)	7.3 ±1.5 (6)

TABLE 12 OXYGEN CONSUMPTION, CARBON DIOXIDE AND NH_3-N

PERCH 15°C RECOVERY

		O_2		CO_2		NH_3-N	
SPEED	DURATION	mg/kg.hr.	ml/kg.hr.	mg/kg.hr.	ml/kg.hr.	NH_3	TOTAL
0.7	60	223.4 ±23.9 (6)	148.5 ±15.9 (6)	277.3 ±50.6 (6)	135.6 ±26.2 (6)	9.3 ±0.8 (6)	8.0 ±1.8 (6)
	120	175.4 ±9.1 (6)	116.6 ± 6.0 (6)	168.2 ±10.8 (6)	81.7 ± 6.1 (6)	9.4 ±1.0 (5)	11.1 ±1.5 (6)
	180	161.9 ± 5.7 (5)	107.3 ± 3.8 (5)	208.5 ±10.9 (4)	98.7 ± 5.0 (4)	11.1 ±0.4 (3)	8.8 ±1.5 (4)
	240	172.4 ±10.4 (4)	114.4 ± 7.3 (4)				

APPENDIX II

Showing the physical characteristics of the fish used in the study.

TABLES 13 and 14

The data is given as the mean ± 1.S.E. (standard error), the number of samples being in brackets.

C.F. = condition factor

$$= \frac{W}{L^3} \times 100$$

TABLE 13 PHYSICAL CHARACTERISTICS OF THE BROWN TROUT USED IN THIS STUDY

TEMP. °C	WEIGHT (g)	LENGTH (cm)	C.F.
5	89.0 ± 6.2 (6)	20.2 ± 0.4 (6)	1.07 ± 0.0 (6)
10	89.5 ± 2.7 (8)	20.4 ± 0.2 (8)	1.05 ± 0.0 (8)
15	95.0 ± 4.4 (7)	20.4 ± 0.3 (7)	1.11 ± 0.0 (7)

TABLE 14 PHYSICAL CHARACTERISTICS OF THE PERCH USED IN THIS STUDY

TEMP. °C	WEIGHT (g)	LENGTH (cm)	C.F.
5	97.1 ± 3.4 (4)	19.3 ± 0.2 (4)	1.34 ± 0.000 (4)
10 Routine	119.5 ± 19.0 (5)	19.7 ± 0.9 (5)	1.50 ± 0.04 (5)
10 Active	101.4 ± 5.9 (7)	19.3 ± 0.3 (7)	1.39 ± 0.00 (7)
15 Routine	116.1 ± 7.0 (4)	19.9 ± 0.5 (4)	1.45 ± 0.05 (4)
15 Active	145.9 ± 11.4 (6)	21.2 ± 0.4 (6)	1.51 ± 0.40 (6)

APPENDIX III

TABLE 15 SOME VALUES OF STANDARD METABOLIC RATE OBTAINED BY EXTRAPOLATION OF ACTIVITY X OXYGEN CONSUMPTION REGRESSION LINE TO ZERO ACTIVITY

SPECIES	TEMP. °C	STANDARD	REGRESSION LINE	AUTHOR	COMMENTS
Oncorhynchus nerka	5	40.7	LOG.Y = 1.61 + 0.34 X	BRETT 1964	Used water tunnel respirometer. Extrapolated to zero activity
	10	60.3	LOG.Y = 1.78 + 0.28 X		
	15	70.8	LOG.Y = 1.85 + 0.27 X		
	20	120.2	LOG.Y = 2.08 + 0.22 X		
	24	195.0	LOG.Y = 2.29 + 0.17 X		
Carassius auratus	20	57.8	LOG.Y = 1.7622 + 0.0733 X	SMIT 1965	Used anular chamber. Calculated from data of Table D I
Perca fluviatilis	10	27.0	LOG.Y = 1.4314 + 0.5560 X	TYTLER (pers.comm.)	Tunnel respirometer (Blazka)
Salmo gairdneri	15	275.0	LOG.Y = 2.4393 + 0.0394 X	KUTTY 1968	Tunnel respirometer (Blazka) Annular chamber.
Carassius auratus	20	164.9	LOG.Y = 2.2172 + 0.1276 X		Calculated from Tables IV and V.

TABLE 15 SOME VALUES OF STANDARD METABOLIC RATE OBTAINED BY EXTRAPOLATION OF ACTIVITY X OXYGEN CONSUMPTION REGRESSION LINE TO ZERO ACTIVITY

SPECIES	TEMP. °C	STANDARD	REGRESSION LINE	AUTHOR	COMMENTS
Oncorhynchus nerka	5	40.7	LOG.Y = 1.61 + 0.34 X	BRETT 1964	Used water tunnel respirometer. Extrapolated to zero activity
	10	60.3	LOG.Y = 1.78 + 0.28 X		
	15	70.8	LOG.Y = 1.85 + 0.27 X		
	20	120.2	LOG.Y = 2.08 + 0.22 X		
	24	195.0	LOG.Y = 2.29 + 0.17 X		
Carassius auratus	20	57.8	LOG.Y = 1.7622 + 0.0733 X	SMIT 1965	Used anular chamber. Calculated from data of Table D I
Perca fluviatilis	10	27.0	LOG.Y = 1.4314 + 0.5560 X	TYTLER (pers.comm.)	Tunnel respirometer (Blazka)
Salmo gairdneri	15	275.0	LOG.Y = 2.4393 + 0.0394 X	KUTTY 1968	Tunnel respirometer (Blazka) Annular chamber.
Carassius auratus	20	164.9	LOG.Y = 2.2172 + 0.1276 X		Calculated from Tables IV and V.

TABLE 15 (contd.)

SPECIES	TEMP.°C	STANDARD	REGRESSION LINE	AUTHOR	COMMENTS
Salmo gairdneri	15	75.5	LOG.Y = 1.878 + 0.47 X	WEBB (1971b)	Tunnel respirometer. Control group.
Salmo	5	30.1	LOG.Y = 1.4785 + 0.5183 X	THIS STUDY	Tunnel respirometer.
trutta	10	82.7	LOG.Y = 1.9175 + 0.2471 X		
	15	105.5	LOG.Y = 2.0233 + 0.2511 X		
Perca	5	12.8	LOG.Y = 0.9395 + 0.9978 X		
fluviatilis	10	63.5	LOG.Y = 1.6278 + 0.2926 X		
	15	54.5	LOG.Y = 1.5581 + 0.5741 X		

TABLE 16 SOME VALUES OF STANDARD METABOLIC RATE OBTAINED BY EXTRAPOLATION OF SPONTANEOUS ACTIVITY TO ZERO

SPECIES	TEMP. °C	STANDARD	AUTHOR	COMMENTS
Salmo gairdneri	15	95	SKIDMORE (1970)	Fish confined in very small box
Salmo gairdneri	5 10 15 20 25	36 42 78 84 138	DICKSON and KRAMER 1971	Used a flowmeter, extrapolated to zero spontaneous activity.
Salmo trutta	10	80.8	BEAMISH 1964 (b)	Used a flowmeter, extrapolated to zero spontaneous activity.
Salvelinus fontinalis	10 15 20	79.3 107.1 147.0		
Cyprinus carpio	10 20 30 35	17.0 48.0 104.5 117.3		
Ictalurus nebulosus	10 20 30	20.0 66.1 106.1		
Catostomus commersonii	10 15 20	34.7 78.5 110.4		
Carassius auratus	10 20 30 35	15.7 30.1 72.0 127.0	BEAMISH and MOOKHERJII 1964	Used a flowmeter, extrapolated to zero spontaneous activity.
Salmo gairdneri	5 15	57.0 112.0	RAO 1968	Annular chamber + photo cells.

APPENDIX IV

Solution of energy substrate simultaneous equations.

$$P = N/0.16$$

$$L = \frac{(\dot{V}O_2 - (\dot{V}CO_2 - 0.75\ P))}{0.57} - 0.94\ P$$

$$C = (\dot{V}O_2 - (\frac{1.96}{1.39}\ \dot{V}CO_2 - 0.75\ P\ )) - 0.94\ P$$

$$(-\frac{1.96}{1.39} \times 0.81) + 0.81$$

All calculated values being in mg/kg.hour.

APPENDIX V

Light induced mortality in Brown Trout

During the laboratory acclimation of the brown trout to exercise conditions prior to use in respiration studies, a fungal infection was seen to develop. The first occasion that this occurred was in early December at a water temperature of about 5°C. The cause was suggested as being a possible hormonal change associated with maturity which rendered the skin susceptible to water or mucous borne fungal spores.

At the end of February when the water temperature in the stock tank was about 8°C, a batch of brown trout including two rainbow trout was transferred from the stock tank into an exercise tank. Over the course of the next seven days the temperature in the exercise tank was raised to 10°C in small increments. The conditions were such that the fish were subjected to water velocities of between 0.5 and 1.0 bls/sec. About twelve days after introduction some of the brown trout darkened in colour and were seen to be infected with fingus. The site of infection did not appear to be specific but tended to arise on the dorsal body on and around the dorsal adipose and caudal fins (Plate III). The entire group of fish were isolated and dosed with malachite green. After three more days all the brown trout had died but the rainbow trout which had not displayed any visible signs of infection, were healthy. This situation was repeated three times in all, each time the rainbow trout did not become infected.

As no fish in the stock tank had developed similar symptoms and no cross infection between diseased and stock brown trout in an isolation tank could be achieved, the indication was that the disease

conditions existed only in the exercise tank. A pathology report (Roberts pers. comm.) indicated that there was no observable difference between the histology of the adrenal or pituitary in the stock and moribund fish, but the skin of the stock fish was healthy and intact whereas that of the dying fish - even on non infected areas - was degenerating and its dermal vessels were congested. As the exercise tank was made of the same material as the stock tank, received water from the same source as the stock tank and the fish density was approximately the same; the fungal infection could only have been induced by either exercise conditions or the physical dimensions of the exercise tank. Although the same shape, the exercise tank was only about half the physical size of the stock tank. Using pieces of plastic drainpipes as cover for the fish, the effect of exercise was found to exert no deteriorous effect.

The only remaining possibility was that of light intensity, both tanks being positioned under fluorescent tube illumination sources. It was found that by reducing the light intensity at the surface of the exercise tank to about 10 lux, by placing a piece of board over the tank, that fungus infection ceased. Although the light insensity at the surface of the stock tank was about 200 lux, the extra depth of water present acted as a light filter. By reducing the intensity of light at the surface of the stock tank to about 100 lux by blanking off part of the illumination, and covering the exercise tank, no further fungal infection was observed.

Plate II. Light induced fungal infection of brown trout.