Intraspecific variation in the metabolism of juvenile Atlantic salmon salmo salar and northern pike esox lucius

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Intraspecific variation in the metabolism of juvenile Atlantic salmon *Salmo salar* and northern pike *Esox lucius*

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Abstract

Basal metabolic rate (BMR), the sum of maintenance energy costs, represents a major component of the energy budgets of ectothermic vertebrates and varies between individuals within a species. Individual ectotherms are generally assumed to have a constant BMR at any given temperature. A strategy of flexibility in BMR might have evolved to cope with differing environmental conditions. Within-individual variation in BMR was examined in two fishes, juvenile Atlantic salmon *Salmo salar* and juvenile northern pike *Esox lucius*, whilst the effects of exercise and ration on BMR, maximum metabolic rate (MMR), enzyme levels and body composition were studied in detail for pike.

In the first part of the study, measurements of BMR were made for first-summer Atlantic salmon parr at rest by respirometry. In 1996, initial measurements of BMR were made for 25 fish held in a stock tank. Fish were then allocated in small groups to channels to represent a change in environment and BMR re-measured after a period of several weeks. The procedure was repeated for 30 fish in the summer of 1997, when fish were given a reduced food ration. Variation in BMR in each experiment was analysed for individual fish, and for all fish using a linear mixed model. There were statistically significant differences in BMR values between the two times, the within-individual, between-time variation representing approximately ± 21% of BMR in 1996 and ± 28% of BMR in 1997. Reduced-ration fish (1997) displayed a significant decrease in the mean elevation of the allometric scaling relationship between body mass and BMR between time periods.

To further explore possible mechanisms for flexibility in BMR and relationships with MMR, juvenile pike were used. Initial measurements of BMR and MMR (following exhaustive exercise) were made and factorial metabolic scope calculated (MMR/BMR). Fish were then split into a high ration no-exercise group (n = 10), low ration no-exercise group (n = 10) and sustained exercise group (n = 13). Initial measurements were termed time 1, with subsequent measurements made after approximately 3 weeks (time 2) and 11 weeks (time 3). Exercised fish had a significantly larger MMR and scope following 3 weeks of sustained swimming. For all fish there were significant correlations between BMR and MMR at times 1 and 3 but not at time 2.

After the oxygen measurements made at time 3 all fish were humanely killed. Maximal enzyme assays were performed on six tissues for each remaining fish (n = 30). Levels of each of two enzymes (citrate synthase, CS, and lactate dehydrogenase, LDH, measured in the direction of lactate oxidation) were found to be similar between treatment groups for respective tissues. Total CS activity levels and LDH levels were highest in the heart and red muscle. In general there was little difference in the relative organ masses of fish exposed to different treatments.

It is concluded that in these two fish species with very different life styles, between- and within- individual variation in BMR (salmon & pike) and MMR (pike only) is apparent and that differences in ration and exercise influence individual physiology.

Declaration

The material contained within this thesis has not previously been submitted for a degree at the University of Durham or any other University. The author has conducted the research reported within this thesis unless indicated otherwise.

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Table of Contents

Glossary of abbreviations used throughout this thesis

LDH	lactate dehydrogenase
CS	citrate synthase
CCO	cytochrome c oxidase
BMR	basal metabolic rate
MMR	maximum metabolic rate
SMMR	sustained maximum metabolic rate
SMR	standard metabolic rate
Scope	factorial metabolic scope (MMR/ BMR)
ASDA	apparent specific dynamic action
wbm	wet body mass
$BMR_{(adj)}$	mass standardised BMR
$MMR_{(adj)}$	mass standardised MMR
$Scope_{(adj)}$	mass standardised metabolic scope
BLs^{-1}	body lengths per second
U_{crit}	critical swimming speed
$\dot{V}O_2$	rate of oxygen consumption
SE	standard error of the mean
log	logarithm to the base 10
$\log_e$	natural logarithm
FAD	Flavin adenine dinucleotide
NAD	Nicotinamide adenine dinucleotide

Chapter One

General Introduction

1.1 What is intraspecific variation in metabolism?

Energy expenditure is often used as a key factor in ecological modelling (Charnov 1976; Sibly & Calow 1986) and has been used in assessments of how animals adjust to the environmental conditions in which they live. Animals often have to make behavioural and physiological adjustments to account for changes in environmental conditions, which reflect alterations in food supply and resource availability. All animals do not necessarily make the same adjustments in response to variable or limiting resources. Animals may migrate to another area, enter a state of torpor, or alter their behaviour to accommodate the limiting resource. The rate of energy expenditure of an ectothermic organism is highly dependent upon various factors, including ambient temperature, nutritional status and levels of activity, leading to the need for defined conditions under which the energy state is measured.

Intraspecific variation in metabolism is defined within this thesis as the difference in metabolic rate that may occur within and/ or between individuals of the same species. Throughout this thesis the term 'plasticity' is used to mean a reversible change in a physiological character such as basal metabolism or organ size of an individual in response to either a physiological or environmental change. The property of variable growth in fish has been referred to as an example of physiological plasticity by Weatherley & Gill (1990).

1.2 Energy allocation

Fish, like all animals, use ingested food to provide molecular precursors for the synthesis of tissue and as the energy supply for all metabolic processes, including the synthesis of body and reproductive tissues (Figure 1.1). The rate at which energy-using metabolic processes utilise energy is termed metabolic rate (Kleiber 1947). A simple energy budget can be used to describe the fate of ingested food:

$$C = P + R + E$$

where C = food consumption, P = somatic and gonadal growth, R = metabolic costs of respiration and E = is the energy contained in waste products (Winberg 1956).

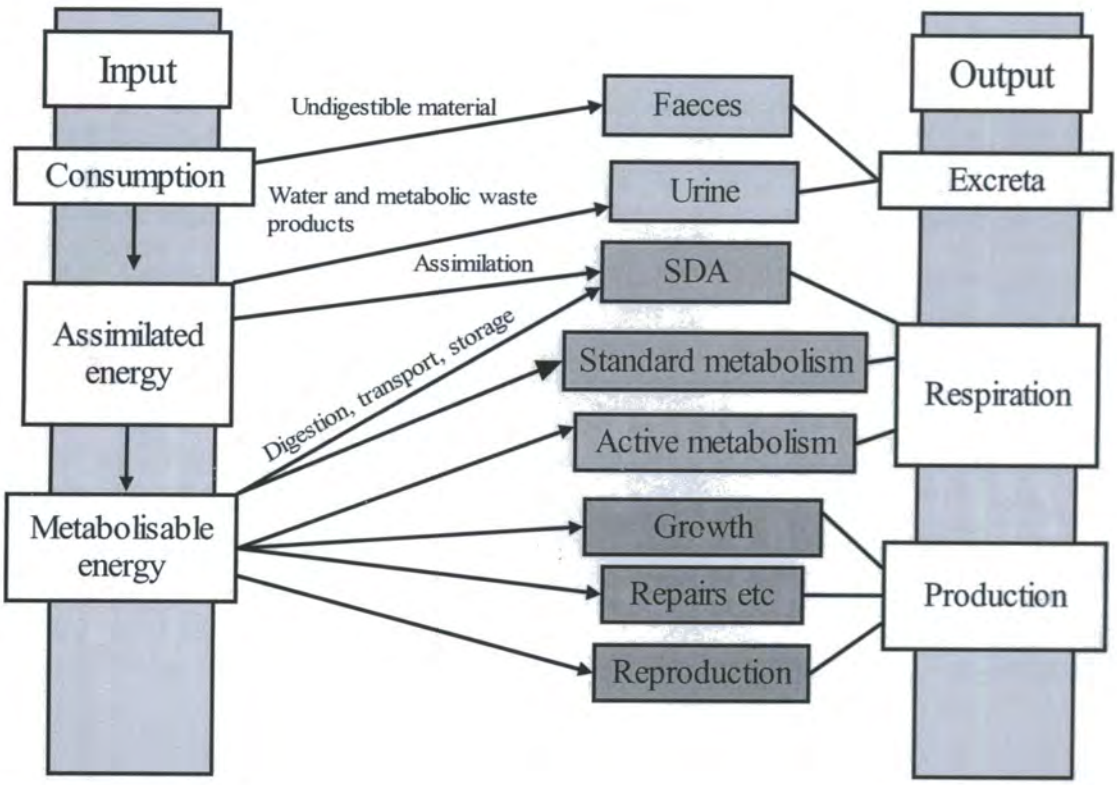


Figure 1.1 The metabolic fate of metabolised energy. Redrawn from Videler (1993).

The basic equation is usually expanded to the form:

$$C = P + R + F + U$$

where F and U represent the energy lost in faeces and excretory products respectively. Total metabolism (R) can be further split into the costs of basal metabolism (Rs), activity (Ra) and digestion of food (Rd):

$$C = P + Rs + Ra + Rd + F + U$$

Following the ingestion of food there is an increase in the metabolic rate. This postprandial increase in metabolism is due to the metabolic costs of digestion in terms of biological and physiological processes, is usually termed heat increment or specific dynamic action (SDA) (Jobling 1981, 1985), or apparent specific dynamic action (ASDA) (Beamish 1974). These costs include the energy used in the absorption, deamination of proteins, mechanical digestion, assimilation and storage of new material. This is thought principally to relate to the metabolic costs of the synthesis of new macromolecules from absorbed precursors, of which protein synthesis seems to be most important (Brown & Cameron 1991). Throughout this thesis the term ASDA will be used. This form of the Winberg equation is the one that is used as the basis of most bioenergetics models today. For fish, the bioenergetics approach is most commonly used to estimate food consumption by fish or the growth of wild fish in different circumstances (Ney 1993).

1.3 Basal metabolic rate

Basal metabolic rate (BMR) is the primary drain on the aerobic energy reserves of both endothermic and ectothermic animals and is the cost of maintaining cells and organs in readiness for higher levels of biological activity (Calow 1985; Hawkins 1991; Ricklefs *et al.*1996). Energy is expended for body maintenance including protein turnover (Hawkins 1991) and supplying oxygen to resting tissues (Farrell & Steffensen 1987). For homeothermic vertebrates, BMR or minimal metabolism represents the minimal rate of heat production in the absence of muscular activity, food consumption and its subsequent processing (Blaxter 1989). Within homeotherms, BMR is therefore measured over an animal's thermoneutral zone. In ectotherms, without physiological control over thermoregulation, BMR will vary with ambient temperature and so can only be referenced to specific temperatures.

In birds and mammals the measurement of both O_2 consumption and CO_2 production enables the calculation of the respiratory quotient (RQ) and therefore the respiratory

substrate being metabolised. In aquatic systems, however, this is considerably more difficult due to the very high solubility of CO_2 in water. In fish, a direct calorimetric measurement of energy expended (heat production) has been considered unsatisfactory due to the relatively low heat production of the fish and the high heat capacity of the water in the system, (Brett & Groves 1979). The usual indirect measurement of metabolism for fish is in the form of oxygen consumption rate. Metabolic rates of fishes, expressed in energy units can be derived from the rates of oxygen consumption ($\dot{V}O_2$) using appropriate conversion factors based on respiratory substrate (Brett & Groves 1979).

1.4 Effects of activity and feeding on metabolism

Many fish species rarely display complete inactivity under natural conditions or when placed in a box respirometer. These movements elevate the oxygen consumption above that of a totally resting fish (Brett 1964; Beamish 1978), complicating measurements. If the spontaneous levels of activity of the fish are not high then the oxygen consumption measurements can be termed 'routine' or 'low routine'. However, the use of routine measurements is usually so ill defined that they may be of limited use, as it is not normally known how active individual fish were. Some species of fish, however, such as walleye *Stizostedion vitreum vitreum* and northern pike *Esox lucius* (referred to as pike throughout the rest of this thesis), may exhibit such low levels of spontaneous activity that the oxygen consumption measurements can be directly equated with BMR (Tarby 1981; Diana 1982a). Even for more active species such as Atlantic salmon *Salmo salar* parr it may be possible to modify the experimental conditions (e.g. by lowering the ambient light intensity) to encourage minimal activity. Implicit in such measurements of resting metabolic rate is that the activity levels must be monitored and data used only when inactivity during and prior to the measurements can be confirmed.

A common approach to account for the metabolic costs of activity and to estimate the BMR of a fish is to force it to swim continuously at a succession of different swimming speeds within a flume respirometer (e.g. Brett 1964; Beamish 1978; Gerhke *et al.* 1990). The relationship between swimming speed and metabolic rate is then extrapolated back to zero swimming speed, at which the metabolic costs are considered not to be associated with any activity. This derived estimate is termed standard metabolic rate (SMR). However, SMR may underestimate the true costs of BMR (e.g.; Forstner & Wieser 1990; Lucas *et al.* 1993). An overestimation of the true cost of BMR can also

occur, in flatfish a comparison of both the resting metabolic rate and extrapolation to zero swimming speed shows resting metabolism corresponds to approximately 60 % of the extrapolated value (Duthie 1982). This is reported to be due to the extra energy exerted in postural swimming effects for flatfish, which have no swim bladder and are negatively buoyant in water (Priede & Holliday 1980; Duthie 1982). There are also fundamental problems with estimating SMR for a number of fish species. For those species of fish that will swim in a respirometer, some individuals may refuse to do so (e.g. Priede & Holliday 1980), or will not consistently and voluntarily swim against a water current (Soofiani & Priede 1985; Axelsson & Nilsson 1986; Adams & Parsons 1998). This means that only those individuals in a population that will swim can be used, hence causing a potential bias in the sample.

A critical feature of the measurement of minimal metabolism is the length of time that animals need to be starved to ensure that the effects of previous meals are negligible and that the animals are in a post-absorptive state.

1.5 Effects of body size on BMR

To assess many of the effects of different variables that affect metabolic rate, the measurement of metabolic rate has to be referred to a base line that takes into account body size. Kleiber (1947) and Brody & Proctor (1932) both showed that minimal metabolism in homeothermic vertebrates were related to body mass (over a wide range of body masses from those of a mouse to an elephant), by the relationship of body mass to the power of 0.734 (Brody & Proctor) and 0.739 (Kleiber). Due to the almost simultaneous timing of both sets of results, the relationship became known as the Brody-Kleiber law. To ease in the aid of calculations, and allowing for the inherent errors in the data, a generally accepted value of 0.75 was taken. This generalisation for homeotherms, however, had limitations as the relationships were measured on a very limited number of bird and mammal species. More recently Hayssen & Lacy (1985) measured the body mass-metabolism relationships for mammals of 293 species and 14 orders of animals and found that minimal metabolism varied within taxonomic orders and that not all orders conformed to the Brody-Kleiber relationship. A good review of the scaling of BMR with body mass is provided by Schmidt-Nielsen (1984).

In fish, which are mostly ectothermic vertebrates, there is less of an effect of size on BMR than seen for homeotherms, with the mean b (slope) values from a plot of log

metabolic rate vs. log body mass falling between 0.8-0.9 instead of the value of 0.75 reported for mammals and birds (Brett & Groves 1979). The rate of decrease in oxygen consumption per unit mass is such that, for instance, in sockeye salmon *Oncorhynchus nerka*, the rate of basal metabolism per unit mass for a 3000 g adult is approximately one fifth of that for a 1g fry (Brett & Glass 1973).

1.6 Effects of temperature

It has long been known that BMR of ectothermic vertebrates is highly dependent on ambient temperature (Krogh 1914; Winberg 1956; Brett 1962). Temperature is also well known to influence the rates of chemical reactions, and the process of metabolism is essentially a series of chemical reactions. Water temperature has been identified as the most important abiotic factor affecting the physiology and growth of fish (Brett 1979; Jobling 1997). Overall, the range of temperatures tolerated by different life forms is quite wide. However, individual species of fish show characteristic temperature preferences and limited tolerances (Schulman & Love 1999). On transferral from one temperature regime to another, fish require time to acclimate to the new temperature, with 'acute' changes leading to an increased BMR compared to a suitably acclimated fish (Cossins & Bowler 1987; Evans 1990).

1.7 Maximum aerobic metabolic rate

In fish, active metabolic rate (AMR) is usually measured as the maximal oxygen consumption for a fish swimming aerobically at its maximum sustainable swimming speed, given adequate metabolic substrates (Brett 1962; Fry 1971; Brett & Groves 1979). However, an increase in oxygen consumption above that measured for AMR during sustained swimming, is found when fish that have a 'low sustained swimming speed' such as cod, are recovering from being chased to exhaustion (Reidy *et al*, 1995, Reidy *et al*, 2000). This indicates that the upper aerobic limit of fish cannot necessarily be measured during swimming. This upper aerobic limit for the fish is termed maximum aerobic metabolic rate (MMR) by various authors (e.g. Bushnell *et al.* 1994). The MMR of an animal is the upper limit of aerobic energy production set by the maximal rate of oxygen transfer to tissues (Weibel *et al.* 1996).

The swimming performance of fishes in relation to aerobic metabolism has traditionally been measured by using a graded series of velocity tests, with a fish having to swim at a set speed that is slowly increased by known increments at set time intervals using a

'Brett type' or similar flume swimming respirometer. The swimming respirometer incorporates a swimming 'tunnel' area in which the test subject is placed. A series of honeycomb meshes provide a rectilinear flow and keeps the fish in the swimming area. The rear end of the swimming tube often has an electrified mesh that gives out a small shock to the fish if it touches it and prevents the fish from resting at the back of the swimming area. The whole apparatus is sealed and a motor used to recirculate the water into the swimming arena, enabling adjustment of the water speed as required. A fish is made to swim at a set water velocity for a specific time period, the duration of which has varied between different studies, including 20 minutes (Kolok 1992), 30 minutes (Schurmann & Steffensen 1997; Reidy *et al.* 2000) or 1 hour (Brett 1964; Brett & Glass 1973; Taylor & Foote 1991; Adams & Parsons 1998). The current velocity and hence swimming speed is increased in a series of steps for a certain period of time until exhaustion occurs (Hammer 1995). The fatigue speed or critical swimming speed (U_{crit}) for increased velocity tests is calculated as:

$$U_{crit} = V_p + \left\{ \left[\frac{t_f}{t_i} \right] \times V_i \right\}$$

Where V_i is the velocity increment in (cm/sec), V_p is the penultimate velocity at which the fish swam before fatigue, t_f is the elapsed time from the velocity increase to fatigue, and t_i is the time between velocity increments (Brett 1964, Brett & Sutherland 1965).

Much effort has been put into calibrating this methodology to standardise results collected (Farlinger & Beamish 1977; Beamish 1978). Ideally U_{crit} is a highly reproducible variable (Reidy *et al.* 2000). However, the size of the water speed increment and time between increments can have a major effect on the results (Davison 1997). The swimming behaviour of fish can fall into one of three general categories: sustained, prolonged or burst. Sustained swimming occurs at relatively slow swimming speeds and utilises predominantly red muscle fuelled from aerobic metabolism (Beamish 1978). Fish are able to maintain sustained swimming speeds for long periods (at least 200 minutes) without tiring (Beamish 1978). Burst swimming involves white muscle and utilises energy from anaerobic processes, enabling high speeds for 15-20 seconds (Beamish 1978). Finally, prolonged swimming involves the use of both red and white muscles and covers a variety of swimming velocities between sustained and burst swimming (Beamish 1978). In fact AMR measurements made in numerous

studies are associated with prolonged rather than sustained swimming speeds, since for logistical reasons, time between speed increments is usually 30-60 minutes.

As the swimming speed increases so there is an increase in the recruitment of white glycolytic (fast) muscle fibres with increasing speed, until the fish finally becomes exhausted (Pearson *et al.* 1990). Because the U_{crit} protocol involves both the use of aerobic and anaerobic fibre contributions to swimming performance (Nelson *et al.* 1994), it may give little information about either system in isolation (Reidy *et al.* 1995). The difference between aerobic and anaerobic performance may have important consequences. Aerobic performance is typically associated with sustained migratory swimming while anaerobic performance, with bursts of speed, is used typically to escape from predators, or to catch prey, or to jump rapids. The swimming performance of salmonids, which are 'athletic' fish, may not really be representative of the majority of other fish species (Goolish 1991). Most fish species generally have a lower swimming ability than salmonids, and do not undertake the large-scale migrations that often occur for salmonids during the course of their life times.

For those fish that do not perform well in a swimming respirometer, an alternative way of eliciting a maximal metabolic response has been to chase the fish to exhaustion (Black 1958, Soofiani & Priede 1985; Armstrong *et al.* 1992) and to measure maximal oxygen consumption (MMR) during the subsequent oxygen debt repayment. On comparison of both a chase protocol and U_{crit} protocol for Atlantic cod *Gadus morhua* a significantly higher rate of oxygen consumption was found following the former (Reidy *et al.* 1995). Soofiani & Priede (1985) reported that in Atlantic cod maximal $\dot{V}O_2$ was reached not during exercise in a flume respirometer, but during recovery from exhaustive exercise, thus suggesting that the aerobic capacity of cod had evolved to meet the energetic demands of recovery rather than activity. The finding was reported frequently due to the unique metabolic strategy that it represented (e.g. Goolish 1991; Lucas & Priede 1992; Armstrong *et al.* 1992). However, the conclusions drawn by Soofiani & Priede (1985) were based on the results of pre and post exhaustion data from two different experiments, with the validity of these results being questioned by Tang *et al.* (1994). Reidy *et al.* (1995), on comparing the two techniques used to exhaust the same individual Atlantic cod, found that $\dot{V}O_2$ was higher for all Atlantic cod on recovery from a chase protocol than that reached during swimming at U_{crit}. Schurmann & Steffensen (1997) confirmed the results of Tang *et al.* (1994), in Atlantic cod, that for

fish swimming at U_{crit}, maximal $\dot{V}O_2$ was measured during swimming and not on recovery from swimming to U_{crit} as had been suggested by Soofiani & Priede (1985). The chase protocol is believed to result in a higher $\dot{V}O_2$ due to the larger metabolic disturbances caused by exhaustive exercise (Milligan 1995).

1.8 Metabolic scope

Metabolic scope, also know as 'scope for activity' (Fry 1947) is defined as the difference between BMR and MMR and reflects the range of aerobic energy expenditure within which an animal must function. This scope has to account for all the requirements of a fish or other animal over and above basal costs including those of digestion, activity, reproduction and food capture. Priede (1985) suggested that generally those fish species with high maximum metabolic rates also have large metabolic scopes , but also high BMRs, reflecting the increased costs of maintaining more metabolic machinery even when the animal is at rest. Usually the increase in maximal metabolic rate is much larger than the increase in overall BMR, so representing a real increase in metabolic scope (Priede 1985).

A major assumption of the aerobic capacity model suggested by Bennet & Ruben (1979) for the evolution of endothermy in vertebrates is that sustained maximal and basal metabolic rate are somehow linked and thus changes in the two traits cannot occur independently (Bennet & Ruben 1979; Ruben 1995). They suggest that the selection for a sustainable maximal aerobic metabolic rate (SMMR) resulted in the evolution of a higher BMR. This increased aerobic capacity would enable the animals to exercise longer and at higher levels, which would be highly advantageous in terms of capturing prey, escaping predators, defending territories or competing for resources (Hayes & Garland 1995). Such animals may also be able to forage over a larger area, due to a higher speed of locomotion (Hayes & Garland 1995). A necessary mechanistic link between both BMR and SMMR has yet to be properly demonstrated in terms of either the degree of proton leakiness of the mitochondrial membrane or a suitable genetic link (Else & Hulbert 1981; Hulbert & Else 1989, 1990; Porter & Brand 1993; Ruben 1995; Swallow *et al.* 1998). Standard metabolic rate, as estimated by the methods described earlier, has been suggested to be positively correlated to MMR and metabolic scope in fish on an interspecific basis (Brett & Groves 1979) although evidence for an intraspecific link between BMR and MMR is weak (Armstrong *et al.* 1992).

For fish there is weak evidence, at an interspecific level, for a positive relationship between relative (factorial) metabolic scope (MMR /BMR) and activity when comparing both low and high activity fish (e.g. Liao & Lucas 2000). Those fish with more sedentary habits and the weakest swimming abilities tend to have the lowest relative metabolic scopes, and they also tend to have the lowest BMR values. In some fishes with poor swimming capabilities such as juvenile Atlantic cod, juvenile pike and common blenny *Blennius pholis*, ASDA can occupy almost the entire metabolic scope (Vahl & Davenport 1979; Soofiani & Hawkins 1982; Soofiani & Priede 1985; Armstrong *et al.* 1992). Armstrong *et al.* (1992) hypothesised that juvenile pike in the wild may operate near to MMR for the majority of the time. Priede (1985) suggested that there were 'cod' type fish in which feeding metabolism may occupy a large proportion of the maximal aerobic metabolic rate and where metabolic scope can just accommodate peaks in digestion metabolism. He suggested that in these 'cod' type fish the metabolic scope is used principally to accommodate visceral metabolism. By contrast in 'trout' type fish maximum aerobic metabolic rate is reached during maximal swimming and the metabolic scope is large. Even at maximal ration, with associated feeding metabolism costs, substantial scope is still available for aerobic fuelling of locomotion.

Priede's (1985) review summarises the general opinion that in fish there must be a continual conflict between the metabolic costs of digestion and the costs of activity. These two metabolic activities are viewed as mutually exclusive and if a fish or other vertebrate is maximally exercising, it will be unable to proceed with any digestion. This assumes that the maximal sustained aerobic metabolic rate of the animal is that attained during maximal swimming (Vahl & Davenport 1979). The basis of apportioning energy between the conflicting demands of swimming and digestion has been referred to as power budgeting by Priede (1985). The energy balance equation of Winberg (1956) assumes that there is independence between the two components, the costs of digestion and costs of swimming (Beamish & Trippel 1990). However, more recent work indicates that there is an interaction between the two (Axelsson & Fritsche 1991; Blaikie & Kerr 1996). Changes in swimming speed were found significantly to affect the duration and magnitude of ASDA in Atlantic cod and the efficiency of digestion were reduced as activity levels increased (Blaikie & Kerr 1996).

The scaling of MMR vs. log body mass has been reported to have an exponent of 1 (isometric) for various fish species. An exponent of 1 or greater observed for sockeye salmon, has been associated with sustainable aerobic activity (AMR) by Brett & Glass 1973; but with recovery from burst exercise (MMR) measured for walleye (Tarby 1981), and pike (Armstrong *et al.*1992); and for rainbow trout *Oncorhynchus mykiss* (Wieser 1985). The values for the allometric scaling of BMR for most fish species are less than 1, typically at about 0.82 (Brett 1979). So long as the scaling exponent for AMR is higher than that for BMR the overall metabolic scope will increase with increasing body size as seen in sockeye salmon (Brett & Glass 1973). Goolish (1991), however, believes that the increase in circulation time of blood with increasing body size as well as other physiological constraints means that it is highly unlikely that the maximum aerobic metabolic rate can either remain isometric or relatively increase with increasing body size. In a recent article Bishop (1999) argues from the combined data of six species of birds and nine species of mammals, that BMR scales at a factor of 0.73, which closely matches the scaling coefficient of 0.75 reflected in the Kleiber-Brody law (1932). Bishop (1999) found that the sustainable maximum aerobic metabolic rate for the same homeotherms scaled at a factor of 0.88.

On the basis of current evidence, power budgeting problems within fish species therefore appear to be at their greatest for small, often young, fish. Wieser *et al.* (1988) have shown that in larval fish, which have a relatively small scope for activity, they may have problems accommodating both high rates of growth and activity at once. Goolish (1991) points out that the total visceral aerobic capacity of small fish is larger than the red muscle aerobic capacity for fast-growing juvenile fish. Hence he suggested that feeding events (ASDA costs) may account for a larger elevation in oxygen consumption than is noted for active aerobic metabolic rate in larval and juvenile fishes. Visceral metabolism as expected follows a pattern of negative allometry (Itazawa & Oikawa 1986; Goolish 1991a). However, to date, rates of metabolism in maximally fed juvenile fish have not been shown to be higher than levels at maximal exercise for most fish species, although in some cases (e.g. juvenile pike) they may closely approach the levels found at maximal exercise (Armstrong *et al.* 1992). Priede (1977) proposed that metabolic rate dependent mortality should occur if fish are forced to work at the upper limits of their metabolic scopes, with animals avoiding working at high metabolic rates to avoid the increased risks of mortality.

1.9 Intraspecific variation in BMR

1.9.1 Seasonal variation in BMR

Large-scale seasonal changes to BMR have been reported in various bird species, this being primarily associated with preparation for migratory flight and moulting (Daan *et al.* 1989, 1990; Piersma *et al.* 1995). In mammals, large-scale seasonal variations in BMR are known, particularly for small mammals that hibernate during detrimental conditions (e.g. Stephenson & Racey 1994; Hosken & Withers 1999), or face seasonal reductions in food quality (Campbell & MacArthur 1998).

Seasonal variation in SMR has also been noticed in various fish species. Facey & Grossman (1990) noted seasonal differences in the SMR of rosyside dace *Clinostomas funduloides* and sculpin *Cottus gobio* that were independent of temperature. In rosyside dace this was a physiological response to spawning; male rosy dace had higher SMR and decreased swimming performance during gonadal development and consequently a lower metabolic scope. Similar findings have also been reported for studies with rainbow trout (Dickson & Kramer 1971) and in pumpkinseed fish *Lepomis gibbosus* (Evans 1984). Koch & Wieser (1983) also reported reduced activity in roach *Rutilus rutilus*, which they suggested allowed partitioning of increased energy reserves into gonadal development. In Arctic charr *Salvelinus alpinus* distinct changes in growth are noted even when kept at constant temperature conditions (Teviten *et al.* 1996). This may not be unexpected, as they inhabit high latitudes where food availability is very seasonal. Beamish (1974) noted differences in the seasonal oxygen consumption between the sexes in brown trout *Salmo trutta*.

Changes in food availability also have profound effects on the energy content of fish and lead to shifts in metabolic capacities of tissues (Sullivan & Somero 1980; Pelletier *et al.* 1993, 1995) and indirectly influence BMR. The reproductive status of the fish also has a profound effect on the metabolic rate, energy content and muscle metabolic capabilities. Reproductive stresses have a significant effect on the BMR and energy content of the fish, as somatic energy stores are converted into gonadal material. Somatic and gonadal growth can be considered to compete for limited resources in the adult fish, with reproduction typically involving the relocation of material previously stored in the body (Bagenal 1969). The liver size in female pike decreases significantly before spawning, probably because energy is reallocated from the liver to the ovaries for final vitellogenesis of eggs (Medford & McKay 1978). By contrast, in Arctic charr, the

energy content of the gonad is directly related to the time during which the gonad is developing (Dutil 1986).

1.9.2 Individual variation in BMR

The majority of work on BMR to date has focused on BMR variation at the interspecific level. This has concentrated mainly on the differences in the allometric scaling of BMR relationships between different species (Hayssen & Lacy 1985) and the relationship between BMR and peak metabolic rates between different species (Koteja 1987, 1991; Hinds *et al.* 1993; Bishop 1999).

Variations in BMR between individuals of a species have only recently been studied and studies of within-individual variation are only now emerging. Differences in BMR between individuals of the same species, but differing in physiological condition have been predominantly studied in birds (Piersma & Gill 1998; Daan *et al.* 1989; Piersma 1994, Piersma *et al.* 1995, 1996; Scott *et al.* 1996). Intraspecific differences in BMR have also been studied in mammals of differing physiological condition (Konarzewski & Diamond 1984; Speakman & McQueenie 1996). Various studies have also examined the extent of differences in BMR between different sub species (Konarzewski & Diamond 1995; Piersma *et al.* 1995; Meerlo *et al.* 1997). Inter-individual differences in BMR have been less well studied in ectothermic vertebrates, except lizards, (Garland 1984; Garland & Else 1987) and in salmonid fish (Higgins 1985; Metcalfe *et al.* 1995; Cutts *et al.* 1998; Yamamoto *et al.* 1998), but with little attempt to examine causation of the differences found between individuals.

A large component of BMR may be the maintenance of the metabolic machinery required in tissues and organs to enable food processing, assimilation and growth (Hawkins 1991). Several studies have been made to assess the contributions of the metabolism of individual tissue masses to overall BMR. It is well known for avian and mammalian tissues that the individual tissues vary considerably in metabolic activity per unit mass (Field *et al.* 1939; Krebs 1950; Hulbert & Else 1981) and in fish (Itazawa & Oikawa 1986). In birds and mammals, feathers and fur are metabolically inactive, with the highest rates of activity usually found in the heart and kidneys (Krebs 1950). The brain, kidney, red muscle and hepatopancreas have the highest metabolic rates in common carp *Cyprinus carpio* (Itazawa & Oikawa 1986).

In carp, the rates of decrease in metabolic activity of different tissues in relation to tissue mass were found to be between $W^{0.94}$ to $W^{0.82}$ (log organ metabolism vs. log organ mass) whilst the whole body rate of decline of metabolic rate related to mass was $W^{0.84}$ (Itazawa & Oikawa 1986). The decline in mass specific metabolic rate with increasing size has been explained partly by the relative increase in those organs with a low metabolic rate (positive allometry, Oikawa & Itazawa 1983) and also a relative decline in those tissues with a high metabolic rate (negative allometry) for fish of increasing size (Itazawa & Oikawa 1986).

In an interspecific comparison of 22 bird species Daan *et al.* (1990) found that lean dry heart and kidney mass were better predictors of BMR than body mass, with approximately half the residual variation in BMR explained by the variation in heart and kidney mass alone. Even though the overall masses of these tissues combined (0.67% body mass) represented a very small proportion of the overall lean body mass, they have a very high mass-specific metabolism and contribute disproportionately to the overall metabolic rate. Similar findings had also been made previously made by Garland (1984) for the iguanid lizard *Ctenosaura timilis* where heart and liver masses together explain 35% of the variation in SMR.

1.9.3 Organ plasticity

In Burmese pythons *Python molurus*, there is phenomenally rapid organ plasticity following consumption of a meal (Secor & Diamond 1995). Within 6 hours of consuming a large meal, a 40% increase in the mass of the intestine was recorded, kidney mass increased by 45% in the first day and lung mass almost doubled by day 14. Less dramatic examples of organ plasticity are seen in many other species including migrating birds. Piersma & Gill (1998) reported very high fat levels (55% fat) for juvenile Alaskan bar-tailed godwits *Limosa lapponica* and reduced nutritional organs (gut length and mass), this being consistent with the suggestion that it is unprofitable and too energetically expensive to carry digestive machinery over thousands of kilometres of ocean (Piersma & Lindström 1997).

Relative organ plasticity of several internal organs of fish has been studied (Weatherley & Gill 1980, 1983; Oikawa & Itazawa 1983; Itazawa & Oikawa 1986; Weatherley & Gill 1990; Schmelzing & Claus 1990) to examine the effects of markedly different growth rates on body composition, but there have been no studies looking at the effects

of different growth rates on BMR, MMR and organ composition in fish. A review of organ plasticity and body composition of fish is provided by Weatherley & Gill (1987).

1.10 Biochemical and physiological basis for BMR

Eukaryotic cells are extremely diverse, covering plant and animal kingdoms as well as fungi. Whether eukaryotes are single celled or complex multi-cellular organisms, the functions and structure of the mitochondria are very similar in all species (Darley-Usmar *et al.* 1987). These cellular organelles are responsible for the consumption of oxygen and production of adenosine tri-phosphate (ATP), . They also contain all the enzymes for oxidative phosphorylation in their inner and cristae membranes, with all the enzymes of the tricarboxylic acid (TCA) cycle being located in the mitochondrial matrix (Mathews & Van Holde 1990).

It has been demonstrated that the difference in BMR between an endothermic mammal and an equally sized ectothermic vertebrate (which has a much lower BMR for its size) is reflected in the differences in total mitochondrial surface area between the two species (Else & Hulbert 1981). Else & Hulbert (1985), on comparing six tissues from six different mammal species, noted that smaller animals had a larger mitochondrial surface area per millimeter of tissue for all tissues examined. This was statistically significant in the brain, kidney, liver and heart, suggesting a decrease in the mitochondrial capacity with increasing body size. Total skeletal muscle mass was the only tissue found to have an allometric exponent greater than 1, with muscle containing 82% of the total mitochondrial volume (Else & Hulbert 1985). At rest in *Homo sapiens*, for example, the liver, heart, brain, and kidneys weigh only approximately 6% of the total body mass but account for a disproportionate 70% of the total O_2 consumption (Drabkin 1950). However, during exercise the major consumption of oxygen will be by aerobic skeletal muscle. On examining the body distribution of the aerobic enzyme cytochrome c oxidase (CCO), Drabkin (1950) found that 83% of the whole body CCO was located in the red skeletal muscle. By contrast in male carp 40-65% of whole body CCO activity is found in the anaerobic white muscle and only 10-15% in the red muscle (Goolish & Adelman 1988). However, in fish, red aerobic muscle constitutes only about 5-6% of the total muscle mass (Johnston & Goldspink 1973; Love 1980; Goolish 1989).

1.11 Energetic processes contributing to the costs of BMR

A recent review of cellular energy demand in mammals suggests that oxygen-consuming processes outside the mitochondrion contribute to about 10% of mammalian BMR (Rolfe & Brown 1997). The major processes that utilise energy in animals are predominantly ATP-consuming reactions. As detailed above, approximately 10% of BMR is not associated with ATP turnover. In a resting state the oxygen consumption of the mitochondrion is not related to ATP turnover, but to maintaining the trans-membrane proton gradient. There is a continual proton leak across the mitochondrial inner membrane. This proton leak, which was originally described in mitochondria of isolated hepatocytes, is now known to be present in mitochondria of all major tissues (Rolfe *et al.* 1994).

Else & Hulbert (1987), measured the mitochondrial density and surface areas in six tissues from six species of mammals. They found that small mammals had a higher mitochondrial surface membrane area per millimetre of tissue than large mammals in all tissues examined, with significant differences noted for the liver, kidney, brain and heart. There was a range of different allometric exponents for mitochondrial surface area ranging from 0.55 for kidney to 0.78 for skeletal muscle.

Liver mitochondria from the bearded dragon lizard *Amphibolurus vitticeps* (an ectothermic vertebrate), which has the same body mass and preferred body temperature as the laboratory rat *Rattus norvegicus*, are five times less proton leaky than those of the rat, an endothermic vertebrate (Brand *et al.* 1991). However, this mitochondrial proton leak represents approximately the same proportion of the resting oxygen consumption in hepatocytes from the livers of both rats and bearded dragon lizards. There is, however, a typically four-fold difference in BMR between the two species (Brand *et al.* 1991). This proton leak is thought to be responsible for approximately 25% of the metabolic costs of BMR (Rolfe & Brand 1996).

Porter & Brand (1993) proposed that some of the differences in decreasing metabolic rate per unit mass for mammals of increasing mass, might be due to the decrease in proton leak noted with increasing size of animals. The liver mitochondria from a large mammal, such as the horse *Equus caballus* are eight times less proton leaky than those from the mouse *Mus musculus*. All the observed variation in mitochondrial membrane leakiness was associated with variations in BMR (Porter & Brand 1993; Porter *et al.*

1996). This mitochondrial proton leak is a highly significant contributor to the overall metabolic rate of resting tissues and hence BMR. However, in highly active tissues it is thought to be a minimal energy cost (Hulbert & Else 1999).

Protein synthesis in mammals is estimated to be responsible for between 20-25% of BMR (Kelly & McBride 1989). The metabolic cost of maintaining the sodium potassium pump in the cell is estimated to be about 20-25% of BMR. The sodium potassium pump enzymes are responsible for the active transport of substrates, maintenance of ionic homeostasis, membrane potential and cell multiplication (Huntington & McBride 1988). Ionic calcium cycling constitutes approximately 5% of total BMR costs, gluconeogenesis 7%, ureagenesis approximately 2.5% and actinomyosin activity approximately 5% (Rolfe & Brown 1997). Other metabolic processes such as substrate cycling and nucleic acid synthesis account for the remainder of BMR (Rolfe & Brown 1997).

1.12 Effects of feeding on protein synthesis

An indication of the protein synthetic capacity of cells can be obtained from measuring the numbers of ribosomes in tissues. As approximately 80% of the cellular RNA is ribosomal RNA (15% transfer RNA, 5% messenger RNA) a measurement of the RNA concentration in the cell will give an indirect estimate of the number of ribosomes present, and hence the protein synthetic capacity (Houlihan 1991). Protein synthesis rates in fish have been shown to decline in animals deprived of food, with white muscle being the most sensitive to fasting (Houlihan *et al.* 1988; Lowery & Somero 1990). The rates of protein synthesis in white muscle were found to decline to a steady plateau after 10-14 days without food in barred sand bass *Paralabrax nebulifer* (Lowery & Somero 1990) and after 4-6 days in cod (Lyndon *et al.* 1992). Upon re-feeding there is an increase in the rates of protein synthesis in the cell and also an increase in the concentrations of ribosomes (Lied *et al.* 1993; Wright & Martin 1985).

Increased food availability can not only lead to an increase in protein synthetic capacity but also to an increase in the rates of breakdown (Houlihan *et al.* 1988). This increase in anabolic protein degradation with increasing synthesis rates is well known in mammals (Millward 1989). In other words there may be an increase in both protein turnover and energy costs for no net increase in the amount of tissue produced (Jobling 1993). Hence it may be argued that the level of energy required for maintenance costs is not constant

but may vary depending upon the level of the food supply and the nutritional state of the animal (see section 4.1.5).

1.13 Intraspecific variation in MMR

In humans repeatable individual differences in maximal exercise performance are known (Bouchard *et al.* 1988) with different maximal rates under different conditions. For example, with increasing altitude there is a decrease in individual maximal performance (Friedman & Bartsch 1997), and decreased MMR with smoking (Sternfield *et al.* 1993). Repeatable intraspecific variation in MMR has been recognised for some time in ectotherms. This has predominantly been observed during maximal locomotor performance, with differences in the maximal oxygen consumption for individual lizards and amphibians being reported (Garland 1984; Pough & Andrews 1984; Wells & Taigen 1984, Garland & Else 1987; Bennett 1987).

Previously, studies examining the critical swimming speed of fishes such as largemouth bass *Micropterus salmoides* concluded that the swimming speed was dependent upon various factors including body mass (Beamish 1970), effects of training (Farlinger & Beamish 1978) and water temperature (Kolok 1991). The variation around the mean of a particular parameter due to variation in individual performance was generally considered to be statistical noise or error (Bennett 1987), with the 'true' central value being used.. Kolok (1992a, 1992b) reported that individual variation in the swimming performance of largemouth bass and northern squawfish *Ptychocheilus oregonensis* was repeatable, with the rank performance of the individual fish found to be the same even at different temperatures (Kolok 1992b; Kolok & Farrell 1994). Similar repeatable measurement of maximal swimming performance of individual fishes has also been found in Atlantic cod (Nelson *et al.* 1992, 1994, 1996; Reidy *et al.* 1995, 2000).

1.14 Factors limiting MMR

Hammond & Diamond (1997) defined sustained maximum metabolic rate (SMMR) rate in humans and animals as the maximal metabolic rate that can be sustained by an animal that maintains constant body mass. On comparing the SusMR of 50 vertebrates, mostly endothermic with a few ectothermic lizards, the ratio between BMR and SusMR was found to approach five for top human athletes but did not exceed seven for any of the species examined (Hammond & Diamond 1997). There appear to be 'metabolic ceilings' that physiologically limit SMMR. Different species excel at different modes

of energy expenditure. For example, SMMR is experienced under lactation by laboratory mice (Hammond & Diamond 1994) and during physical activity in humans (Friedman & Bartsch 1997). Maximal metabolic rate is highly dependent upon the development of specialised structures such as lungs, heart and mitochondria (Weibel *et al.* 1991) and the level of MMR depend upon the extent of flexibility of these individual components (Hammond & Diamond 1997). These organs need to increase in mass (and in metabolic capacity) during conditions of high food intake or greatly elevated exercise levels as at rest they provide only modest functional reserve capacities (Hammond & Diamond 1992, 1994).

Factors limiting maximum aerobic metabolic rate will only become limiting when the organism is working at its maximal rate. For fish, these factors include gill surface area (Gray 1954; Hughes 1966; Palzenberger & Phola 1992), a decreased gill surface area being associated with a decreased rate of maximal oxygen consumption ($\dot{V}O_{2max}$). Since at rest there is no effect of gill surface area reduction upon BMR, the whole of the gill surface area is only perfused during maximal exercise (Duthie & Hughes 1987). A reduction in gill surface area reduces U_{crit} and AMR of the fish. Other factors that relate to the respiratory and circulatory system may also have a limiting effect on $\dot{V}O_{2max}$.

Maximum cardiac performance has been correlated with maximum aerobic swimming performance (Farrell & Steffensen 1987). The fish heart consists of a single venous atrium, ventricle and bulbous arteriosus in series (Santer & Walker 1980). The critical swimming velocity of rainbow trout was reduced by 32% following occlusion of the coronary vessels (Farrell & Steffensen 1987. That maximal rates of oxygen uptake occur when fish are maximally exercised, was demonstrated by Duthie & Hughes (1987) who showed that removing 30% of the gill tissue from rainbow trout resulted in an associated reduction in $\dot{V}O_{2max}$. Oxygen consumption at rest and at sub-maximal swimming speeds were not affected. This corroborated suggestions that the whole of the gill surface area was only fully perfused with blood at maximum aerobic demand (Hughes 1984). Experiments in mammals have also altered the $\dot{V}O_{2max}$ of subjects by either increasing the oxygen concentration in inspired air (Welch & Pederson 1978) or by an infusion of red blood cells (Ekblom *et al.* 1976; Buick *et al.* 1980) and induced a reduction in $\dot{V}O_{2max}$ by reducing the blood volume (Ekblom *et al.* 1972). It has been concluded by Di-Prampero (1985) that $\dot{V}O_{2max}$ in terrestrial animals at sea level is limited essentially by the O_2 transport system, that is cardiac output and blood carrying

O_2 capacity. Gal laugher (1994, as cited in Farrell 1996), used blood doping to alter the haemoglobin concentration and examine the effects on $\dot{V}O_{2max}$ in salmonids. Marked reductions in $\dot{V}O_{2max}$ were noted in anaemic fish (haematocrit <23%). Smaller increases in $\dot{V}O_{2max}$ were noted with an increased haematocrit (up to 42%).

Brett (1964) pointed out that fish could vastly exceed their active aerobic metabolic rate during short bursts of anaerobic activity, building up intracellular levels of lactate. This anaerobic activity allows a temporary increase in the upper limit of maximal metabolism (Wokoma & Johnston 1981). Reidy *et al.* (1995) on comparing different methods of measuring post-exercise exhaustion, found that a chase method produced the highest $\dot{V}O_2$ and that this was significantly higher than that measured during U_{crit} protocols. The low amounts of aerobic (red) muscle that are typically found in fish, comprising approximately 5% of wet body mass (Greer-Walker & Pull 1975; Love 1980), make a relatively large contribution to the overall aerobic capacity of the whole animal. However, Goolish (1991a) suggests that recovery from exhaustive exercise seems the most appropriate technique. Pike are ambush predators and have small amounts of red muscle, estimated to be 3.8% of wbm for a 1 kg pike (Schwalme & Mackay 1985) but measured as approximately 2.4% wbm for a standard pike of 45g in the present study (Appendix 6). In exhaustive exercise there is full recruitment of white muscle, a situation that may be more common than prolonged swimming for sedentary fish species.

As fish become larger there is an increasing reliance on anaerobic metabolism (Goolish 1991a), a similar trend to that seen in reptiles (Coulson 1987). During exercise there is a large peripheral dilation of blood vessels that is facilitated by the increase in cardiac output observed with increasing exercise (Kicenuick & Jones 1977). In fish, the blood vessels under the skin form part of the secondary blood circulation, and contain, essentially, plasma with little or no erythrocytes (Vogel 1985, as cited in Randall & Wright 1995). A certain proportion of the oxygen consumption will be derived through the skin of an animal and will decrease with increasing size and the relative decrease in surface area.

1.15 Metabolic consequences of exercise

After exhaustive sprint exercise there is a rapid increase in the whole body oxygen consumption rate in salmonids, with the levels remaining above SMR for several hours

(Hochachka 1961; Brett 1964; Wieser *et al.* 1985; Scarbello *et al.* 1992; Milligan 1995). A similar trend has been reported in pike (Schwalme & McKay 1985; Armstrong *et al.* 1992). The large post-sprint exercise increase in oxygen consumption rate (oxygen debt) of fishes is associated with a large increase in whole body lactate, and near total depletion of whole body glycogen, ATP and creatinine phosphate in trout (Scarbello *et al.* 1991). Larger fish have a higher white muscle lactate concentration after exhaustive exercise (Ferguson *et al.* 1993). A significant proportion of lactate formed during strenuous exercise in plaice *Pleronectes platessa* is known to be held within the muscle and converted to glycogen by gluconeogenesis in the recovering muscle (Batty & Wardle 1979; Girard & Milligan 1992).

Although white muscle has a low oxidative potential per unit mass, through the sheer bulk of white muscle mass, it is estimated to be the major contributor to lactate oxidation. The lactate that has entered the blood after exhaustive exercise in rainbow trout is oxidised, with the proportion of blood lactate oxidised increasing with $\dot{V}O_2$ of the fish (Milligan & Girard 1993). In their study hepatectomised rainbow trout had a larger blood lactate content after exercise but also exhibited faster metabolic recovery than non-hepatectomised fish. This implies that glycogen resynthesis in trout muscle may actually be retarded as blood lactate is metabolised by the liver. The actual large increase in oxygen consumption following exhaustive exercise has been suggested to be not fully explained by the metabolic costs of lactate clearance or glycogen resynthesis alone and probably reflects additional costs of ionic shifts and cellular water disturbances (Wood 1991).

1.16 The theory of symmorphosis

The principal of symmorphosis assumes that animals incur a selective penalty for maintaining structures in 'excess' of immediate demand. This idea implied by Aristotle was summed up by Darwin in his 'Origin of the species' (1859, as cited in Linstedt and Jones 1987): '...natural selection will tend in the long run to reduce any part of an organism as soon as, through changed habits, it becomes superfluous.' Taylor & Weibel (1981) predicted that the peak performance of the components of the respiratory system should match the peak power of the system as a whole, and as such, should not waste energy maintaining spare capacity. Weibel *et al.* (1991) found that the structures supporting the pathway of oxygen were to a large extent adjusted to the functional capacity of the system. That is, no more structure is built and maintained than is

required to meet the functional demands. A probable consequence of the optimal design of any system, however, is a reduced effectiveness when it is used for more than one function (Dudley & Gans 1991). Natural selection does not act to produce perfect rate matching between parts of the system, as selective forces tend to act on different parts of the system, are multifactorial and vary greatly in magnitude and character. Under such circumstances there is no reason to expect a correlation between performance levels at different levels of a physiological cascade (Dudley & Gans 1991). This is what symmorphosis suggests and why its widespread occurrence in nature would be surprising.

1.17 Consequences of a high BMR for Atlantic salmon parr: social dominance

In those fish species, such as salmonids, that form linear dominance hierarchies, there would appear to be a greater advantage to having a relatively high mass-specific BMR. A strong relationship between social status and SMR (defined by Metcalfe *et al.* 1995 as the O_2 consumption of a quiescent fish) was found for hatchling Atlantic salmon by Metcalfe *et al.* (1995), who proposed that high ranking fish are also likely to have a high MMR, providing a means by which to maintain their status. Confirmation of these results has been demonstrated by Cutts *et al.* (1998, 1999a) for Atlantic salmon parr and for masu salmon parr *Oncorhynchus masou* (Yamamoto *et al.* 1998). A higher SMR has been associated with a higher MMR due to the purported increase in metabolic costs of supporting the tissues needed for a higher MMR to be achieved (Brett & Groves 1979; Priede 1985). On this basis a fish with a high SMR may be predicted to have a larger scope and have more energy for energetic activities such as aggression (Puckett & Dill 1985; Metcalfe 1995). Dominant salmonids with faster growth and higher metabolic rates were also noted to have larger otoliths (ear bones), than subordinate fish (Titus & Mosegaard 1991; Metcalfe *et al.* 1992). Otolith size tends to more closely reflect metabolic rate than somatic growth (Mosegaard *et al.* 1988; Wright 1991). These interactions may affect the life history of salmonids and other fishes through the social status of the animals (Simpson & Thorpe 1976; Thorpe 1977; Metcalfe 1985). It was noted that during dominance testing between pairs of juvenile Atlantic salmon, the dominant individual was the largest only for 54% of the time, which indicates that the large size was a consequence of dominance and not the cause (Huntingford *et al.* 1990).

As salmon fry hatch and emerge from the redd, the tendency is for most fry to set up home ranges which they may or may not defend (Jenkins 1969; Fausch 1984; Nakano

1995), with more profitable sites being occupied by the most dominant individuals (Fausch 1984; Nakano 1995). Having selected the best feeding territories, dominant individuals often attain larger body sizes than subordinates (Li & Brocksen 1977; Fausch 1984; Metcalfe 1989). Those subordinate fish that do not achieve sufficient growth rates emigrate from the local habitat, and are displaced downstream to less profitable feeding positions (Elliott 1990).

The social status of fish that form linear dominance hierarchies is relatively stable once it has been established (Abbott *et al.* 1985). Dominant fish use various tactics to ingest more of a limited food supply and these include: acquiring a limited ration first, preventing a subordinate's food acquisition or behaviourally inhibiting a subordinate's food acquisition (Koebel 1985). Growth is limited by both food acquisition and social status and as a result dominant individuals often attain a larger size than subordinates (Huntingford *et al.* 1990; Fausch 1984; Metcalfe *et al.* 1989). In a high competition environment, fish that exhibit a high tolerance to a crowd of conspecifics are expected to grow faster than those fish that that have either no preference or less tolerance (Ruzzante & Doyle 1990).

At a specific stage of a salmonids development the parr undergoes internal and external transformations that preadapt the fish for survival in the marine environment. The fish becomes a smolt for a critical and short freshwater phase that brings it to the status of a seawater fish. At the end of this smoltification stage, the fish migrates to the estuary, where it remains in brackish water to adapt completely, prior to migrating to the open ocean feeding areas. The marine environment enables rapid growth of the fish (for a review see Boeuf 1993). Bimodality in the length of juvenile salmon is noted by the late summer / early autumn of the first year of freshwater growth (Simpson & Thorpe 1976; Thorpe 1977). The upper modal group (larger fish) become S1 smolts and undergo their seaward migration in the following spring, while the lower modal fish (S2) normally smolt the subsequent year. All individuals are capable of becoming S1 smolts. However, whether they do or not depends upon decisions made towards the end of the first year (Metcalfe *et al.* 1988; Wright *et al.* 1990; Metcalfe 1994). After this time the appetites and growth patterns of the fish diverge. The lower modal growth fish have a reduced appetite, whilst the appetite of upper modal growth fish increases (Metcalfe *et al.* 1986, 1988; Metcalfe 1994). Both upper and lower modal groups contain fish of both sexes (Thorpe 1977).

Thorpe *et al.* (1980) suggested that a certain level of plasma thyroxine must be obtained before smolting takes place, the actual level being determined genetically. Those that fall bellow the required level lose appetite and stop growing for 4-6 months (Metcalfe & Thorpe 1992). Dominant individuals are more likely to migrate early (Metcalfe *et al.* 1989, 1990), possibly due to being better competitors for food. Fish that delay migration are more likely to be intimidated by the presence of a competitor (Metcalfe 1989, 1991). Highly stressed fish have been observed to stop feeding and subordinate fish observed to voluntarily reduce food intake (Abbott *et al.* 1985). Koebel (1985) found that the differences in growth rates of fish removed from social interaction whilst feeding were lower than those that interacted during feeding.

By contrast to juvenile salmonids pike exhibit no dominance hierarchy formation. However, cannibalism between conspecifics is considered to be a major regulating force within pike populations (Grimm, 1981). A review of cannibalism in pike is presented by Grimm & Klinge (1993). Mann (1982) found that in the River Frome, in southern England older pike (aged 4 years and above) had the largest numbers of conspecifics in their stomachs. A pike that becomes cannibalistic may rapidly increase its growth rate by consuming large meals compared to non-cannibalistic pike and reduce its individual chances of being preyed upon (Diana 1996).

1.18 The citric acid cycle, citrate synthase and the role of lactate dehydrogenase

The citric acid cycle is the metabolic pathway responsible for the release of energy from fuel molecules such as glucose, amino acids and fatty acids. The cycle starts with the initial breakdown products of fuel molecules, pyruvate and acetyl-Co-A, and oxidises them with a series of reactions, with the release of carbon dioxide and the synthesis of high-energy molecules. These high-energy molecules provide the driving force for the synthesis of ATP. The energy rich products are formed from the reduction of nicotinamide adenine dinucleotide (NAD) to NADH and flavin adenine dinucleotide (FAD) to $FADH_2$ (Alberts *et al.* 1998) .

The enzyme CS catalyses the first reaction of the citric acid cycle, regulating the whole process and is regarded as a key indicator of aerobic potential (Torres & Somero 1988). Citrate synthase (see Figure 1.2a) is a site of regulation for the whole of the citric acid cycle. The citric acid cycle only occurs in the presence of oxygen. The oxygen is not

required for any of the reactions of the cycle, but it is needed as an electron acceptor after the oxidation of NADH and FADH.

A temporary shortage of oxygen prevents the citric acid from taking place, so the muscle cells have to rely on the energy provided by the break down of glucose to pyruvate. Lactate dehydrogenase catalyses the conversion pyruvate to lactate. Lactate dehydrogenase is most commonly assayed with anaerobic conversion of pyruvate to lactate (see Figure 1.2b). However, under differing conditions (high lactate) lactate dehydrogenase can also oxidise lactate to pyruvate. Lactate dehydrogenase was assayed in the lactate oxidation mode, as this could indicate differences in the rates of recovery of individual fish from exhaustive exercise (Hulbert & Moon 1978). Finally an attempt was made to determine how much of the inter-individual variation in BMR and MMR of pike could be explained by the total activity of the key indicator enzymes assayed.

1.19 Aims of the present study

A niche has been defined as the place of an organism within the ecosystem or all of the components of the environment with which an organism or population interacts (Stiling 1996). There is a mass of literature on physiological and morphological adaptations of animals to individual niches, with fish utilising different niches at different sizes (e.g. Forseth *et al.*1994; Piet 1998; Heggenes *et al.*1999). There has, however, been less exploration as to the metabolic condition of individual fish responding to differing environmental conditions. This thesis is concerned with the level of flexibility in metabolic response only in fish to alteration in environmental conditions, principally food availability, social interaction and exercise.

Atlantic salmon parr were chosen as an experimental subject because in the context of their life history strategy, it has been argued that SMR is fixed early in life, with those fry that emerge first from the redd tending to have a higher SMR than later emerging fry (Metcalfe 1989; Metcalfe *et al.* 1992, 1995). It has been proposed that Atlantic salmon with a higher SMR for their size maintain this at least during the freshwater phase of the fish's development (Metcalfe *et al.* 1995). Metcalfe *et al.* (1995) postulated that within a heterogeneous environment salmon parr with either a high or low SMR would occur together since neither strategy would alone be the fittest option.

(A)

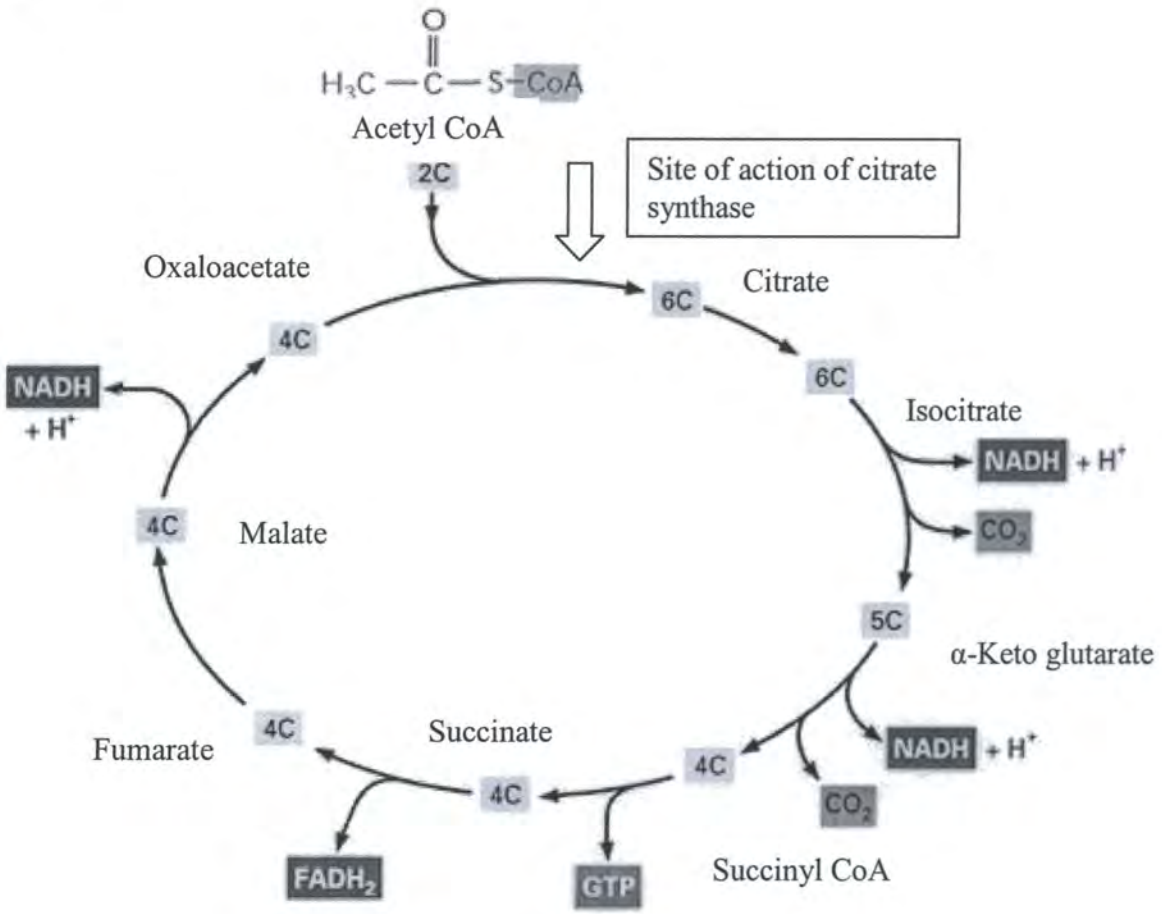


Figure 1.2(a) The metabolic site of action of the enzyme citrate synthase (CS), modified from Alberts *et al.* 1998. The initial reaction in the cycle is the irreversible formation of citrate by CS. The reaction is strongly exergonic and is the site of regulation of the whole cycle.

(B)

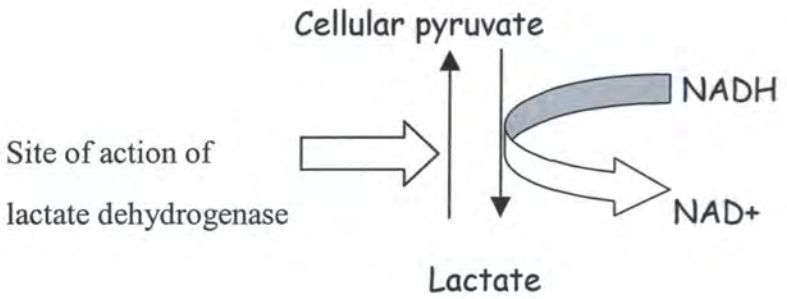


Figure 1.2(B) The metabolic site of action of the enzyme lactate dehydrogenase (LDH), which catalyses the reversible reaction of pyruvate to lactate under anaerobic conditions. The NAD+ generated is a necessary substrate required to keep glycolysis functioning and ATP production under anaerobic conditions.

The present study set out to examine possible flexibility in BMR for Atlantic salmon parr, and possible flexibility in BMR, MMR and factorial metabolic scope in juvenile pike. These two fish species have contrasting life histories. Atlantic salmon have a high metabolic capacity and may migrate great distances at sea to different feeding areas, this change in niche enabling rapid growth. Salmon parr form a social dominance hierarchy, suggested to be dependent on relative SMR (see above). Sockeye salmon typically have a high BMR, MMR and high factorial metabolic scope with MMR being approximately 14 times BMR for a 300 g salmon (Brett & Glass 1973). Pike are solitary, ambush top predators, often showing extended periods of inactivity (Diana 1982). Pike rely on a fast burst of acceleration to capture prey (Frith & Blake 1995). The high percentage of body musculature and the large proportion of white muscle favour a high power output and short duration burst of activity (Webb 1978).

Pike have been reported to have a low level of aerobic red muscle (Schwalme & McKay 1985) apparently reflecting a low capacity for sustained activity (Goolish 1991a), with a low relative metabolic scope of approximately 2 to 4 times BMR (Armstrong *et al.* 1992). The juvenile pike used in the present study would not swim reliably in flumes and so the only way to measure maximal aerobic capacity was that which occurred during oxygen debt repayment (Armstrong *et al.* 1992). Oxygen debt repayment probably represents the genuine cause of a metabolic ceiling under natural conditions.

The first aim of the thesis was to identify and quantify flexibility in BMR of salmon parr, prior to their first winter at which point those fish with a relatively high BMR (for their body mass) tend to continue feeding with an increased appetite over the winter months. Initial experiments with salmon parr explored the extent of individual flexibility in BMR of fish held in small groups. Possible mechanisms for any observed plasticity in BMR and MMR between individuals were then further explored using juvenile pike. Pike were used for several reasons. Firstly, the pike were of increased size than parr; secondly pike were known to be much less active in a box respirometer than salmon parr; thirdly it was known that an accurate measurement of maximal metabolic rate could be attained following exhaustive exercise (Armstrong *et al.* 1992). Also of critical importance the food consumption of individual pike could be monitored accurately. The increased size of the study animals was important as a detailed organ weight and enzyme analysis of tissues was desired. Preliminary dissection of the approximately 3 g salmon parr failed to provide the sufficient tissue to measure enzyme

activities, as the visceral organ mass were approximately 7% of the body mass and was complicated to perform.

It is known that the activity per gram of enzymes in tissues can vary as well as the overall organ mass (e.g. Kleckner & Sidell 1985). So a comparison of the tissue enzyme activities of two key indicator enzymes citrate synthase (an indicator of aerobic capacity) and lactate dehydrogenase (an indicator of the rate of recovery from exhaustive exercise, when assayed in the lactate oxidation mode) were carried out.

Chapter Two

Measurements of BMR in Atlantic salmon parr

2.1 Introduction

The basic principal of bioenergetics is that all energy ingested as food is lost either as waste in faeces or excretion, used as an energy source for metabolic processes or laid down as new body tissue (section 1.2). The minimum rate of energy expenditure by a post-absorptive, homeothermic animal, at rest in its thermoneutral zone, is usually termed resting or basal metabolic rate (BMR). In ectothermic vertebrates BMR is temperature-dependent and normally regarded as specific for a fixed temperature (Brett 1979). A large component of BMR may be the maintenance of homeostasis by the sodium potassium pumps (Kelly & McBride 1989) and counter acting the proton leak across the mitochondrial surface (Rolfe & Brand 1996, section 1.11). These basal costs represent a significant proportion of the total energy costs to the organism (Priede 1985).

Mechanisms exist for economising on basal metabolism in response to extreme adverse conditions. During sustained unfavourable conditions 'metabolic rate depression' may be elicited, for example by *Artemia* in the absence of oxygen (Guppy *et al.* 1994), and lungfish (Dipnoi) in the absence of water (Smith 1939). This occurs through the controlled relaxation of normal homeostatic processes. Basal metabolism in ectotherms is further influenced by environmental temperature and thermal history. Although extensive studies have examined economy of reducing activity metabolism in optimising foraging (Weihs 1975; Wieser 1991; Sogard & Olla 1996) there has been little exploration of the scope for BMR in ectotherms to vary in response to relatively unpredictable and short-term variations in their environment. This is despite the fact that small adjustments to BMR could have profound effects on the overall energy budget. For example, it has been suggested by Priede (1985) that the metabolic costs of routine activity (not including BMR) in lake-dwelling brown trout represent an eighth of the total metabolic cost of BMR. The potential for variation in BMR depends critically on two factors: the extent of possible flexibility in BMR to vary within individuals and the range over which BMR can vary between individuals.

It is now relatively well known that BMR varies between individuals of similar body mass within populations of both ectothermic and endothermic vertebrates, including

birds (e.g. Daan *et al.* 1989; Piersma *et al.* 1996), mammals (Konarzewski & Diamond 1995; Meerlo *et al.* 1997), lizards (e.g. Garland & Else 1987) and salmonid fishes (Metcalfe *et al.* 1995; Cuffs *et al.* 1998, 1999a; Yamamoto *et al.* 1998). Individual variation in BMR appears to reflect different relative masses of highly metabolically active organs such as heart, kidney, liver and intestines (Field et al. 1939; Itazawa & Oikawa 1986). In birds (Hogstadt 1987; Bryant & Newton 1994) and salmonid fishes (Metcalfe *et al.* 1995; Cutts *et al.* 1998, 1999a; Yamamoto *et al.* 1998) a positive correlation between BMR and social status has been demonstrated.

Atlantic salmon were chosen as the study species for the work described in this chapter because a substantial body of information exists relating their metabolism to life histories strategies . Those individual salmon that have a relatively high BMR at the time they start to feed tend to be most dominant (Metcalfe *et al.* 1995). Dominant salmon tend to grow most quickly when fed *ad libitum* in laboratory tanks (Huntingford *et al.* 1990). They also tend to smolt at an earlier age than lower ranking fish (Thorpe *et al.* 1992) and at this stage have a relatively high BMR (Higgins 1985, section 1.13). Hence, the available data suggest that the relative magnitude of BMR of an individual salmon is set early in life and is maintained at least over the period of growth in freshwater. In other words, variation in BMR between individuals subsumes any variation within individuals over this period.

Given the apparent advantages of having a high BMR, the question has arisen as to why a low BMR and subordinate strategy persists in salmon parr. Metcalfe *et al.* (1995) suggested that dominant fish with high metabolic rates would be expected to prosper when and where food is abundant but may lose out to individuals with low metabolic rates when food is scarce. They suggested that strategies of fixed high and low metabolism would be included within a population since neither would always be the fittest option. However, such strategies could result in substantial genetic fitness costs to individual animals over time. Therefore, this part of the thesis investigated how much the relative BMR of individual salmon parr can vary over time in response to simple environmental perturbations.

2.1.1 Accounting for the activity of individual fish

In determining useful measurements of BMR, an assessment of the levels of activity of the fish is critical. Some species of fish, such as pike, may exhibit such low levels of

spontaneous activity that it is relatively easy to measure resting levels of metabolism, which equate directly with BMR (Diana 1982). However, many species of fish are active routinely and this complicates measurement of BMR. Atlantic salmon parr are a good example of a fish that exhibits random periods of activity when enclosed in a box respirometer.

A common approach to account for activity in estimating BMR of fish is to force an individual to swim continuously at a range of speeds within a flume respirometer (Brett, 1964; Beamish 1978; Gerhke *et al.*, 1990; section 1.4). The relationship between swimming speed and metabolic rate may then be extrapolated to zero activity to provide the standard metabolic rate (SMR). Although SMR is a useful estimate of basal metabolism and is a repeatable measurement, it may underestimate the true BMR (section 1.4). On the other hand, it is conceivable that fish, which are forced to swim continuously, may be stressed and so exhibit metabolic rates elevated above the costs of BMR and locomotory activity alone. For many species of fish there are fundamental problems in estimating SMR. Even for those species that can swim in a flume respirometer, some individuals may refuse to do so (e.g. Priede & Holliday 1980; Adams & Parsons 1998). Some stream-dwelling species, such as Atlantic salmon parr can remain static against the streambed at no metabolic cost even in high flows, using their large pectoral fins as depressors (Rimmer *et al.* 1985; Arnold *et al.* 1991). This makes juvenile Atlantic salmon parr difficult to assess for swimming ability as they have such a good substratum-holding ability at high current velocities. Recently, in assessing the swimming performance of juvenile salmonids, Peake & McKinley (1998) found that Atlantic salmon parr (4.8-13.1 cm in length) were able to anchor themselves to the substratum almost indefinitely (i.e. above 200 minutes) at water current velocities of up to 0.86 ms^{-1}.

There are further practical limitations on the derivation of SMR; flume respirometers are expensive, and are complicated to build and may be difficult to use with very small or large fishes. The measurement of oxygen consumption of small fish is a potential problem due to the large volumes of water generally found in flume respirometers. Approaches that have not relied on flume respirometers have also been used to estimate BMR. Oxygen consumption of anaesthetised fish has been measured as a surrogate for BMR (De Silva *et al.* 1986; Bennetti *et al.* 1995). However, the exact physiological state of anaesthetised fish is hard to determine, making the interpretation of the readings

more difficult. A more common approach, which has been applied to a wide variety of fish species, is to use a box-type respirometer chamber in which the water circulates (e.g. Higgins 1985), or flows at a constant speed through a chamber holding an individual fish (e.g. Wieser 1985). If the level of spontaneous activity by fish in such a system is not high, then $\dot{V}O_2$ measurements may be termed 'routine' or 'low routine', and if activity is absent then the measurements may be termed 'resting' and approximate to BMR. When using such respirometers, it is necessary to choose periods of time when the fish are inactive to estimate BMR measurements. Estimates of activity levels must be made with caution because a common response of species such as juvenile Atlantic salmon to disturbance from a potential predator is to freeze motionless (Gotceitas & Godin 1993). Hence an individual fish that is routinely active may appear to have been resting when visually inspected intermittently.

Routine activity is generally so ill defined that measurements of metabolism that are not of true resting fish or of fish at known sustained swimming speeds may be of limited value and were inadequate for this thesis. For fish such as Atlantic salmon parr there are no obvious methods of establishing SMR due to their 'clinging' behaviour in flows (although a tilting-type swimming respirometer could be used) and a degree of spontaneous activity is inevitable when attempting to measure BMR. Therefore, it is critically important to know the degree of activity and use only true resting periods for the determination of BMR.

2.1.2 Experimental aims

The initial aims of the experiment were to assess the validity of using $\dot{V}O_2$ data from unfed Atlantic salmon parr, together with measurements of spontaneous activity, in order to obtain accurate measurements of BMR, as it was initially not known how the parr would react to the respirometer system. Once it was known that reliable measurements could be obtained, the aim was to assess possible flexibility in BMR by transferring the parr to an altered environment and subsequently re-measuring the BMR values. If individual BMR is fixed early in life, with different salmon parr having a BMR occurring on a continuum from relatively high to low values as has been suggested by Metcalfe *et al.* (1995) then little change in the BMR of individual fish would be expected between the different measurement periods.

However, if BMR were flexible then some if not all fish would be expected to be able to modify their relative BMR from one time period to the next. To examine this, the BMR of each fish was carefully measured. To encourage changes in BMR, the habitat was altered, by placing the fish from that were held as part of a large group in a holding tank in to small groups held in individual flumes (see section 2.2.9), and after about one month the BMR were re-measured. It was believed that a change to an unfamiliar environment for the parr would cause the most 'pressure' as dominance hierarchies are re-established, and the most likely situation under which to observe possible temporal flexibility in metabolism. As all the fish were moved to the new environment at the same time, fish would need to re-establish territories and dominance hierarchies. These measurements of BMR were then used to estimate variation in BMR between individuals, within individuals but one month apart, and within individuals on the same day. An initial experiment was performed in the summer of 1996, with a modified experiment performed the following year (summer of 1997). These were termed Experiments 1 and 2 respectively.

2.2 Material and methods

2.2.1 Experimental animals

These experiments used juvenile Atlantic salmon parr, which were full sibling offspring of adult sea-run Atlantic salmon caught from the River Almond, Perthshire, Scotland. The eggs were incubated at the Freshwater Fisheries Laboratory's salmon rearing unit at Almondbank, Perth. The parr (Plate 2.1) were transferred to the research station at Faskally, Pitlochry, and were held at a density of approximately 130 fish m^{-3} in a tank of 0.67 x 0.61 x 0.90 m. Water for all aquaria and the experimental facilities was supplied from Loch Faskally at ambient temperatures of (mean ± SD) 15.6 ± 1.0°C for the experiments in 1996 and 14.3 ± 1.2°C for the experiment carried out in 1997. Fish in the stock tank were fed *ad-libitum* with pellet food at approximately 5% wet body mass (wbm) per day. The water velocities were kept constant between both the holding tanks and the experimental channels at approximately 5 cm s^{-1}. In both experiments fish were held in natural daylight conditions prior to and during the experiments. Salmon parr were in their first summer of growth when used for experiments (Experiment 1 (1996), initial mass [$\bar{x}$ ± SE] = 2.02 ± 0.11 g and fork length 58.8 ± 0.61 mm (n = 25) final mass = 2.14 ± 0.13 and fork length 59.2 ± 1.01 (n = 24); Experiment 2 (1997), initial mass 1.62 ± 0.06 g and fork length 55.3 ±0.7 mm, final mass = 1.83 ± 0.09 and fork length = 57.4 ± 0.86, (n = 22).

2.2.2 Closed system respirometry

Rates of oxygen consumption were measured with a multiple closed chamber respirometer system, consisting of six chambers in series. Each holding chamber of the system was constructed using a clear 300 ml polycarbonate flask (Sartorius, UK), with an O-ring sealed lid and inlet tubing holes (Plate 2.2). A section of small plastic pipe lead from the inlet at the top of the chamber to the bottom and was used to create a uni-directional flow against which the salmon parr could orientate its self. A small plastic shelter (3 x 3 cm) was provided in each respirometer chamber. The provision of both an area of shelter and a unidirectional flow was found to make the salmon parr settle better and be less active in the chamber. Individual fish were selected from the stock holding tank at random, using a dip net and placed into a bucket. A single fish was then chosen at random from the bucket for an initial measurement of BMR. This was done so as to try to select fish as randomly as possible, since first-captured fish from the stock tank might be those with the lowest swimming abilities. Prior to each measurement, the fish was lightly anaesthetized with ethyl-m-amino benzoate methane sulphonate (MS222) at 0.1 g l^{-1}, weighed to the nearest 0.01 g and the fork length measured to the nearest millimeter. The whole respirometry system was submerged in a constant temperature glass water bath (240 l volume) maintained at $15.0 \pm 0.1^{\circ}C$.

Each fish was introduced to an individual chamber at least 16 hours prior to the oxygen consumption measurement, giving the fish adequate time to recover from handling stress (Higgins 1985). After approximately 12 hours in the respirometer the rate of oxygen consumption declined no further indicating that the fish had fully recovered from handling stress. Fish were starved for 12-16 hours prior to being placed in the respirometer, thus ensuring complete evacuation of the gut, which took approximately 20 hours (Higgins & Talbot 1985). During the acclimation period the respirometer chamber was flushed continuously with fully oxygenated water using centrifugal pumps (Eheim). The fish was not fed whilst in the chamber. Dark screens visually isolated the fish from external disturbances. Fish were provided with natural lighting in a photoperiod regime of approximately 16L: 8D.

Plate 2.1 Atlantic salmon parr showing the distinctive parr marks along the side of the body.

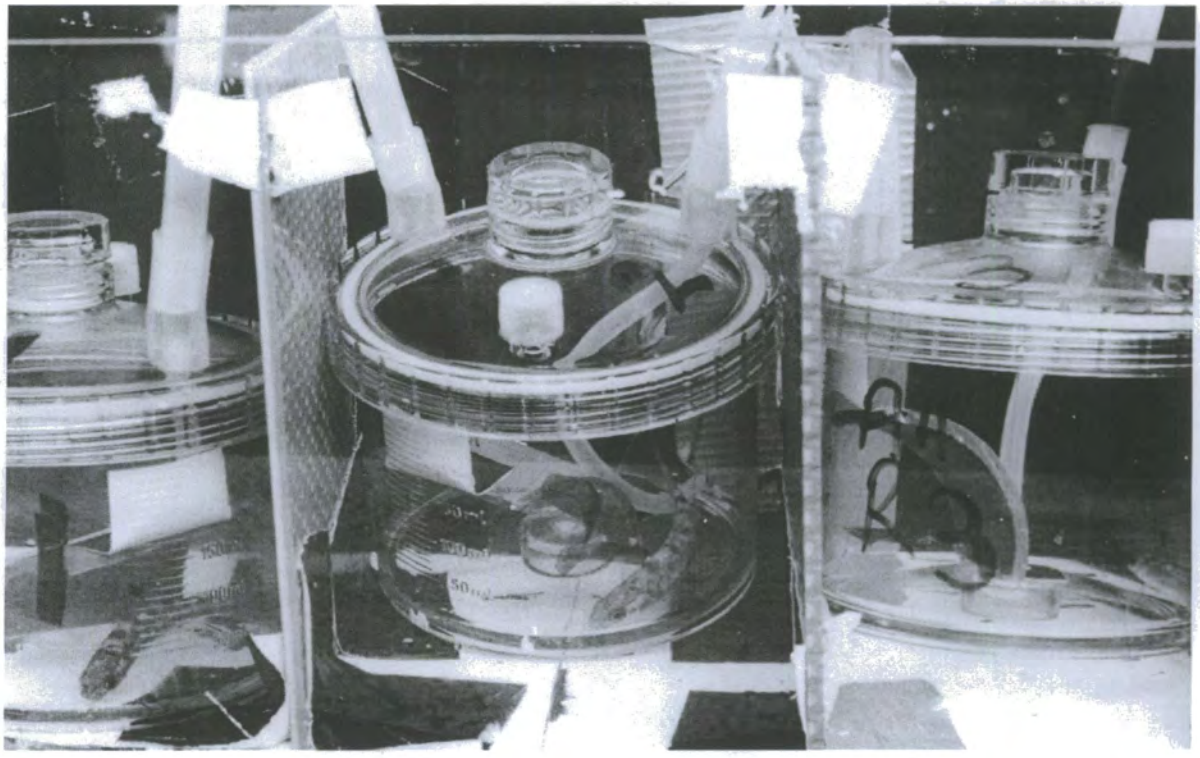


Plate 2.2 Atlantic salmon parr within the respirometer chambers. The viewing screen has been removed for photographic purposes.

During each measurement of oxygen consumption rate ($\dot{V}O_2$) a system of taps was closed, circulating water between a single chamber and an oxygen electrode (model 1302, Strathkelvin Instruments, Glasgow) mounted in a cuvette. The decline in water oxygen content was recorded on a pen recorder (Kipp & Zohnen) and a data logger (Picolog ADC 12, Pico Technology Limited, Cambridge, UK). Oxygen consumption measurements were corrected for both barometric pressure and vapour pressure changes. The oxygen electrode was calibrated throughout the day, as necessary using 100% air saturated water and a zero oxygen solution, containing an excess solution of sodium dithionite, whilst held in the constant temperature water bath. This was critical as the calibration is highly temperature specific.

The oxygen saturation of the respirometry chamber was not allowed to fall below 80% of the air-saturated value at any time. Blank experimental runs were performed at least once a day to measure the background microbial respiration rate. This rate was then subtracted from the actual rate of oxygen consumption recorded to provide corrected oxygen consumption data. In an attempt to minimise microbial respiration, the water was changed regularly (every second day) and a filter (Fluvial 303) and UV steriliser (Uvaq 30/3P) were also employed.

2.2.3 Measuring the lag time of the respirometer

In the context of respirometry the lag time is the time taken for a change in the respirometer environment to be detected by the sensor, in this case the oxygen electrode. The lag time of the respirometer was measured by injecting a known volume (5 ml) of deoxygenated water into the system (oxygen was removed by bubbling nitrogen through it). The lag time taken for a drop in oxygen content to be recorded by the system was found to be less than a minute (approximately 40 seconds). Blocks of 10 minutes of time were considered an appropriate period suitable for oxygen consumption measurement, and analysis in relation to activity for both years of experiments. Ten-minute blocks of time allowed for approximately 15 complete flushes of the respirometer and considered to be a sufficient number for a single oxygen consumption measurement.

2.2.4 Removing effects of activity

During the oxygen consumption measurements, the fish was viewed continuously through a slit in the screen surrounding the chamber, with the observer sat in semi-

darkness against a black background to prevent shadows. Initial experiments used a video camera to record the behaviour using a slit in a screen. However, the low light levels proved to be a problem and also analysis of a sometimes poor image proved difficult. It was not possible to film the salmon parr from above due to a combination of both a large volume of water to film through and possible disturbance to the parr of an object overhead. The most reliable technique was simply to sit and watch the fish, as this provided immediate records without the need for subsequent analysis and possible wasted time due to extended periods of fish activity.

Basal metabolic rate measurements were taken only from fish that were observed to be totally quiescent, that is the only observable activity was opercular movements and very minor pectoral or caudal fin adjustments, with the fish remaining stationary. Infrequent repositioning of the body position occurred occasionally within the respirometer, and was deemed acceptable and included for a quiescent fish if it occurred for less than 5 seconds in duration in any ten-minute period (less than 1% of the time) and was associated with a tail beat frequency of less than 1 Hz. Over 40 % of the readings taken were discarded. However, this underestimates the total activity of the fish, as when a period of sustained activity was observed for an individual the readings were halted until later on in the working day.

Because this rigorous procedure for making genuine BMR measurements was adopted the numbers of BMR measurements varied between individual fish depending on the extent of their random activity. It is possible that intrinsic diel variations in BMR could affect the BMR measurements if fish tended to be more active at particular times of the day. To examine this possibility, BMR was measured in eight fish at approximately two-hour intervals between 09.30-19.30, covering the entire experimental day. A comparison of measurements made through out day and night SMR by Fallon-Cousins (1999) in Atlantic salmon parr failed to find any significant differences between the two periods.

2.2.5 Calculation of oxygen consumption rates

To calculate the oxygen consumption of the individual animals, the following formula was used, giving the total oxygen consumption per animal as mg O_2 h^{-1}:

$$\dot{V}O_2 = \{[(O_2)_I-(O_2)_f)/100)] * [(BP - WVP)/760)] * [(O_2)_{sat}] * [Vol]\} /T$$

$(O_2)_I$	Initial % saturation
$(O_2)_f$	Final % Saturation
BP	Barometric pressure * (mmHg)
WVP	Water vapour pressure (mmHg)
$(O_2)_{sat}$	Oxygen at 100% saturation **(mgO_2 l)
Vol	Volume (Litres)
T	Time (hours)

* BP was read as mbar and converted to mm Hg by the following 1 mbar = 0.7501 mm Hg.

** The oxygen content of the water for 100% saturation at a specified temperature was expressed as ml/l to convert this to mg/l the following was used mg/l = ml/l * 1.428.

To calculate the oxygen consumption per kg the above value was divided by the mass of the fish in kg to give the units as mgO_2 kg^{-1} h^{-1}

Throughout this thesis the units are expressed as mg O_2 kg^{-1} h^{-1}, however to convert these to µmol , use the conversion $1mgO_2 = 31.251$ µmol O_2.

2.2.6 Removing the effects of body size (standardising mass)

Large fish generally consume more oxygen than small fish, but in terms of the oxygen consumption per unit mass, smaller fish consume more oxygen than larger fish, due to a larger proportion of more highly metabolically active tissues (Oikawa & Itazawa 1983; for a detailed explanation see section 1.9). The general allometric scaling relationship can be described as:

$$\dot{V}O_2 = (a)\ W^{(b)}$$

where $\dot{V}O_2$ is the rate of oxygen consumption in mgO_2 h^{-1} per animal, W is the mass of the fish in grams, and (a) and (b) are constants.

To remove the effects of body size the mean log $\dot{V}O_2$ during periods of minimal activity was plotted against log body mass. This relationship was used to provide an allometric

scaling coefficient (b) for standardising oxygen consumption to those fish of a mean mass. These values were termed adjusted metabolic rates ($\dot{V}O_{2(adj)}$) and calculated as below

$$\dot{V}O_{2(adj)} = (Ws/W)^{b} \times \dot{V}O_{2(exp)}$$

where $\dot{V}O_{2(adj)}$ is the corrected oxygen consumption in $mgO_2\ h^{-1}$ for a standard fish of mass Ws grams (2.1g for fish in Experiment 1 (1996), and 1.7g for fish in Experiment 2 1997), W is the mass of the experimental animal in grams, and $\dot{V}O_{2(exp)}$ is the observed oxygen consumption of the animal in $mg\ O_2\ h^{-1}$(Soofiani & Priede 1985). This approach was used to provide directly comparable quantitative information on metabolic rates which could be plotted and visualised easily.

2.2.7 Removing the effects of body size (residual analysis)

Another approach suitable for assessing variations in individual metabolic rate is to analyse the residuals around the mean data. For a comparison of data for individual fishes between experimental times, the regression of log $\dot{V}O_2$ against log body mass was plotted, and then the associated residuals from the regression line used in analyses. The regression line is the expected $\dot{V}O_2$ for a fish of a certain size. Those fish that have a higher than expected $\dot{V}O_2$ for their size have a positive residual and those fish that have a lower than expected $\dot{V}O_2$ for their size have a negative residual (Metcalfe *et al.* 1995). Individual residuals for each fish were compared between the individual experimental times by the use of Mann-Whitney *U* tests due to the small sample sizes and heterogeneous variances.

2.2.8 Statistical analysis

Unless otherwise stated, all statistical analyses were used in this thesis were performed using SPSS (Norusis, 1994). In all cases where statistical comparisons were made between samples, tests of homogeneity of variance (Levenes' test) were carried out. Where variances were homogenous parametric tests were used. Following transformations of the data, where the variances were not homogenous non-parametric equivalent tests were performed. Where an ANOVA was performed on multiple comparisons, the significance of pair wise comparisons was subjected to Bonferroni correction (Devore & Peck 1993).

As an additional approach to the individual comparisons made between the same fish approximately one month apart, a linear mixed modelling approach was performed to analyse the different sources of variation in the data between and within different fish. This is an evolving statistical technique and was performed by R. Fryer, a statistician from the Fisheries Research Services, Marine laboratory, Aberdeen, using the statistical package Genstat for Windows.

2.2.8.1 Linear mixed model approach

The BMR measurements were analysed using linear mixed models (Hocking 1996). The models generalised standard linear regression models by allowing for both fixed effects, the explanatory variables typically encountered in linear regression, and random effects, which represent different sources of variation in the data. Metabolic rate data were first $\log_e$ transformed to homogenise variances, and then fitted to the following mixed model.

$$\log_e \text{BMR} = \text{fixed effects} + \text{random effects}$$

where

$$\text{fixed} = \log_e \text{mass} + \text{time} + \log_e \text{mass} \,.\, \text{time}$$

$$\text{random} = \text{between-fish} + \text{within-fish between-time} + (\text{within-fish within-time} + \text{measurement error})$$

The fixed part of the model has three terms that allow $\log_e$ BMR to vary linearly with $\log_e$ mass, $\log_e$ BMR to vary with time, and the slope of the relationship between $\log_e$ BMR and $\log_e$ mass to vary with time (the $\log_e$ mass. time interaction). The random part of the model has four random components, which are most easily described in reverse order. Measurement error was simply the error associated with taking each BMR measurement. The within-fish within-time term represented variation in ($\log_e$) BMR measurements made on the same fish on the same day, having accounted for any measurement error. In practice, it was only possible to estimate the combined effect of these two components from the data, but it is useful to separate them here to understand all the processes involved. The within-fish between-time term represented variation in BMR measurements made on the same fish in different times, having accounted for any within-fish within-time variation and measurement error. Thus, it measured the *extra* variation in BMR that occurred by looking at the same fish one month apart. Finally, the between-fish term represented the *extra* variation in BMR that occurred by examining different fish, having accounted for any within-fish variation and measurement error. Strategies of fixed low and high metabolism would correspond to a

large between-fish variance component and negligible within-fish variance component. The effects of fish being placed into different channels were also incorporated in the mixed model as either a fixed or a random effect (although not both at the same time).

The mixed model was fitted by residual maximum likelihood (Robinson 1987). The significance of the various fixed and random effects was assessed by likelihood ratio tests (Hocking 1996; Welham & Thompson 1997). Although it is possible to obtain approximate standard errors on estimates of variance components, their utility is sometimes limited because the estimators can be quite skewed. Instead, R. Fryer computed the approximate 95% bootstrap confidence intervals for each of the variance components (Efron & Tibshirani 1993).

2.2.9 Experimental design

2.2.9.1 Experiment 1 (summer 1996)

Fish were removed from the holding tank at random and starved for 12-16 hours. After measuring BMR throughout a single day, the individual fish were then allocated at random to one of five groups of five fish (total $n = 25$). Each group was then allocated to an individual channel (section 2.2.9.3) and individual BMRs were measured again after 3-4 weeks. The first set of measurements (termed time 1) were made between 5-13 August, and the second set (time 2) were made between 30 August - 5 September. Food pellets (EWOS Salmon Starter, number 2) were offered at approximately 4.5% wbm per day.

2.2.9.2 Experiment 2 (summer 1997)

Fish were removed from the holding tank at random and starved for 12-16 hours. After measuring BMR throughout a single day, the individual fish were then allocated to one of six groups of five fish (total $n = 30$) of similar log residual metabolic rate (i.e. positive or negative) and body mass. This provided six groups of comparably matched individual fish for which more intense competition in developing social hierarchies was expected. Individual BMRs were measured again after 4-5 weeks. The first set of measurements (time 1) were made between 5-18 August and the second set (time 2) were made between 17 September-1 October. Food pellets (EWOS Salmon Starter, number 2) were offered at approximately 2.3% wbm per day.

In Experiment 2 there was mortality between times 1 and 2, with a single mortality noted in most of the groups, but two and three mortalities in the low body mass groups (groups 6 and 5 respectively). There was no evidence of disease.

2.2.9.3 Allocation to individual channels

Prior to the start of both experiments, each fish was anaesthetised and given a unique alcian blue dye mark on the dorsal surface. Each group was then allocated to a single channel (0.8 x 0.15 x 0.15 m) within a system of six channels arranged in parallel. The channels were constructed from bonded sheet plastic and supplied with a continuous flow of water from Loch Faskally, at a constant water depth of 0.15 m. In all cases the fish were held in the channels at a higher density than in the holding tank (the initial density in the channels was 420 fish m^{-3}, approximately three times higher than stock tank density). Fish in both experiments were fed twice daily and once a day at the weekends throughout the experimental period. Uneaten food was removed as necessary.

2.3 Results

In the 1996 experiment there were no significant differences between any slopes or elevations of the allometric BMR- body mass relationships for the experimental times (MANOVA, $p > 0.05$); pooled data gave a regression equation of:

$$\log \dot{V}O_2 = 0.959 \log W - 0.814 , r^2 = 0.818, n = 49, p<0.001$$

where VO_2 is the oxygen consumption rate in mgO_2 per animal h^{-1} and W is the body mass in g. This scaling factor (0.959) was used to adjust all the BMR values to those for a fish of mean mass of 2.1g ($BMR_{(adj)}$). In the second experiment (1997), although there was no significant difference between the gradients of the slopes (MANOVA, $p> 0.05$) there was a significant difference between the elevations of the slopes (MANOVA, $p< 0.001$, $F = 54.98$), indicating a substantial time effect (Figure 2.1):

$$\text{Time 1 } \log \dot{V}O_2 = 0.828 \log W - 0.740 , r^2 = 0.744, n = 32, p<0.001$$
$$\text{Time 2 } \log \dot{V}O_2 = 0.825 \log W - 0.834 , r^2 = 0.568, n = 22, p<0.001$$

The $BMR_{(adj)}$ for the majority of fish declined between the time 1 and time 2 in Experiment 2 (1997). Therefore the mean value of the slopes (0.826) was used as the

scaling factor. Since in the second experiment, the fish were given a lower food ration they grew less than in 1996, and were scaled to a mean mass of 1.7 g.

2.3.1 Fixed effects using the linear mixed model

The results of the mixed model approach confirmed the results of the MANOVA analysis with very similar results to those above, but with some additional information. In both experiments there was, as expected, a significant positive relationship between $\log_e$ BMR and $\log_e$ mass ($p < 0.0001$ in each), with no evidence of a $\log_e$. mass time interaction, so the slope of the relationship was the same in both times. There was evidence of a time effect in Experiment 2 ($p < 0.0001$) but not in Experiment 1. The fitted relationships are given below, the numbers in parentheses are standard errors.

Experiment 1: $\log_e \text{BMR} = -1.875\ (0.047) + 0.966\ (0.063)\ \log_e \text{mass}$

Experiment 2:

time 1: $\log_e \text{BMR} = -1.711\ (0.048) + 0.831\ (0.088)\ \log_e \text{mass}$

time 2: $\log_e \text{BMR} = -1.929\ (0.059) + 0.831\ (0.088)\ \log_e \text{mass}$

There was no evidence of a channel effect, regardless of whether it was treated as fixed or a random effect indicating that all the channels provided a replicate environment.

2.3.2 Random effects using the linear mixed model

Estimates of the variance components with their bootstrap 95% confidence intervals are given in Table 2.1. In both experiments, all the variation in the data is explained by within-fish variation and measurement error. There is variation in BMR measurements made on the same fish on the same day, and additional variation when BMR is components are larger than the within-fish within-time variance components, but both are of the same order of magnitude.

There is no evidence of any additional between-fish variation. Of course, such variation must exist, but it is swamped by the natural within-fish variation. In other words, fish that have a high BMR in time 1 are just as likely to have a low BMR in time 2 as a high BMR. For illustration, the mean $\log_e$ BMR measurement on each fish each time was

calculated, adjusted to the mean mass observed in that time through the BMR – mass relationships described above. These data are plotted in Figure 2.2, with the mean $\log_e$ BMR measurements back-transformed to the original scale to aid interpretation, and with the values for each fish joined by a straight line to show the progression from time 1 to time 2. Large between-fish variation would result in a series of nearly parallel lines. However, the lines are clearly not parallel, indicating considerable within-fish, between-time variation.

The estimates in Table 2.1 measure variation in BMR on the logarithmic scale. The variation on the original scale was assessed by bootstrap computing the approximate coefficients of variation (CV). For example, the estimate of the within-fish between-time variance component from experiment 1 is about 0.013 on the $\log_e$ scale, which corresponds to a CV of about $100 \times \sqrt{0.013} = 11\%$ on the original scale. Thus, the BMR of an individual fish could vary between times by up to approximately $\pm 2 \times 11 = \pm 22$ % at the 95% level. The full set of CV is shown in Table 2.2.

2.3.3 Diurnal variations in BMR

Slight variations of measurement in BMR were noted for individual fish throughout the day, as denoted by the small standard errors of the mean values (Tables 2.3 and 2.4). These individual variations were small because great care was taken to ensure that the BMR data incorporated a negligible activity component (Figure 2.3). Although replicate readings for individual fish were made throughout the sampling period, to check both the repeatability of the results and to assess the effect if any, of diel variation on individual BMR, one-way ANOVA were carried out for individual fish between times of day. No differences were found (one-way ANOVA, $p>0.05$). The occurrence of diel variability in BMR was also analysed using a linear mixed model approach (see above), but revealed no significant diel effects ($p>0.1$) and the issue was considered no further.

2.3.4 Analysis of residuals

Analysis of relative mass standardised BMR ($BMR_{(adj)}$) for individual fish in Experiment 1, carried out by comparing the magnitude of residuals about the BMR - body mass regression, demonstrated that substantial within-individual variation in the mean log residual values for each fish occurred between the two sampling times (Table 2.3). Examples of the substantial between-individual differences for several salmon parr

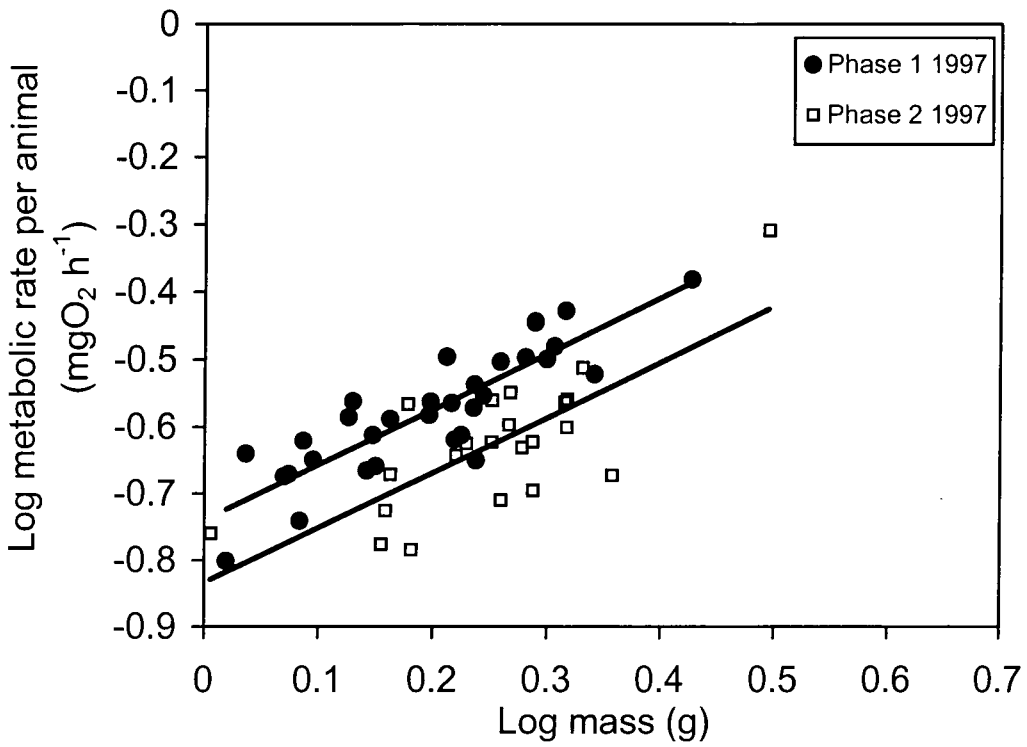


Figure 2.1 The allometric scaling relationship between log body mass and log BMR for all fish in Experiment 2 (1997). There was a statistically significant difference in the mean elevation in BMR for fish (MANOVA $F = 54.98$, $p<0.001$) between time 1 ($n = 30$) and time 2 ($n = 22$).

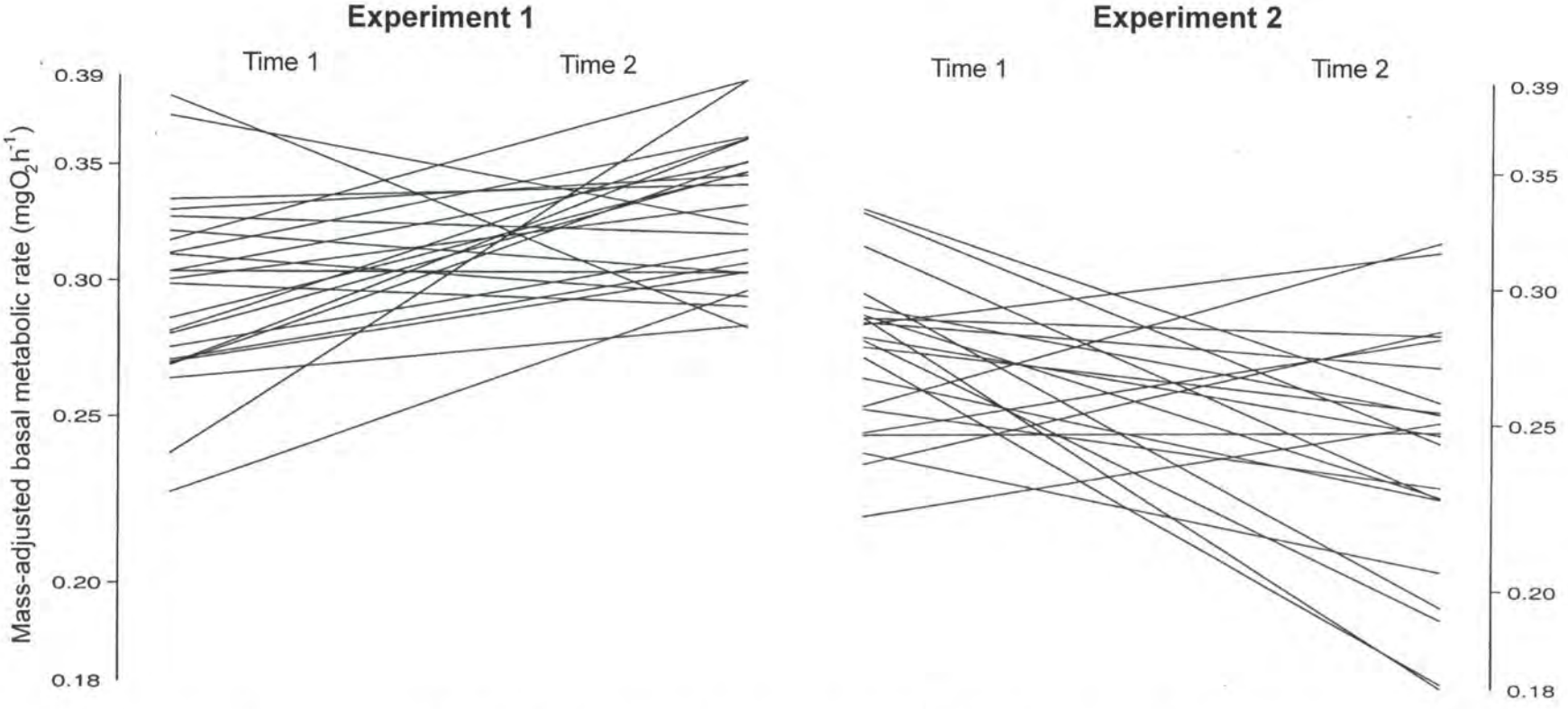


Figure 2.2 A comparison of the mean log BMR measurements of fish at times 1 & 2 for Experiment 1 (1996) and Experiment 2 (1997). The means were adjusted to the mean mass of the fish at each time, and are back transformed for presentation. The values for each fish are connected by a straight line to show the progression from time 1 to time 2. Large between fish variation and little within fish variation would have lead to a number of parallel lines. The non-parallel lines indicate a considerable amount of within-fish variation between the experimental phases.

are shown in Figure 2.3. There were also substantial inter-individual differences between experimental times (Table 2.3). These between-time intra-individual variations were much larger than the intra-individual variability observed over any one sampling day (Figure 2.3).

The lack of any correlation between the mean log residual $BMR_{(adj)}$ measured at time 1 and time 2 in the 1996 experiment ($r^2 = 0.013, p > 0.05$) and in the 1997 experiment ($r^2 = 0.020, p > 0.05$) provides further evidence of individual plasticity in $BMR_{(adj)}$. Had individual $BMR_{(adj)}$ been fixed, then the $BMR_{(adj)}$ values at each experimental time would have been positively correlated with one another.

In both salmon parr experiments (1996 and 1997), BMR measurements at time 1 and at time 2 were compared for each individual fish, between each of the experimental times. Due to the low sample sizes non-parametric analyses were performed. Mann-Whitney *U*-tests were performed to compare the residual BMR values for each individual fish between the two experimental times. Almost 42 % of the fish (10/24) exhibited a statistically significant difference between the two experimental times in the 1996 experiment and 59% of the fish (13/22) exhibited a statistically significant difference between the two experimental times in the 1997 experiment (Tables 2.3 and 2.4). Both experiments revealed a large degree of flexibility in the $BMR_{(adj)}$ of individual fish.

In 1997 (Experiment 2) there was an increase in the overall coefficient of variation of BMR as the experiment progressed (Table 2.4). This, however, was expected as the groups were initially chosen to have as little within group variation as possible and effects of competition and establishment of a dominance hierarchy might be expected to cause an increase in variation between the groups. Had the $BMR_{(adj)}$ been inflexible, this variation would have been expected to be similar throughout the experiment.

For Experiment 1, a comparison of the mean adjusted metabolic rate for those fish that initially had negative and positive residuals at time 1 revealed a significant difference in mean metabolic rates between those fish with positive and negative residuals (repeated measured ANOVA; $F_{1,21} = 11.08, p < 0.01$), and between the individual experimental times ($F_{1,21} = 5.47, p < 0.05$). More importantly, there was a significant interaction effect which indicated that those fish with initially negative residuals increased their

Table 2.1 Estimates of the bootstrap 95% confidence intervals for various components of the linear mixed model. Note that the within-fish within-phase component and the measurement error effects cannot be separated from the data, so the estimates represent their combined effect.

	Experiment 1			Experiment 2		
		95% confidence limits			95% confidence limits	
Variance component	estimate	lower	upper	estimate	lower	upper
Between-fish	0.000	0.000	0.003	0.000	0.000	0.003
Within-fish between-phase	0.013	0.004	0.023	0.020	0.010	0.027
Within-fish within-phase + measurement error	0.0078	0.0060	0.0098	0.0066	0.0053	0.0080

Table 2.2 The coefficient of variation for each experimental phase.(The mean coefficient of variation for each experiment is displayed along with the 95% confidence limits for each value).

	Experiment 1			Experiment 2		
		95% confidence limits			95% confidence limits	
Variance component	Estimate	lower	upper	estimate	lower	upper
Between-fish	0	0	5	0	0	5
Within-fish between-phase	11	6	15	14	10	16
Within-fish within-phase + measurement error	28	24	31	26	23	28

Table 2.3. Variation in metabolic rates for individual fish between times 1 and 2 (Experiment 1, 1996) The mean values are expressed for individual fish (each fish being standardised to 2.1g in weight) as $mgO_2\ kg^{-1}\ h^{-1}$. The statistical significance for each individual fish between the separate experimental phases was tested using a Mann-Whitney *U* test of the log residual values. Key - = no value, ns = no statistical significance ($p>0.05$), * $p<0.05$, ** $p<0.01$, *n* = the number of observations, SE = standard error, Cv = coefficient of variation and Gm=group mean, group number in parentheses).

	Time 1			**Time 2**			
Animal No.	*n*	Mean Phase 1	SE	*n*	Mean Phase 2	SE	**Sig (1-2)**
1	2	183.98	7.70	5	151.75	5.98	*
2	5	129.49	3.68	7	133.09	7.17	**ns**
3	5	153.97	7.88	6	170.57	8.67	**ns**
4	3	138.23	5.21	2	169.84	6.93	**ns**
5	4	140.62	4.42	4	162.41	-	**ns**
Gm(I)/Cv	5	149.26/14.27		5	157.53/9.92		
6	3	188.97	5.07	6	133.22	9.01	*
7	3	149.71	5.70	6	164.66	5.99	**ns**
8	3	150.29	10.27	6	142.08	2.82	**ns**
9	2	156.50	0.00	6	184.57	3.14	*
10	5	148.17	5.49	6	136.32	1.88	*
Gm(II)/Cv	5	158.73/10.84		5	152.17	/ 14.39	
11	2	111.64	-	4	138.77	3.32	*
12	4	135.71	5.72	4	146.39	3.86	**ns**
13	2	132.88	5.06	4	142.88	6.92	**ns**
14	2	133.65	9.14	4	144.43	7.01	**ns**
15	4	159.27	10.070	5	142.71	4.81	*
Gm(III)/Cv	5	134.63/ 12.54		5	143.04/ 1.96		
16	6	117.49	3.10	7	181.79	5.78	**
17	3	137.66	0.00	5	162.45	4.59	*
18	3	162.20	5.76	4	161.86	7.32	**ns**
19	3	132.11	5.05	6	165.74	9.39	**ns**
20	2	116.39	6.56	-	-	-	-
Gm(IV)/Cv	5	133.17/ 14.00		4	167.96/ 5.58		
21	2	132.24	6.92	5	170.71	9.04	**ns**
22	4	161.33	6.52	5	149.63	5.23	*
23	3	153.27	4.26	5	137.68	4.49	*
24	3	164.79	4.01	4	160.41	9.96	**ns**
25	3	148.72	9.01	5	155.93	5.83	**ns**
Gm(v)/Cv	5	152.07/ 8.40		5	154.87/ 7.94		
Total mean	25	145.57	3.81	24	154.57	3.08	**ns**
Coeff. of variation		13.08			9.77		

Table 2.4 Variation in metabolic rates for individual fish between times 1 and 2 (Experiment 2, 1997). The mean standardised values are expressed for individual fish (each fish being standardised to 1.7g in weight) as $mgO_2\ kg^{-1}\ h^{-1}$. The statistical significance for each fish between the separate experimental phases was tested using a Mann-Whitney *U* test of the log residual values. Key - = no value, ns = no statistical significance ($p>0.05$), * $p<0.05$, ** $p<0.01$, *n* = the number of observations, SE = standard error , Cv = coefficient of variation an Gm = group mean, group number in parentheses).

	Time 1			**Time 2**			
Animal No.	*n*	Mean Phase 1	SE	*n*	Mean Phase 2	SE	**Sig (1-2)**
1	4	168.29	3.01	6	174.67	3.14	**
2	4	186.77	6.21	3	125.28	4.33	*
3	4	188.77	3.39	-	-	-	-
4	4	170.30	6.59	5	126.19	7.03	**ns**
5	4	175.07	3.60	5	108.67	5.31	*
Gm(I)/Cv	5	177.84	/5.30	4	133.70	/21.30	
6	4	145.49	8.64	5	136.92	3.18	*
7	4	168.53	3.89	5	149.50	4.75	**ns**
8	4	163.47	7.67	4	137.68	8.49	**ns**
9	4	160.60	3.71	6	98.23	4.69	**
10	4	129.97	4.35	6	139.14	5.74	**
Gm(II/Cv	5	153.61	/10.26	5	132.29	/14.90	
11	4	169.27	3.78	5	155.73	2.95	**
12	4	164.94	3.89	5	136.66	6.21	**ns**
13	4	194.73	3.96	8	134.87	2.78	**
14	5	171.75	3.21	5	140.12	3.03	**ns**
15	5	172.79	3.46	-	-	-	-
Gm(III)/Cv	5	174.70	/6.64	4	141.85	/6.70	
16	5	156.43	5.62	9	125.88	5.23	**ns**
17	5	145.21	3.11	6	155.20	4.32	**
18	4	144.68	3.92	-	-	-	-
19	4	164.34	4.04	5	106.54	3.37	*
20	6	150.35	7.45	5	127.00	3.55	**ns**
Gm(IV)/Cv	5	152.02	/5.44	4	128.66	/15.57	
21	4	187.65	8.59	-	-	-	-
22	4	195.45	-	5	142.74	6.56	**n/s**
23	4	185.28	6.30	-	-	-	-
24	4	169.16	4.91	5	106.18	10.63	*
25	4	196.08	9.11	-	-	-	-
Gm(V)/Cv	5	186.72	/5.84	2	124.46	/20.77	
26	4	168.22	7.26	-	-	-	-
27	5	150.25	3.75	6	176.63	6.14	**
28	5	141.22	4.93	5	113.69	5.66	**ns**
29	4	169.15	5.16	-	-	-	-
30	5	138.97	4.56	5	156.91	7.51	**
Gm(VI)/Cv	5	153.56	/9.40	3	149.08	/21.59	
Total	30			22			
mean	30	166.41	4.94	22	135.20	5.21	***
Coeff. of variation		6.13			8.84		

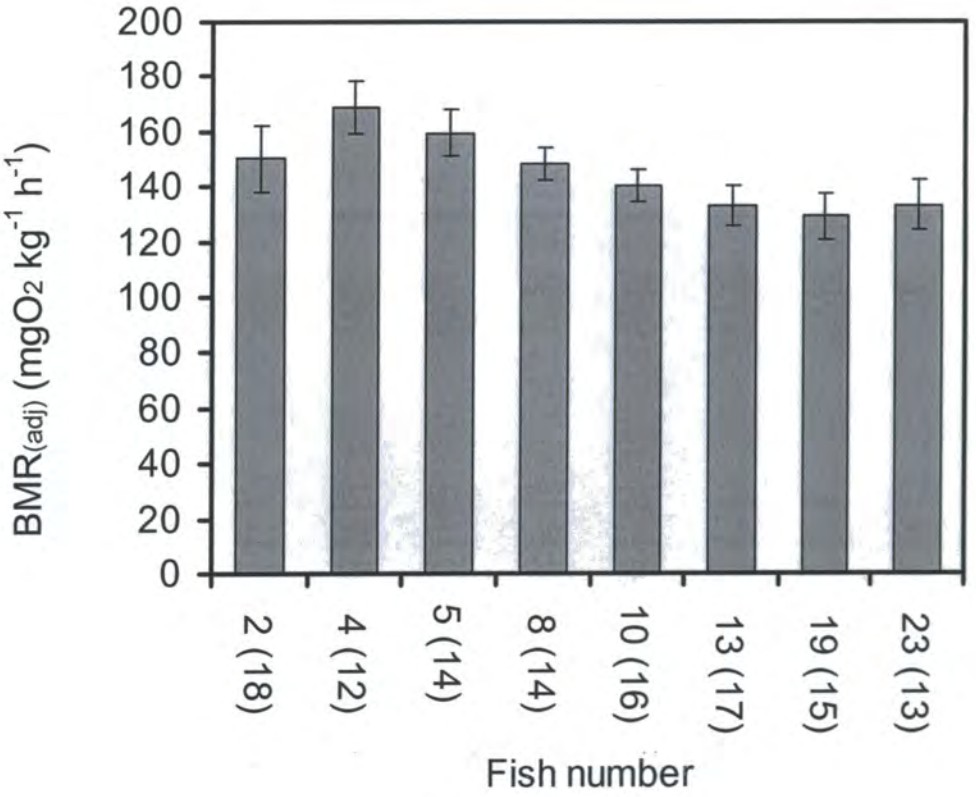


Figure 2.3 Mean ± standard deviation (SD) of BMR readings for eight fish made at approximately two-hour intervals (between 09.30 –19.30), to cover the entire experimental working day. The readings were standardised to the mass of a 3 g fish. The fish number and the total number of readings are given in parentheses. Of note is the variation in BMR between individuals compared to the variation within individuals.

mean BMR compared to those fish that initially had positive residuals ($F_{3,47} = 16.88, p <$ 0.001). The group mean values for both groups at time 2 were similar for fish with initially positive and negative results ($\bar{x} \pm$ SE, 153.2 ± 4.2 $mgO_2\ kg^{-1}\ h^{-1}$, 156.2 ± 4.7 $mgO_2\ kg^{-1}\ h^{-1}$ respectively). These are displayed in Figure 2.4a.

For Experiment 2 (1997), a comparison of the mean adjusted metabolic rate of those fish that initially had positive residuals from the log $\dot{V}O_2$ - log body mass regression (Groups I, III and V, Table 2.4), and those with negative residuals (Groups II, IV, VI, Table 2.4) in 1997, was performed using a repeated measures ANOVA. This revealed, as expected, significant differences in mean metabolic rates between those fish with initial positive and negative residuals ($F_{1,21} = 29.76, p<0.001$) and between the individual experimental times ($F_{1,21} = 5.72, p<0.05$). There was again a significant interaction effect, which indicated that those fish with initially positive residuals decreased their mean BMR more than those fish that initially had negative residuals ($F_{1,21} = 5.87, p<0.05$). The group mean values for groups with initially positive and negative residuals dropped to very similar values at time 2 ($\bar{x} \pm$ SE, 135.3 ± 6.5 $mgO_2\ kg^{-1}\ h^{-1}$ and 135.1 ± 6.5 $mgO_2\ kg^{-1}\ h^{-1}$ respectively). This is displayed in Figure 2.4b.

2.3.5 Relationship of BMR with growth rate

Since a large component of BMR may be the maintenance of metabolic machinery for enabling food processing, assimilation and growth (Hawkins 1991), the individual growth rate of fish might be expected to be correlated with individual BMR. Plots of $BMR_{(adj)}$ at time 2 against percentage growth per day, the increase in growth between experimental times 1 and 2, revealed a significant positive correlation between those fish that initially had negative residuals and growth rate. In experiment 1 the fish with the highest $BMR_{(adj)}$ at time 2 grew the fastest ($n = 12, r^2 = 0.49, p<0.05$, Figure 2.5a). In 1997 there was also a significant negative correlation only between those fish that initially had negative residuals and growth rate (Figure 2.5b) ($n = 12, r^2 = 0.58$ $p<0.005$). The only exception was a single fish that initially had a positive residual and maintained a high relative growth rate whilst also maintaining a high BMR.

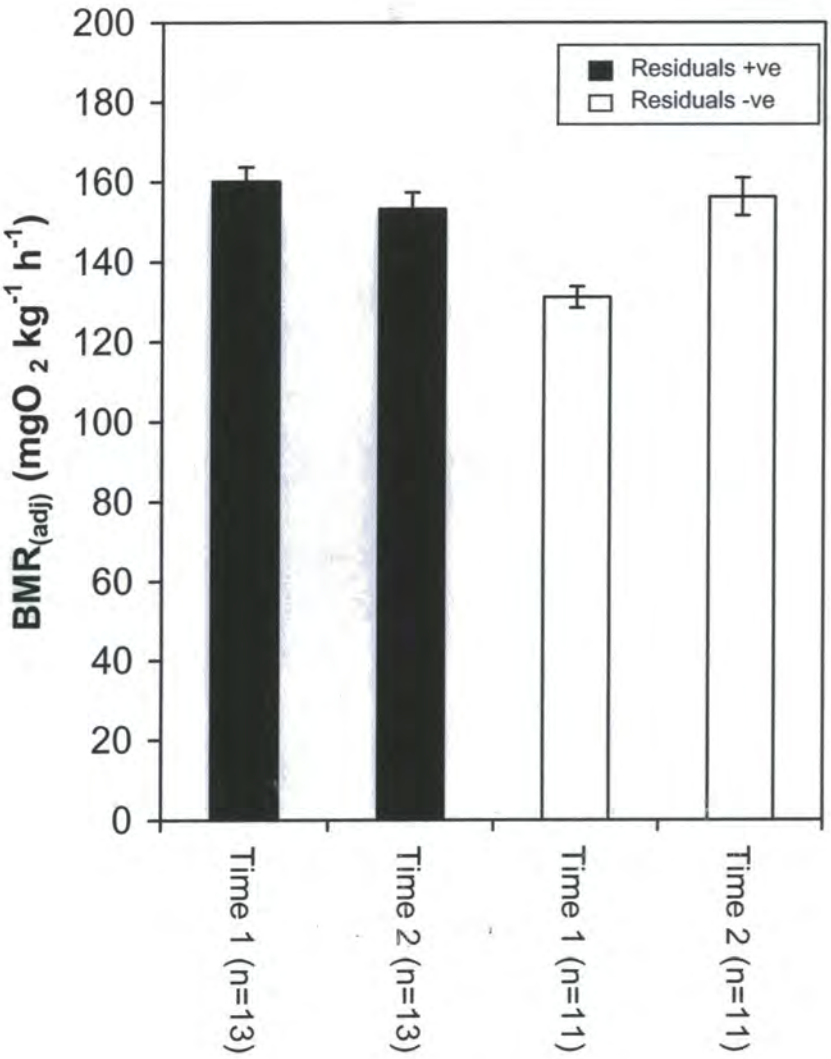


Figure 2.4a A comparison of the change in BMR (mean and SE) between those fish in Experiment 1 (1996), that initially had positive or negative residuals from the log VO_2 log body mass relationship. Metabolic rates are standardised to those of a 2.1g fish. A statistically significant increase in BMR was noted for those fish with negative residuals at time 1 (prior to the start of the experiment) compared to the same fish at time 2, after 3-4 weeks in flumes (Repeated measures ANOVA, $p<0.01$.)

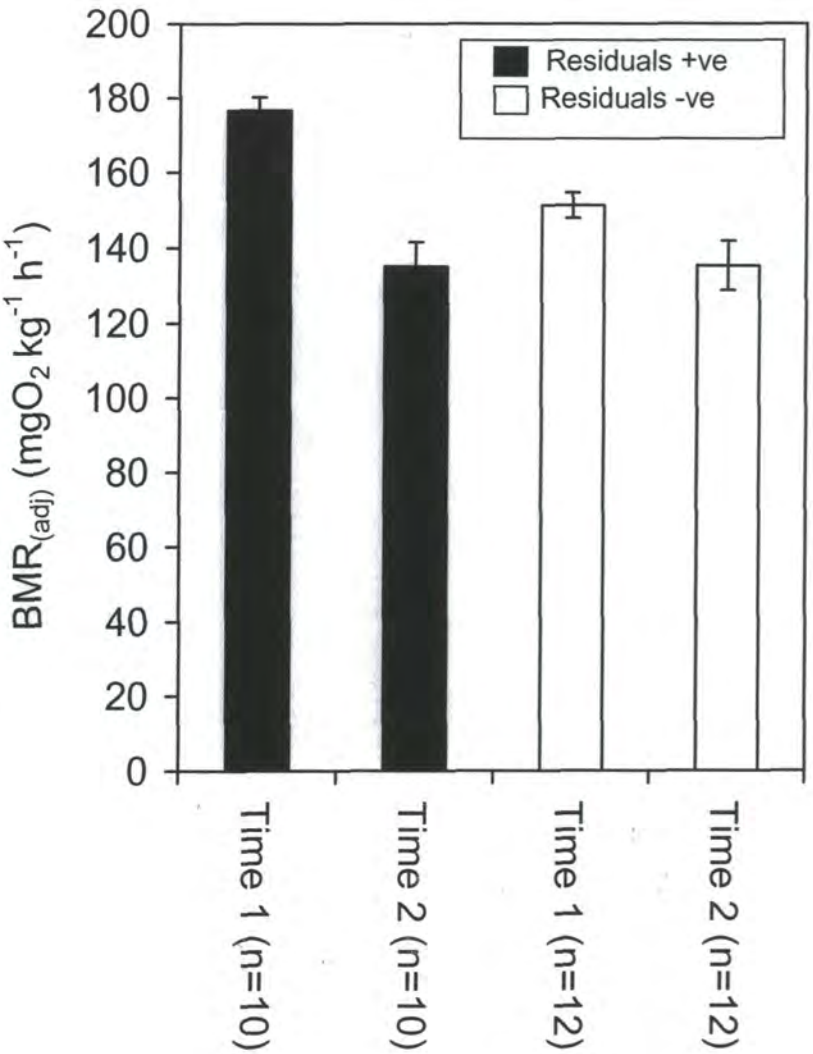


Figure 2.4b A comparison of the change in BMR (mean and SE) between those fish in Experiment 2 (1997), that initially had positive or negative residuals from the log VO_2 log body mass relationship. Metabolic rates are standardised to those of a 1.7 g fish. A larger statistically significant decrease in BMR was noted for those fish with positive residuals at time 1 (prior to the start of the experiment) compared to the same fish at time 2, after 4-5 weeks in flumes (Repeated measures ANOVA, $p<0.05$.)

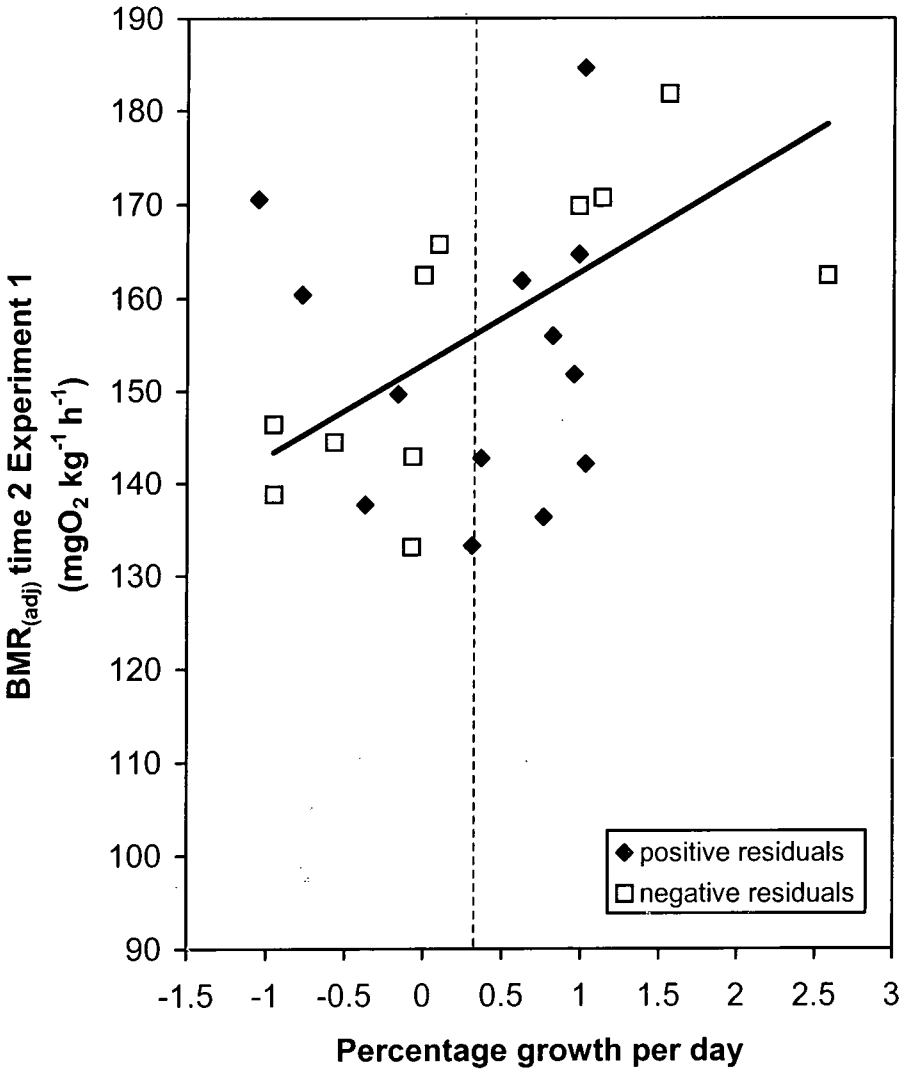


Figure 2.5a BMR at time 2 for all fish in Experiment 1, 1996 (mass standardised to a fish of 2.1g) versus the rate of growth between time 1 and time 2. A significant positive correlation was found for those fish that had negative residuals at time 1 prior to the start of the experiment (n =12, r^2 = 0.49, p<0.05). Those fish with the higher $BMR_{(adj)}$ at time 2 (after 3-4 weeks in flumes) tended to have the highest growth rates. A line indicating the mean growth rate of the fish has been added (0.34 % wbm day^{-1}).

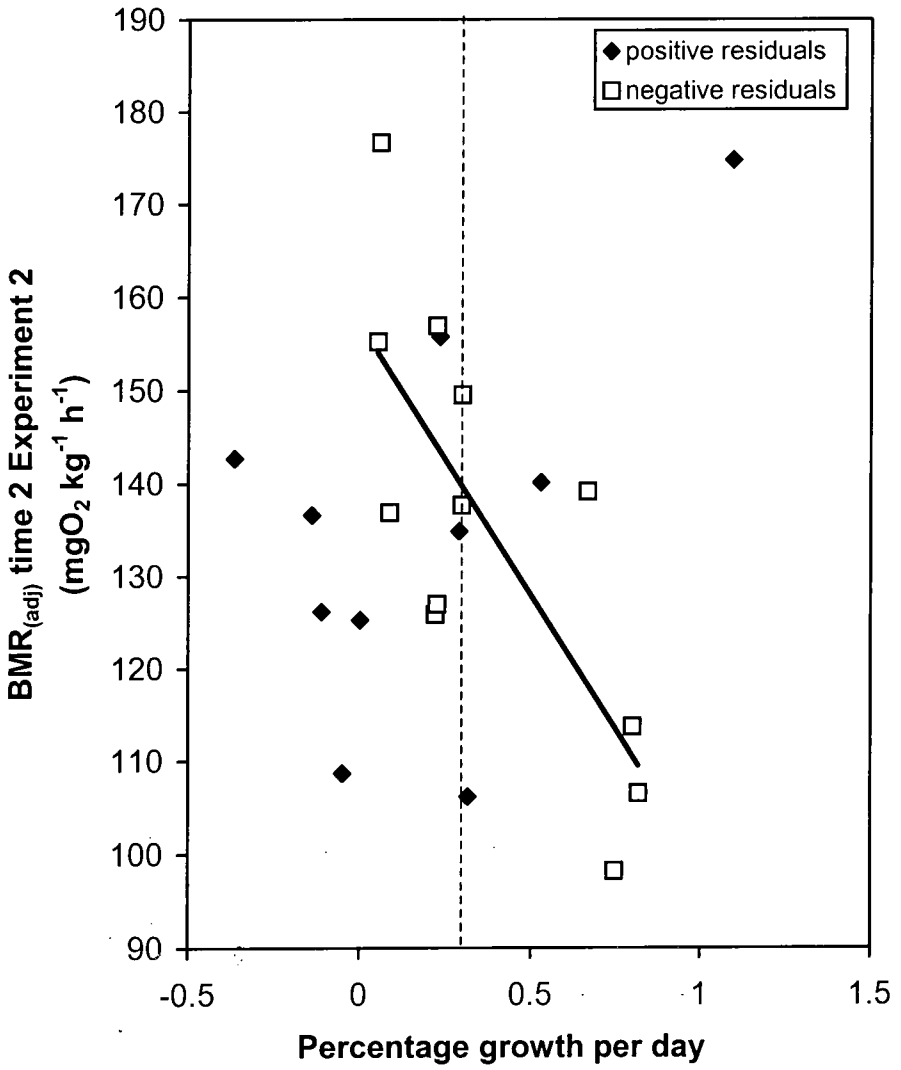


Figure 2.5b BMR for all fish in Experiment 2, 1997 at time 2 (standardised to a fish of 1.7 g) versus the rate of growth between time 1 (prior to the start of the experiment) and time 2 (after approximately 4-5 weeks in flumes). A statistically significant negative correlation was found for those fish that had negative residuals at time 1 (n =12, r^2 = 0.58, p<0.01). Those fish with the lowest $BMR_{(adj)}$ at time 2 tended to have the greatest growth rates. A line indicating the mean growth rate of all fish has been added (0.29 % day^{-1}).

2.4 Discussion

The results clearly show that the BMR of an ectothermic animal, Atlantic salmon parr, can vary, whilst held at stable temperature, within a time period of 3-4 weeks. Therefore, BMR of Atlantic salmon parr is a flexible trait and is not fixed as has previously been assumed. Variation in BMR was recorded both at the population and individual level. In a population exposed to a variation in the environment (Experiment 1), the relative individual BMR could change from relatively low to relatively high and *vice versa* while the group mean BMR (adjusted for mass) did not vary between times. In a population exposed to a low-food, high-competition environment (Experiment 2), mean BMR (adjusted for mass) decreased. This was not a response to starvation as most fish gained mass despite the conditions. The results of comparisons between the metabolic rates of individual fish between the different experimental times were confirmed by the alternative use of a linear mixed model approach performed by R. Fryer. The major source of variation was within fish between the individual times; and was larger than the variation seen for individual fish during a measuring period. These differences swamped the individual variation seen between individual fish which, although not apparent in the model, is known clearly to exist. The within-individual between-time variation in Experiment 2 (about ± 28%) was similar to that recorded in Experiment 1 (about ± 22%).

The mean BMR for Atlantic salmon in this study (Experiment 1, Table 2.3), scaled to that of a 2.1g fish was 145 mg O_2 $kg^{-1}h^{-1}$. This is similar to the value of 139 mg O_2 $kg^{-1}h^{-1}$ reported for a 2 g sockeye salmon at 15°C (Brett & Glass 1973). Brett & Glass (1973), however, measured their SMR in a flume respirometer, which is known to underestimate the actual cost of SMR on extrapolating back to a swimming speed of zero. A possible reason for reduced metabolic costs in a flume respirometer may be the switch to the use of ram ventilation (Roberts 1975) whilst in a swimming flume.

Locomotor activity is usually considered to be the most flexible component of an animal's energy budget (Boisclair & Tang 1993). Natural levels of activity may be expanded or contracted depending on conditions. For example, roach decrease their swimming activity levels when the extra seasonal costs of gonadal development occur (Koch & Wieser 1983), and decreased activity has been seen in several fish species following large meals where ASDA can utilise most of the available metabolic scope (e.g. Soofiani & Hawkins 1982). Since locomotor activity is such a variable component

of total metabolic rate, an accurate assessment is essential if a realistic measure of BMR is to be obtained. A major factor influencing activity costs is the complexity of movement. Swimming in a straight line, or orientation, is less expensive than turning and other complicated movements, which are characteristic of foraging behaviour under natural conditions (Webb 1991; Boisclair & Tang 1993; Krohn & Boisclair 1995). Forstner & Wieser (1990) found that the swimming behaviour of juvenile roach could be separated into two types of spontaneous activity: low complexity and high complexity swimming patterns. They found that higher energetic costs were associated with increased complexity of swimming patterns. However, the total costs of activity may be low compared to the metabolic costs of BMR. Puckett & Dill (1984) estimated that the cost of burst activity for juvenile coho salmon was 5 $mgO_2\,kg^{-1}$ per single charge. They estimated that a juvenile coho salmon had a routine metabolic rate of 350 $mgO_2\,kg^{-1}h^{-1}$ and that a territorial fish made 14 activity bursts an hour (Puckett 1983 as cited in Puckett & Dill 1984). Assuming that during the 16 hours of daylight the fish exhibits 14 activity bursts per hour, this gives an oxygen consumption of 960 $mgO_2\,kg^{-1}day^{-1}$ as opposed to a total routine metabolic cost for 24 hours of 8400 $mgO_2\,kg^{-1}day^{-1}$, resulting in an 11% increase on the routine costs. A metabolic saving due to a decrease in BMR of 22% (Experiment 1) or 28% (Experiment 2), if this was applicable to juvenile coho salmon, would represent 2-3 times the total costs of activity.

Metcalfe's prediction (1995) was that those fish with a relatively high SMR will profit when conditions are good. If SMR is inflexible, under conditions of food shortage, fish with a low SMR have an advantage since their metabolic costs are lower (Metcalfe 1986). He also suggests that the nursery streams of salmon are heterogeneous and suitable for a spectrum of SMR values rather than an optimum one. According to Metcalfe's prediction for the salmon parr in Experiment 1 (1996), with an abundance of food, the highest growth rates would be expected for those fish with an initial positive residual BMR. Those fish with a relatively low residual BMR would show low growth rate, with relative metabolism translating into growth performance. However, in Experiment 1 the high scatter of the data is evidence of a change in BMR at time 2 compared to the values measured at time 1. The majority of fish that had initially positive residuals had positive growth, but tended to have a lower $BMR_{(adj)}$ than initially. In Experiment 2 with reduced food rations (2.3% wbm day^{-1} offered), those

fish with the lowest $BMR_{(adj)}$ at time 2 grew the fastest, except for a single individual with both a high $BMR_{(adj)}$ and high growth rate.

Conclusive evidence for flexibility in BMR comes from comparing the group mean $BMR_{(adj)}$ values in both experiments at time 2. In both experiments there was initially a significant difference in the group means between those fish that had positive and negative residuals. By time 2 in both experiments, both fish had very similar group mean values and standard errors. A possible reason for the observed flexibility in BMR might be that introduction of all the fish in each experiment to their respective channels causes a re-establishment of a dominance hierarchy. Those fish that may have been subordinate in a laboratory tank may become dominant under different conditions. However, behavioural assessments were not carried out in this study and so it is not known whether this was the case.

During measurements of $\dot{V}O_2$ for fish exhibiting spontaneous activity, bursts of anaerobically-fuelled activity have the potential to provide a false measurement of basal $\dot{V}O_2$ from fish that appear to be at rest but are repaying a previous oxygen debt. In this study, vigorous burst activity, with tail beat frequencies > 4 Hz, was extremely rare, because the relatively confined nature of the respirometer precluded it. It is suggested that for Atlantic salmon parr, a continuous recording of activity is the safest method of making true measures of basal metabolism from quiescent fish. Furthermore, it became clear early in this study that activity periods of salmon parr showed no consistent pattern, and that continual monitoring was needed to assess whether the fish had remained quiescent over the whole monitoring period or not.

It is widely accepted that animals tend to invest energy in activity to avoid predators and acquire more energy by catching food. The balance between energy investments and gains has been considered within the framework of optimal foraging theory (Charnov 1976). Analogous optimisation arguments can be applied to the investment in BMR. It has been speculated that some individuals have a relatively high BMR because they have a large metabolic scope, allowing the fish to compete more aggressively (Titus & Mosegaard 1991). High BMR may relate, at least in part, to the costs of supporting the relatively large mass of respiratory and cardiovascular tissue needed to provide a large metabolic scope (Priede 1985) and such tissue is expensive to maintain (Houlihan *et al.* 1988). The data presented in this chapter indicate that large changes in BMR can occur

in salmon parr and organ masses might also need to be flexible. It is known that the mass of highly metabolically active organs can vary substantially. The relative heart ventricle mass, for example, can increase by c.60% in male Atlantic salmon parr preparing to spawn (Armstrong & West 1994) and can double in sexually maturing male rainbow trout (Franklin & Davie 1992; Clark & Rodnick 1998). However, it is not clear how quickly such changes in organs can occur. The significantly larger decrease in metabolic rate for those fish with initially positive residuals (Experiment 2) may imply that these fish have a greater proportion of highly metabolically active tissue that can possibly be reduced. Effectively they may begin at a higher base line than the low residual fish, which may have less scope for metabolic plasticity in the first place. Another large component of BMR may constitute maintenance of enzyme systems associated with processing food. These systems are most likely to respond to short term variations in growth potential since there would be no requirement for massive structural modifications to the organs (e.g. Pelletier *et al.* 1994, section 4.4.2).

Previous work linking respiratory physiology to behaviour and life histories of salmon parr has suggested that BMR is set very early in life (Metcalfe *et al.* 1992). Those fish with high BMR tend to be most dominant (Metcalfe *et al.* 1995; Cutts *et al.* 1998; Yamamoto *et al* 1998) and to grow most quickly (Fausch 1984; Metcalfe *et al.* 1989), and hence life histories of high and low BMR fish would be expected to diverge. The results in this chapter suggest that while BMR varies early in life and may correlate with early dominance, BMR later in life is not fixed and may depend on the performance of fish in their local environment. Hence, BMR later in life may correlate with initial BMR only if conditions favour relatively fast growth of dominant fish (as is usually the case in laboratory studies when fish are reared in tanks). However, these results suggest that a high BMR may be a handicap in a poor growth environment only during the period that the BMR can change, which can be less than three weeks. If there is a direct causal link between BMR and dominance, then dominance status, like BMR, is not a fixed individual trait, but is variable and perhaps is dependent on the local environment.

The data presented in this chapter suggest a need for revision of our understanding of the energy budgets of ectothermic vertebrates. Respiratory metabolism is generally considered as the sum of BMR, ASDA and locomotion. Apparent specific dynamic action has been considered to be a fixed proportion of energy intake in pike (Lucas and Armstrong 1991). Basal metabolic rate has been considered to be constant, while

activity costs have been viewed as the fundamental varying parameter in the respiratory budgets of ectothermic vertebrates at any given temperature (Boisclair & Leggett, 1989). However, present experiments show that within three weeks of exposure to a lower-food, higher-density environment salmon parr can vary BMR by as much as ± 28 %, having a major implication for the overall energy budget. Further work could usefully tease out the scope for BMR to vary in response to each of a range of environmental variables, including various locomotory activities, feeding regimes, water flow regimes and season, as well as exploring the effects of ontogeny.

Due to the time constraints of a short growing season for Atlantic salmon parr, and insufficient time to construct a working miniature flume respirometer it was not feasible to measure MMR on the salmon fry as well. Due to the small size (approximately 2 g), it was not possible to measure organ tissues or enzyme activities with high accuracy either. The aims of the next two chapters were to use an appropriate fish model to identify possible metabolic flexibility in BMR, MMR and metabolic scope in relation to food ration and an exercise regime. If substantial metabolic plasticity could be demonstrated in another fish species, then the possible causes would be examined by studying between-individual variations in body composition and enzyme activities.

Chapter Three

The effects of exercise and food ration on basal metabolic rate (BMR) and maximum aerobic metabolic rate (MMR) in juvenile northern pike *Esox lucius*.

3.1 Introduction

3.1.1 Pike biology and metabolic capacity

Esocids are often considered to be of interest to scientists and fisheries managers due to both the large size attained by individuals and the predatory nature of the species. The pike is one of the most economically important freshwater fish species in many parts of the north temperate zone, supporting important recreational fisheries as well as commercial fisheries elsewhere. Esocids are piscivorous top predators, preferring temperatures of 20-25°C, this being dependent upon latitude of distribution. The piscivorous nature of pike usually develops during the first year of growth, with the availability of suitable prey determining growth rates of fish (Kipling 1983; Hart & Connellan 1984). Pike have also been described as opportunistic in their feeding habits (Mann 1982), with small pike being vulnerable to intraspecific predation by larger pike, in particular during the winter months when vegetation cover diminishes in size and quality (Grimm 1981).

Measurements of BMR for pike have been made previously (Dolinin 1973; Diana 1980, 1982; Bevelhimer *et al.* 1985; Armstrong 1988; Armstrong *et al.* 1992) and for muskellunge (Clapp & Wahl 1996; Chipps *et al.* 2000). The majority of bioenergetics models used assume that the BMR of these fish is relatively constant with season though dependent on temperature (e.g. Diana 1983; Bevelhimer *et al.* 1985). Armstrong *et al.* (1992) were unable to induce sustained aerobic activity in pike held in a swimming flume. Therefore they concluded that the only way they could estimate the maximum aerobic metabolic rate, was to measure the rate of oxygen consumption during the recovery from exercise, in individuals that were exercised to exhaustion by chasing them with a net. Jones *et al.* (1974) measured U_{crit} for pike with a size range of 12 – 62 cm, and found that they had a very low critical swimming velocity (c. 0.8 BL s^{-1} for a

fish of 50 cm). Diana (1996) commented that all of the esocid taxa probably have poor sustained swimming capacity and that evaluation of their swimming capacity is difficult.

In those fish with relatively poor swimming capabilities such as juvenile cod and pike, ASDA may occupy almost the entire metabolic scope. Small pike fed at a high ration (following a meal of c. 10% wbm) operate near to their MMR (Armstrong *et al.* 1992). This situation has also been reported in cod (Soofiani & Hawkins 1982) and in common blenny (Vahl & Davenport 1979).

3.1.2 Growth rate, temperature and nutritional state

Growth governs the length of time for which fish are susceptible to predation, as well as the types and sizes of food items eaten (Hart & Hamrin 1988). Those pike that can regularly feed on higher rations, often through cannibalism, may have a growth advantage and reduce their own susceptibility to predation (Stein *et al.* 1981). In the laboratory environment, daylight and temperature have been shown to previously affect the growth rates of pike (Cassleman 1978).

It has been demonstrated that growth rate is affected by a fish's previous nutritional history (Weatherley & Gill 1981; Talbot *et al.* 1984). Periods of starvation in fish can affect intestinal morphology and enzyme activity, and thereby influence future growth rates and food consumption (Jobling 1993). In juvenile pike, the physiological optimum temperature for growth was measured to be approximately 19-21°C (Cassleman 1978) for pike fed maximum rations. Bevelhimer *et al.* (1985) found that the maximum growth of pike in their experiments occurred at 25°C. A possible explanation for the difference, suggested by Diana (1996), could be due to the inadvertent selection of pike that grew faster in warmer waters.

A tentative growth-ration curve was put forward by Diana (1996), combining data from both Diana (1982) and Bevelhimer *et al.* (1985) for a 20g pike held at 15°C. The losses due to starvation have then been estimated to be 0.82% wbm day^{-1}. They also suggested a maintenance ration of 1.72% of wbm day^{-1}, with an optimum ration of 3.2 % wbm day^{-1} and a maximum ration of 5.7 % wbm day^{-1}. In pike a fasting period of 7 days at 15°C was found to be sufficient to remove any ASDA effects that may be present after a large meal (Lucas 1989; Lucas & Armstrong 1991).

Previously pike have also been reported to have a relatively high growth conversion efficiency. When comparing the mass of food eaten to the body mass increase, growth conversions of between 0.15 (Mann 1982) and 0.38 have been found, with the majority of values being approximately 0.29 (Johnson 1966; Diana 1982) for fish of a similar size to those used in this thesis.

3.1.3 Effects of exercise on growth rate

Growth rates have been improved by exercise training in several salmonid species including rainbow trout (Farrell *et al.* 1990; Gamperl *et al.* 1991), Arctic charr (Christiansen *et al.* 1989, 1991, 1993), and in Atlantic salmon (Jorgensen & Jobling 1993). However, growth in chinook salmon *Oncorhynchus tshawytscha* has been reported to either be unaffected (Kiessling *et al.* 1994) or decreased by exercise (Thorarenson *et al.* 1993). Hammer (1994) found an increase in the growth rate of exercised whiting *Merlangius merlangus*, while Young & Cech (1993a, 1993b, 1994a, 1994b) observed increased growth rate and swimming performance in exercised striped bass *Morone saxatilis*.

Exercise at a fish's preferred swimming speed has been reported to increase the growth conversion efficiency of some fishes, particularly in salmonids (Davison & Goldspink 1977; Leon 1986; Christiensen & Jobling 1990; Jorgensen & Jobling 1993). Chinook salmon has been shown to have a decreased growth efficiency (Thorarenson *et al.* 1993; Kiessling *et al.* 1994) when swimming at their preferred speed. In cyprinid fishes, which are typically poorer swimmers, Davison & Goldspink (1978) showed that the growth of goldfish *Carassius auratus* was impaired at any exercise speed. Specific investigations on the effects of exercise on growth and metabolism of pike have not been carried out because of difficulties in stimulating pike to swim in a flume or swimming respirometer for even short periods, as well as a general consensus that pike have a very limited sustained swimming ability (Jones *et al.* 1974; Diana 1982; Armstrong *et al.* 1992; Diana 1996).

3.1.4 Muscle composition

Muscular tissue forms a large part or the majority of fish body mass, representing 40-60% of the wet mass (Love 1978). In most fish species slow, oxidative (red) fibres are located in a superficial wedge-shaped layer just below the lateral line (Johnston 1981),

whilst fast, glycolytic (white) fibres form the majority of the muscle mass. Red fibres tend to have slow shortening speeds, a high haemoglobin content, a dense vascular capillary bed and high mitochondrial volume reflecting their high aerobic capacity. This enables continuous contractions allowing sustained swimming at low swimming speeds (Bone 1966; Johnston 1981). White fibres function mainly anaerobically, using large amounts of ATP rapidly to provide the power for burst speed acceleration and are typically used when escaping from a predator (Johnston 1981). Several other intermediate muscle types, with intermediate properties to red and white fibres have also been described (Johnston *et al.* 1974), with the numbers of fibres and types differing between fish species (Johnston 1981). Numerous studies have shown that with increasing swimming speed increasing numbers of white fibres are recruited (Bone 1966; Johnston & Moon 1980a,b; Rome *et al.* 1990; Rome 1994). At lower cruising speeds the propulsive power is provided almost exclusively from red fibres. These fibres are typically non-fatiguing and propel the fish at low, sustainable swimming speeds, although these fibres typically have little power (Webb 1994). At higher swimming speeds there is a gradual switch in teleosts from red fibres to white fibres, characterised by low mitochondrial density, myoglobin content and fuelled primarily by glycolysis (Bone 1978; Johnston 1981). These fibres have a high power output but are limited in endurance since they are non-sustainable (Bennett 1985; Rome *et al.* 1988).

The basic composition of pike muscle consists of a small superficial layer of red muscle covering a deeper layer of white muscle (Schwalme & McKay 1985). Pike are a typical 'sprinter-type' fish (Webb 1978), with a large percentage of wet body mass consisting of white muscle mass – c. 54% wbm for a 1 kg pike (Schwalme & McKay 1985). Pike have a relatively low red muscle mass compared to fish with sustained swimming ability such as trout (Goolish 1989), and are reported to have poor sustained swimming abilities (Diana 1996). The only data for critical swimming speeds of pike are data obtained by Jones *et al.* (1974) for pike of 11 – 62 cm at 12 °C that enabled production of the equation $U_{crit} = 4.9\ L^{0.55}$. Jones *et al.* (1974), state that the speed that could be maintained by pike for 100 minutes was 0.6 U_{crit}.

3.1.5 Effects of exercise on swimming musculature

There has been a substantial amount of work on the effects of exercise on muscle morphology, which has been reviewed by Davison (1986). To summarise, in fish made

to swim at sustainable swimming speeds, the proportion of red muscle has been shown to increase relatively when expressed as a percentage of the total myotomal muscle. This increase has been associated with an increase in both cell numbers (e.g. Davison & Goldspink 1977, 1978; Weatherley & Gill 1984) and an increase in cell diameters (Greer Walker & Pull 1973; Davison & Goldspink 1977, 1978). In fish unlike mammals, the number of muscle fibres is not fixed. White muscle has also been shown to increase in fibre diameter with exercise in some fish species such as Atlantic salmon (Virtenan & Forsman 1987; Hinterleitner *et al.* 1992; Davison 1994) but not in others such as Danube bleak *Chalcalburnus chalcoides mento* and nase *Chondrostoma nasus* (Hinterleitner *et al.*1992; Sanger 1992).

3.1.6 Experimental aims

The key aims of the experiments described in this chapter were to:

- To examine if continual sustained swimming of the exercised group had any effects on, MMR, BMR, relative metabolic scope (MMR/BMR) or upon measured growth conversion efficiency?
- To examine the affects of feeding a high ration, and hopefully achieving a high growth rate may have on BMR, MMR and relative scope?
- Was there any relationship between BMR and MMR, was there any evidence of an increasing metabolic scope with increasing size for pike as had been previously suggested by Armstrong *et al.* (1992)?
- If there was a treatment associated change in either BMR or MMR was there any repeatability of individual maximal performance as has been previously suggested for some other fish species by (Kolok 1992a; Kolok & Farrell, 1994; Gregory & Wood, 1998,1999)?

Pike are sit-and-wait predators, relying on ambush and speed to capture prey. As a result they are often motionless in a respirometer for substantial periods of time, with measurements of oxygen consumption, under these circumstances equating directly to BMR measurements (Diana 1982). It is also almost certainly the case that maximal aerobic metabolic rates are elicited in pike following maximal burst exercise (Armstrong *et al.* 1992).

3.2 Materials and Methods

3.2.1 Experimental fish

Juvenile one year old pike were used in the present study (Plate 3.1), the majority were caught in a smolt trap on the River Conan near Inverness in the Scottish Highlands, in late May - early June (1997 and 1998). Fish were removed from the smolt trap daily and held in a single trough in the hatchery. Fresh water was supplied directly from the river. Fish were held under natural lighting conditions. A few additional wild pike, obtained by netting were also supplied from Framlington Fisheries, near Ipswich, Suffolk. When collected, the pike were transported in sealed clear polyethylene sacks. The sealed sacks each contained approximately 5-6 fish of similar size and were filled up with oxygen from an oxygen cylinder, prior to being transported to the aquarium facilities at Durham University.

On arrival at Durham, the pike were held at constant temperature (15 ± 0.1 °C) and on a constant 12h light, 12h dark cycle. The fish were initially separated into a series of large, static-water tanks (1.5 x 1.2 x 0.9 m), with fish of a similar size grouped together in an attempt to reduce cannibalism. Fish were provided with drain-pipe sections for refuge. Initially fish were fed once a week on rainbow trout. The trout were supplied from Costa Spring hatchery, near Pickering, Yorkshire. Each pike on capturing a food item required a finite food handling time before swallowing it. Trout were generally caught from the side and turned in the mouth until they could be swallowed head first (Bucke 1977). The finite handling time prevented most pike from eating more than a single fish during the limited feeding session. Initially pike only accepted live food. Subsequently they were gradually moved on to a diet of dead fish as they became accustomed to laboratory conditions. Once thawed, dead fish were skimmed along the water surface and were readily taken as they fell through the water column and were moved by water currents in the tanks. Uneaten fish were removed the following day.

Prior to the start of any experiment, pike were held in captivity for a minimum of two months in a constant temperature (15± 0.02°C), with a 12 h light: 12 h dark cycle in order to become fully acclimated to laboratory conditions. Tank water was changed once a week from a dechlorinated water supply and was continually filtered with a filter pump (Eheim, 2112). Only fish, which were healthy and feeding, were used in experiments, and the acclimation period was necessary to enable a stable environment to

be developed. Stock fish were fed a ration of approximately 1.5% wbm day^{-1}, which achieved a low rate of growth.

3.2.2 Construction of respirometers

A selection of flushing, closed-system respirometers with capacities of 1.6-5.8 l were constructed from 6 mm thick PVC (Plate 3.2). The respirometers were large enough to allow the fish sufficient space to move and prevent undue stress from restricted space, but sufficiently small to allow the measurement of an adequate drop in oxygen concentration with time. Each respirometer was sealed using a rubber o-ring attached to the lid to provide a water-tight seal, held tight in place by a series of wing nuts. The respirometers were tested for leaks by using deoxygenated water. The constant temperature water bath was deoxygenated by bubbling nitrogen through it until the water became approximately 40% saturated with oxygen for the temperature and pressure. The respirometer was then sealed and the water bath reoxygenated. Any leaks would be apparent from an increase in the oxygen saturation in the respirometer. No leaks were found after test periods of 16 hours.

Each respirometer was surrounded by black plastic on the sides to prevent possible visible interaction between the fish and resultant influence on metabolism. Dark sides also helped to reduce any disturbance by the experimenter. Respirometers were kept in a constant temperature water bath (15± 0.2°C) in a constant temperate room (Plates 3.2 and 3.3) held also at 15± 0.2°C. The constant temperature water bath was also constructed from tinted plastic providing a shaded environment in which to monitor the pike. The high walls of the water bath prevented any disturbance from the experimenter whilst in the same room. The immediate overhead florescent lighting was blacked out. The dim lighting appeared to encourage pike to settle more quickly and reduced activity within the respirometers.

Plate 3.1 Juvenile pike illuminated for photographic purposes.

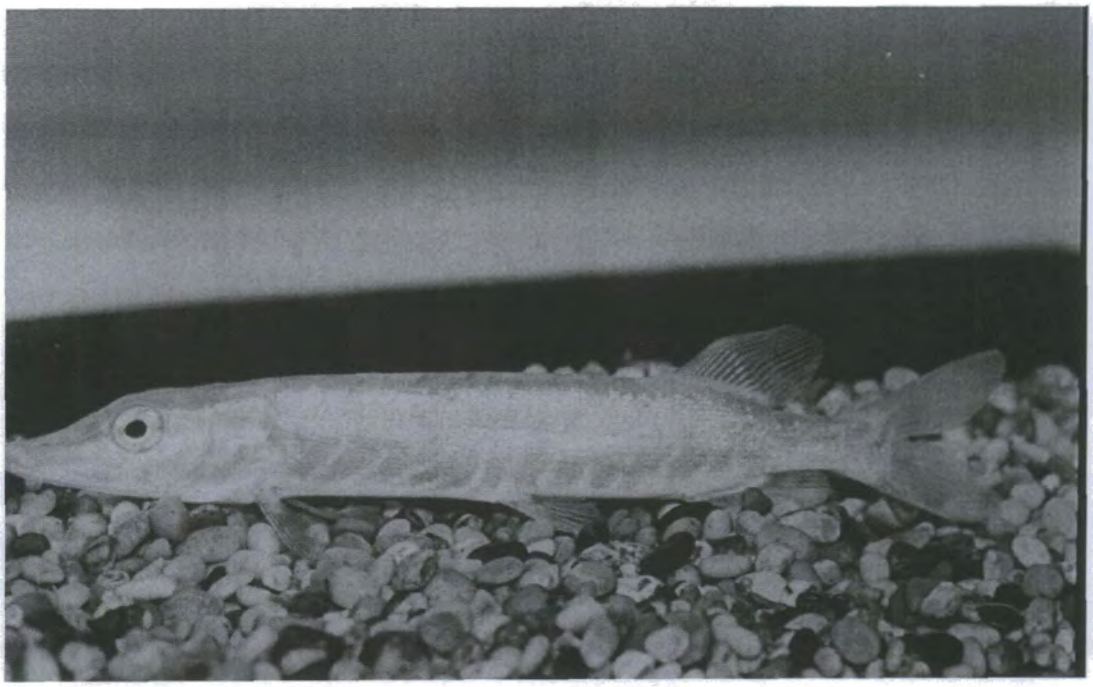
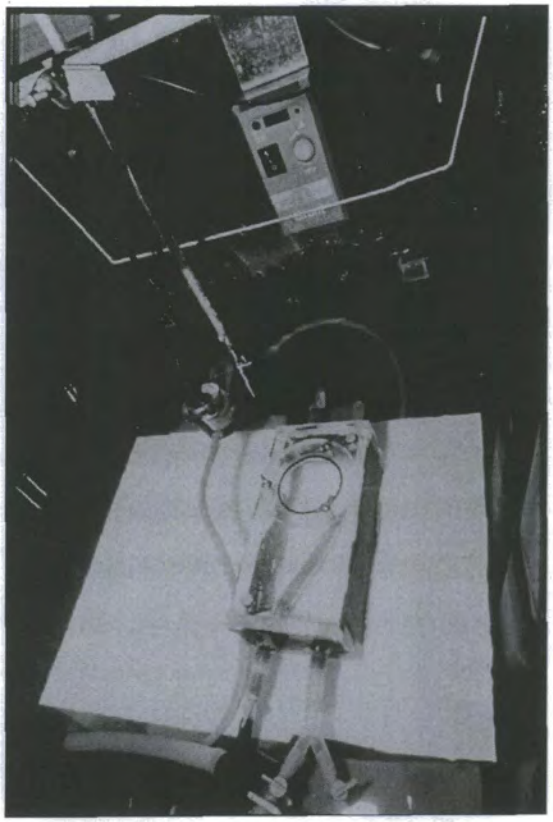


Plate 3.2 Single pike in a respirometer in the 'open' mode. Artificially illuminated for photographic purposes.



3.2.3 Measuring the respirometer lag time and background respiration

The decline in oxygen saturation of the water was recorded on a chart recorder and an ADC 16 data logger (Pico Technology, Cambridge UK), with a sampling rate for the logger set at every 15 seconds. The water within the constant temperature water bath was vigorously aerated using air from a compressed air line system. The lag times of the respirometers used were tested by injecting a known volume of deoxygenated water (obtained by bubbling nitrogen through it), into the respirometer and measuring the lag time between injection and a response by the oxygen electrode. In all cases the response time of the electrode was found to be less than 1.35 minutes in the largest respirometer used, so periods of ten minutes were considered a satisfactory period for the measurement of oxygen consumption in relation to activity measurements. Blank experimental runs were performed at least once a day, in order to take into account background respiration. These were subtracted from the pike oxygen consumption measurements.

3.2.4 Construction of a swimming arena for continuously exercising pike

An initial study to test the feasibility of getting pike to swim continuously was carried out in summer 1997 using three-spined sticklebacks *Gasterosteus aculeatus* as a proxy for pike, to assist in the design of suitable swimming apparatus. At the time sticklebacks were readily available, and were known to have a similar reluctance to swim in a flow of water as pike. They were successfully forced to swim continuously within a half section of a drain pipe, fitted with a mesh false floor to ensure that they were kept in the main water flow and denied access to boundary areas where the water velocity was reduced. The half section of the pipe was divided longitudinally into subsections in which individual sticklebacks could be placed. Areas of shelter (black polyethylene) were placed over the upstream-most third of each section of the pipe, with the rest of the water illuminated. The sticklebacks were found to swim continuously at a slow speed (approximately 0.5 BL s^{-1}) under the areas of shelter. The fish rarely left the proximity of the shelter and fed well on flaked fish food. The experiments achieved continual slow, sustained swimming in sticklebacks for up to four days at which point they were stopped.

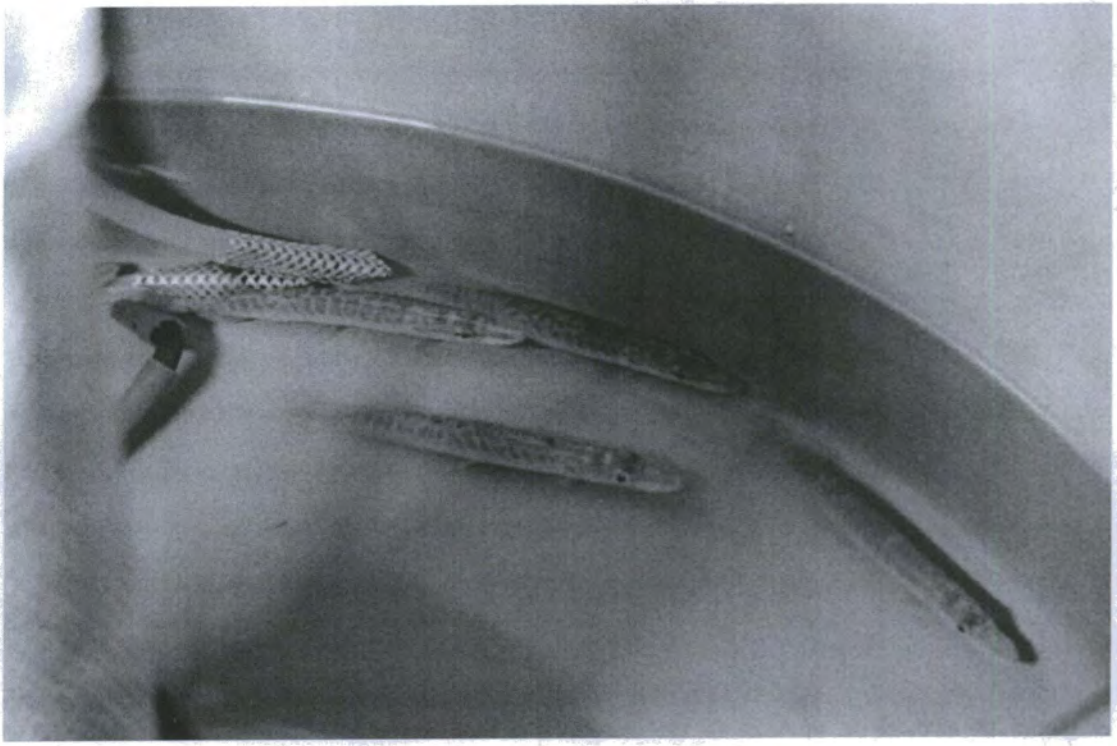
Despite the success of preliminary swimming trials with sticklebacks, there was substantial difficulty in encouraging pike to swim continuously. Various flumes were

constructed, the first of which was a box-type flume, but the fish tried to avoid the water flow by positioning themselves as close as possible to the sides of the flume in low velocity areas. A slight improvement was achieved by the addition of a semi-circle of pipe sealed in to the bottom of the flume to reduce low-velocity 'edge' areas. Finally a coarse mesh floor was introduced to try to keep the pike in the flow of water. A fine mesh floor was avoided since in preliminary observations it was noted that pike were able to hook their fin rays in to the mesh and so remain stationary. If the flow was greater than about 2 BL s^{-1} pike tended to burst swim within the confines of the chamber. Up to a maximum of three pike could be placed in separate sections of a single channel. All three pike could be made to swim for an hour or so at a velocity of about 0.5 BLs^{-1}, but within an hour and a half, fish were found to be trying to exploit any slower water at the sides of the trough. The large space needed for numerous flumes also made the use of this system impractical.

The final construction of the exercise apparatus is displayed in Plate 3.3. It was found that pike swam quite readily in a circular flow. In a circular tank using a relatively low constant water flow from two filter pumps (Eheim 2213), an even circular flow could be produced in the tank (1.2 m x 0.7 m, water depth 0.18 m). The outflow and inflow of each pump were placed at the bottom of the tank to help prevent slow areas of water occurring at the water level used by the fish. The outlets for each pump were placed approximately 90° apart to further help prevent any slow velocity areas being formed and exploited by the pike. The use of dye and a velocity meter failed to find any areas of slow water movement.

The water velocity in the tank was measured using a TT C2 current meter (Kempton, Germany). A mean water velocity of 0.1 ms^{-1} (~0.5 BLs^{-1}) was recorded throughout the tank, which was sufficient to stimulate the pike into swimming slowly and continuously against the water current. Faster water velocities than this were found to preclude sustained swimming. For pike the initial size used in the study (~18 cm) U_{crit} from Jones *et al.* (1974) was calculated to be 1.3 BLs^{-1} and U_{100} minutes as 0.6 x U_{crit} for pike (Jones *et al.* 1974) or 0.8 BLs^{-1}. The swimming velocity achieved for the pike in the present study is therefore believed to be near the maximum that could be achieved over a period of weeks. Faeces were removed using either a siphon and by filtration.

Plate 3.3 Pike exhibiting sustained swimming in the exercise tank



The water was part-changed every 2-3 days using a dechlorinated tap water supply. Additional aeration was supplied via air stones from a compressed air supply.

3.2.5 Procedure for oxygen consumption measurements

An initial measurement of the maximum metabolic rate (MMR) was made for pike exercised in a non-toxic foam lined tank (1.14 x 1.51 x 0.9 m with a water depth of 0.2m). The foam lining prevented damage to pike during exercise. The tank water was kept at the same constant temperature (15± 0.2^{o}C) as the rest of the experimental apparatus. On transfer of a pike to the exercise tank, a foam-covered stick was used to stimulate it to swim. The stick was swiftly placed within 0.3 m of the fishes head, to initiate a 'c' type startle response typical of pike escaping from a predator (Frith & Blake 1995) and resulting in rapid acceleration of the fish over a short period of time (Frith & Blake 1991). The number of burst swimming responses and the time taken to reach total exhaustion was recorded for each fish. Once exhausted the fish was then removed with a hand net. Exhaustion was deemed to be reached when the fish no longer responded to either having a stick placed within 0.3 m of its head, being followed with a hand net or when it did not struggle whilst in the net. At this stage a fish was also noted to be ventilating its gills heavily. In species with a low sustained swimming ability such as pike and cod the recovery from oxygen debt is considered to reflect MMR (Armstrong *et al.* 1992; Reidy *et al.* 1995). All exercise to exhaustion procedures and subsequent measurement of maximum oxygen uptake were carried out under Home Office licence (PPL 60/2312, PIL 60/180) by M. Lucas, but the author made all the observations and data records.

On reaching exhaustion, the fish was transported swiftly in the net, placed into the respirometer (which took less than 10 seconds), the respirometer sealed and an oxygen consumption reading begun immediately. Oxygen consumption readings continued for a period of up to 1 - 1.5 hours (usually <1 h), until it was clear that a maximum oxygen uptake rate had been achieved. On no occasion was the oxygen saturation of the chamber allowed to fall below 80% of the air saturated value. The fish was then allowed to rest for a minimum of 18 hours prior to a BMR measurement being taken; at which point the effects of previous handling stress on the fish had been removed. Initial experiments with four pike showed that after approximately 12 h VO_2 readings were stable and declined no further for at least 34 hours, indicating that the fish had recovered

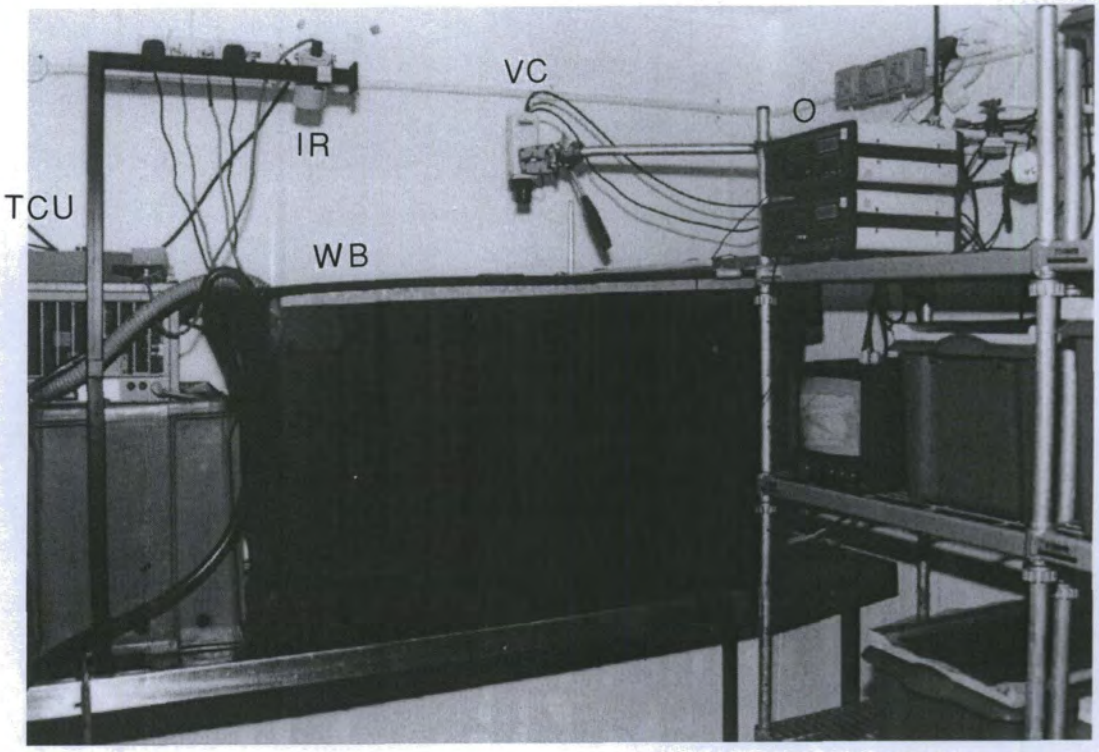
from the previous handling stresses by 12 h post-exercise (Figure 3.1). Very similar results were also noted by Armstrong *et al.* (1992) whose pike were fully acclimated after approximately 12 hours.

Measurements of BMR were made from oxygen consumption readings made between 09.30 and 15.00 during daylight and associated with inactivity. Instead of continual observation of the fish, an overhead camera recorded activity during each experimental run onto a time-lapse video. The time-lapse video allowed correlation in time between fluctuations in activity and oxygen consumption to be assessed. The experimental set up of the apparatus is displayed in Plate 3.4. All oxygen consumption determinations categorised as BMR measurements were defined as ten minute periods containing no activity other than opercular movements and minor fin movement. Following a satisfactory BMR measurement, the fish was anaesthetised with MS222. Once completely anaesthetised the fish was weighed to the nearest 0.01g and fork length measured to the nearest mm. Each fish was given a unique code by tattooing one or more marks on the underneath of the fish using alcian blue injected into the dermis. For the exercised group only, each fish was given an additional tattoo on the dorsal surface to identify individual fish from above. Pike were not fed whilst in the respirometer and were starved for a week prior to metabolic rate readings to ensure complete evacuation of the gut (Lucas 1989; Lucas & Armstrong 1991) and the elimination of any ASDA effects, which may last several hours after gut evacuation (Jobling & Davies 1980).

3.2.6 Diel variation in BMR

To determine whether diel variation in BMR occurred in juvenile pike, measurements were made from four individual pike at 2-4 h intervals throughout a day and night as a pilot experiment. After a period of 16 h to allow for acclimation to the respirometer, the median BMR was not different for any of the four pike between the day and night periods (Mann-Whitney U, all $p>0.05$). An assessment of activity during measurements in the dark (19.00-07.00) was made using an infrared light source, that was detectable by the camera system (Plate 3.4). All subsequent MMR and BMR measurements were made during the day between 08.30 and 19.00.

Plate 3.4 Experimental set up of respiratory apparatus for pike; TCU, temperature control unit; WB, water bath; VC, video camera; O, oxygen meters; IR infra red light source.



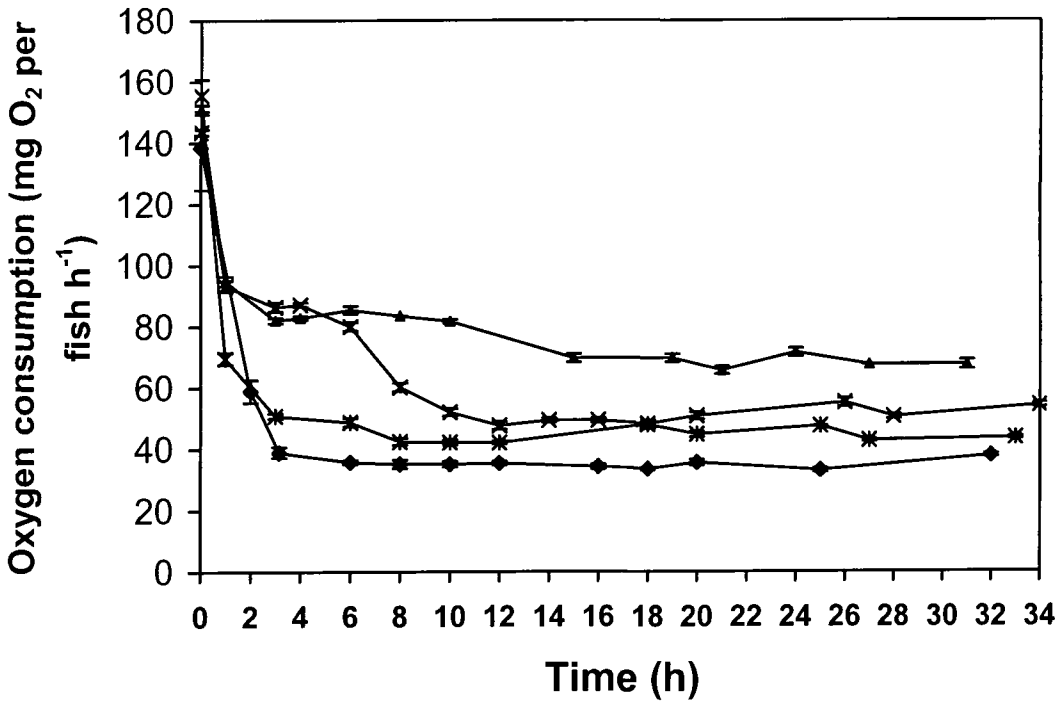


Figure 3.1 Rates of oxygen consumption for four pike at 15°C, the values for each reading corresponding to the mean and SE of several measurements made at each time. Of note is the rapid acclimation to the respirometer.

3.2.7 Ration and timing of experimental measurements for exercised fish

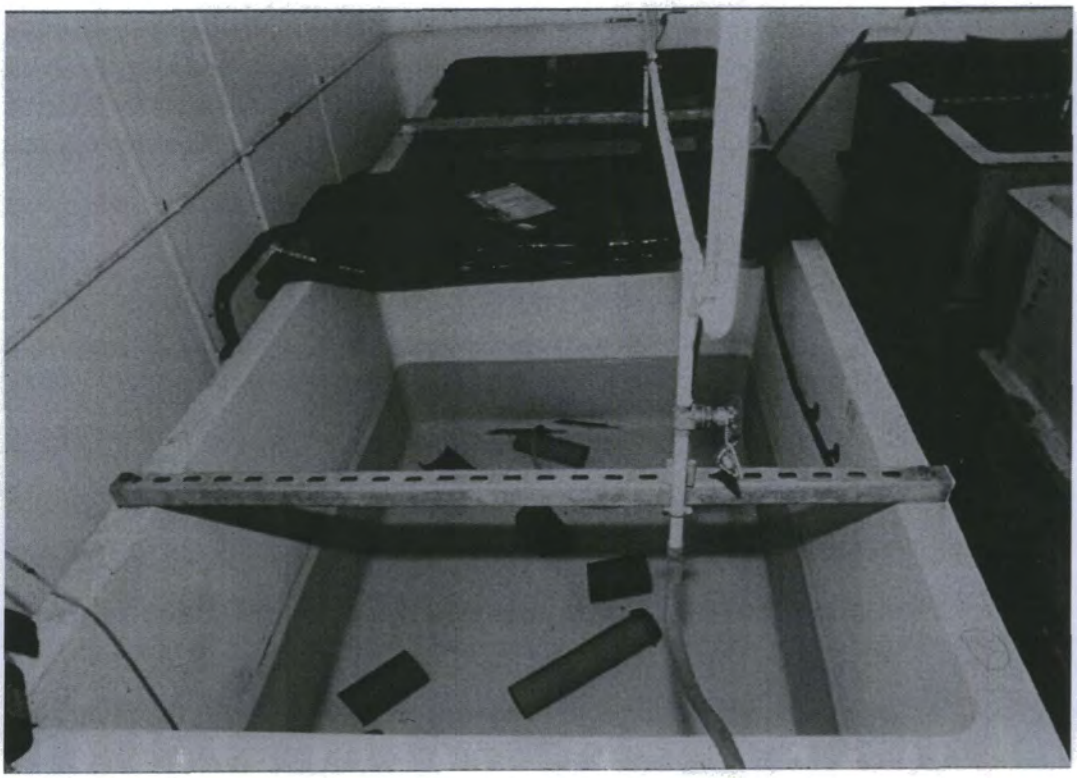
All pike on which BMR and MMR measurements were made were collected in late May – early June 1998 from the same River Conan source (section 3.2.1). The first sets of measurements on thirteen fish removed from the holding tanks (Plate 3.5), prior to any experimental regimes, (10-19 August 1998) was termed time 1. A second set of measurements was made approximately 3 weeks later (3-10 September 1998) and was termed time 2. A third set of readings made approximately 11 weeks after the initial measurements was termed time 3 (20 October – 5 November 1998). For exercised fish, the mean mass (± SE) and body length at the start of the experiment were 32.05 ± 1.42 g and 17.86 ± 0.22 cm (n =13). Although each period of metabolism measurements occurred over 1-2 weeks, variation in the intervals between measurements for individual fishes was minimised by making oxygen determinations for individual fishes in approximately the same order at each sampling time.

Exercised fish were initially fed once a week, equating to a predicted low-growth ration of approximately 2.1% wbm day^{-1} at 15°C (Diana 1996) until the second set of BMR and MMR readings. The majority of fish lost mass whilst offered this ration during this period (section 3.3). True ration was not as high (mean ration, 0.7% wbm day^{-1}, Appendix 1) between time 1 and time 2, since rations were calculated on the basis of initial pike body masses, not all fish fed on all occasions, and fish had to be starved for a week before respirometry to ensure removal of ASDA effects. Therefore at this point the ration was increased to 2 meals per week (equivalent to approximately 4% wbm day^{-1} offered). The actual mean ration consumed was 2.0% wbm day^{-1}. To feed pike in the exercise tank an individual trout was weighed to the nearest 0.01g, and then added to the swimming tank. The tank was then observed to determine which pike consumed the prey item. Due to the handling time (c. 1 minute) for each prey item (trout were generally manipulated in the jaws to be swallowed headfirst), pike rarely consumed more than a single fish during a given feeding session. Fish were introduced one at a time until all pike had fed. Occasionally a single pike showed little interest in the introduced fish. If this pike failed to feed within an hour of the introduction of a prey item, it was recorded as having not fed during that session and the prey fish was removed. Due to the size of trout used for feeding, (approximately 10-15% of pike wbm) fed fish were quite easily distinguished from unfed fish due to a

Table 3.1 The timing of experimental pike BMR/MMR measurements and protocols followed in the present study. Time 1 BMR and MMR measurements were made before the appropriate treatment regime started. A pilot study was carried out between May-July 1998, with the pike held on the same ration and exact conditions as the low ration pike. Pike were all killed after a satisfactory MMR and BMR measurement was taken, at time 3. Organs were used for organ weight analysis and measurement of enzyme activities (see Chapter 4).

Treatment group	Number of pike	Time 1 BMR/MMR prior to treatment	Time 2 BMR/MMR after 3 weeks	Time 3 BMR/MMR after 11 weeks
Pilot Study*	9	-	-	-
Exercised fish	13	10-19/08/98	3-10/09/98	20/10-5/11/98
High ration fish	10	19/08-2/11/98	11-17/09/98	12/11-2/12/98
Low ration fish	10	19/08-2/11/98	11-17/09/98	12/11-2/12/98

Plate 3.5 One of the several holding tanks used to acclimate pike to laboratory conditions prior to the start of the experiment.



noticeably extended stomach and increased opercular movements, both as the fish was being swallowed and immediately afterwards. Occasionally a fish took a second prey item, but the occurrence of this was very rare.

3.2.8 Static-water high and low ration pike experiments

Other groups of pike were held in static water and offered 'high' (5.7 % wbm day^{-1}) or 'low' (2.1 % wbm day^{-1}) food rations. The 'low' food ration group received similar food intake and exercise regime to fish held in stock tanks and may therefore be regarded as a control in terms of the changes in food and exercise received by the other groups. These food rations were based on initial body masses of pike, and so the absolute rations (Appendix 1) were somewhat lower. This was also influenced by the period of 7 days starvation prior to respirometry. Ration levels were chosen from the a tentative growth-ration curve presented by Diana (1996), for a 20g pike at 15°C. Actual mean ration for the low ration group was c. 1.1% wbm day^{-1} and for the high ration group was 2.6% wbm day^{-1}. After an initial BMR and MMR measurement, two groups of ten pike from the stock tanks were held individually in opaque plastic tanks and fed either a near maximum or low-growth ration of trout. The holding tanks were 22 l opaque, plastic tanks (0.31 x 0.43 x 0.27m). Each tank contained a combined aerator and carbon filtration unit (Interpet, IPF1-duo), which filtered up to 175 lh^{-1}.

At the start of the experiment the mean body mass (± SE) and body length for the high food ration group was 24.69 ± 2.03g, and 16.53 ± 0.65cm (n =10) respectively and for the low ration group was 31.04 ± 3.42g and 17.38 ± 0.62cm (n = 10) respectively. Following an initial measurement of MMR and BMR at time 1 (19 August –2 September 1998), these measurements were repeated approximately 3 weeks later (time 2, 11-17 September 1998) and at approximately 11 weeks after time 1 (time 3, 12 November –2 December 1998).

High ration fish were fed three times a week, the equivalent to approximately 5.7 % of critical wbm day^{-1}, whilst the low food ration group were fed once per week, at a level of approximately 2.1 % wbm day^{-1}. Each food item (trout) was weighed to the nearest 0.01g prior to being placed into the tank, and were generally consumed rapidly after introduction. Those fish that were not consumed within 24 hours were removed, this was however, very rare (<5% of food items). The wet mass of any regurgitated fish

was also subtracted from the mass of the food placed into the tank and the mass excluded from food intake and growth efficiency calculations (Appendix 1).

3.2.9 Statistical analysis

All data was tested for any departure from normality or homogenous variances prior to analysis. Data that was not normally distributed or homogenous and uncorrected by transformation (predominantly unequal variance between groups), was subjected to the appropriate non-parametric equivalent test . Where ANOVA was used, pair wise multiple comparisons were Bonferroni corrected before assessing the significance of each pair.

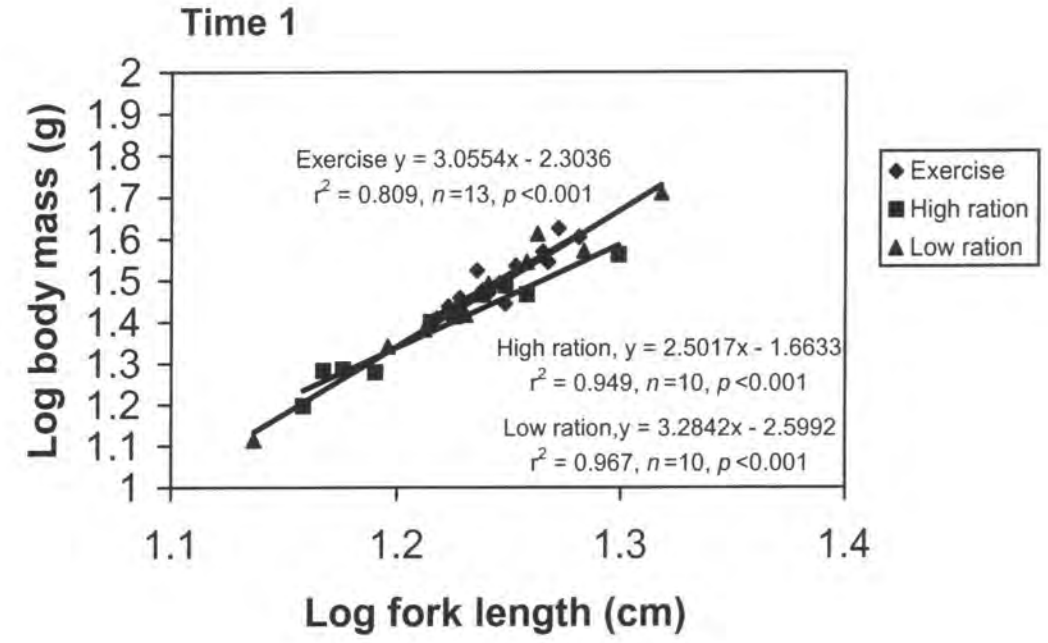
3.3 Results

3.3.1 Growth

An initial comparison of the length-mass relationships (Figure 3.2) between each of the treatment groups revealed good correlations between length and body mass. The high r^2 values indicate tight relationships between body mass and length for all fish throughout the study.

A comparison of growth rate (Table 3.2), calculated as the increase in wet body mass per day (% wbm day^{-1}), compared to the initial mass at the start of the experiment showed a negative growth rate (i.e. a loss in mass) for most of the exercised fish (10/13) between time 1 and time 2 measurements. Minor mass losses were also noted for pike 22 (high ration) and pike 25 and 30 (low ration). Pike 23 (high ration) has also lost mass between times 1 and 2 reflecting poor food consumption over this period, and subsequently died. To counter the negative growth of exercised fish fed at the low ration (approximately 2.1% wbm day^{-1} offered) between times 1 to 2, the mean food ration for exercised fish was increased to approximately 4.5% wbm day^{-1} offered between times 2 and 3. It is believed that the recorded mass loss for the exercised group between times 1 and 2 must have been related to the increased metabolic costs of continual swimming, since the exercise group received the same ration as the static water low ration group but exhibited negative growth.

(A)



(B)

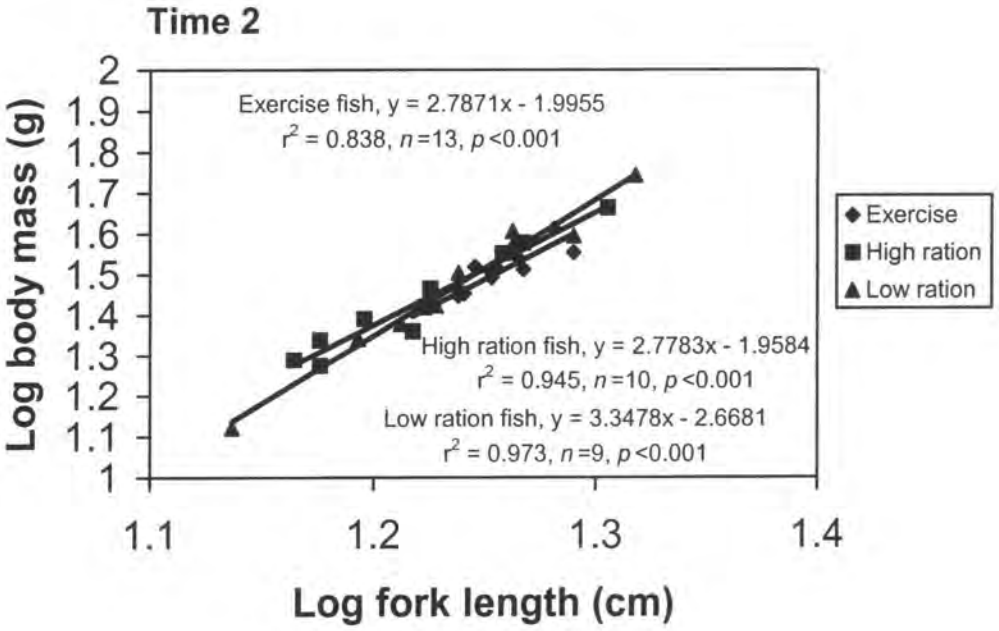


Figure 3.2 Length-mass relationships for groups of pike at (a) time 1, prior to the start of the experiment (above) and at (b) time 2, after c. 3 weeks of treatment (below).

(C)

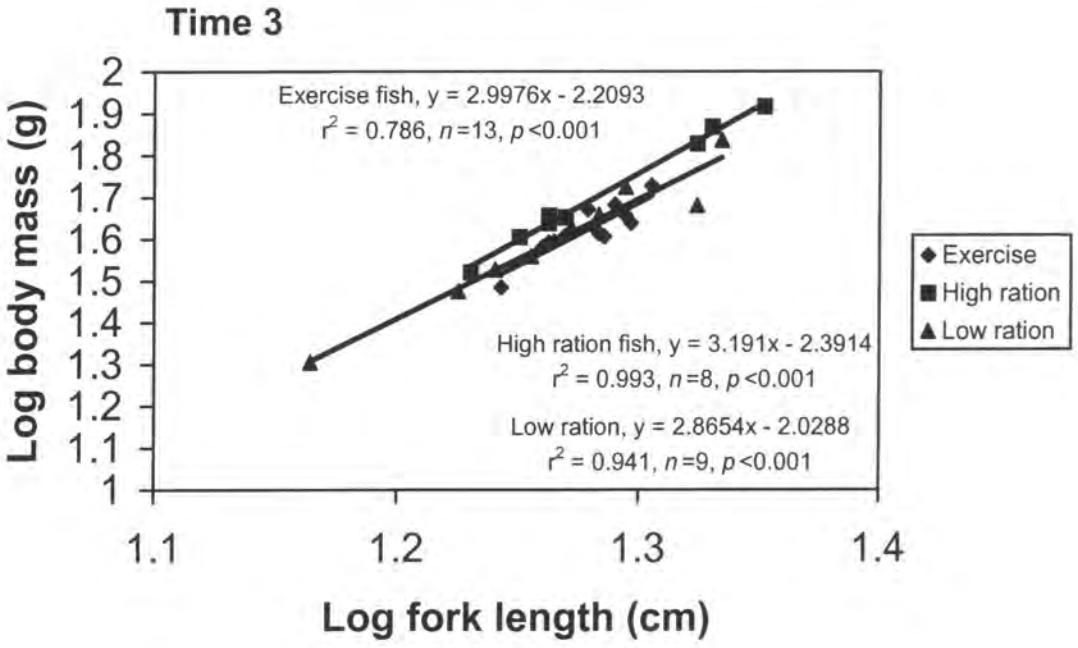


Figure 3.2c Length-mass relationships for groups of pike at time 3 after c. 11 weeks of treatment.

Table 3.2 A comparison of the individual growth rate and % conversion efficiency of individual pike. The body mass (g) for each pike at each time of the experiment is denoted by the respective mass number. Growth was calculated as the percentage change in mass per day. Percentage efficiency was calculated as the increase in wet body mass (g) for the mass of wet fish consumed (g). # = fish refused to feed.

	Time 1 to Time 2				Time 2 to Time 3		
Pike No.	Mass 1	Mass 2	% Growth	% Conversion	Mass 3	% Growth	% Conversion
Exercised pike							
1	33.46	33.04	-0.047	-8.25	46.87	0.775	31.37
2	25.63	22.98	0.382	-39.32	30.49	0.536	22.71
3	27.44	28.06	0.069	7.42	39.07	0.912	31.43
4	31.12	32.71	0.159	17.75	40.38	0.460	23.75
5	27.83	31.99	0.452	51.11	41.09	0.490	28.37
6	29.52	28.39	-0.143	-20.36	40.53	0.764	32.83
7	35.08	32.55	-0.267	-31.43	45.33	0.785	29.87
8	28.67	26.90	-0.220	-44.36	38.09	0.756	36.79
9	37.09	34.67	-0.241	-43.37	48.07	0.966	33.96
10	29.96	28.8	-0.143	-13.50	39.24	0.906	28.99
11	34.33	31.12	-0.406	#	42.75	0.795	32.27
12	42.21	40.98	-0.135	-19.68	53.40	0.651	30.07
13	40.23	35.86	-0.493	-83.08	43.58	0.399	22.11
Mean	32.51	31.39	-0.160	-18.92	42.22	0.707	29.58
High ration fed pike							
14	29.25	37.86	1.338	38.57	73.67	1.168	43.24
15	36.53	46.15	1.197	35.64	82.23	0.965	39.27
16	19.01	24.6	1.337	31.60	44.81	1.014	29.25
17	25.15	29.18	0.801	30.16	dead	dead	dead
18	30.78	35.62	0.828	38.65	67.1	1.133	38.27
19	19.18	21.85	0.773	24.47	40.25	1.186	27.48
20	25.97	26.28	0.066	2.36	43.44	0.933	25.00
21	15.76	19.47	1.385	35.95	45.24	1.864	37.64
22	19.33	18.86	-0.174	-5.39	33.17	1.186	32.53
23	25.97	22.91	-0.842	-29.45	dead	dead	dead
Mean	24.69	28.28	0.671	20.26	53.74	1.185	34.08
Low ration fed pike							
24	37.23	39.40	0.324	24.97	47.87	0.331	29.74
25	40.96	40.44	-0.063	-7.40	52.98	0.403	27.41
26	35.03	37.28	0.378	33.78	45.44	0.313	36.59
27	31.05	31.90	0.152	15.45	42.39	0.427	31.71
28	29.24	dead	dead	dead	dead	dead	dead
29	51.34	55.38	0.437	36.76	68.53	0.308	27.86
30	24.10	23.91	-0.066	-7.63	33.62	0.625	33.88
31	26.15	26.51	0.106	8.49	36.17	0.561	34.26
32	21.93	21.94	0.004	0.45	29.83	0.571	32.24
33	13.01	13.22	0.005	-21.52	20.15	0.672	29.29
Mean	31.00	32.22	0.156	9.26	41.89	0.468	31.44

The group mean growth of exercised fish between times 2 and 3, was intermediate to both the static-water low ration and high ration group mean values. Another possible reason for mass loss was the short duration in time between readings and the fact the fish had to be starved for a week prior to BMR measurements to remove any possible ASDA effects. Initial (time 1) and final (time 3) group mean lengths and masses for each group of fish are presented in Table 3.2.

3.3.2 Gross conversion efficiency

Gross conversion efficiency was calculated for all fish as the total mass of fish consumed divided by the increase in wet body mass over that period and expressed as a percentage (Table 3.1). All fish that lost mass between times 1 and 2 had a corresponding negative gross conversion efficiency. Of those fish with a positive gross conversion efficiency between times 1 and 2, 88% (14/16) tended to increase their gross conversion efficiency between times 2-3. A direct comparison of the gross conversion efficiency between each treatment group at the end of time 3 revealed no significant differences between the individual treatment groups (arcsine transformed data, 1-way ANOVA ($F_{2,26} = 1.96, p>0.05$). However, the fact that exercised fish were almost certainly expending much more energy than static-water low and high ration fish, strongly suggests that they must have developed a higher net growth conversion efficiency.

3.3.3 Condition factor

Condition factor of each fish was calculated as the body mass (g) / length^3 (cm) (Jensen 1979). A comparison of the condition factor of pike was made between the individual treatment groups over time (Table 3.3) using 1-way ANOVA with Bonferroni correction. The largest increase in condition factor was for those fish fed at the highest ration over the course of the experiment (1-way ANOVA, high ration times 1-3 , $F_{2,27} = 46.33, p<0.001$). Exercised fish exhibited a significant decrease in condition factor between times 1-2 (1-way ANOVA, $F_{2,36} = 11.03, p<0.001$) whilst held at the low ration, but regained condition between times 2-3 whilst fed at a higher ration. The higher increases in condition factor over times 2-3 ($p<0.001$) indicated the larger time period covered by that time period (approximately 8 weeks). Low ration fish had a significant increase in condition factor although as expected, less than for the other groups ($F_{2,27} = 4.86, p<0.05$).

Table 3.3 A comparison of the group mean mass and lengths of fish of the three treatment groups, an exercised treatment group and fish fed at a high ration and at a low ration. Measurements reported are those of pike at time 1 (at the start of the experiment) and time 3 (after 11 weeks).

	Time 1			Time 3		
Treatment Group	Initial mass (g) $\bar{x} \pm$ SE	Initial length (cm) $\bar{x} \pm$ SE	*n*	Final mass (g) $\bar{x} \pm$ SE	Final length (cm) $\bar{x} \pm$ SE	*n*
Exercise	32.05 ± 1.42	17.86 ± 0.22	13	42.21 ± 1.54	18.98 ± 0.20	13
High ration	24.69 ± 2.03	16.53 ± 0.65	10	53.74 ± 6.33	19.37 ± 0.71	8
Low ration	31.04 ± 3.42	17.38 ± 0.62	10	41.88 ± 4.71	18.57 ± 0.73	9

Table 3.4 A comparison of the individual condition factors (body mass/ length^3) for juvenile pike within each treatment group between each of the three different measurement times(time 1 prior to the start of the experiments, time 2 after c. 3 weeks and time 3 after c. 11 weeks). Statistical comparisons were made using 1-way ANOVA with Bonferroni correction. C.F. = condition factor, SE= standard error, ns = no statistical significance, (p>0.05), * p<0.05, ** p<0.01, *** p<0.001. The numbers in parentheses marks each experimental time.

Treatment	C.F. (1)	SE	Sig time 1-2	C.F. (2)	SE	Sig time 1-3	C.F. (3)	SE	Sig time 2-3
Exercise	0.584	0.01	*	0.547	0.01	ns	0.614	0.01	***
High ration	0.542	0.01	*	0.591	0.01	***	0.715	0.01	***
Low ration	0.569	0.01	ns	0.580	0.02	*	0.634	0.02	ns

Table 3.5 A comparison of the pike condition factors (body mass/ length^3) between each treatment group, at the same time of the experiment (time 1 prior to the start of the experiments, time 2 after c. 3 weeks and time 3 after c. 11 weeks). Statistical comparisons were made using 1-way ANOVA with Bonferroni correction. C.F. = condition factor, SE= standard error, ns = no statistical significance, (p>0.05), * p<0.05, ** p<0.01, *** p<0.001.

	Time 1			Time 2			Time 3		
Treatment	C.F.	SE	Sig time 1	C.F.	SE	Sig time 2	C.F.	SE	Sig time 3
Exercise vs. (High ration)	0.584	0.01	ns	0.547	0.01	*	0.614	0.01	***
High ration vs. (low ration)	0.542	0.01	ns	0.591	0.01	ns	0.715	0.01	**
Low ration vs. (Exercise)	0.569	0.01	ns	0.580	0.02	ns	0.634	0.02	ns

A comparison of the condition factors between individual treatment groups at each time point (Table 3.4) revealed no statistically significant difference in the body condition between fish at time 1 ($p>0.05$). At time 2 there was a significant difference in condition factor between high ration and exercise fish, (1-way ANOVA, $F_{2,31} = 3.84$, $p<0.05$), the difference being attributed largely to the decrease in mean condition factor for all exercised fish and a relative increase in condition factor for all the high ration fish. By time 3 the high ration fish had a significantly greater condition factor compared to both exercise and low ration fish (1-way ANOVA, $F_{2,29} = 16.31$, $p<0.001$).

3.3.4 Allometric scaling coefficients

A comparison of the allometric scaling of BMR and MMR relationships between treatment groups showed no significant differences in either slope or elevation for either BMR or MMR values at time 1 (MANOVA all $p>0.05$, Figure 3.3a). This was broadly expected as these readings were taken prior to the commencement of treatment and involved fish of similar size drawn at random from the same tanks and fed the same ration. There was no significant difference between any of the slopes (allometric scaling coefficients) when comparing BMR and MMR values at any time. For all fish, mass was standardised to that of a 35 g pike using the equation of Soofiani & Priede (1985; section 2.2.6). Individual values of the slopes b from the allometric relationships were used to scale the MMR and BMR values at time 1 using the respective allometric scaling equations. The scaling values used at time 1 were 0.81 for BMR and 0.90 for MMR. There were no significant differences between the slopes of BMR and MMR for any of the individual treatment groups (MANOVA, $p>0.05$).

At time 2, (Figure 3.3b) there was a highly significant increase in the elevation of the MMR allometric scaling equation for exercised fish, compared to both high ration and low ration fish (MANOVA; exercise vs. high ration, $F_{1,20} = 26.25$, $p<0.001$; exercise vs. low ration, $F_{19,1} = 46.73$, $p<0.001$). There was no significant difference between the elevations of allometric scaling equations of MMR for high and low ration fish ($p>0.05$). On comparing allometric scaling equations for BMR between groups of pike, there was a significant difference in the elevations between high and low ration fish only (MANOVA; $F_{1,16} = 6.15$, $p<0.05$). Mean b values used to scale fish masses at time 2 were 0.69 for BMR and 0.89 for MMR. There was no significant difference between

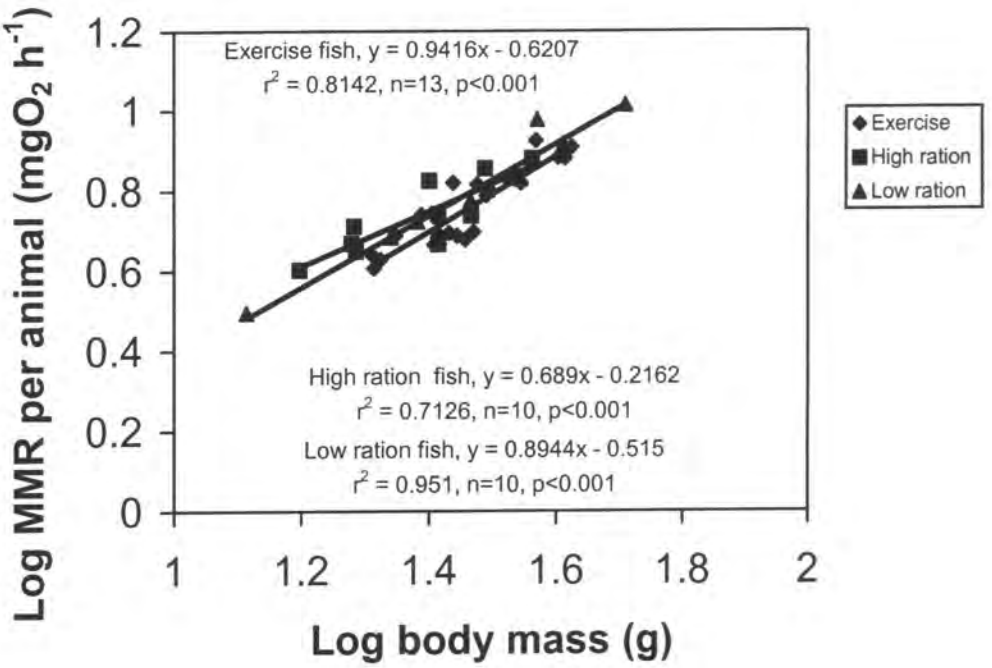
the slopes of BMR and MMR for any of the three treatment groups (MANOVA, $p>0.05$).

At time 3 (Figure 3.3c) there remained a highly significant difference in the elevations of MMR allometric scaling equations between exercise fish and both high and low ration fish (MANOVA; exercise vs. high ration, $F_{1,18} = 54.14$, $p<0.001$; exercise vs. low ration $F_{19,1} = 98.55$, $p<0.001$). There was no significant difference in MMR between the high and low ration (MANOVA; $p>0.05$), or in slopes (MANOVA; $p>0.05$). A comparison of the BMR allometric scaling equations revealed a significant difference between the elevations of the regressions for exercise and low ration fish (MANOVA; $F_{19,1} = 16.35$, $p<0.01$) and high and low ration fish (MANOVA; $F_{14,1} = 6.42$, $p<0.05$). There were no significant differences in elevation of the BMR allometric equations between exercise and high ration fish (MANOVA; $p>0.05$). Mean scaling values b used to scale fish to a constant mass at time 3 were 0.79 for BMR and 0.78 for MMR. There was found to be no significant differences between the slopes of MMR and BMR for any of the three treatment groups (MANOVA, p>0.05).

3.3.5 Condition factor and growth rate

A good correlation may be expected between condition factor and growth rate. There was a highly significant correlation between condition factor at time 2 and percentage growth rate between times 1 and 2 ($F_{30,1} = 17.81$, $r^2 = 0.372$, $p<0.001$). A similar highly significant correlation was also found between the condition factor at time 3 and percentage growth rate between times 2 to 3 ($F_{28,1} = 21.01$, $r^2 = 0.428$, $p<0.001$).

A comparison of hepatosomatic index (the mass of the liver divided by the somatic mass) and condition factor revealed a highly significant positive correlation ($F_{1,28} = 54.06$, $r^2 = 0.659$, $p<0.001$). As all the pike were still juveniles it was crudely assumed that excess energy remaining after the metabolic costs of BMR, ASDA and activity etc would be used for somatic growth with no energy channelled in to gonadal growth. The lack of gonadal growth in any fish was confirmed at necropsy.



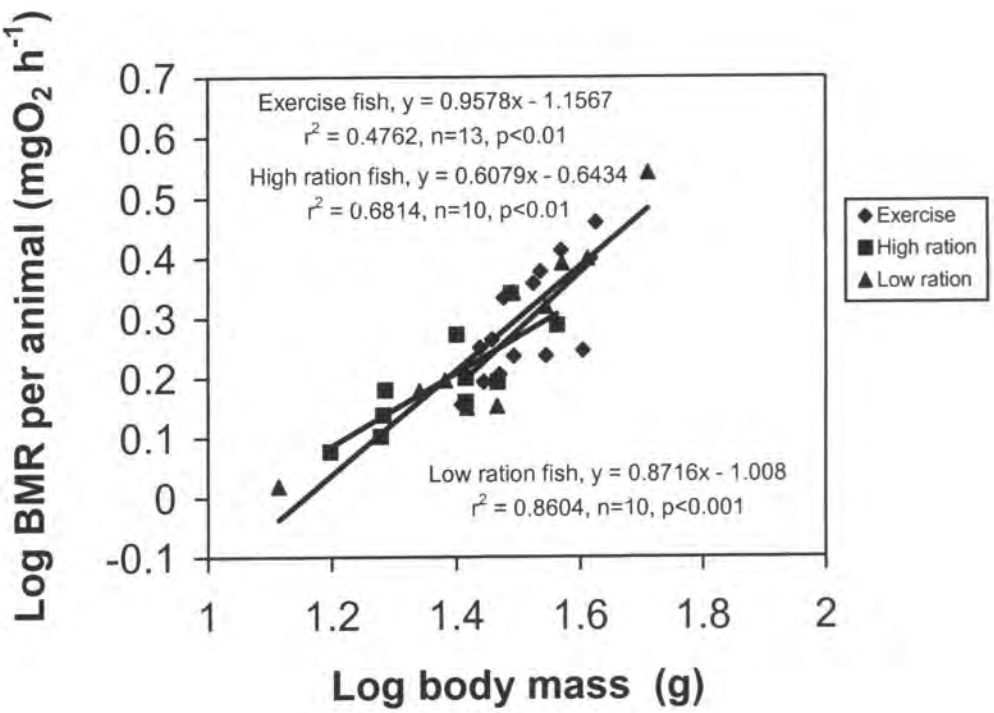
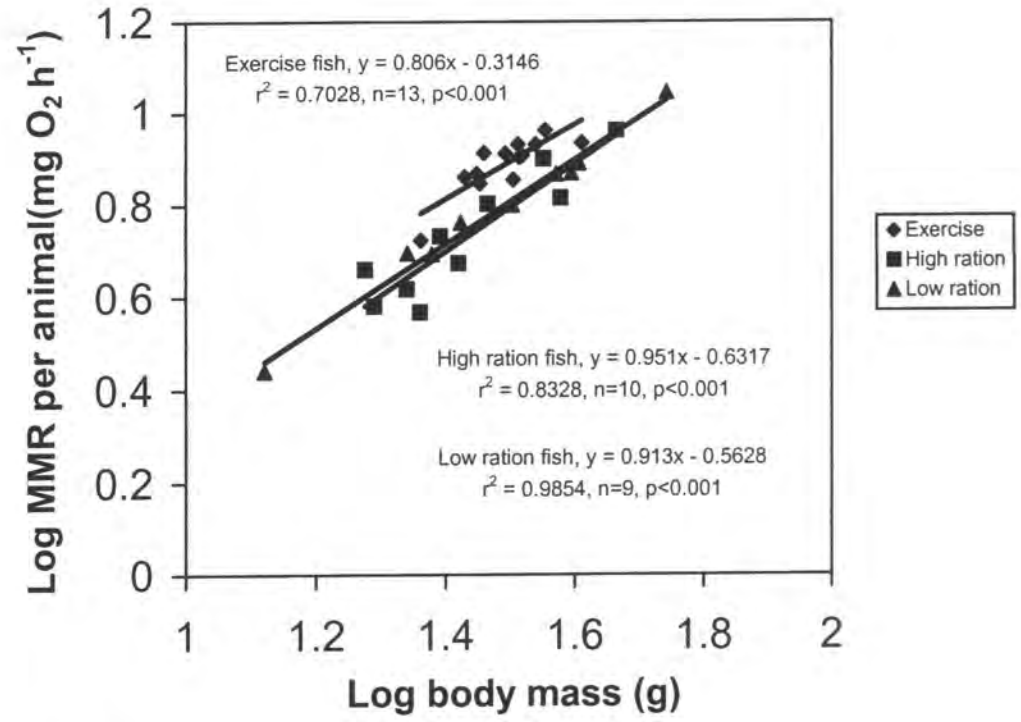


Figure 3.3a Allometric relationships for BMR and MMR for juvenile pike at time 1, prior to the start of the experiment. Points for BMR represent the mean of several measurements for an individual, while points for MMR represent single determinations for each fish.



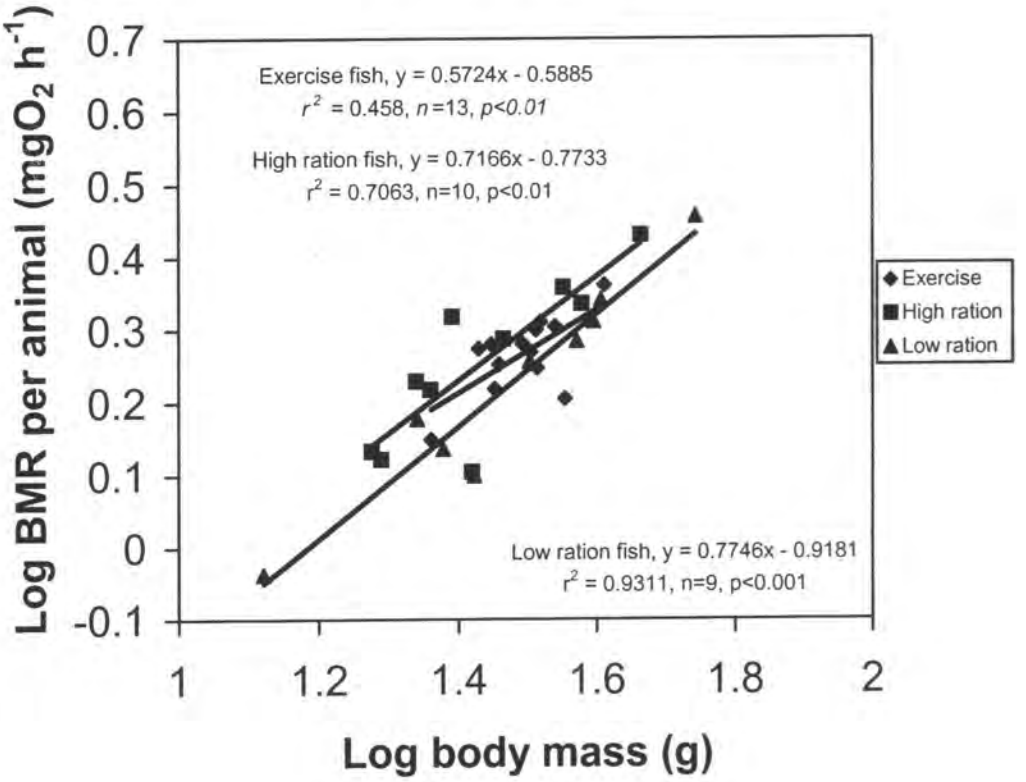
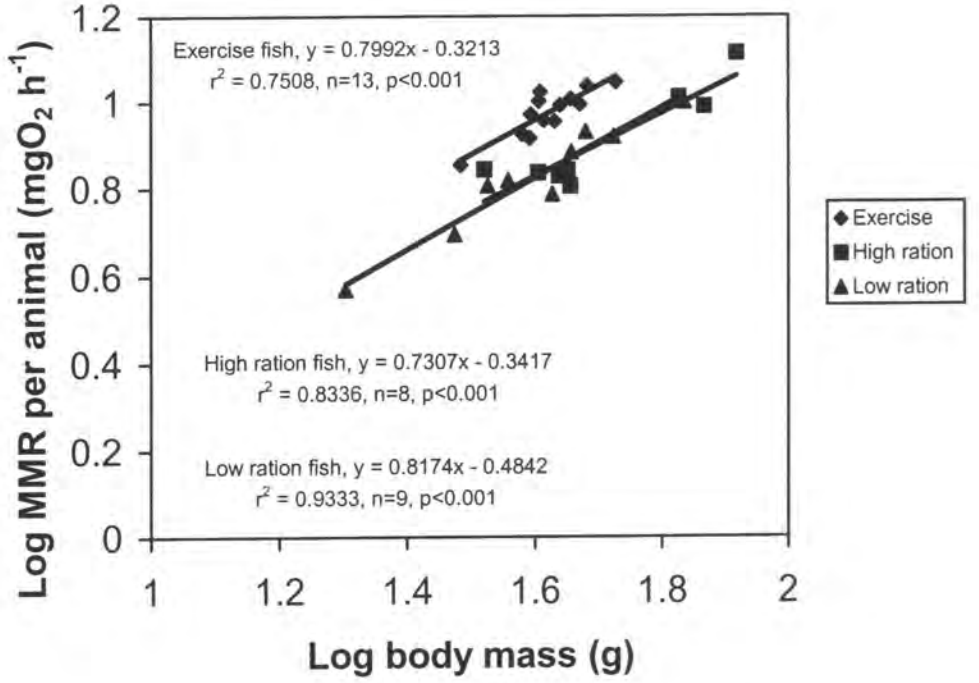


Figure 3.3b Allometric relationships for BMR and MMR for juvenile pike at time 2, after c. 3 weeks under experimental conditions. Points for BMR represent the mean of several measurements for an individual, while points for MMR represent single determinations for each fish



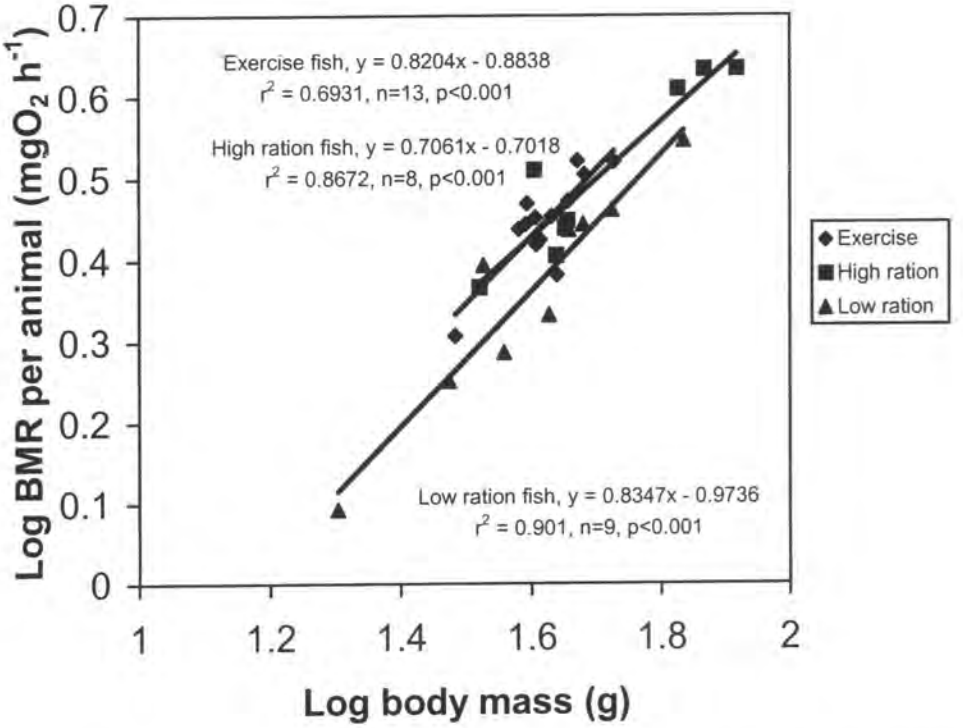


Figure 3.3c Allometric relationships for BMR and MMR for juvenile pike at time 3, after c. 11 weeks under experimental conditions. Points for BMR represent the mean of several measurements for an individual, while points for MMR represent single determinations for each fish.

3.3.6 BMR and growth rates

A significant correlation was noted between mass adjusted BMR values (standardised to a 35 g pike) at time 3 and the growth rate between times 2 and 3, for both exercise fish and high ration fish ($F_{1,11} = 8.57$, $r^2 = 0.438$, $p<0.05$ and $F_{1,5} = 7.63$, $r^2 = 0.604$, $p<0.05$ respectively). Those fish with the highest growth rate, having the highest mass adjusted BMR values. There was no significant correlation for those fish held at a low ration. No correlations were noted between mass adjusted BMR values at time 2 and the growth between the period of time 1 to time 2 for any of the treatment groups.

3.3.7 MMR and time taken to reach exhaustion

The number of swimming bursts required for each fish to reach exhaustion prior to measurement of MMR (Table 3.5) was compared for treatment groups and experiment times. Body mass may be expected to strongly influence burst swimming performance (e.g. Coulson 1987; Goolish 1991a) of fishes and therefore this was taken into account. Analysis of Covariance (ANCOVA), using body mass as the covariate, revealed no significant differences between any of the treatment groups at each of the times, 1, 2 and 3 ($p>0.05$). On comparing each treatment between times, again using body mass as the covariate, there was a significant increase in the number of bursts made prior to reaching exhaustion for all treatments (exercise fish, $F_{3,35} = 7.76$, $p<0.01$; high ration fish, $F_{3,24} = 13.32$, $p<0.001$; low ration fish, $F_{3,24} = 5.15$, $p<0.05$). This demonstrates that as well as an effect of body mass (although this was not statistically significant in some comparisons) on the performance attained (number of swimming bursts) by pike, there was a strong effect of time of measurement for all treatment groups, but no significant difference at any given time between the treatment groups. This result appears to have been due to a specific training effect of the exhaustion protocol, since fish exposed to sustained swimming over the whole period ('exercise' treatment) showed no significant difference in burst swimming performance compared to the other groups (Table 3.5).

3.3.8 Rates of recovery from exhaustive exercise

A comparison of individual rates of recovery from exhaustive exercise in terms of $\dot{V}O_2$ for exercised fish is presented in Appendix 1. Similar information, presented as group means (± SE) is displayed in Figure 3.4. At time 1, before experimental treatments, the recovery profiles were similar between groups and characterised by progressive declines in $\dot{V}O_2$ with time. The group mean recovery profiles for the exercised group were

Table 3.6 Mean number of swimming bursts to reach exhaustion for each treatment group at each experimental time. The group mean body mass of fish in each group is expressed in grams, with the time taken to reach exhaustion expressed in minutes. The standard errors for each variable are in parentheses.

	Time 1				Time 2				Time 3			
Treatment	*n*	Body mass	No of bursts	Time (min)	*n*	Body mass	No. of bursts	Time (min)	*n*	Body mass	No. of bursts	Time (min)
Exercise	13	32.50 (1.41)	53.7 (1.9)	5.08 (0.24)	13	31.38 (1.25)	52.6 (2.7)	5.00 (0.20)	13	42.22 (1.54)	80.0 (4.3)	7.03 (0.46)
High ration	10	24.69 (2.03)	48.9 (2.9)	5.10 (0.23)	10	28.28 (2.82)	61.0 (3.5)	5.20 (0.26)	8	53.73 (6.33)	90.9 (5.4)	7.11 (0.38)
Low ration	10	31.00 (3.41)	59.5 (5.14)	5.50 (0.16)	9	32.22 (4.17)	55.2 (4.1)	5.06 (0.27)	9	41.89 (4.71)	79.0 (2.9)	7.26 (0.17)

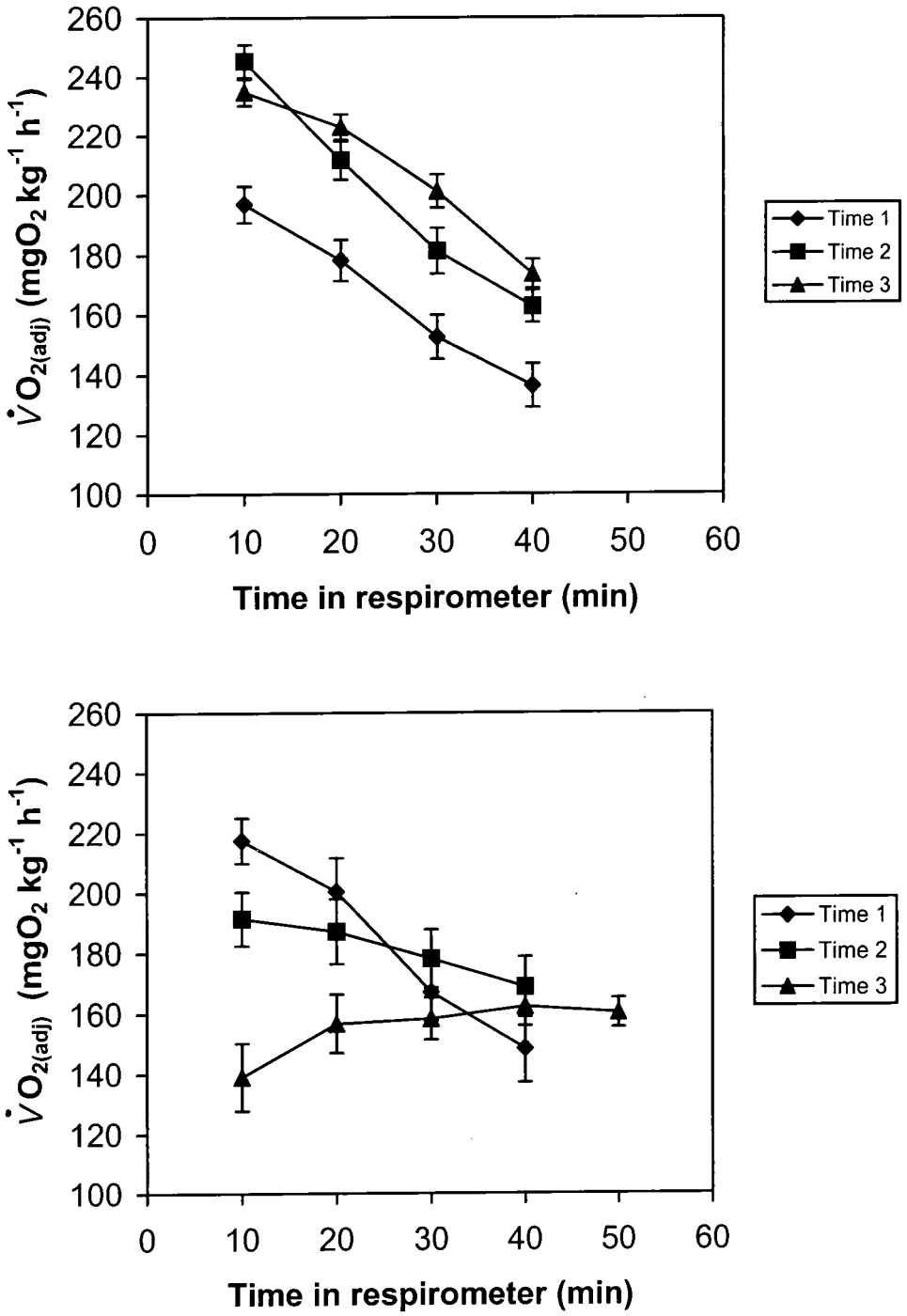


Figure 3.4 The mass standardised rate of oxygen consumption following exhaustive exercise for exercise fish (above) and high ration fish (below) at each experimental time period (time 1, prior to the start of the experiment, time 2 after c. 3 weeks and time 3 after c. 11 weeks). The group mean values of mass standardised rate of oxygen consumption for each ten minute time period are displayed with standard error bars.

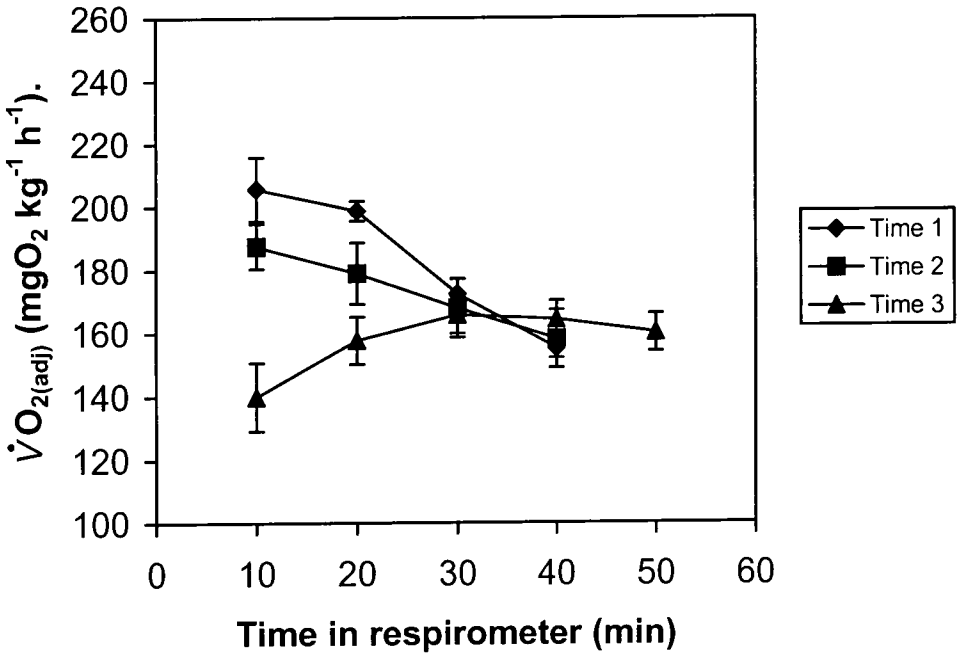


Figure 3.4 The mass standardised rate of oxygen consumption following exhaustive exercise for low ration fed fish at each experimental time period (time 1, prior to the start of the experiment, time 2 after c. 3 weeks and time 3 after c. 11 weeks). The group mean values of mass standardised rate of oxygen consumption for each ten minute time period are displayed with standard error bars.

similar at times 1, 2 and 3, although VO_2 was higher for both the time 2 and 3 observations, reflecting the increased MMR (Figure 3.4). Between the first record (10 minutes) and the fourth record (40 minutes) exercise group mean $\dot{V}O_2$ declined by approximately 25-35% for the three experimental times. It is clear that both the high and low ration fish exhibited somewhat different $\dot{V}O_2$ recovery profiles from the exercise group at times 2 and 3, particularly the latter (Figure 3.4). For high ration fish there was a 12 % decline in mean $\dot{V}O_2$ between 10 minutes and 40 minutes post-exercise at time 2 and a 15 % increase over the same period at time 3. For the low ration group there were -16 % and +15 % changes in mean $\dot{V}O_2$ over the same period for times 2 and 3 respectively.

3.3.9 Individual BMR, MMR and relative scope measurements

Individual mass adjusted BMR ($BMR_{(adj)}$) values, standardised to a 35g pike (Figure 3.5), showed minimal individual variation, reflected in the small standard error bars (group mean values are presented in Table 3.6). Also of note is the large degree of variation in mass adjusted BMR values between individuals in each of the three treatment groups. There was no statistical difference between the group mean $BMR_{(adj)}$ at time 1 (prior to the start of treatment) (Table 3.7 1-way ANOVA, $p>0.05$).

3.3.10 Effects of treatments on BMR

Data on $BMR_{(adj)}$ displayed in Figure 3.5 are ranked from left to right in order of the magnitude of $BMR_{(adj)}$ at time 1. Exercise fish with initially low mean $BMR_{(adj)}$ values (to the left hand side) tended to increase their mean $BMR_{(adj)}$ values by time 2 and those with higher $BMR_{(adj)}$ reduced them, forming a more homogenous group. This is reflected by the decrease in standard error between the two times (Table 3.6). By time 3, those exercise fish with the lowest $BMR_{(adj)}$ values had increased their $BMR_{(adj)}$ in excess of the time 1 value. For those fish with the highest $BMR_{(adj)}$ values at time 1, by time 3, values had returned to similar time 1 values. Statistical analysis was performed using Wilcoxon's signed ranks test, a non-parametric equivalent of a paired *t*-test. For exercise fish there was a statistically significant increase in $BMR_{(adj)}$ by time 3, compared to time 1 (Wilcoxon, $z = 3.180$, $p<0.01$), see Table 3.6.

For high ration fish, $BMR_{(adj)}$ followed a similar trend to that seen for exercised fish between times 1 and 2, those with initially higher $BMR_{(adj)}$ tended to decrease the

$BMR_{(adj)}$ by time 2 whilst those with initially lower $BMR_{(adj)}$ tended to increase them by time 2. By time 3 $BMR_{(adj)}$ values were either similar to those recorded at time 1 or higher (7/8 pike). High ration fish had a significantly higher $BMR_{(adj)}$ by time 3 compared to time 1 values ($z = 1.960$, $p<0.05$), see Table 3.6.

Low ration fish had significantly lower $BMR_{(adj)}$ values between times 1 and 2 ($z = 2.666$, $p<0.01$). These fish tended to have similar or lower $BMR_{(adj)}$ by time 3 (8/9) compared to the time 1 values, see Table 3.6.

At time 1 (prior to commencement of treatment) there was no significant difference between the treatment group $BMR_{(adj)}$ values (1-way ANOVA, $p>0.05$). By time 2 there was a significant increase in $BMR_{(adj)}$ between groups ($F_{2,29} = 4.02$, $p<0.05$), with high ration compared to low ration (Table 3.7). By time 3 there was also a statistically significant difference between $BMR_{(adj)}$ values for all groups (1-way ANOVA, $F_{2,27} = 8.10$ $p<0.01$) with significant differences seen between exercise and low ration fish ($p<0.01$) and high ration and exercise fish ($p<0.05$).

3.3.11 Effects of treatment on MMR

For exercised fish there was a highly significant increase in MMR adjusted to a pike of body mass of 35 g ($MMR_{(adj)}$), between times 1 and 2 and times 1 and 3, (Kruskall Wallis; $z = 3.810$, $p<0.01$, and $z = 2.970$, $p<0.01$ respectively, Table 3.8). The increase in $MMR_{(adj)}$ for exercised fish observed at time 2 was continued at time 3 (Figure 3.6).

For high ration fish there was a significant decrease in $MMR_{(adj)}$ at times 2 and 3, with the values at time 3 being the lowest (Kruskall Wallis; $z = 2.479$, $p<0.05$, and $z = 2.521$, $p<0.05$ respectively). A very similar trend was also seen for low ration fish with a significant decrease in $MMR_{(adj)}$ at time 2, having further decreased by time 3 (Table 3.8). Both high and low ration fish had very similar group mean ± SE values by time 3 of 172.4 ± 6.7 and 172.7 ± 2.1 $mgO_2\,kg^{-1}h^{-1}$ respectively.

Table 3.7 A comparison of group mean BMR values for fish within experimental treatments between each experimental time (time, prior to the commencement of the experiments, time 2 after c. 3 weeks and time 3 after c. 11 weeks. Statistical tests were performed using Wilcoxon signed rank test of individually matched mean BMR standardised to a 35g pike and expressed as mg O_2 $kg^{-1}h^{-1}$. Group mean values are expressed in the table with the number of pairs in parentheses. SE=standard error, ns = no statistical significance, (p>0.05), * p<0.05, ** p<0.01, *** p<0.001. z values are given below the level of significance.

Treatment	Mean BMR 1 $\bar{x}$ ± SE	*n*	Sig time 1-2	Mean BMR 2 $\bar{x}$ ± SE	*n*	Sig time 1-3	Mean BMR 3 $\bar{x}$ ± SE	*n*	Sig time 2-3
Exercise	59.9 (±2.5)	13	ns	57.4 (±1.4)	13	** 2.97	69.6 (±1.4)	13	** 3.18
High ration	61.3 (±2.3)	10	ns	61.6 (±2.4)	10	* 1.96	67.9 (±2.4)	8	ns
Low ration	62.0 (±2.5)	10	** 2.66	53.7 (±1.7)	9	ns	59.7 (±2.1)	9	ns

Table 3.8 A comparison of the group mean BMR values (mgO_2 kg^{-1} h^{-1}) between different treatment groups at each experimental time (time 1 prior to the commencement of the experiments, time 2 c. after c. 3 weeks and time 3 after c.11 weeks). Statistical tests were performed using 1-way ANOVA with Bonferroni correction between each treatment group, for pike standardised to 35g body mass. SE = standard error, ns = no statistical significance (p>0.05), * p<0.05, ** p<0.01, *** p<0.001.

Treatment comparison	Mean BMR 1 $\bar{x}$ ± SE	*n*	Sig time 1	Mean BMR 2 $\bar{x}$ ± SE	*n*	Sig time 2	Mean BMR 3 $\bar{x}$ ± SE	*n*	Sig time 3
Exercise & (High ration)	59.9 (±2.5)	13	ns	57.4 (±1.4)	13	ns	69.6 (±1.4)	13	ns
High ration & (Low ration)	61.3 (±2.3)	10	ns	61.6 (±2.4)	10	*	67.9 (±2.4)	8	*
Low ration & (Exercise)	62.0 (±2.5)	10	ns	53.7 (±1.7)	9	ns	59.7 (±2.1)	9	**

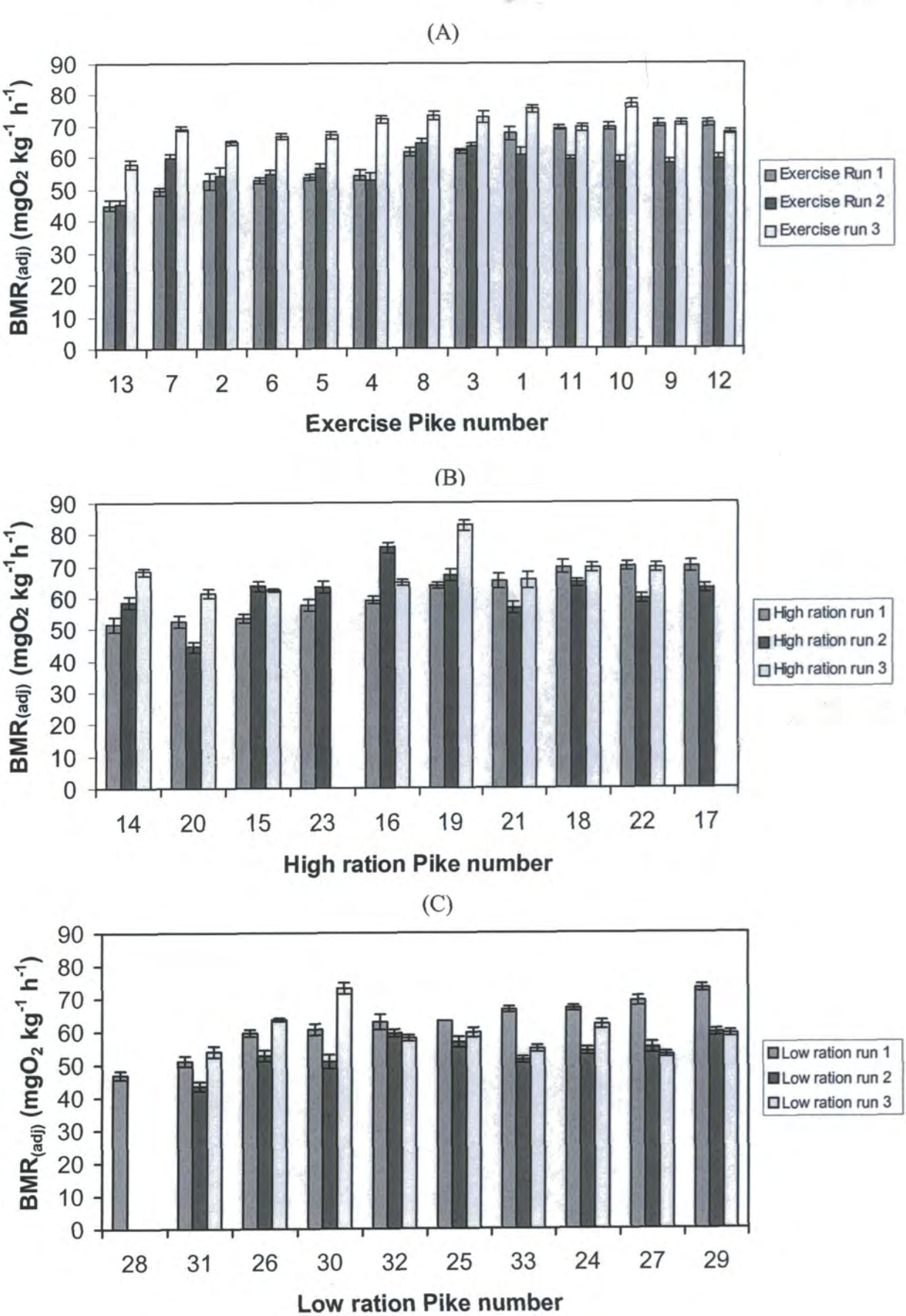


Figure 3.5a-c, Mean BMR (± SE) of individual mass standardised pike (35 g) at each treatment for each experimental time (time 1 prior to the start of treatment, time 2 after c. 3 weeks and time 3 after c. 11 weeks).

Table 3.9 A comparison of the mean MMR values ($mgO_2\,kg^{-1}h^{-1}$) for pike within a single treatment group between experimental times. Statistical tests were performed using Wilcoxon signed rank tests between individually matched mean MMR for the same individual pike standardised to 35g body mass. Group mean values are expressed in the table with the number of pairs in parentheses. SE = standard error, ns = no statistical significance($p>0.05$), * $p<0.05$, ** $p<0.01$, *** $p<0.001$, z values are given below the level of significance.

Treatment	Mean MMR 1 $\bar{x} \pm$ SE	*n*	Sig time 1-2	Mean MMR 2 $\bar{x} \pm$ SE	*n*	Sig time 1-3	Mean MMR 3 $\bar{x} \pm$ SE	*n*	Sig time 2-3
Exercise	195.17 (±6.3)	13	** 3.18	246.21 (±5.2)	13	** 2.97	234.88 (±4.2)	13	ns
High ration	219.53 (±8.6)	10	* 2.49	194.57 (±7.8)	10	* 2.52	172.42 (±6.8)	8	* 2.52
Low ration	210.70 (±5.5)	10	ns	200.36 (±3.2)	9	** 2.67	172.65 (±4.5)	9	** 2.67

Table 3.10 A comparison of the mean MMR values ($mgO_2\,kg^{-1}h^{-1}$) for fish between different treatment groups at each experimental time. Statistical tests were performed using 1-way ANOVA with Bonferroni correction between each treatment group for pike standardised to 35g body mass. SE = standard error, ns = no statistical significance ($p>0.05$), * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

Treatment comparison	Mean MMR 1 $\bar{x} \pm$ SE	*n*	Sig time 1	Mean MMR 2 $\bar{x} \pm$ SE	*n*	Sig time 2	Mean MMR 3 $\bar{x} \pm$ SE	*n*	Sig time 3
Exercise & (High ration)	195.2 (±6.3)	13	*	246.2 (±5.2)	13	***	234.9 (±4.2)	13	***
High ration & (Low ration)	219.5 (±8.6)	10	ns	194.6 (±7.8)	10	ns	172.4 (±6.7)	8	ns
Low ration & (Exercise)	210.7 (±5.5)	10	ns	200.4 (±3.2)	9	***	172.7 (±2.1)	9	***

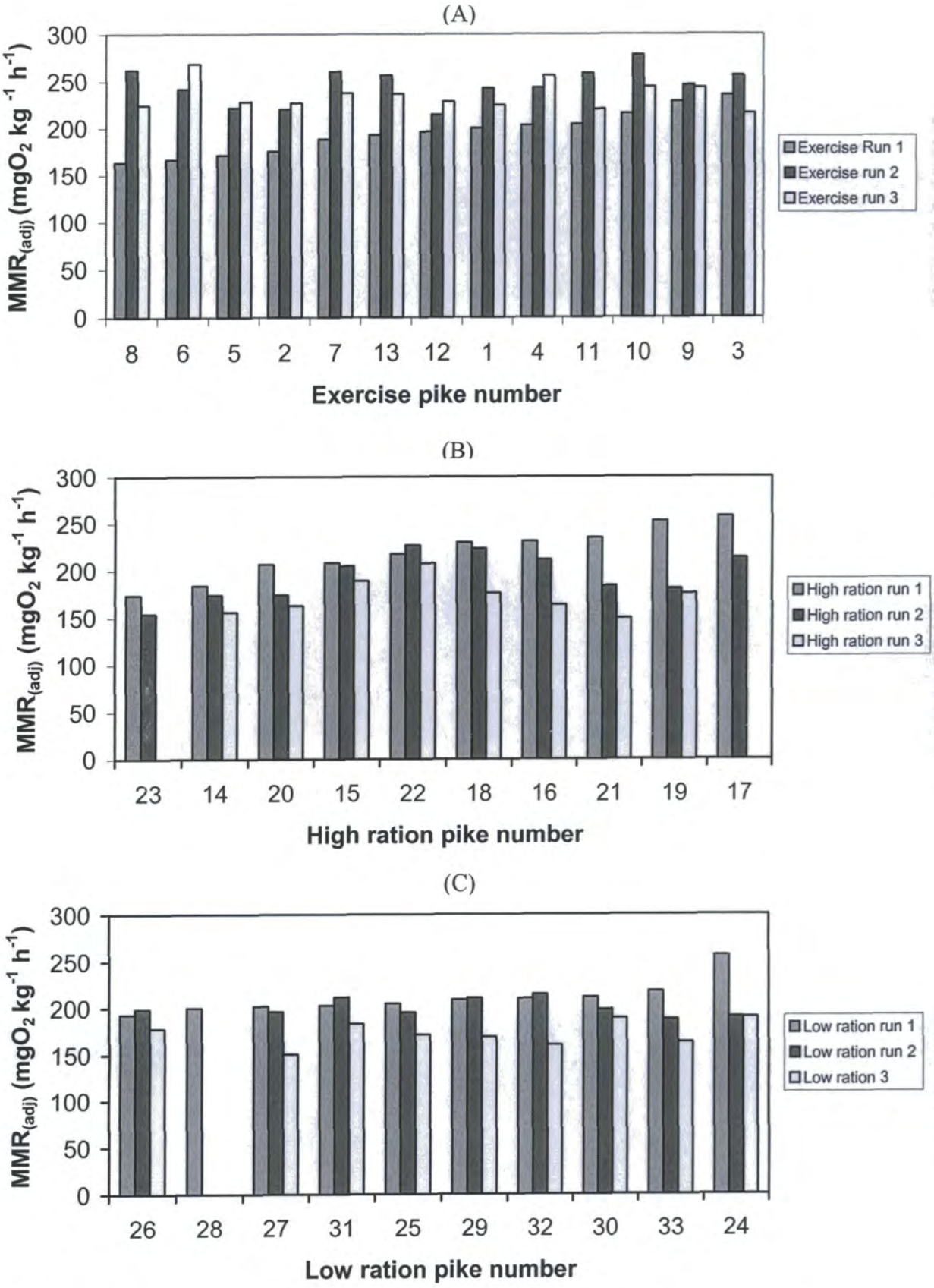


Figure 3.6 a-c, MMR of individual mass standardised pike (35 g) at each treatment for each experimental time (time 1 prior to the start of treatment, time 2 after c. 3 weeks and time 3 after c. 11 weeks).

A comparison between the individual groups at each experimental time revealed significantly lower $MMR_{(adj)}$ for exercised fish at time 1 (1-way ANOVA, $F_{2,30} = 3.4$, $p<0.05$), prior to the commencement of treatment, compared to high ration fish, ($p<0.05$, Bonferroni correction, Table 3.9). At times 2 (time 2 , $F_{2,29} = 26.07, p<0.001$) and 3 (time 3, $_{2,27} = 57.64, p <0.001$)., exercised fish had a significantly higher $MMR_{(adj)}$ than either high ration or low ration fish.

3.3.12 Repeat maximal performance

A comparison between the rank order performance of $MMR_{(adj)}$ for all fish was tested using Spearman rank order correlation (r^s) to see if those fish that had the highest $MMR_{(adj)}$ for example at time 1 were also the highest at times 2 and 3 . Was the maximal performance of fish repeatable over subsequent time periods? The only group of pike found to have a significant correlation between rank order performances, were the high ration fish between the time periods 2 and 3 ($r^s = 0.714, p<0.05$). Graphical presentation of changes in $MMR_{(adj)}$ of individual fish between the different times (1, 2 and 3) are plotted in Appendix 3.

3.3.13 Effects of treatment on relative metabolic scope

The relative mass-adjusted metabolic scope ($scope_{(adj)}$) was calculated as the $MMR_{(adj)}$ divided by $BMR_{(adj)}$. This relative value was considered to be more useful to plot than the actual change in scope, as changes in the relationship between BMR and MMR would be more obvious as a ratio than being expressed as the actual change in total scope.

For exercised fish only it is clear that there was a large statistically significant increase in $scope_{(adj)}$ at time 2 (Figure 3.7, Table 3.10), compared to that at time 1 or 3 (1-way ANOVA; $F_{2,36} = 20.24, p<0.001$). However $scope_{(adj)}$ at time 3 returned to a group mean value similar to that observed at time 1. This was the result of a stable MMR between times 2 and 3, but an increase in BMR over the same period.

For high ration fish there was a significant decrease in $scope_{(adj)}$ between times 1 and 3 exercise group mean values (1-way ANOVA; $F_{2,25} = 15.44, p<0.001$). Low ration fish exhibited a significant difference in scopes between times 1 and 2 ($F_{2,27} = 11.22$, $p<0.001$) with eight out of nine fish displaying an increase in $scope_{(adj)}$. A significant

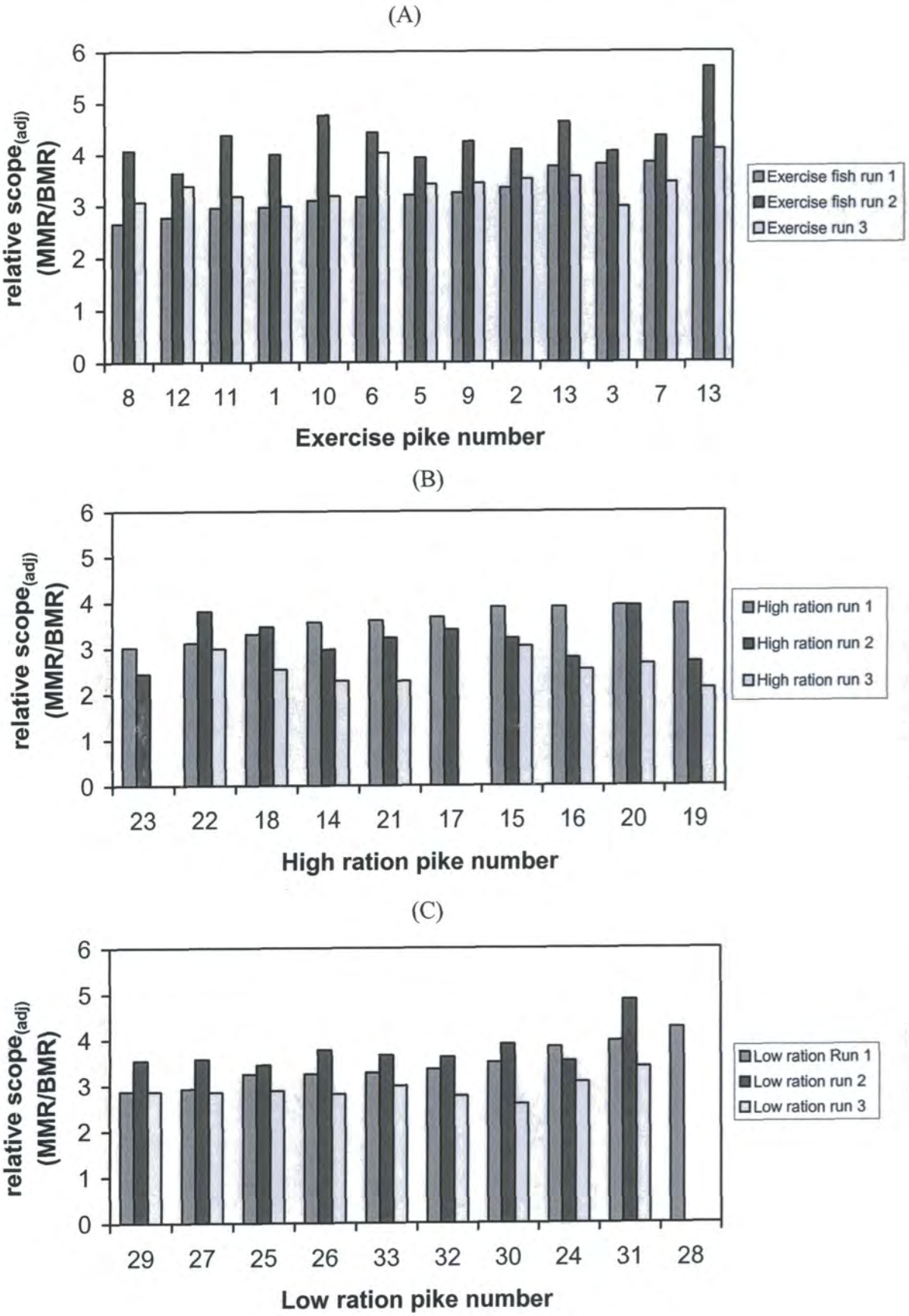


Figure 3.7 Mass standardised relative individual metabolic scopes (MMR/BMR) adjusted pike (35 g) at each treatment for each experimental time (time 1 prior to the start of treatment, time 2 after c. 3 weeks and time 3 after c. 11 weeks).

Table 3.11 A comparison of the mean relative metabolic scope (MMR/BMR) for within treatment groups between experimental times. Statistical tests were performed using 1-way ANOVA with Bonferroni correction with fish standardised to 35g body mass. Values are expressed as the relative metabolic scope. Group mean values are expressed in the table with the number of pairs in parentheses. SE = standard error, ns = no statistical significance ($p>0.05$), * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

Treatment	Rel. Scope (1) $\bar{x} \pm$ SE	*n*	Sig time 1-2	Rel. Scope (2) $\bar{x} \pm$ SE	*n*	Sig time 1-3	Rel. Scope (3) $\bar{x} \pm$ SE	*n*	Sig time 2-3
Exercise	3.3 (±0.1)	13	***	4.3 (±0.1)	13	ns	3.4 (±0.1)	13	***
High ration	3.6 (±0.1)	10	ns	3.2 (±0.2)	10	***	2.6 (±0.1)	8	**
Low ration	3.4 (±0.1)	10	ns	3.8 (±0.1)	9	*	2.9 (±0.1)	9	***

Table 3.12 A comparison of the mean relative metabolic scope (MMR/BMR) for individual fish between treatment groups at each experimental time. Statistical tests were performed using 1-way ANOVA with Bonferroni correction for pike standardised to 35g body mass. SE = standard error, ns = no statistical significance ($p>0.05$), * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

Treatment	Rel.Scope (1) $\bar{x} \pm$ SE	*n*	Sig time 1	Rel. scope (2) $\bar{x} \pm$ SE	*n*	Sig time 2	Rel.Scope (3) $\bar{x} \pm$ SE	*n*	Sig time 3
Exercise & (High ration)	3.3 (± 0.1)	13	ns	4.3 (±0.1)	13	***	3.4 (±0.1)	13	***
High ration & (low ration)	3.6 (±0.1)	10	ns	3.2 (±0.2)	10	*	2.6 (±0.1)	8	ns
Low ration & (Exercise)	3.4 (±0.1)	10	ns	3.8 (±0.1)	9	*	2.9 (±0.1)	9	**

decrease in $scope_{(adj)}$ for low ration fish was evident between times 1 and 3, and time 2 and 3 (Table 3.10).

A comparison of $scope_{(adj)}$ between treatment groups at each of the experimental times revealed no significant difference in $scope_{(adj)}$ between any groups of fish at time 1 (1-way ANOVA; $p>0.05$) (Table 3.11). By time 2 there was a significant difference in $scope_{(adj)}$ between groups ($F_{2,29} = 15.67$, $p<0.001$) for exercise and high ration fish ($p< 0.001$, Bonferroni correction). There was a significant difference between treatment groups at time 3 (1-way ANOVA $F_{2,29} = 18.64$, $p<0.001$), for exercise and high ration fish ($p<0.001$, Bonferroni correction). At time 2 there was a statistically significant difference in $scope_{(adj)}$ between high and low ration fish and exercise and low ration fish (Table 3.11). At time 3 exercise fish had a significantly higher $scope_{(adj)}$ than low ration fish ($p<0.05$), and low ration fish had a significantly higher $scope_{(adj)}$ than high ration fish ($p<0.01$).

3.3.14 Variations of BMR for individual pike between experimental times

A comparison of $BMR_{(adj)}$ over time for individual pike was statistically tested using the log residuals from the log body mass vs. log $\dot{V}O_2$ per relationships. If there were a significant difference in $BMR_{(adj)}$ of individual pike over time (indicating metabolic plasticity), then there would be expected to be a difference in the relative mean log residuals for an individual between times.

Comparisons between the BMR(adj) values for the same individual pike were statistically tested using a 1-way ANOVA with Bonferroni correction of the log residual $BMR_{(adj)}$ values, between each of the three experimental times. Statistically significant differences in $BMR_{(adj)}$ for individual fish between at least two of the experimental times, were detected for individual fish in all of the groups (see Table 3.12). For exercised pike, 54% of fish showed a statistically significant difference between at least two of the three experimental times, with 80% of the high ration pike and 77% of the low ration pike. This indicates a high degree of plasticity in the BMR of pike held at any of the treatment regimes including the static-water 'low ration' group, which was instituted as a control. Had there have been no plasticity in BMR then we would have expected no change in the relative individual residuals of any of the pike.

3.3.15 A relationship between BMR and MMR?

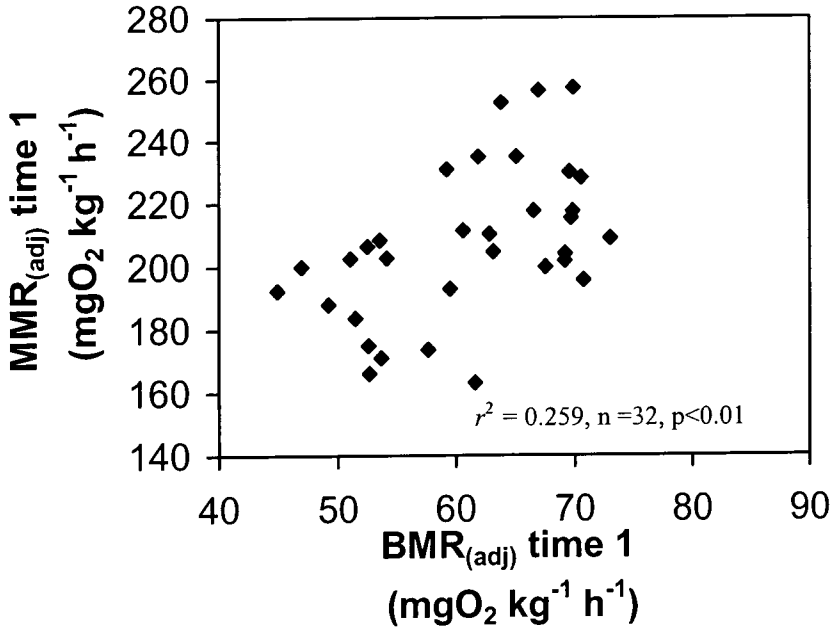
At time 1 there was found to be a significant positive correlation between $BMR_{(adj)}$ and $MMR_{(adj)}$ for all fish removed from the stock conditions. However, the relationship explained only 26% of the variation (Figure 3.8) ($r^2 = 0.259$, $p<0.01$).

At time 2 (after approximately 3 weeks, see Figure 3.8b), there was a totally random distribution of points for both high and low ration fish; no correlation between BMR and MMR was present. Exercise fish were located towards the top of the graph in a cluster reflecting the increased MMR for this group between times 1 and 2. There was no significant correlation between BMR and MMR for all data combined. By time 3 there was again a significant positive correlation between BMR and MMR ($F = 7.19$, $r^2 = 0.204$, $p< 0.05$) (Figure 3.9). Inspection of the data showed that the highest MMR values were associated with exercise fish for which 100% of MMR were higher than the overall mean, and for which 84% of BMR were higher than the overall mean.

Table 3.13. Individual variation in mean BMR of individual pike between the different experimental times. Mean values are expressed as $mgO_2\,kg^{-1}\,h^{-1}$ for a fish standardised to a mass of 35 g. The statistical significance for each individual fish between the individual times was tested using a 1-way ANOVA with Bonferroni correction of the log residual unadjusted BMR values. ns = no statistical significance ($p>0.05$), * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

Pike no.	*n*	Mean	SE	Sig (1-2)	*n*	Mean	SE	Sig (2-3)	*n*	Mean	SE	Sig (1-3)
	Time 1				Time 2				Time 3			
Exercise												
1	7	67.5	2.2	ns	9	60.8	2.1	ns	8	75.4	1.2	ns
2	6	52.6	2.63	ns	10	53.9	2.0	ns	8	64.7	1.2	ns
3	8	61.9	0.8	ns	6	63.4	0.1	ns	10	72.8	1.7	ns
4	8	54.1	1.7	ns	7	52.7	2.3	ns	7	72.2	1.2	*
5	9	53.7	1.1	ns	8	56.5	1.2	ns	7	66.9	1.4	ns
6	8	52.7	1.0	ns	7	54.6	1.4	ns	9	66.7	1.1	ns
7	8	49.2	2.8	***	6	59.9	1.0	ns	6	69.1	0.7	***
8	7	61.6	3.4	ns	8	64.4	1.4	ns	9	73.2	1.2	ns
9	10	70.6	1.3	***	9	57.9	1.4	ns	9	71.1	0.9	**
10	7	69.7	1.3	***	8	58.5	1.5	*	10	76.9	1.5	*
11	8	69.2	0.8	***	11	59.2	1.0	ns	9	69.3	1.2	ns
12	7	70.8	1.1	*	9	59.0	1.5	*	9	67.8	0.6	***
13	7	44.9	1.5	ns	8	45.1	1.6	ns	9	57.9	1.5	*
Mean	-	59.9	2.5	-	-	57.4	1.4	-	-	69.5	1.4	-
High												
14	6	51.5	2.3	ns	13	58.6	1.8	ns	8	68.2	1.1	*
15	7	53.6	1.5	**	6	63.6	1.7	*	8	62.2	0.5	ns
16	8	59.3	1.2	***	7	75.8	1.7	***	9	64.6	0.8	ns
17	6	69.9	1.7	**	7	62.7	1.3	-	-	-	-	-
18	7	69.6	2.0	**	6	64.5	1.0	ns	7	69.5	1.3	**
19	7	63.8	1.0	*	7	67.1	1.7	*	7	82.8	1.7	***
20	7	52.5	1.7	**	9	44.4	1.8	**	6	61.3	1.4	ns
21	10	65.1	2.3	ns	7	56.8	2.0	ns	6	65.4	2.5	ns
22	6	69.8	1.4	**	7	59.6	1.2	ns	8	69.4	1.2	**
23	9	57.7	1.9	ns	9	63.2	1.9	-	-	-	-	-
Mean	-	61.3	2.3	-	-	61.6	2.5	-	-	67.4	2.4	-
Low												
24	7	66.9	0.8	*	6	54.0	1.2	ns	7	62.0	1.3	ns
25	7	63.1	0.0	ns	9	56.7	1.6	ns	9	59.6	1.4	ns
26	9	59.5	1.0	ns	8	52.7	1.7	*	7	63.6	0.5	*
27	10	69.2	1.5	*	7	55.0	1.7	**	8	52.9	0.8	***
28	10	46.9	1.3	-	-	-	-	-	-	-	-	-
29	8	73.0	3.1	*	8	59.5	1.1	*	6	59.1	0.9	*
30	7	60.6	1.7	ns	8	50.9	2.1	***	10	73.1	1.8	**
31	9	51.1	1.5	ns	8	43.5	1.4	ns	9	53.9	1.7	ns
32	9	62.8	2.3	ns	8	59.4	1.1	ns	8	57.9	0.9	*
33	7	66.5	0.8	**	7	51.4	1.0	**	8	54.7	1.0	***
Mean	-	62.0	2.5	-	-	53.7	1.7	-	-	59.6	2.1	-

(A)



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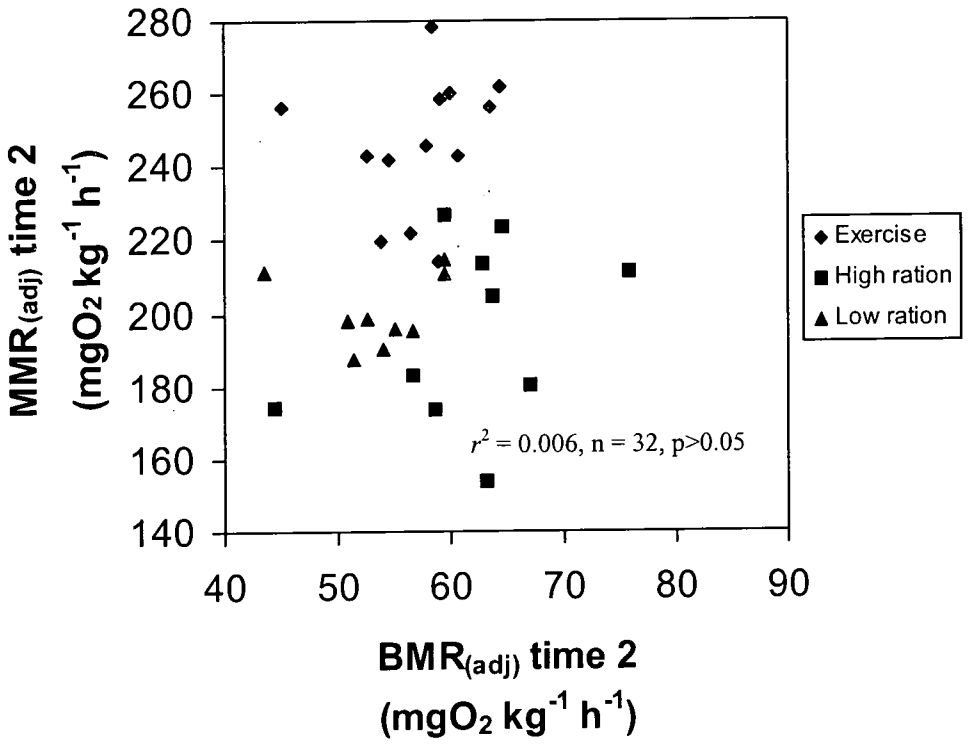


Figure 3.8a and 3.8b Correlations between the mass standardised (35 g) BMR vs. MMR for all pike at experimental time 1 (above, prior to the start of experiments) and all pike at experimental time 2 (below, after approximately 3 weeks).

(C)

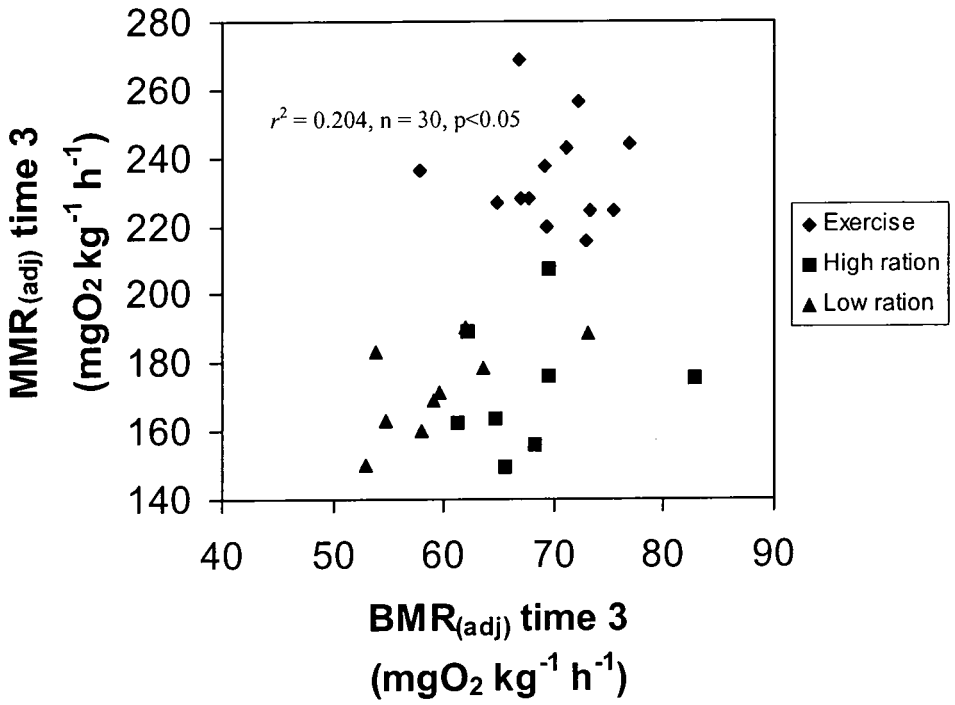


Figure 3.8c A correlation between the mass standardised (35 g) BMR vs. MMR for all pike at experimental time 3, after approximately 11 weeks.

3.4. Discussion

3.4.1 The effects of treatment upon BMR and MMR

There was no difference between the $MMR_{(adj)}$ of all the treatment groups at time one, however, with the exercised group of pike there was a clear increase in $MMR_{(adj)}$ for all fish at time 2 which remained elevated at time 3. Several previous studies have shown that exercise training results in an increase in U_{crit} of various fish such as species such of trout, (Hochachka 1961; Johnston & Moon 1980; Houlihan & Laurent 1987; Farrell *et al.* 1990) and striped bass (Young & Cech 1993a, 1994a) but others have shown no effect at all upon U_{crit} with trout (Farrell *et al.* 1991; Gamperl *et al.* 1991) chinook salmon; Thorarensen *et al.* 1993) and leopard sharks *Triakis semifasciata* (Gruber & Dickson 1996). Few studies have directly measured the simultaneous response of U_{crit} and $\dot{V}O_{2max}$ (Kolok 1999), but it is implied that a direct link exists between the two and they have previously been assumed to be analogous. The majority of the fish above are considered to have a substantially superior swimming ability than pike, being found predominantly in fast flowing water.

Pike are known to have a low relative body mass percentage of aerobic red muscle compared to more active fish such as trout (Goolish 1991a), however could adapt well to sustained exercise. Pike are not often found in areas of fast flowing water in the wild (Harrison & Hadley, 1978), however they are not excluded from these areas due to a poor sustained swimming ability. Both the high ration group and the low ration group showed a decrease in the $MMR_{(adj)}$ by the end of the experiment (time 3) possibly related to the isolated environment, with a lack of interaction with other fish.

For both the high ration and exercised fish there was an increase in $BMR_{(adj)}$ by the end of the experiment (time 3). For the high ration fish the increase in group mean $BMR_{(adj)}$ did not coincide with higher rations fed between weeks 1-3, which may have been predicted due to an expected possible increase costs of protein synthesis, through improved growth rates (see section 4.1.5.). For exercised fish there was no increase in $BMR_{(adj)}$ between times 1 and 2, but a significant increase by week 11. Between times 2 and 3 there was a positive correlation between $BMR_{(adj)}$ and growth rates, for high ration and exercise fish only, those fish with the highest $BMR_{(adj)}$ growing the fastest rate.

3.4.2 The extent of plasticity of BMR in pike

If there was no plasticity in BMR of pike as has been suggested to be the case for salmon parr (Metcalfe *et al.* (1995) see section 2.1), then clearly we would have expected there to be no variation in $BMR_{(adj)}$ of pike over the experimental period. There was clearly a large number of individual pike, which had statistically significant differences in $BMR_{(adj)}$ between at least two of the experimental phases. With at least 54-80% of the pike having a significant difference in $BMR_{(adj)}$ between at least two of the different time periods, indicates that there is substantial plasticity in $BMR_{(adj)}$ and not the 'fixed' SMR that has been suggested for salmon parr.

3.4.3 The effects of treatment upon relative metabolic scope

The apparent lag in an increase in $BMR_{(adj)}$ for exercised fish at time 2 and the increase in MMR for exercised fish at time 2, led to a significant increase in the relative scope for all exercised fish, which had returned to the similar relative scope at time 3 as that for the fish at time 1. At time 1 all three groups had similar group mean relative scopes, which declined for both the high and low ration fish between times 2- 3. Again possibly relating to the more sedentary life style of these fish whilst being held in individual tanks.

The significant increase in relative metabolic scope observed for exercised fish at time 2 was due to an increase in the group mean MMR without a simultaneous increase in group mean BMR (n=13). This apparent lag in BMR indicates either that the metabolic costs associated with an increased MMR are not very large, or that BMR and MMR are not closely coupled at the same time scale. The increase in mean relative scope for low ration fish between times 1 & 2 was due to a significant decrease in group mean BMR at time 2. Those studies reporting an increase in U_{crit} or $\dot{V}O_{2max}$ with exercise do not comment on whether the BMR is also increased (Houlihan & Laurent 1987; Farrell *et al.* 1990; Young & Cech 1993b, 1994b), this having obvious effects on the usable metabolic scope of the fish.

3.4.4 Is there a link between MMR and BMR ?

There was a positive correlation between $BMR_{(adj)}$ and $MMR_{(adj)}$ for pike at time 1 of the present study. Such a relationship has been predicted for animals by Bennett and Ruben (1979) when comparing BMR and SMMR, and has been postulated for fish on comparing SMR and AMR (Priede 1985). However, for fish at an intraspecific level,

there has been no examination of whether this occurs. At time 2, after a period of metabolic change, there was a lack of a relationship suggesting a lack of direct coupling of BMR and MMR during this period of flux. However, by time 3 (11 weeks) there was again a significant correlation between $BMR_{(adj)}$ and $MMR_{(adj)}$ for all fish combined. At time 3 the distribution of points for treatment groups was non-random. Points for the exercised fish were clustered, reflecting the higher $MMR_{(adj)}$ and (mostly) $BMR_{(adj)}$ of exercised fish. Figure 3.3b, shows that an increase in $MMR_{(adj)}$ of exercise-trained pike had occurred by time 2, but with no accompanying increase in $BMR_{(adj)}$. However, by time 3 although $MMR_{(adj)}$ of exercised pike remained stable, $BMR_{(adj)}$ significantly increased (Figure 3.3c). This suggests that these two metabolic characteristics may have different temporal responses, with BMR responding more slowly than MMR. There is a general consensus of opinion that training increases the haematocrit of fish (Davison 1987). Maximum metabolic rate might change rapidly through an increase in haematocrit. Young & Cech (1993a) demonstrated a large increase in haematocrit of trained vs. untrained fish (3% increase in controls vs. 60% increase in exercised trained fish).

Increases in MMR can also occur through increased circulation, although an increase in heart mass has rarely been reported (Hochachka 1961; Greer Walker & Emerson 1978). Increased numbers of blood capillaries in the red muscle has been associated with increased exercise (Sanger 1992). Davie *et al.* (1986) investigated the ratios of LDH and isoenzymes and concluded that training produced a shift to those isoforms concerned with lactate oxidation in rainbow trout. The activities of lactate dehydrogenase between the treatment groups, in various tissues were explored in Chapter 4 (see section 4.3.6).

3.4.5 Allometric scaling of BMR, MMR and metabolic scope

At each of the experimental times there was no significant difference between any of the slopes of log BMR per animal vs. log mass. However, there were significant differences in elevations of the slopes between the different experimental times. At each time, due to the fact that there were no significant differences between each of the individual slopes, the mean slope was taken. The mean scaling *b* values for $BMR_{(adj)}$ were 0.81 at time 1, 0.69 at time 2 and 0.79 at time 3. The corresponding mean $MMR_{(adj)}$ scaling values were 0.90 at time 1, 0.89 at time 2, and 0.78 at time 3. Allometric scaling

exponents for BMR of 0.81 and 0.79 are similar to those reported for pike by various other authors; 0.82 (Diana 1982) and 0.80 (Armstrong *et al.* 1992). Armstrong *et al.* (1992) reported an MMR exponent of 0.99 for pike, which is near to unity and is much higher than that found in the present study. More importantly also was that there was found to be no significant difference in the slopes between BMR and MMR for any of the treatment groups at any of the time periods in contradiction to the findings of Armstrong *et al.* (1992).

With the equations that Armstrong *et al.* (1992) provided for the scaling of BMR and MMR with body mass, the BMR for a 40g fish is calculated as 77.5 $mgO_2kg^{-1}\ h^{-1}$, with an MMR of 156.1 $mgO_2\ kg^{-1}\ h^{-1}$ and an overall relative scope (MMR/ BMR) of 2.0. For a 1291g pike, BMR is calculated as 38.7 $mgO_2\ kg^{-1}\ h^{-1}$ and MMR as150.8, $mgO_2\ kg^{-1}\ h^{-1}$ with an overall relative scope of 3.9. Thus pike have a larger overall scope as they increase in body mass. In the narrow mass range used in this present study (15-82 g), there was no evidence of an increasing scope with increasing pike mass.

Using the equations of Armstrong *et al.* (1992) the BMR and MMR for a 35g pike is 79.5 and 136.8 $mgO_2\ kg^{-1}\ h^{-1}$ with an overall scope of 1.7. Their BMR value (Armstrong *et al.* 1992) is approximately 23% higher than that measured at the start of the present study, with a MMR approximately 34% lower than those measured in the present study. On examining the allometric relationship reported by Armstrong *et al.* (1992), the measurements at the lower end of the size range (and having a large influence on the overall regression) were from a single 40 g and a single 180 g fish only (the majority of pike >600 g). It is likely that their allometric equation for MMR may be somewhat erroneous. The BMR measurements, due to the high stability of the readings over time, however, should be directly comparable.

A recent re-examination of the allometric scaling of gill surface area in pike found that for pike of 87-1870 g gill surface area increased by the slope of 0.65 with increasing body mass (Jakubowski 1993) being approximately one sixth those reported by Byczkowska-Smyk (1959) for similar-sized pike. The relative decrease in gill surface area with increasing body size is faster than that for BMR (c. 0.8). Larger pike must, therefore, have to rely increasingly on anaerobic energy production with increasing size, as has been suggested by Goolish (1991a).

3.4.6 Individual maximal performance and rates of recovery from exercise

Correlations of maximal aerobic performance of individual pike (MMR) were generally not found to be repeatable under the altered conditions between time 1 and time 2, with a positive rank order correlation of MMR for high ration fish only between times 2 and 3. Significantly repeatable rank order maximal burst performance has been reported lizards snakes and amphibians under constant abiotic conditions. A repeatable rank order of U_{crit} has been demonstrated in five fish species under constant abiotic conditions (see review by Kolok 1999).

As the experiments progressed form time 1 to 3, there was a significant increase in the number of swimming bursts to reach exhaustion for all groups, the largest differences being noted for exercised and high ration fish. Although allometric scaling of anaerobic metabolism has been noted previously in fish (Goolish 1991b) with larger fish taking longer to become exhausted, the analysis accounted for body mass as a covariate, and still demonstrated a clear time effect which can only be interpreted as a training effect.

The group mean rate of recovery from exhaustion, displayed in terms of $\dot{V}O_2$ profiles, were similar for exercised fish between all three experimental times, with no apparent effect of training. The $\dot{V}O_2$ profiles (rates of recovery) for both the high and low ration groups declined as the experiment progressed (see Figure 3.4). This may have been due to the sedentary lifestyle exhibited by these groups in their isolated tanks. It is assumed that the increase in $\dot{V}O_2$ after exercise was due to physiological disturbances, including the reoxidation of lactate.

Improved ability to recover from exhaustive exercise has been demonstrated in several long-term training studies (Hochachka 1961; Hammond & Hickman 1966; Lackner *et al.* 1988; Scarbello *et al.* 1992), with trained fish having improved rates of lactate clearance (Hammond & Hickman 1966; Lackner *et al.* 1988; Pearson *et al.* 1990). Although lactate clearance was not measured in the present study due possible problems with infection, the specific activities of the enzyme lactate dehydrogenase (LDH), which catalyses the reversible conversion of pyruvate to lactate and also enables lactate oxidation, was examined between the treatment groups (Chapter 4). The LDH activity

was measured in the oxidation direction, the reactions being driven using favourable conditions, by using lactate as the substrate and a high pH.

3.4.7 Effects of treatment upon growth rate and growth conversion efficiency

For both high ration and exercised fish there was found to be a positive correlation between $BMR_{(adj)}$ and growth rate, between times 2 and 3, those fish with the highest $BMR_{(adj)}$ having the fastest growth rates.

Although there was a large individual variation in growth rate for pike between the different treatment groups between times 2 and 3 (Table 3.1), the overall mean gross conversion efficiencies for each of the pike treatment groups were not statistically different from one another. The group mean values were: exercise, 30%; high ration, 34%; low ration, 31%. Very similar gross conversion efficiencies have also been reported previously for pike, Willemsen (1965) reported gross efficiencies of between 18-33% for 13 pike between 20-60 cm in length. For 1 year old pike an average food conversion efficiency of 29% being reported by both Johnson (1966) and Weitman & Anderson (1977). Exercised pike fed at an intermediate ration had a growth rate between both the high and low ration fish between times 2 and 3 and a similar gross conversion efficiency, although they were presumed to have much larger metabolic costs, due to continual sustained swimming.

Gross conversion efficiency in fish has been reported to either increase in various trout species (Greer Walker & Emerson 1978; Leon 1986; East & Magnan 1987) and Arctic charr (Christiansen & Jobling 1990; Christiansen *et al.*1992;) decrease in the gold fish *Carassius auratus*, (Davison & Goldspink 1978) or be unchanged in rainbow trout (Davie *et al.* 1986) by swimming activity. Possible reasons put forward for improved conversion efficiency in salmonids include reduced aggression, due to the break down of dominance hierarchy with exercise. These reasons obviously do not apply to pike, which are typically solitary and do not form linear dominance hierarchies.

Other reasons suggested for improved growth conversion efficiency include the increased production of growth hormones, which has been previously demonstrated in steelhead trout (Barrett & McKeown 1988a, 1988b). Some of the increase in growth efficiency has also been attributed to switching of swimming mode and the use of ram ventilation at increased water velocities (Roberts 1975; Steffenson 1985).

efficiency has also been attributed to switching of swimming mode and the use of ram ventilation at increased water velocities (Roberts 1975; Steffenson 1985).

3.4.8 Conclusion

In conclusion pike although previously reported to have a low sustained swimming ability, the exercised group were found to have a high capacity for sustained swimming for up to 11 weeks with no deleterious effects. The exercised fish demonstrated a highly significant increase in $MMR_{(adj)}$ in response to training which was coincident with the start of the exercise, which was maintained until the end of the experiment. The lack of an increase in $BMR_{(adj)}$ for both high ration fish and exercised fish until time 3 hints at an independence between mechanisms driving BMR and MMR, possibly acting over different physiological time scales. With pike kept in isolation, there was a reduction in the $MMR_{(adj)}$ of both the high and low ration fish. Both groups also had a decrease in their recovery from exhaustive activity profiles indicating a possible physiological response to a lack of training. A high degree of individual variability in $BMR_{(adj)}$ was found in response to the different the different treatment regimes.

Chapter Four

Pike organ masses and analysis of tissue enzyme activities

4.1 Introduction

4.1.1 The importance of growth rates

One of the most important factors affecting life history strategies of fish is growth rate (Weatherley & Gill 1995). Growth can be considered as a process that is ongoing or has occurred in the recent history of the organism. The most obvious growth is that occurring at the whole organism level, typically as an increase or decrease in wet mass and/or an increase in body length. As body length does not tend to decrease under periods of starvation, it is considered to be more permanent than increased mass (Couture *et al.* 1998). Positive growth typically involves two sets of processes, an increase in mass and an increase in length or, most commonly, a combination of the two. The relative importance of increasing length and increase in mass is dependent upon the developmental stage of the fish. A rapid increase in length is more important for juvenile fish to decrease the time that they are susceptible to predation by larger fish (Mann 1982). An increase in mass is more important as fish become sexually mature, with stored energy (protein and lipid) being seasonally converted into gonadal material (Ricker 1979). Mass can vary to a large extent with season, reflecting the reproductive state and food abundance (e.g. Jensen 1979). More subtle and rapid changes in growth occur at lower levels of biological organisation at the organ, tissue or cellular level and these are examined in later sections.

Biologists often measure the growth of fishes in terms of several parameters, the most common of which include wet mass and length. Other parameters have included various indices of condition of the fish using Fulton's condition factor (Bagenal & Tesch 1978) and the use of the liver-somatic (hepatosomatic) index (Bulow *et al.* 1978). Also several different bony structures have been used to indirectly measure growth. Otoliths of fish have been used to study the age, growth and metabolism relationships in fishes (Cassleman 1987, Wright 1991). In pike the cleithrum bone in the pelvic girdle has been used to make quantitative studies of age and growth, using tetracycline administration (Treasurer *et al.* 1992). Other methods have routinely used scale readings, and the rate of increase in body mass over a fixed period of time. The

nutritional condition and growth rates of fishes have also been determined over much shorter time scales using various biochemical indices, including enzyme activities and nucleic acid concentrations (Bullow 1987). Activities of enzymes of metabolic pathways that generate energy have been used as indicators of the nutritional status of larval and adult fish (e.g. Moon 1983; Lowery *et al.* 1987; Lowery & Somero 1990; Pelletier *et al.* 1993a, 1993b, 1995; Overnell & Batty 2000).

4.1.2 Indices of body composition in fish

Historically, various indices have been used to evaluate the condition and nutritional state of fish caught both in the field and held in laboratory conditions. These indices include the relative condition factor (Le Cren 1951), gut index (Jensen 1980) the hepatosomatic index (Dehaunty & de Vlaming 1980, Adams & Mclean 1985), and the visceral somatic index (Dehaunty & de Vlaming 1980).

Unlike higher vertebrates, fish exhibit very little storage of carbohydrate (e.g. glycogen) within the body. However, glycogen is used as an immediate source of energy in the liver or in the muscle and represents 1-2% and 0.2-0.4% of the mass of these tissues respectively (Connor *et al.* 1964; Love 1980). Fish undergoing long-distance migration and spawning may utilise stored energy reserves during periods of starvation and use predominantly muscle protein as an energy source (Love 1980, Diana 1982). In cod there is a change with age in the ability to mobilise protein. In small cod an increase in the water content of the white muscle of the fish (reflecting protein depletion) from 80% to above 86 % is lethal. However in older cod an increase in the water content from 80% to 95% can be tolerated under natural conditions (Schurmann & Love 1999).

Of all the organs, the liver has received most attention as an important and sensitive indicator of the nutritional status and energy condition of fish (Jensen 1979; Adams & McLean 1985). In those fish with localised fatty deposits, the liver is known to be the major site of fat storage (Love 1970, 1980; Jobling *et al.* 1991; Lamburt & Dutil 1997). The liver has also been shown to be highly sensitive to pollution and it is known that exposure of the animal to xenobiotic compounds often leads to liver enlargement, which is coincident with induction or stimulation of hepatic enzyme activities (Schulte-Herman 1979).

4.1.3 Flexibility in organ masses of vertebrates

It is becoming apparent that even when fully grown, the anatomical and physiological configuration of vertebrates does not remain constant over time. The functional sizes of organs and amount of metabolic machinery may show great variations with seasons, food supply and under differing environmental conditions (Chapter 1, section 1.9). A fixed or constant mass of organs and associated metabolic parameters reflected in BMR would handicap an animal exposed to continually fluctuating environmental conditions. Large-scale skeletal muscle changes associated with migration have been reported in various bird species (Klaassen *et al.* 1997; Piersma & Lindström 1997). In mammals examples of organ hypertrophy have been found to be associated with food processing i.e. during lactation (Speakman & McQueenie 1996). They reported that 71.8 % of the variation in BMR between lactating and non-lactating mice was explained by the increases in the mass of alimentary tract and associated organs.

4.1.4 Scaling of metabolic machinery (the mitochondrion) in tissues

Mammals have been shown to decrease enzyme activites with increasing body size, associated with decreasing numbers of mitochondria per gram of tissue (Smith 1956). Drabkin (1950), examined porphyrin chromoproteins in mammals and found that the haemoglobin content was proportional to body size and that the activity of the enzyme cytochrome c oxidase was inversely proportional to body mass. The higher enzyme content of the rat per gram as opposed to the horse was in accordance with its higher mass specific metabolic rate. Mathieu *et al.* (1981) measured the mitochondrial content of four muscle types from 13 different species of mammals and found that the rate of decline in mitochondrial numbers was closely paralleled by the rates of maximal oxygen consumption. Mitochondria contain all enzymes associated with the process of oxidative phosphorylation in both of their inner cristae membranes and also contain all the enzymes of the tricarboxylic acid cycle in their matrix (Darley-Usmar *et al.* 1987).

4.1.5 Protein synthesis, oxygen consumption and growth

There is a good correlation between rates of protein synthesis and oxygen consumption. An increase in oxygen consumption occurs following ingestion of a meal (ASDA) and is associated with the observed increase in protein synthesis rates (McMillan & Houlihan 1988; Brown & Cameron 1991) (section 1.11). Different tissues were found to respond at different rates with the liver and stomach respondingmost rapidly (Brown

and Cameron 1991; Lyndon *et al.* 1992), and other tissues taking longer to respond (McMillan & Houlihan 1988).

In many different animal species the rates of food intake have been shown to drive the rates of protein synthesis, with increased food consumption elevating the rate of protein synthesis. It has been estimated that metabolic costs of protein synthesis represent 20-25% of the total energetic costs of BMR (Kelley & McBride 1991; Rolfe & Brown 1997).

At a level of food consumption that maintains mass, protein synthesis can be described as being at a maintenance value. For fish fed with lower rations or starved, rates of protein synthesis initially decline , then do not decline further (Houlihan *et al.* 1993). Any increase in the consumption of protein bringing about the stimulation of protein synthesis is postulated to be via amino acid and/or hormonal stimulation (Houlihan 1991; McMillan & Houlihan 1991). Protein degradation rates also appear to increase in fish with increased protein consumption (Houlihan *et al.* 1988). Hence the overall balance of protein synthesis will depend considerably on feeding conditions.

Reductions in food intake lead to a decrease in protein synthesis rates (Houlihan 1991; Lyndon *et al.* 1992), with white muscle being the most sensitive tissue to fasting (Loughna & Goldspink 1984; Houlihan *et al.* 1988; Lowery & Somero 1990). The time taken for animals to decrease whole body protein synthesis rates during starvation differs between species (McMillan & Houlihan 1992; Lyndon *et al.* 1992; McCarthy *et al.* 1999; Smith *et al.* 1999). Down regulation of protein synthesis on a tissue-specific basis has been shown in crucian carp *Carassius carassius* and suggested to be a significant mechanism for energy conservation, helping to promote survival during anoxia (Smith *et al.* 1996). It has been suggested that the decreased oxygen consumption that accompanies starvation is partly due to the reduction in protein synthesis rates (Lied *et al.* 1982) although few, if any, studies have clearly shown a decline in activity independent SMR or BMR in starved fish. Growth hormone implants in rainbow trout have been shown to increase rates of growth and protein synthesis, with little increase in the rate of protein degradation. This has been shown to lead to significant differences in the protein synthesis rates of liver, gill, ventricle and stomach in growth hormone implanted fish by comparison to controls (Foster *et al.* 1991).

As body size increases there is a decrease in the fractional rate of protein synthesis, the rate of decline having a similar exponent to that of oxygen consumption (Houlihan 1991). Total rates of protein synthesis, increase with increasing body mass, but vary between tissues (Houlihan *et al.* 1986; Fauconneau *et al.* 1995; Lyndon & Houlihan 1998). With increasing body size four tissue types showed the same ranking in both fractional traits of protein synthesis and degradation rates, the highest rates occurring in the order gill, heart, red muscle and white muscle (Houlihan *et al.* 1986). Those tissues that had the highest maintenance values also had the highest increases in protein synthesis for a given increase in growth rate (Houlihan *et al.* 1988).

Individual variations in the rates of protein turnover and growth efficiency have been reported in rainbow trout, demonstrating that fish having a similar food consumption could exhibit a two to three fold difference in fractional protein synthesis rates and fractional protein growth rates (McCarthy *et al.* 1994). In individual Atlantic salmon large differences in protein growth efficiency (protein growth divided by protein consumption) have been reported, the differences being related to different rates of protein retention of newly synthesised protein (Carter *et al.* 1993).

4.1.6 Glycogen resynthesis and the fate of lactate

It is known that vertebrate muscle is not capable of *in situ* glycogenesis. In mammals lactate produced in muscle is released into the blood and converted into glucose in the liver (the Cori cycle), which is then converted into pyruvate at the site of the muscle (Stryer 1988). The Cori cycle has little or no use in the role of glycogen resynthesis from lactate in fish skeletal muscle (Wardle 1978; Fournier & Guderley 1992). Muscle resynthesis of glycogen happens *in situ* in the muscle using the lactate produced as the primary substrate. This has been supported by a number of studies demonstrating a high level of retention of lactate within the muscle itself (Turner *et al.* 1983; Pagnotta & Milligan 1991), with the clearance of lactate coincident with the replenishment of muscle glycogen (Milligan 1996). The primary fate of lactate released into blood is probably oxidative, representing a fuel source in the heart and red muscle (Bilinski & Jonas 1972; Lanctin *et al.* 1980; Milligan & Farrell 1991). The use of lactate-based *in situ* muscle glycogenesis by fish enables effective recycling of lactate. This would not be possible if substantial lactate was transferred for glycogenesis at the liver (Milligan

1996), since movement of lactate to the liver via the blood could mean that other aerobic tissues could use the lactate as a fuel source, leading to a slower muscle recovery time. Milligan & Girard (1993) indicated this to be the case by the faster recovery from exercise of hepatectomised rainbow trout.

4.1.7 Relationships between growth rate and enzyme activity

Increased growth rates have been linked to the activities of certain aerobic enzymes. Goolish & Adelman (1987) noted that cytochrome c oxidase (CCO), an aerobic mitochondrial enzyme reflecting aerobic metabolic capacity, was significantly correlated with whole body growth rate in largemouth bass. Increased CCO activity was observed for increased growth rates in white muscle and CCO activity was reduced during starvation (Sulivan & Somero 1983) and increased again after feeding (Goolish & Adelman 1987). Oxidative enzymes in white muscle are also possibly related to levels of activity in rainbow trout (Kiessling *et al.* 1991).

Goolish & Adelman (1988) used CCO activity to describe changes in the aerobic potential of tissues with increasing size of common carp. The tissue contributing the most to whole body CCO activity was found to be white muscle. With increasing size of fish, there was an increase in the white muscle CCO as a percentage of whole body CCO activity, from approximately 50% for a 2 g fish to 80% for a 2200 g fish. Red muscle aerobic capacity is approximately four times that of white muscle (Oikawa & Itazawa 1983). However, red muscle constitutes only approximately 6% of the total muscle mass (Johnston & Goldspink 1973; Love 1980), resulting in a distribution of 40-65% of the total whole body CCO activity being distributed in the white muscle and 10-15% in the red muscle (Goolish & Adelman 1988). Activity of citrate synthase (CS), a key aerobic enzyme, has been shown to be either related (Courture *et al.* 1998) or unrelated (Pelletier *et al.* 1994) to growth rates in Atlantic cod.

Glycolytic enzyme activity (those enzymes associated with glycolysis, the anaerobic degradation of glucose) in fish has been noted to scale with size in fish (Somero & Childress 1980; Sullivan & Somero 1983). Increased activity of glycolytic enzymes, particularly lactate dehydrogenase (LDH) has also been noted for fish feeding and growing at higher rates (Mathers *et al.* 1992; Pelletier *et al.* 1993; Pelletier *et al.* 1995; Dutil *et al.* 1998). Lactate dehydrogenase activity has been shown to decrease in the white muscle of fasted scorpion fish *Scorpaena guttata* (Yang & Somero 1996).

4.1.8 Biochemical responses to sustained exercise

Exercise training has been reported to increase rates of protein synthesis in tissues including muscle and liver in rainbow trout (Houlihan & Laurent 1988). However, the increased rates of protein synthesis were only maintained as long as the fish were still being exercised. Trained rainbow trout were also found to have higher levels of aerobic enzymes than untrained trout, with an increase in those enzymes for lipid metabolism (Farrell *et al.* 1990; Farrell *et al.* 1991). Only slight changes in the aerobic enzymes were seen for the swimming muscles of exercised brook trout *Salvelinus fontinalis* and coalfish *Pollachius virens* (Johnston & Moon 1980a, 1980b). In response to training Danube bleak *Chalcalburnus chalcoides mento* and nase *Chondrostoma nasus* showed little or no change in the activities of aerobic or glycolytic enzymes in the red, white or cardiac muscles. The only trend was a slight increase in the glycolytic enzymes in the white muscle (Hinterleitner *et al.* 1992). Citrate synthase and LDH activity in the leopard shark were found to increase as a result of six weeks training (Gruber & Dickson 1997).

4.1.9 Factors affecting BMR and limitations to MMR

Those factors thought to contribute to individual differences in BMR include differences in the masses of highly metabolically active tissues (e.g. Daan *et al.* 1990). In fishes the relative decrease in the mass of highly metabolically active organs and relative increase in those tissues with a low metabolic rate results in a decline in mass specific BMR with increasing body mass (e.g. Oikawa & Itazawa 1983, section 1.12). The rate of protein synthesis and degradation have also been found to decrease with increasing size (Fauconneau 1984; Houlihan *et al.* 1986) with rates of protein synthesis varying between tissues (Fauconneau 1985).

Those factors that may have a large influence upon BMR such as highly metabolically active tissues and associated cellular processes, maintaining the sodium/potassium pumps, and costs of maintaining ion balance and protein synthesis represent a much smaller percentage of total MMR costs. It has been estimated that the cost of ventilation for a rainbow trout at rest is approximately 10% of SMR (Jones & Schwarzfeld 1974). At higher swimming speeds the costs of ventilation move from the branchial muscle to those of the myotomal muscle as the fish switches to the use of ram ventilation (Roberts

1978). Fish are also able to alter the gill perfusion rate by altering the gape of the mouth or adjusting the swimming speed, responding to changes in tissue oxygen demand (Brown & Muir 1970).

Factors limiting maximum aerobic metabolic rate will only become limiting when the organism is working at its maximal rate. For fish one of these possible-limiting factors includes gill surface area (Gray 1954; Hughes 1966) with a decrease in gill surface area associated with a reduced rate of maximal oxygen consumption (Duthie & Hughes 1987). Blood circulation time may also limit MMR (Goolish 1991). Farrell & Steffensen (1987) pointed out that the costs of oxygen transport under resting conditions are approximately 14.6% of SMR in rainbow trout. However, improved efficiency of the heart with increasing swimming speed and the switch to ram ventilation at high speed, result in a reduction of the cost of oxygen convection at U_{crit} to between 2.4- 6.9%. Maximum cardiac performance has been correlated with maximum aerobic swimming performance (Farrell & Steffensen 1987) and U_{crit} of rainbow trout was reduced by 32% following occlusion of the coronary vessels (Farrell *et al.* 1990).

4.1.10 Chapter aims

The aim of this chapter was to explore possible mechanisms that could account for the variation and flexibility in BMR and MMR that were demonstrated for individual pike in Chapter 3. Basal metabolic rate is known to vary with different masses of highly metabolically active organs (section 1.9), but it was not known to what extent organ masses of individual pike might vary under the different treatment conditions. Another important issue was to measure how well individual variation in organ masses might explain individual differences in BMR, MMR and factorial metabolic scope. The enzyme concentrations were also of interest: how well might differences in enzyme concentrations explain variations in maximal performance or BMR? The relevant literature has few studies examining any of these areas for fishes.

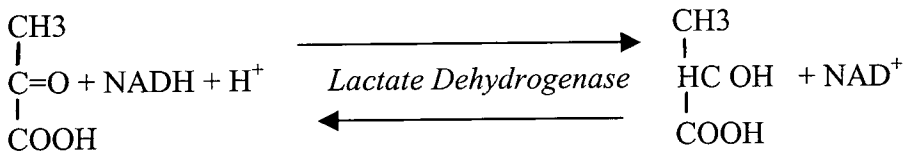
4.1.11 Choice of enzymes

Variation in BMR and MMR of fish may be associated with differing body composition and/or differences in the metabolic activity per unit mass. The latter represents the capacity to increase metabolic capacity without large increases in tissue mass through increased cell numbers. For this reason it was thought pertinent to examine the activity

of a representative aerobic enzyme and an enzyme thought to limit the rate of metabolic recovery from exhaustive exercise in relation to metabolic rate. Only two enzymes were chosen due to the time taken to actually run the assays in all the required tissues.

The two enzymes chosen were citrate synthase (CS) and lactate dehydrogenase (LDH). In the mitochondrion citrate synthase is found attached to the mitochondrial wall (Darley-Usmar *et al.* 1987). Citrate synthase catalyses the conversion of oxaloacetate to citrate, which is the first committed step in the citric acid cycle. This first step is highly exergonic and considered to be a site of regulation for the whole metabolic pathway (Mathews & van Holde 1990). It is considered to be a representative enzyme of aerobic synthesis with cellular levels being indicative of the aerobic capacity of the tissue in question and of the organism as a whole (Torres & Somero 1980). Citrate synthase is found in all eukaryotic organisms, and in higher eukaryotes is considered to be one of the best markers of the matrix fraction of mitochondria (Darley-Usmar *et al.* 1987).

Lactate dehydrogenase (LDH) is an enzyme commonly distributed through out the cytosol of most tissues and is involved in glycolysis, which is the anaerobic degradation of glucose phosphate to yield pyruvate. Lactate dehydrogenase also catalyses the reversible reaction of lactate to pyruvate in the presence of oxygen and using NAD+ . Pyruvate represents a major and important metabolic branch point. What happens next crucially depends upon cellular conditions.



Pyruvate Lactate

The direction of the reaction can be altered by the use of alkaline pH (pH 10), which removes the proton shown in the equation above, favouring the formation of pyruvate. Lactate is a dead end in metabolism and must be converted back into pyruvate before it can be metabolised. Pyruvate is generated by a number of metabolic processes including glycolysis, and under aerobic conditions pyruvate would normally enter the mitochondria to be oxidised to acetyl -Co A. This can then be oxidised by the TCA

cycle or the electron transport chain in the mitochondrion to produce energy. However, in rapidly contracting muscle an insufficient oxygen supply makes the rapid oxidation of pyruvate in the mitochondria impossible. Muscle glycogen stores are degraded by anaerobic glycolysis to pyruvate, which is then reduced to lactate by LDH in the muscle cytoplasm. The enzyme utilises NADH, which provides the reducing power by donating a hydride ion, generating lactate and NAD^+. The NAD^+ generated by this method is required by a previous enzyme in glycolysis (gluteraldehyde phosphate dehydrogenase), so under anaerobic conditions the main function of LDH is to generate NAD^+, and allow glycolysis to continue.

Lactic acid is produced by the anaerobic respiration of vertebrate tissue. The reversal of the LDH reaction then occurs to regenerate pyruvate, which has at least three possible fates. These include a) direct transfer back to the white muscle for redox shuttling; b) oxidation of the pyruvate to CO_2 and water and energy via the Krebs cycle; and finally c) entry to the oxidative phosphorylation system and/ or recycling to glucose and glycogen (Hulbert & Moon 1978). Since MMR of pike was measured during recovery from exhaustive exercise it was postulated that levels of LDH available to oxidise lactate during recovery might influence achieved MMR.

4.1.12 Isoenzymes of lactate dehydrogenase

It has been known for some time that lactate dehydrogenase occurs as various isoenzymes (Kaplan & Ciotti 1961). Lactate dehydrogenase is a tetramer of two types of subunits; the H type predominates in the heart and the M type in the skeletal muscle and liver. These subunits associate to form five types of tetramer isoenzymes: H_4, H_3H, H_2M_2, H_1M_3 and M_4 (for a full review see Stryer 1988). The most widely held theory is that the M isoforms of the enzyme are especially geared to reduction of pyruvate to lactate, and are located in those tissues that are dependent upon aerobic glycolysis. The properties of the H-type isoenzyme make the enzyme more efficient for the oxidation of lactate in tissues with an aerobic metabolism (Everse & Kaplan 1973). The differences between the H_4 (Heart) isomer and M_4 (skeletal muscle) isomer have been shown to be clear cut, with the H_4 isomer strongly inhibited by pyruvate whilst the M_4 isoenzyme is insensitive to pyruvate inhibition. The other isoenzymes have intermediate properties depending on their exact composition (Everse & Kaplan 1973). The H_4 isoenzyme has a higher affinity for lactate than the M_4 form (Stryer 1988).

The distributions of the different isoenzymes of LDH have been studied extensively in various fish species (Bouck & Ball 1968; Lim *et al.* 1975; Panepuci *et al.* 1984; Wieser *et al.* 1987). It was not the intention of this thesis to study the different isoforms of LDH in pike, but rather to examine the lactate oxidation capabilities of different tissues. High rates of such activity would be expected to be associated with an increased capacity for recovery from anaerobically fuelled exercise.

4.2 Methods

After the final set of BMR and MMR measurements from each pike in each of the treatments (time 3) taken after approximately 11 weeks at each of the three treatment regimes, pike were sacrificed for tissue analysis. Any pike that died during the course of an experiment were not processed due to an unknown time of death and possible poor physical condition. Three treatment groups of pike were set up, the first, was an exercised group. This group underwent sustained swimming at approximately 0.5 BLs^{-1} (n = 13) and were initially fed 0.7% wbm day^{-1} between time 1 (prior to the start of the exercise regime) and time 2, after approximately three weeks of sustained swimming. Due to initial feeding problems, and a slight weight loss, the food ration was increased to approximately 2.0% wbm day^{-1} between time 2 (and 3 (after approximately 11 weeks of sustained swimming). The other treatment groups were a high ration group (n = 10) offered approximately 2.7% wbm day^{-1} and a low ration group (n = 10), offered approximately 1.1% wbm day^{-1}.

4.2.1 Statistical analysis

Prior to statistical analysis all data were checked for normality visually and using a Kolmogorov-Smirnov goodness of fit test (Norusis 1994), with a Levene test for homogeneity of variances. Those data that could not be suitably transformed to fulfil the criteria of normally distributed data with homogenous variances were subjected to the appropriate non-parametric equivalent test. Where multiple comparisons were made following ANOVA, pair wise comparisons were subjected to Bonferroni correction.

4.2.2 Carcass and organ mass analysis

Individual pike were killed quickly and humanely by a sharp blow to the back of the head, followed by brain destruction. Each pike was killed within a maximum of 36 h of

the last experimental BMR measurement for that fish. Fish were not refed subsequent to the last BMR measurement and prior to death. A paper towel was used to remove excess surface water and the fish weighed to the nearest 0.01g using a top pan balance (Oertling GC-32). Fork length was measured to the nearest millimetre. The following tissues were removed, weighed and samples taken for enzyme analysis: gill filaments, heart, intestine, kidney, liver, red muscle, stomach and white muscle. The following tissues were weighed only: brain, spleen and carcass. The carcass consisted of all the remaining tissues including gall bladder, bones, skin, head, fins and any body fat.

For those organs that were required for enzyme analysis, a representative sub sample of approximately 0.5-1.0 g was taken, except for the heart (which was used whole) and the gill filaments (which were taken from the first two-gill arches on the left hand side of the fish). The gill filaments were removed from the cartilage using scissors prior to weighing. These tissues were sampled immediately on dissecting the fish. Each sample was placed onto a pre-weighed petri dish, and weighed to the nearest 0.001g using a torsion balance (Torbal ET-1). Once weighed, all samples were placed on to labeled card and immersed in liquid nitrogen (-196°C). All tissue samples used for enzyme analysis were frozen within 7 minutes of the pike's death. The intestine immediately adjacent to the stomach was used for enzyme analysis as activity of the enzyme alkaline phosphatase has been shown to decrease along the length of the intestine in pike, roach and bream *Abramis brama* (Kuz'mina & Smirnova 1991). Tissue samples from all fish were taken from the same area each time to standardise the sampling technique.

Following removal of tissue for enzyme analysis, the remainder of the visceral organs and brain were removed, then rapidly dissected free of any adherent fat (if present) and weighed to the nearest 0.001 g. Samples of tissue containing the white muscle, red muscle and skin were taken by removing a small fillet from the posterior part of the trunk musculature between the dorsal and anal fins. Red muscle at the base of the paired fins, and in the head region associated with the opercular apparatus was added to the total of red muscle (Schwalme & McKay 1985). The remaining carcass was also then immersed in liquid nitrogen and removed once frozen. It was noted previously in a preliminary experiment that as the frozen carcass thawed out, the white muscle blocks tended to split up allowing separation of the superficial red muscle layer that remained attached to the underside of the skin, from the white muscle beneath. The red muscle

runs laterally along each of the muscle blocks approximately following the lateral line system. Once thawed sufficiently, the backbone was removed from the fish and the remaining intact flank dissected. Having been removed using a scalpel, the red muscle layer from this flank was then weighed and the value doubled to estimate the total present. All remaining white muscle was dissected free from the carcass and weighed.

4.2.3 Measurement of tissue water content

The water content of the tissues from fish used in the pilot study was measured as the decrease in the water content of fresh wet tissue samples dried at 50°C for at least a week until a constant dry mass was reached. The water content was measured for all those organs that were to be used for enzyme analysis for those pike in the main set of experiments. This pilot group of pike was held in exactly the same experimental conditions as the low ration group (n = 9) in the later experiment, (section 4.2.6) to provide an indication of typical values (Table 4.1). The whole of the heart was used in the enzyme assays. Due to the low degree of variation in tissue wet water content in most tissues, the water content was measured only for those tissues that were known to change the most with feeding and exercise, white muscle and liver (Medford & McKay 1978). The water content of the white muscle was measured for all fish, and in addition the liver samples for most fish.

Protein content was measured (see section 4.2.5.) for the white muscle of all pike used in the growth and exercise experiments and liver samples for those animals that had sufficient tissue remaining after enzyme assays had been carried out (Appendix 7). Liver water content was also measured in a limited number of pike, predominantly in the high and low ration groups.

4.2.4 Measurement of enzyme levels

4.2.4.1 Citrate synthase

In higher eukaryotic organisms citrate synthase (CS) is regarded as the best enzyme marker for indicating intact mitochondrial function, and more specifically the matrix fraction. The enzyme is bound to the inner membrane *in situ*. The disruption techniques described below solubilise the enzyme.

Samples of organs previously collected as described above, were stored at –80°C for 2-3 months prior to determination of the enzyme levels. The method followed was that of Srere (1969) with slight modifications by O'Connor (1995, 1996). Approximately 20-100 mg of thawed tissue was minced finely with scissors and then homogenised on ice, using a 1ml glass-glass homogeniser, and added to ten volumes of ice-cold buffer (0.02% Triton X-100, 100 mM KH_2PO_4, 2 mM EDTA, pH 7.3). All equipment was cleaned between use on different tissues to prevent contamination of subsequent samples. Triton X 100 was added as it has been shown that many mitochondrial enzymes are 'activated' by detergent treatment, which removes the constraints on enzyme activity imposed by mitochondrial membrane structure (Schnaitman & Greenawalt 1968). Triton X-100 treatment of mitochondria has been shown to increase the activity of Cytochrome C Oxidase (CCO) by up to 90% on some previous estimates (Tyler & Nathanailides 1995). The homogenate was then sonicated for three x 15-s pulses separated by 45-s pauses on ice, to further disrupt the mitochondrial membranes. The homogenate was diluted 10 fold using a serial dilution of the original homogenate with the reaction buffer (200 mM Tris-HCL, 5 mM EDTA, pH 7.5). The pH of the reaction buffer is highly temperature specific, as Tris buffers have a substantial temperature coefficient. The pH of the buffer was set for a reaction temperature of 24°C, which is near to the maximum growth temperature for northern pike (Bevelhimer *et al.* 1985) and the maximal temperature assay conditions for the enzymes.

A volume of 0.6 ml of the reaction buffer (0.1 ml of 1 mM DTNB, 0.1 ml of 0.2 mM Acetyl-CoA, and 0.1 ml of the diluted homogenate) was placed into each cuvette. A control absorbency reading was read at 412 nm for 2-3 minutes using a dual beam Pye-Unicam SP8100 ultraviolet spectrophotometer, with a thermostated water bath set at 24°C. Finally 0.1 ml of 50 mM oxaloacetic acid was added to the cuvette to start the reaction. The cuvette was covered in parafilm and inverted rapidly to mix the contents. The absorbency increase was then read for a further 4-5 minutes. Each tissue assayed was run in duplicate or triplicate as required (i.e. if there was a discrepancy between the first and second readings of ± 10% a third reading was taken). Homogenised samples were kept on ice at all times whenever possible and used within an hour of the tissue having been homogenised. However CS is known to be a relatively stable enzyme if kept on ice for up to 24 hours (Darley-Usmar *et al.* 1987).

The rate of change in absorbency was calculated as the change in absorbency per minute. The steepest part of the slope was used, having subtracted the rate of acetyl CoA hydrolase activity. The equations of O'Connor (1995, 1996) were used:

$$\text{Enzyme activity} = (\text{Change in Abs min}^{-1}) \times 0.07353\# \times \frac{(\text{volume of buffer} + \text{mass of tissue}) \times \text{dilution factor}}{\dfrac{\text{Mass of tissue}}{0.1\text{ml diluted homogenate}}}$$

4.2.4.2 Aerobic lactate oxidation by lactate dehydrogenase

The method followed was that of Berger & Broida (1957). Lactate dehydrogenase catalyses the reversible reaction of the conversion of pyruvate to lactate and *vice versa.*

$$\text{Pyruvate} + \text{NADH} + \text{H}^+ \rightleftharpoons \text{Lactate} + \text{NAD}^+$$

Pyruvate represents a major and important metabolic branch point. The initial problem with the reaction is that at a neutral pH 7.0 and at 25°C the reaction equilibrium is about $3\text{x}10^{-12}$ (Weiser *et al.* 1987). The only way to push the reaction in the lactate oxidation mode is when there is a high concentration of NAD^+ and lactate (as occurs in cells during recovery from exhaustive exercise). When used to determine the rate of lactate conversion it is usual to pull the reaction to the left by trapping both the H^+ and pyruvate produced with an alkaline buffer (glycine –NaOH at a pH of 9.0-10.0) containing hydrazine or semicarbazide. Certain isoforms of LDH are known to be sensitive to pyruvate inhibition (Wieser *et al.* 1987). The conversion from lactate to pyruvate was the reaction of interest (lactate oxidation) for the current study since it provides an indication of the rate of recovery from exhaustive exercise of fish.

Approximately 20-100 mg of thawed tissue was minced finely with scissors and then homogenised on ice using a 1ml glass-glass homogeniser in 10 volumes of ice-cold buffer (67 mM KH_2PO_4, 2 mM EDTA, pH 7.3). All homogenising equipment was thoroughly cleaned between samples to avoid possible cross contamination of other tissues. Samples of homogenate were then spun in a refrigerated centrifuge at 4°C at a speed of 15,000 rpm (approximately 10,000g) for 10 mins (Avanti bench top centrifuge with an F306 rotor). The centrifuge was switched on approximately an hour before use

to allow the rotor and chamber to cool to 4°C. The resulting supernatant was then removed and the pellet containing cell organelles discarded. Samples were kept on ice.

The homogenate was diluted using the appropriate serial dilution of the original homogenate with the reaction buffer (200 mM Tris-HCL, 5 mM EDTA, pH 7.5). Initial experiments with different organs at different dilutions were run to check that the organ LDH activities fell on the linear region of the standard curve. The final serial dilutions of the 1:10 initial dilution for each organ were liver 1:5, gill 1:50, intestine 1:100, heart, red muscle and white muscle 1:200. As mentioned above, the pH of the reaction buffer is temperature specific as Tris buffers have a substantial temperature coefficient. The pH of the buffer was set for the same reaction temperature of 24°C as that used for CS.

Assay tubes were individually labeled and set up in a water bath set to 24°C. The tubes contained 0.5 ml of distilled water, 0.5 ml of 3.6 mM NAD (β-nicotinamide adenine dinucleotide) and 0.5 ml of 0.54 M DL lactate glycine buffer (made from 0.54M DL sodium lactate dissolved in 0.2M glycine buffer, pH 10.0). These tubes were then incubated for 5 min. The addition of 0.5 ml of the diluted enzyme extract then started the reaction. The tubes were then incubated for exactly 10 min, at which point 2 ml of 2:4 dinitro phenol hydrazine (DNPH) was added, stopping the enzyme reaction. The assay tubes were then incubated for exactly 20 min, at which point the reaction was stopped on addition of 3 ml of 1.33 M sodium hydroxide. The absorbency was read after 5 min and before 30 min at 440 nm, using a dual beam Pye-Unicam SP8100 ultraviolet spectrophotometer, with a distilled water blank and a thermostated water bath set at 24°C. Each tissue was assayed in duplicate in the first instance or repeated if there was a discrepancy of more than ± 10%. Two blanks were run with each group of tissues assayed. The value of the blank was then subtracted from each reading and the reading converted to an amount of product, by comparison to a standard curve constructed from known pyruvate concentrations (Figure 4.1). The blanks on each occasion contained the same constituents as above except for 0.5 ml of enzyme, which was replaced, by 0.5 ml of distilled water.

A standard curve was made up from different concentrations of 0.5mM pyruvate made up to 2 ml in volume with the buffered lactate solution, but without enzyme. These solutions were then treated exactly the same as in the experimental assays, by the

addition of 3 ml of 1.3M sodium hydroxide and 2 ml of 2:4 DNPH, to give the exact same volume as those tubes in the assay (7 ml). The amount of enzyme was then calculated from the amount of pyruvate created, with the concentration of pyruvate being derived from the standard curve constructed above. On all occasions the mean assay values for each tissue were taken.

The amount of pyruvate produced was calculated as follows:

Total abs – blank value produced in 10 minutes, divided by ten to give the absorbance change per minute (Abs min^{-1}). The enzyme activity was then calculated in μmol g^{-1} min^{-1}, as:

$$= ((\text{Abs min}^{-1}) - 0.035^*)/0.0112^* \frac{\text{x (vol of buffer + mass of tissue)}}{\text{mass of tissue}} \text{ x dilution factor} = \mu\text{mol g}^{-1}\text{ min}^{-1}$$

where * values were derived from the constructed standard curve of known quantities of pyruvate (Figure 4.1).

4.2.5 Measurement of protein content

The measurement of the soluble protein content of the tissues was performed using a Bio-Rad protein assay (Bio-Rad Laboratories Ltd., Hemel Hempstead UK.). The Bio-Rad protein assay is based on the observation that the absorption maximum for an acidic solution of Coomassie brilliant blue (G250) shifts from 465 nm to 595 nm when binding to protein occurs.

An initial standard curve was constructed for the assay by using suitably diluted bovine serum albumin (Sigma Chemicals) assayed at room temperature to give a range of protein concentrations between 2-20 μgml^{-1} (Figure 4.2) with the dye reagent concentrate. The bovine serum albumin was diluted with the homogenising buffer to create the standard curve. Test tissue samples were then diluted suitably until they fell in the middle range of the standard curve.

For protein measurements the organs were first homogenised in 10 volumes of ice-cold phosphate buffer (67 mM KH_2PO_4, 5 mM EDTA, pH 7.3) using a 1 ml glass-glass homogeniser. The sample was then spun on a bench top centrifuge at 1,500 rpm for ten

minutes to remove any particulate matter that might interfere with the assay and the pellet containing the cell organelles was discarded. The remaining homogenate was diluted to the appropriate degree using the homogenising buffer. To 0.8 ml of the suitably diluted homogenate, 0.2 ml of the dye reagent concentrate was added. At least two blanks were run with each group of samples. These were composed simply from 0.8 ml of homogenising buffer added to 0.2 ml of the dye concentrate. The absorbance value was read after a period of 5 minutes but before an hour had elapsed on a spectrophotometer set at 595 nm.

The protein content of the sample, once the blank value has been subtracted, was read from the standard curve created (Figure 4.2). The calculations used to calculate the protein concentration of an organ using a standard curve were similar to the method described for LDH.

4.2.6 Pilot study

Prior to the main study, a pilot study was performed with nine pike kept in static water in individual tanks between April and July 1998. These were fed exactly the same rations as the low ration group in Chapter 3 at (approximately 1% wbm day^{-1}) and held in exactly the same experimental conditions as those fish used in later experiments (i.e. 12L:12D cycle and at 15.0°C). An initial comparison of the regression slopes from the log organ mass vs. log body mass graphs for individual organs for both the pilot study group and low ration group showed a very good overlap in data for all tissue masses and that the relationship were not statistically significantly different form one another (MANOVA, $p>0.05$). Hence, since the treatment conditions were similar and there were no statistical differences in the organ mass- body mass relationships the two data sets were combined. These additional fish ($n=9$) were used for the analysis of the organ mass data only to increase sample size. There were no enzyme assays performed on these tissues from the group, tissue was used in order to provide additional data on variation in water content (Table 4.1).

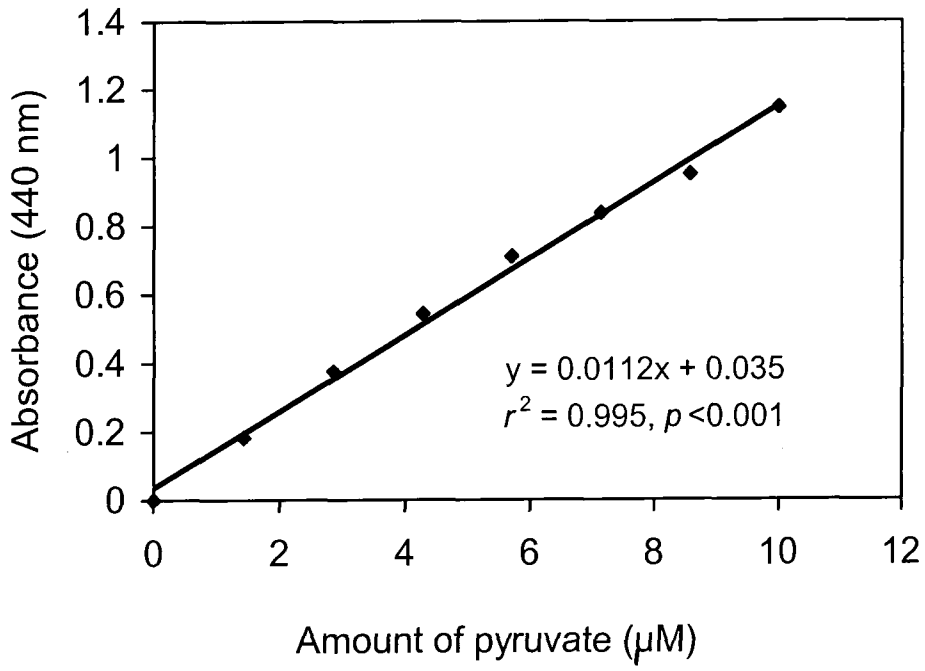


Fig 4.1 Standard curve for pyruvate. Absorbance readings for known concentrations of pyruvate.

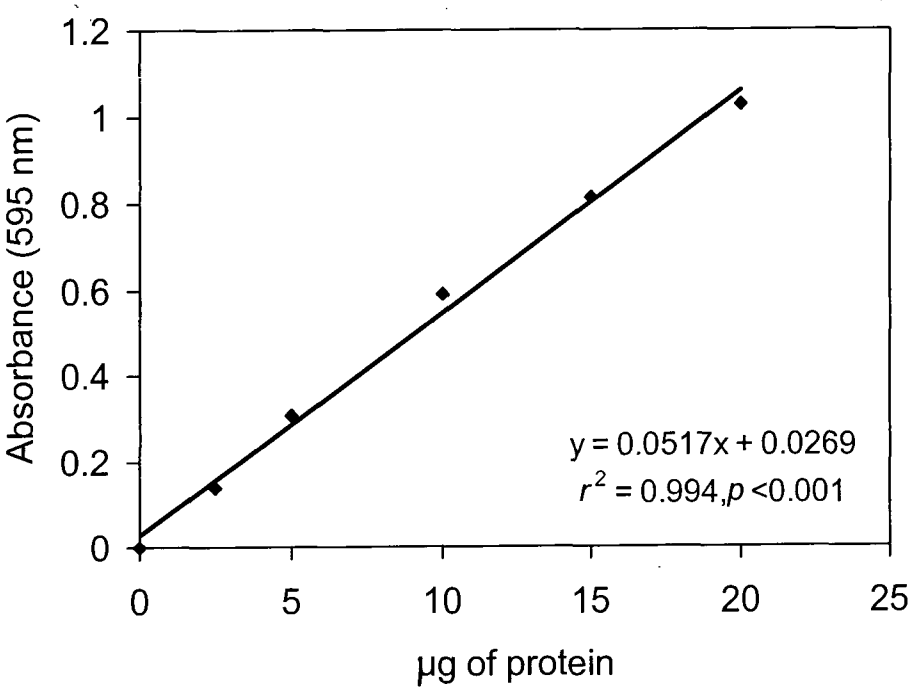


Figure 4.2 Standard curve for organ protein content, for known concentrations of bovine serum albumin.

Table 4.1 mean water content of tissues from individual pike on the pilot study. Pike were fed at approximately 1% wbm day^{-1}, the same ration as the low ration fish. Wet tissue samples were dried for at least a week at 50°C, until a constant dry mass was reached, (see section 4.2.6).

Organ	Mean water content (%)	Number	Standard error (±)
Gills	84.68	9	0.94
Heart	82.65	8	0.30
Intestine	76.92	9	0.51
Kidney	79.47	9	0.58
Liver	64.65	9	0.71
Stomach	75.64	9	0.92
White Muscle	78.29	9	0.53

4.2.7 Standardisation of body mass

To compare the relative organ masses for individual fish and between each of the three treatment groups (exercise, high and low ration), the organ masses for each individual fish were standardised to the mean mass of pike at the end of the experimental period (45 g). This was done using the relevant organ allometric scaling coefficient, which was derived from the plot of log organ mass vs. log body mass graph, and inputting to using the equation below:

$$\text{Adjusted organ mass} = (\text{bm}/45)^{b}$$

where bm is the original body mass of the fish in grams, 45 is the required body mass in g, and b is the organ allometric scaling exponent.

4.2.8 An exploration of the organ mass data using principal components analysis (PCA)

Principal components analysis (PCA) is a method of data reduction designed to clarify the relationships between two or more characters and divide the total variance of all the characters into a limited number of new correlated variables. These are termed principal components (Norusis 1994). Principal components analysis operates on the variables to maximise the differences between individuals (Dytham 1999). An exploratory analysis of the mass adjusted organ mass data (scaled to the mass of a 45 g pike) was performed for all pike for which all organ masses were recorded ($n = 36$) including those fish used in the isolation experiment ($n = 9$), using principal components analysis (SPSS). As it is known that PCA maximises the differences between individuals in this study, the principal component would usually reflect the differences in mass between the different organs of the fishes body, which is not highly informative. Therefore, prior to the analysis, the mass standardised organs were scaled relative to one another, by taking the heaviest mass of each of the organ types, assuming that its value represented 100%. All the other organs were then standardised to those of the heaviest organs of the same type as a percentage. The scaled organ percentages were arcsine transformed and logged prior to statistical analysis (Zar 1984).

4.3 Results

4.3.1 A comparison of mass adjusted fish and organ masses

Body organs of juvenile pike had different allometric relationships (Figure 4.3). To enable a comparison of the relative organ mass between individual fish and treatment

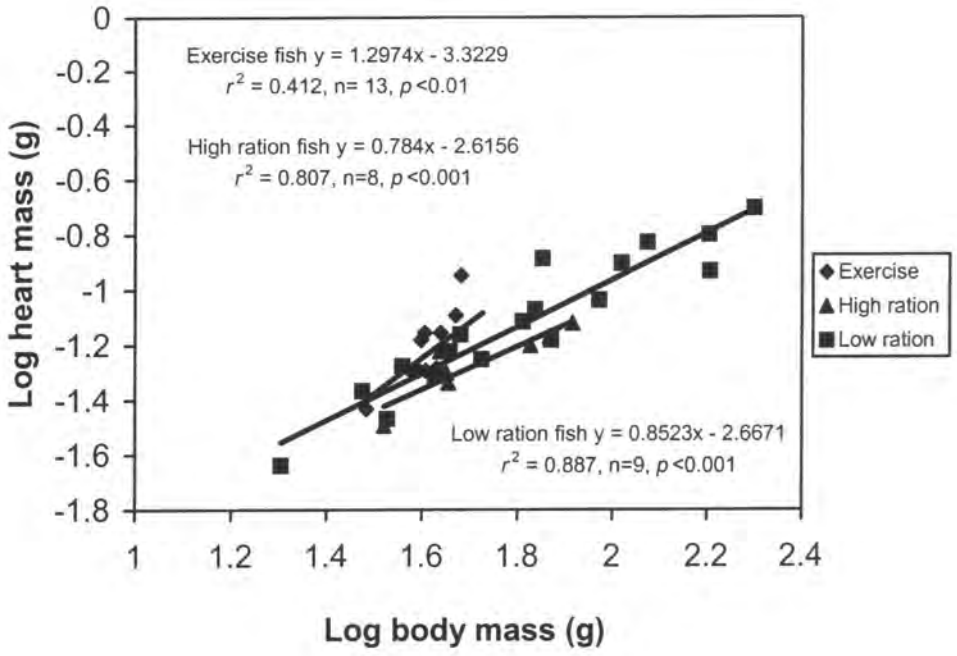
groups, the organ masses for individual fish were standardised to those of a 45 g pike using the correct organ allometric scaling relationship. Unadjusted organ masses for each fish are displayed in Appendix 4. Significant differences between the slopes of the different treatment groups were found only in kidney between high and low ration fish ($F_{1,16} = 7.59, p < 0.05$) and in the carcass between exercise and low ration fish ($F_{1,21} = 5.36, p < 0.05$). For the rest of the organs there was no significant difference between the slopes (MANOVA, $p > 0.05$), and for these the mean slopes was then taken as the organ-scaling exponent (Table 4.1). The individual regression slopes for each organ were compared between the treatment groups using MANOVA. Statistically significant differences in elevations of the slopes between the different treatments are summarised in Table 4.2. The relative wet mass of most visceral organs declined with increasing size of fish. Brain mass exhibited the greatest relative decrease in size with increasing body mass. A high r^2 value was noted for all regressions of log organ mass vs log body mass for all organs for all treatment groups, except for the kidney with low correlation for both exercise fish and high ration fish.

As an initial exploration of the data, the mass standardised organs for each fish were summed and compared to the expected total adjusted mass of 45 g. All summed organ and tissue masses were close to the expected mass (mean 98.2%). The total sum of the organs was not 100% due to loss of blood and body fluids during the dissection although care was taken to minimise these losses (Appendix 4). Relative organ masses for each fish (expressed as the percentage of adjusted body mass, Appendix 5) are plotted in Figures 4.4a and 4.4b. Statistical analysis was performed using a 1-way ANOVA between the treatment groups with Bonferroni correction of the log adjusted organ mass. The following organs had statistically significant differences between at least two of the treatment groups for brain, liver, kidney, red muscle, white muscle and carcass. In this study mean liver mass for mass standardised fish as a percentage of wet body mass (mean ± SE) was significantly higher for high ration (n=8), 1.84 ± 0.028%, compared to exercise fish (n=13) 0.83 ± 0.018% and low ration fish (n=18) 0.88 ± 0.210%. These are summarised in Table 4.4.

4.3.2 Principal components analysis (PCA)

The analysis revealed four principal components for standardised organ masses that accounted for 69.4% of the total variation between individual pike. The components and

(A)



(B)

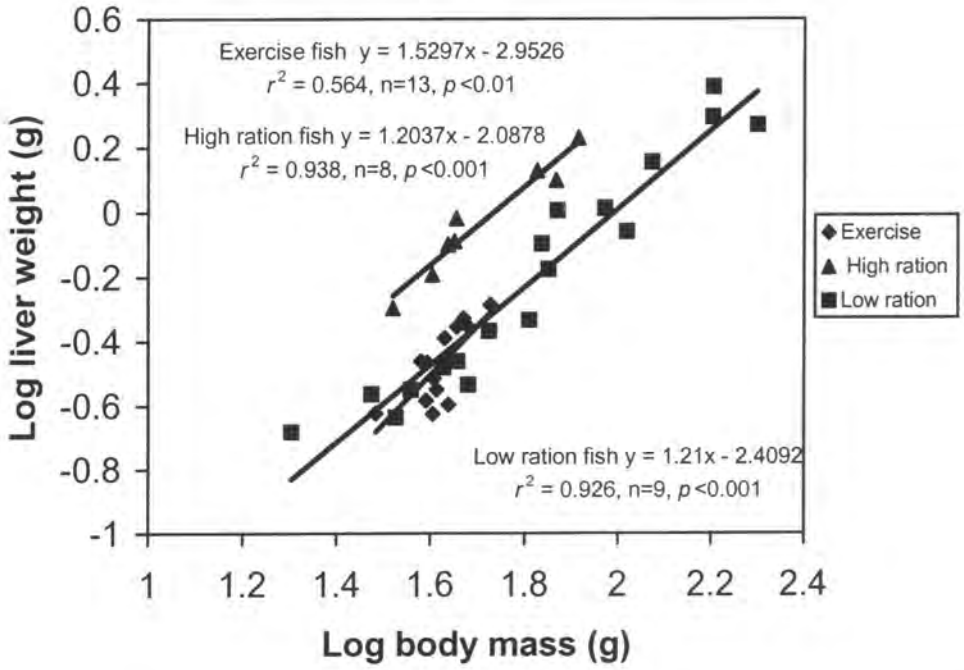
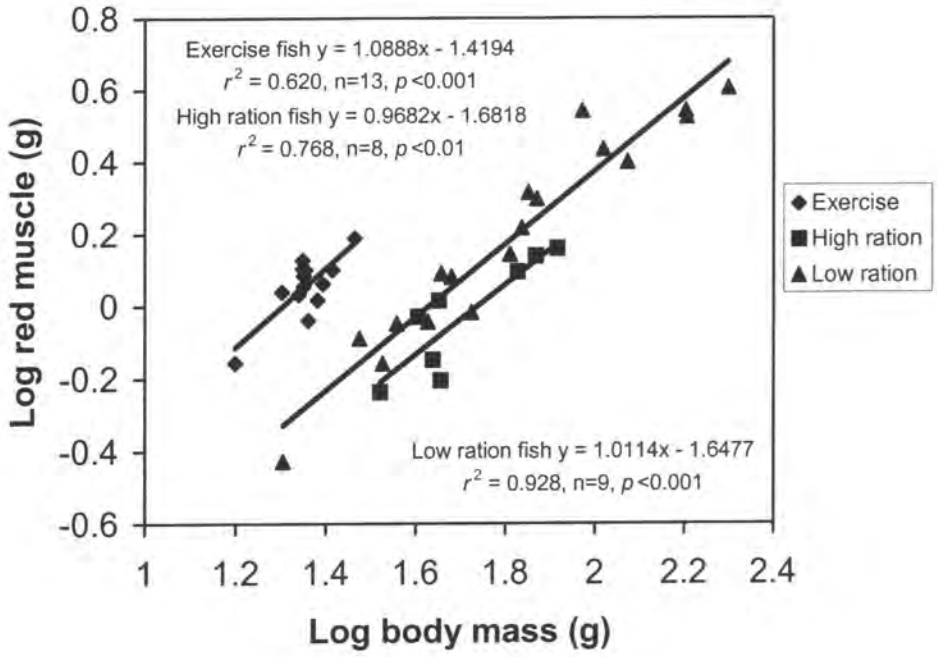


Figure 4.3 Allometric relationships between log organ mass and log body mass for individual juvenile pike after 11 weeks of continual exercise (n=13), or being held in static water tanks at either a high (n=8) or low ration (n=9) for the heart (a) and liver (b).

(C)



(D)

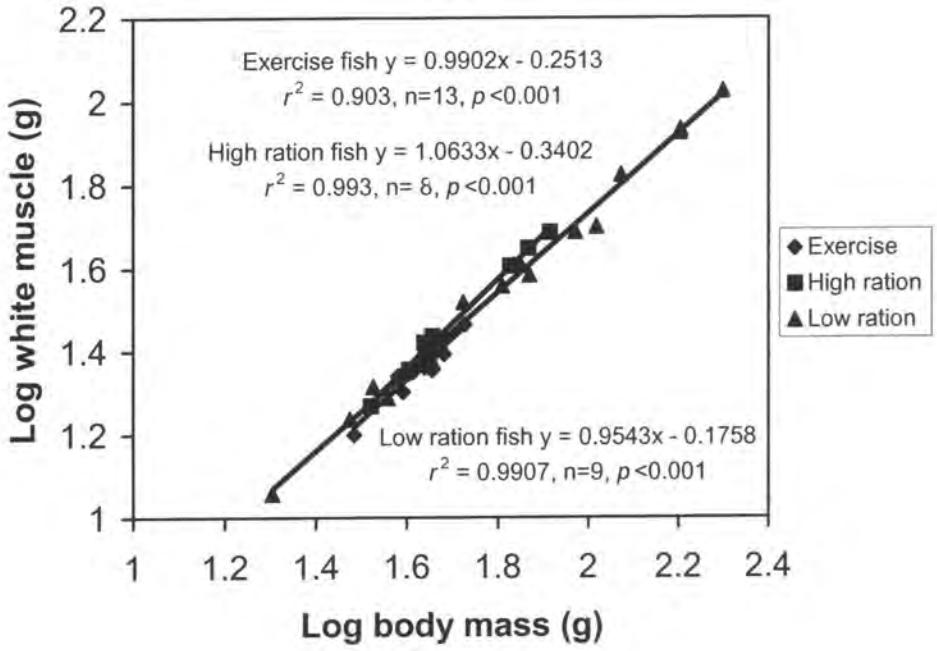


Figure 4.3 (cont) Allometric relationships between log organ mass and log body mass for individual juvenile pike after c.11 weeks of continual exercise (n=13), or being held in static water tanks at either a high (n=8) or low ration (n=9) for the red muscle (c) and white muscle (d).

Table 4.2 Allometric scaling exponents (b) measured for different organs in pike, used to scale fish to a standard mass of 45 g. The results are compared to those measured in the channel catfish *Ictalurus punctatus* (Schultz *et al.* 1999) and carp *Cyprinus carpio* (Itazawa & Oikawa 1986). In this study, for those organs where there was no significant difference between the slopes (tested using MANOVA), a mean allometric scaling value was taken. Organ masses were measured for 39 pike (20.2 –199.3 g), 23 channel catfish between 63-1873g in weight (Schultz *et al.* 1999), and carp from 0.07-1900g. Values in all cases were obtained from a plot of log tissue mass (g) vs. log body mass (g).

Organ	b (This study)	SE	r^2	b (Schultz *et al.* 1999)	b (Itazawa & Oikawa 1986)
Brain	0.48	0.04	0.75	0.32	0.41
Heart	0.81	0.07	0.78	0.81	0.82
Gill	0.83	0.07	0.79	-	0.84
Kidney	Exercise 0.565* High 0.472* Low 1.27*	0.22 0.20 0.10	0.32 0.47 0.91	0.87	0.92
Liver	1.2	0.13	0.74	0.95	0.92
Spleen	1.17	0.16	0.64	-	0.90
Stomach	0.65	0.07	0.72	1.16	-
Intestine	0.88	0.06	0.85	0.81	0.84
Red muscle	0.97	0.07	0.84	-	-
White muscle	0.98	0.02	0.99	-	1.04
Carcass	High and Low 1.045* Exercise 1.01*	0.03 0.15	0.97 0.80	-	1.02

* statistically significantly different slopes

Table 4.3 A statistical comparison of the adjusted organ mass, regression slope elevations, for the three treatment groups of pike (exercised pike, low ration pike and high ration pike). A comparison of the organ mass vs body mass scaling regression slopes between each of the three treatments groups, were compared for each individual tissue using MANOVA, with the level of statistical significance given as, ns = no statistical significance, * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

Organ	Exercise and high ration	Exercise and low ration	High and low ration
Brain	**	**	ns
Heart	*	ns	ns
Gill	ns	ns	ns
Liver	***	ns	***
Kidney	ns	ns	ns
Spleen	ns	ns	ns
Stomach	ns	ns	ns
Intestine	ns	ns	ns
Red muscle	***	**	*
White muscle	**	ns	*
Carcass	***	***	***

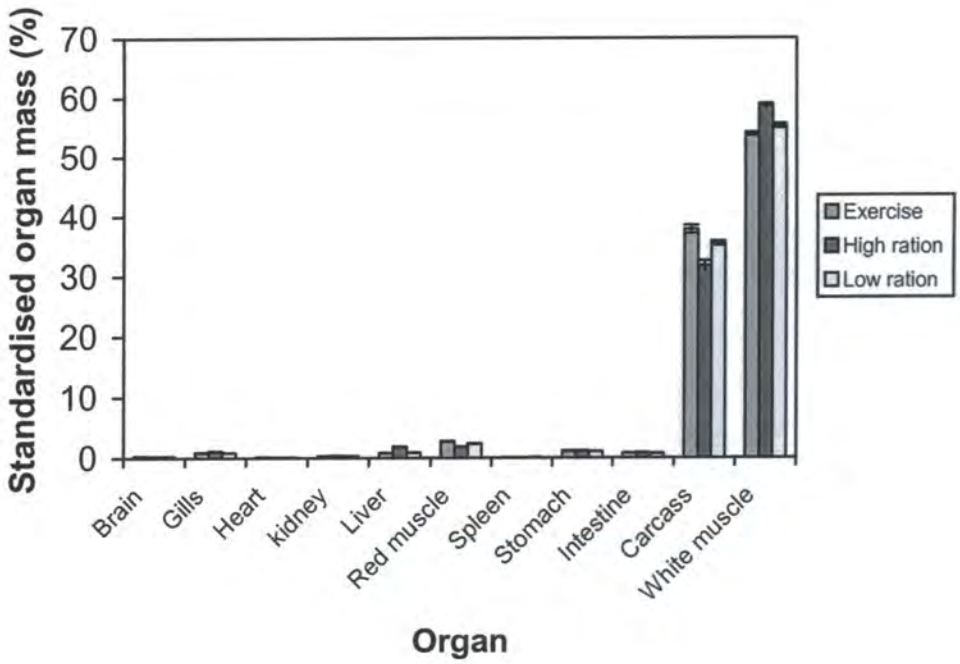


Figure 4.4a Standardised organ masses (mean and SE) adjusted to those of a 45 g pike and expressed as a relative percentage of adjusted body mass, (exercised fish (n=13), high ration fish (n=8), low ration fish (n=18)).

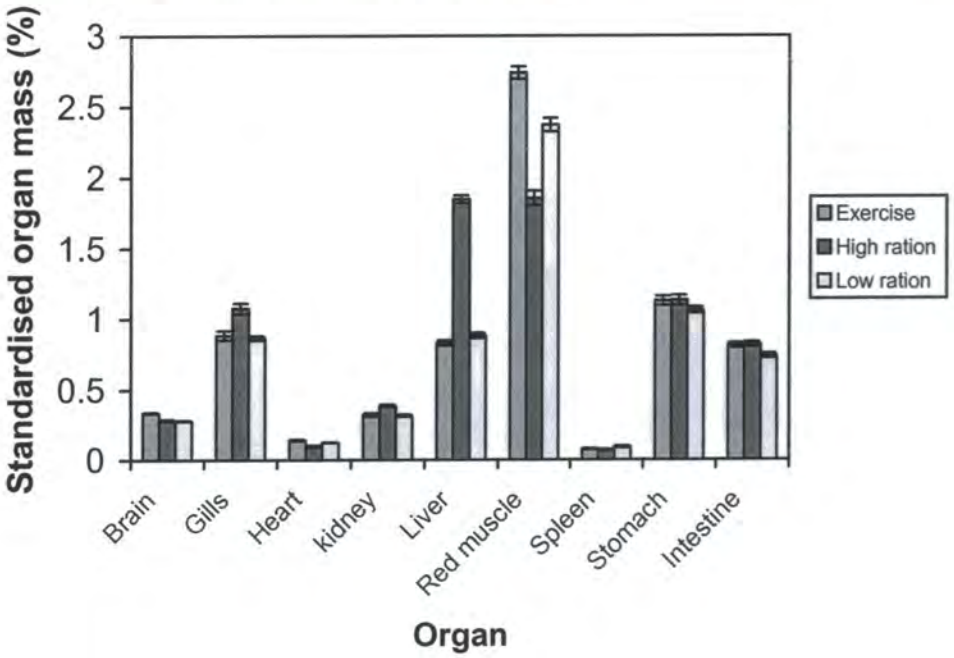


Figure 4.4b Standardised organ masses (mean and SE) adjusted to those of a 45 g pike and expressed as a relative percentage of adjusted body mass for all tissues except carcass and white muscle, (exercised fish (n=13), high ration fish (n=8), low ration fish (n=18)).

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extent of the percentage variation explained by each is summarised in Table 4.5, with high Eigen values noted for principal components one and two. The PCA showed for the principal axis a strong positive correlation for red muscle carcass and heart mass and a strong negative correlation for white muscle and liver mass. This indicates that these organs varied the most between individual fish. Principal component 2 had a strong positive correlation for kidney and intestine masses. Component axis 3 was strongly positively correlated to the stomach mass, with a strong negative correlation for the spleen mass. Component axis 4 was strongly positively correlated to the brain mass. Gill tissue mass was not strongly correlated to any of the four principal factors and consequently had the lowest communality value.

4.3.3 Step-wise discriminant analysis

A stepwise discriminant analysis was performed on the log individual adjusted organ masses of a total of $n = 36$ pike, that is all individuals with complete set of organ masses (Appendix 4). The technique is designed to produce new axes that minimise variation within designated groups and maximise differences between designated groups (Wiley 1981). This technique was used to assess how well pike could be assigned to the individual treatment groups in terms of their adjusted organ mass values. Both the primary and secondary discriminant functions were highly statistically significant (Table 4.6). The primary function explained 86.6% of the total variance and the secondary function the remaining 13.4%. The primary discriminant consisted of a strong positive correlation with the liver. The secondary discriminant was described by a positive correlation with brain mass and red muscle mass. There was a high overall success rate (84%) of determining individual treatment group membership for pike from the individual organ masses, using the discriminant functions developed in the analysis. Exercise fish were correctly classified 61.5% of the time, being wrongly classified 38.5% of time as low ration fish only. High ration fish were correctly classified 100% of the time, and low ration fish 94.4% of the time with a single individual wrongly classified as an exercised fish. The overlap of the individual groups and positions of the group centroids are represented in a graph of function 1 vs function 2 (Figure 4.5).

Table 4.4 Individual one-way ANOVA with Bonferroni corrections of individual log mass adjusted organ masses standardised to a pike of 45g. Liver was tested using a Kruskal-Wallis test due to a statistically significant Levene's test. Levels of statistical significance are ns = no statistical significance, * $p<0.05$, ** $p<0.01$, *** $p<0.001$.)

Organ	*n*	df	F	Significance	Exercise and High	Exercise and Low	High and Low
Brain	39	2	6.99	$p<0.01$	*	**	ns
Heart	37	2	4.61	$p<0.05$	*	ns	ns
Gill	39	2	2.02	$p>0.05$	ns	ns	ns
Kidney	38	2	3.57	$p<0.05$	*	ns	ns
Spleen	39	2	0.21	$p>0.05$	ns	ns	ns
Stomach	39	2	0.47	$p>0.05$	ns	ns	ns
Intestine	39	2	1.85	$p>0.05$	ns	ns	ns
Red muscle	39	2	14.53	$p<0.001$	***	*	**
White muscle	39	2	6.46	$p<0.01$	**	ns	*
Carcass	39	2	11.39	$p<0.001$	***	ns	**

Organ	*n*	df	χ^2	Significance	Exercise and High	Exercise and Low	High and Low
Liver	39	2	14.18	$p<0.01$	***	-	-

Table 4.5 Principal components analysis of morphological variation in proportions of 11 mass standardised tissues, standardised to those of a 45 g pike (Arcsine transformation of the relative percentage weight of each tissue measured was performed prior to analysis) for 36 pike.

	PC1	PC2	PC3	PC4
Eigen value	3.46	1.83	1.30	1.04
Proportion	.315	.166	.166	.094
Cumulative	.315	.481	.481	.694
Eigen vectors:				
Brain	.372	.001	.009	.667
Carcass	.903	.085	.152	-.172
Gill	-.410	.053	.441	.233
Heart	.530	-.183	.237	.497
Intestine	.147	.831	.228	.119
Kidney	.147	.817	.093	.119
Liver	-.757	.392	.163	-.056
Red muscle	.835	.029	.068	-0.99
Spleen	.033	.127	-.744	.376
Stomach	-.271	-.455	.592	.165
White muscle	-.822	-.223	-.178	.228

Table 4.6. Summary of the canonical discriminant function and standardised canonical discriminant coefficients.

	Function 1*	Function 2*
Eigen value	3.60	0.56
Cumulative variation	86.62	100.0
Canonical correlation	0.88	0.60
Stand. canon. discrim. coeff		
Brain	0.06	0.86
Liver	0.88	0.47
Red muscle	-0.39	0.53

Test of functions, 1-2: Wilks $\lambda = 0.139$, $\chi^2 = 62.94$, df =6., $p<0.001$; function 2: Wilks $\lambda = 1.64$, $\chi^2 = 14.14$, df =2, $p<0.001$.

4.3.4 Tissue water and protein content

Tissue water content was measured for the white muscle of all pike used in the main experiments (n = 30) and water content for the liver for most pike in the high ration (n = 7) and low ration (n = 8) groups only, as it is know that these tissues vary the most in water content under normal growing conditions (Medford & McKay 1978). The liver and white muscle are most sensitive to changes in growth rates (Houlihan 1993). There was found to be no significant difference in the in the water content of the white muscle for pike growing at any of the growth rations (ANOVA, $F_{2,29} = 0.37, p > 0.05$) with a similar range of values found in each group. The average water content of the white muscle of individual pike varied independently of growth rate being between 78.4 - 81.7 % (mean ± SE = 79.3 ± 0.18) of the wet mass of the tissue. There was found to be significant variation in protein content of the white muscle between groups (ANOVA, $F_{2,27} = 3.89, p = 0.03$) with a significant difference occurring only between high ration fish and other groups (Bonferroni correction $p < 0.05$). The protein content of the liver was also found to be significantly different between the groups ($F_{2,18} = 5.86, p < 0.05$), being significantly higher for the high ration fish only ($p<0.05$, Bonferroni correction) with large individual variations in the protein content of the liver noted for low ration fish. There was no significant difference in the liver water content between high and low ration fish (ANOVA, $p> 0.05$), exercised fish were excluded from the analysis, as there was only a single sample for an exercised fish (Appendix 6).

4.3.5 A comparison of the specific activity of the aerobic enzyme citrate synthase

A measurement of the activity of CS per gram of tissue was made for six tissues (heart, gill, liver, intestine, red muscle and white muscle) in each of 30 pike. Those tissue with the highest CS activities per gram were the heart and red muscle, with the lowest activities per gram being found in the liver and white muscle (Figure 4.6). A comparison of the log CS activity per gram between different treatment groups was performed using a 1-way ANOVA with a Bonferroni correction. The only organ to have a highly statistically significant difference in the activity per gram of CS was the liver ($F_{2,29} = 38.95, p < 0.001$). After Bonferroni correction there were significant differences between exercise and high ration groups ($p < 0.001$), exercise and low ration groups ($p<0.01$) and high and low ration groups ($p < 0.001$).

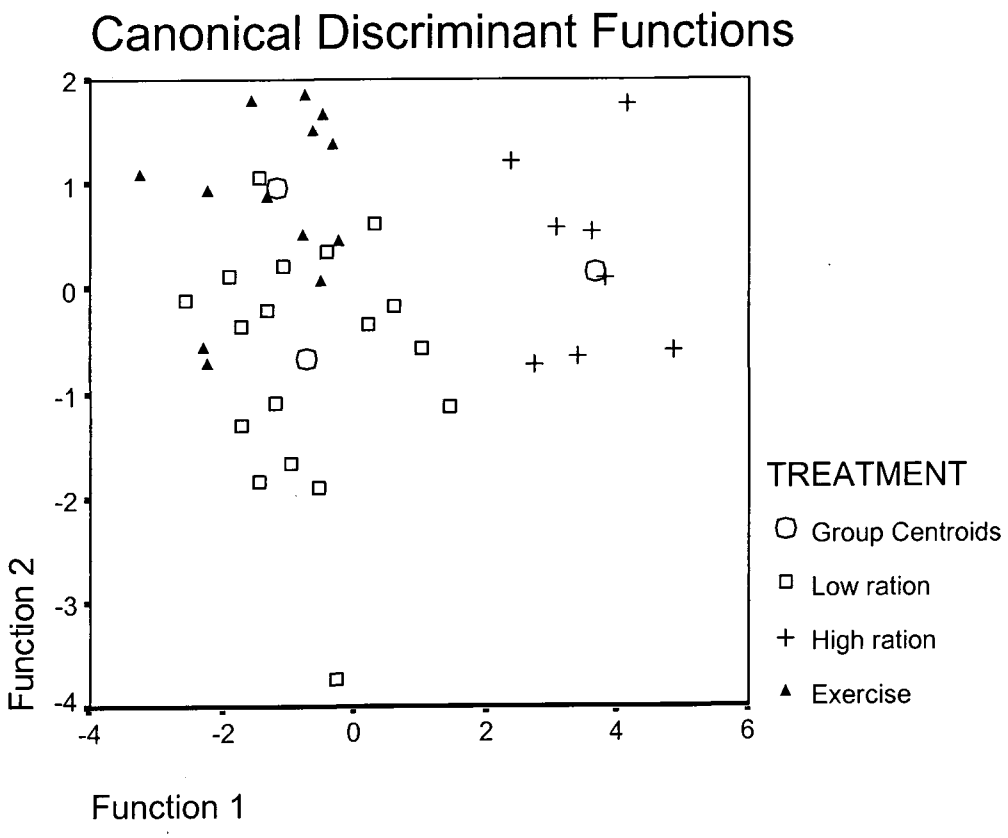


Figure 4. 5 Discriminant analysis used to classify mass standardised pike (45g) into their respective treatment groups on the basis of individual relative organ masses.

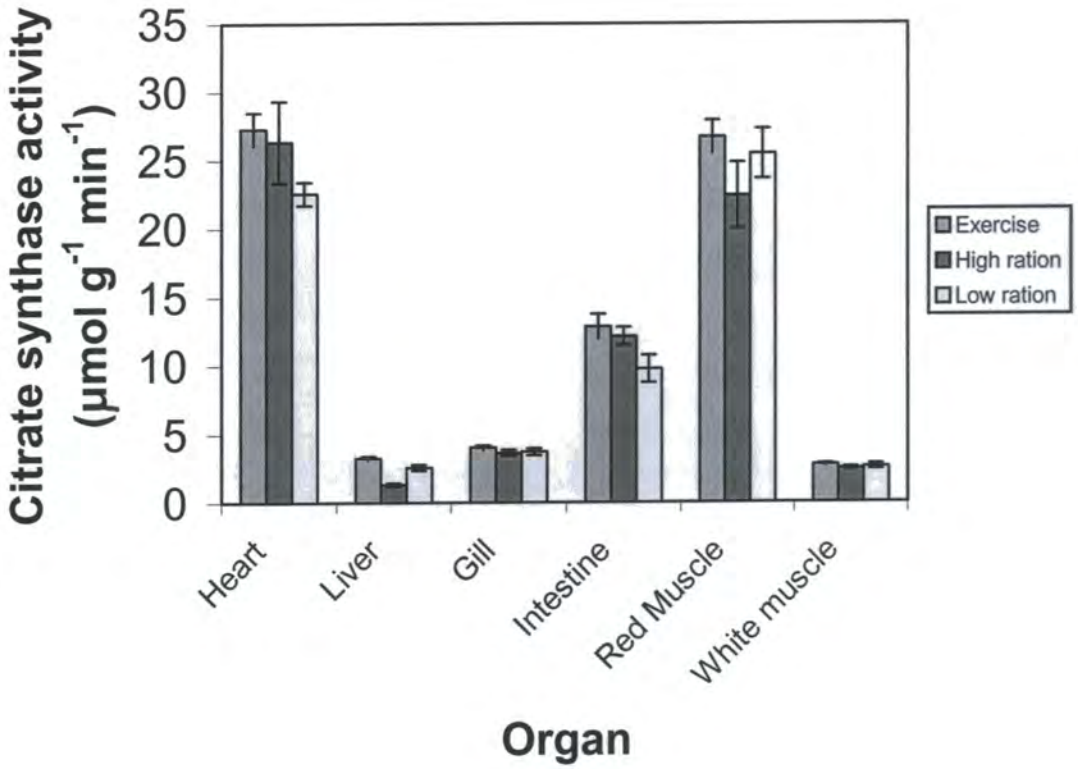


Figure 4.6 Mean ± SE of citrate synthase activity per gram in different organs of individual pike, held at one of three treatment regimes for approximately 11 weeks. Pike were either exercised continually, or held in individual static water tanks at a high or low food ration (n = 30).

Significant relationships between log CS activity per gram and log body mass were found for several tissues reflecting whole body allometric relationships. There was a significant decrease in the activity per gram of CS in the gill for low ration fish, (F_7 = 8.39, r^2 = 0.58, p< 0 .05); with a significant increase in CS activity per gram for the heart of low ration fish (F_7= 8.12, r^2 = 0.58, $p < 0.05$) and red muscle of low ration fish (F_7 = 7.15, r^2 = 0.44, p<0.05). A statistically significant increase in activity per gram of CS was noted for the intestine of exercised fish (F_{12}= 9.36, r^2= 0.41, $p < 0.05$).

Plots of log total activity per organ (activity per gram multiplied by the organ mass), vs. log organ mass revealed significant linear relationships (Figures 4.7a-4.7f, Table 4.7). These plots indicated that in most cases there were significant positive correlations between total organ activity and organ mass, indicating that for a given mass of organ, the enzyme activity were constant. Statistical comparison of the log total CS activity vs log total organ mass relationships between treatment groups for each organ was performed using MANOVA. There were found to be no differences in the slopes between any of the treatment groups for any of the organs. The elevation of the regression slopes of log liver CS activity and log liver mass in the pike were highly statistically significant different between each of the treatment groups (slope, $F_{2,29}$ = 0.25, p>0.05; elevation, $F_{2,29}$= 15.29, $p < 0.001$). High ration fish had a significantly lower elevation than both exercise and low ration fish ($p < 0.001$), with exercise fish having a significantly higher elevation than low ration fish (p<0.05). There was found to be a negative correlation between estimated whole body CS activity (activity per gram multiplied by the tissue mass and summed for the six organs that enzyme activity was measured in), and growth rate for low ration fish only (F = 6.54, n = 8, r^2 = 0.48, p < 0.05, Figure 4.8)

4.3.6 A comparison of the specific activity of lactate dehydrogenase (LDH) in the oxidation of lactate to pyruvate

Lactate dehydrogenase activity (in its oxidative mode of action) was found to be the highest in the heart, followed by red muscle and white muscle, with the lowest activity measured in the liver (Figure 4.9). A comparison of the log activity per gram of LDH was performed for each organ between different treatment groups (1 way ANOVA). As with CS, the only organ found to have a significant difference in LDH activity per gram between the different groups was the liver ($F_{2,29}$ = 24.13, $p < 0.001$).

Table 4.7 Statistical significance of individual regressions of log total organ activity of citrate synthase vs log organ mass and log total organ lactate dehydrogenase activity vs. log organ mass. (The levels of statistical significance were as follows, ns = no statistical significance, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

		Citrate synthase				Lactate dehydrogenase			
Organ	Treatment group	df	*F* value	r^2	Sig.	df	*F* value	r^2	Sig.
Heart	Exercise	11	11.93	0.54	**	10	22.06	0.71	**
	High ration	7	5.78	0.49	ns	7	19.78	0.73	**
	Low ration	7	299.32	0.98	***	7	93.68	0.94	***
Gill	Exercise	12	91.45	0.89	***	12	12.46	0.53	**
	High ration	7	24.62	0.80	**	7	30.69	0.86	**
	Low ration	7	132.8	0.96	***	6	209.95	0.98	***
Liver	Exercise	12	38.21	0.78	***	12	75.07	0.87	***
	High ration	7	12.31	0.67	*	7	1.67	0.22	ns
	Low ration	8	12.85	0.65	**	8	48.42	0.87	***
Intestine	Exercise	12	28.59	0.77	***	12	5.84	0.35	*
	High ration	7	21.66	0.78	**	7	2.68	0.31	ns
	Low ration	8	8.91	0.56	*	8	15.85	0.69	**
Red muscle	Exercise	12	15.19	0.58	**	12	26.00	0.73	***
	High ration	7	5.40	0.47	ns	6	45.36	0.91	***
	Low ration	8	111.65	0.94	***	7	69.29	0.91	***
White muscle	Exercise	12	17.60	0.615	**	12	8.62	0.44	*
	High ration	7	67.38	0.92	***	7	10.68	0.64	*
	Low ration	8	8.66	0.55	*	8	60.22	0.90	***

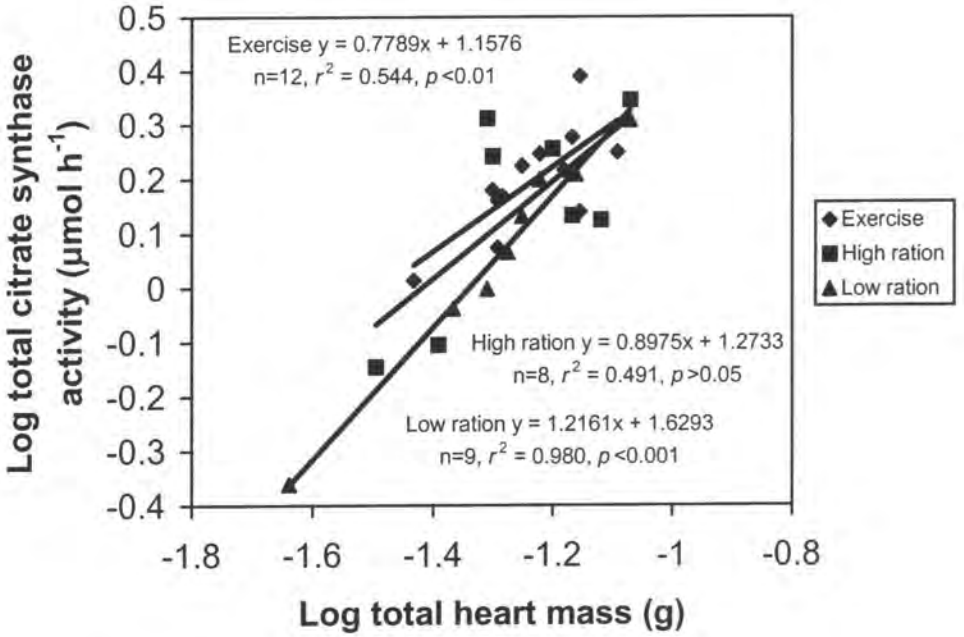


Figure 4.7a Relationship between total log heart citrate synthase activity and log heart mass in individual pike, after c.11 weeks at one of the three experimental regimes. These were sustained exercise fish (n=12), or pike held in static water tanks at either a high food ration (n=8) or low ration (n=9).

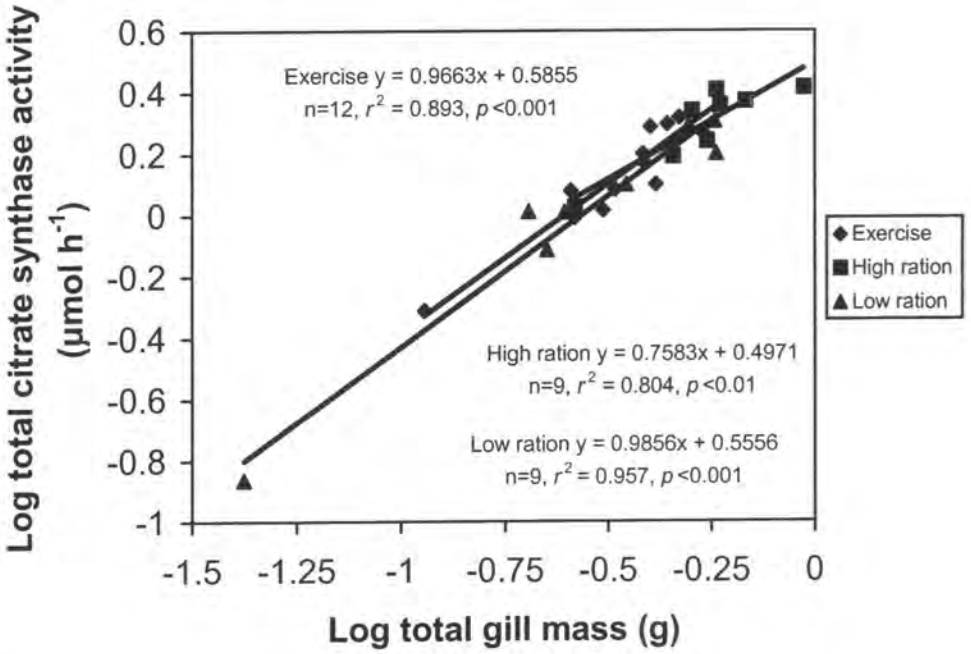


Figure 4.7b Relationship between total log gill citrate synthase activity and log gill mass in individual pike, after c.11 weeks at one of the three experimental regimes. These were sustained exercise fish (n=12), or pike held in static water tanks at either a high food ration (n=8) or low ration (n=9).

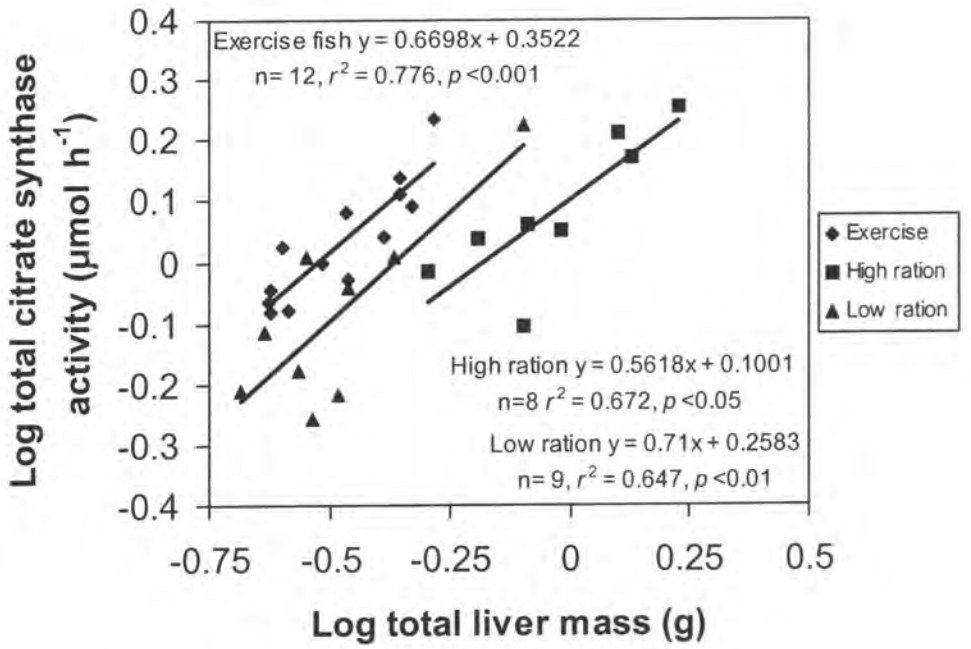


Figure 4.7c Relationship between log liver citrate synthase activity and log liver mass in individual pike, after c.11 weeks at one of the three experimental regimes. These were sustained exercise fish (n=12), or pike held in static water tanks at either a high food ration (n=8) or low ration (n=9).

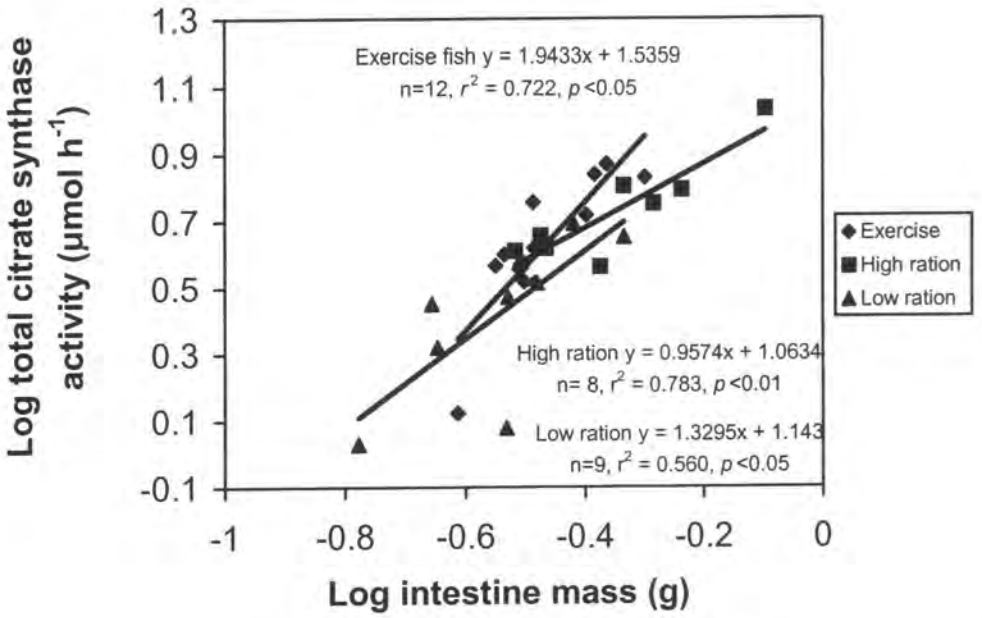


Figure 4.7d Relationship between log intestine citrate synthase activity and log intestine mass in individual pike, after c.11 weeks at one of the three experimental regimes. These were sustained exercise fish (n=12), or pike held in static water tanks at either a high food ration (n=8) or low ration (n=9).

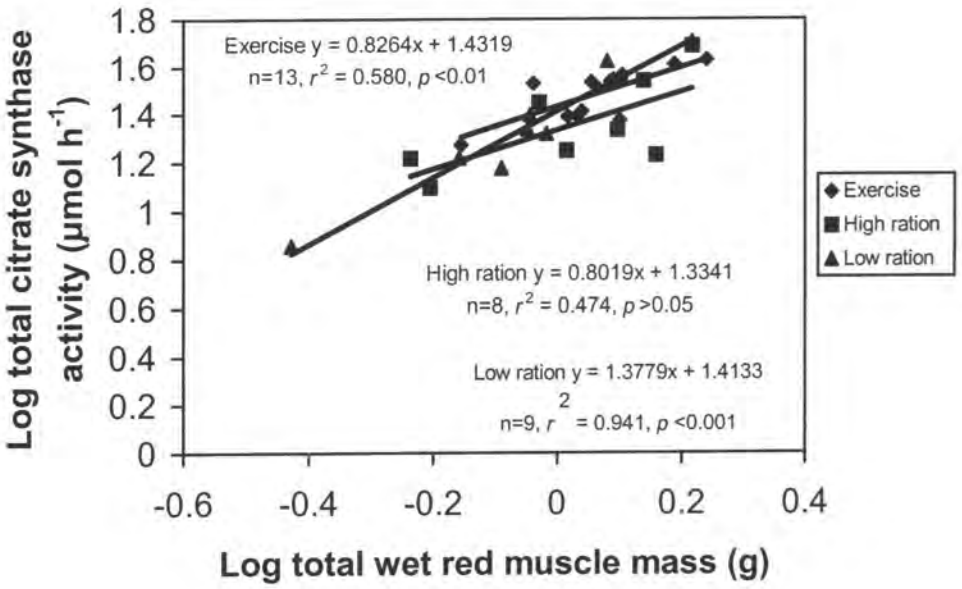


Figure 4.7e Relationship between log red muscle citrate synthase activity and log red muscle mass in pike, after c.11 weeks at one of the three experimental regimes. These were sustained exercise fish (n=12), or pike held in static water tanks at either a high food ration (n=8) or low ration (n=9).

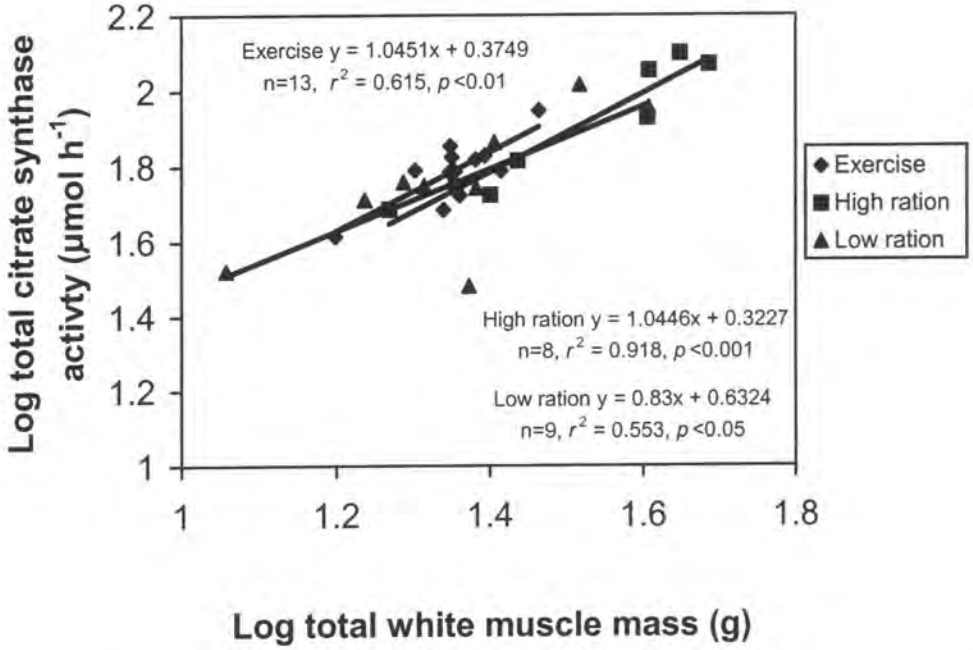


Figure 4.7f Relationship between log white muscle citrate synthase activity and log white muscle mass in pike, after c.11 weeks at one of the three experimental regimes. These were sustained exercise fish (n=12), or pike held in static water tanks at either a high food ration (n=8) or low ration (n=9).

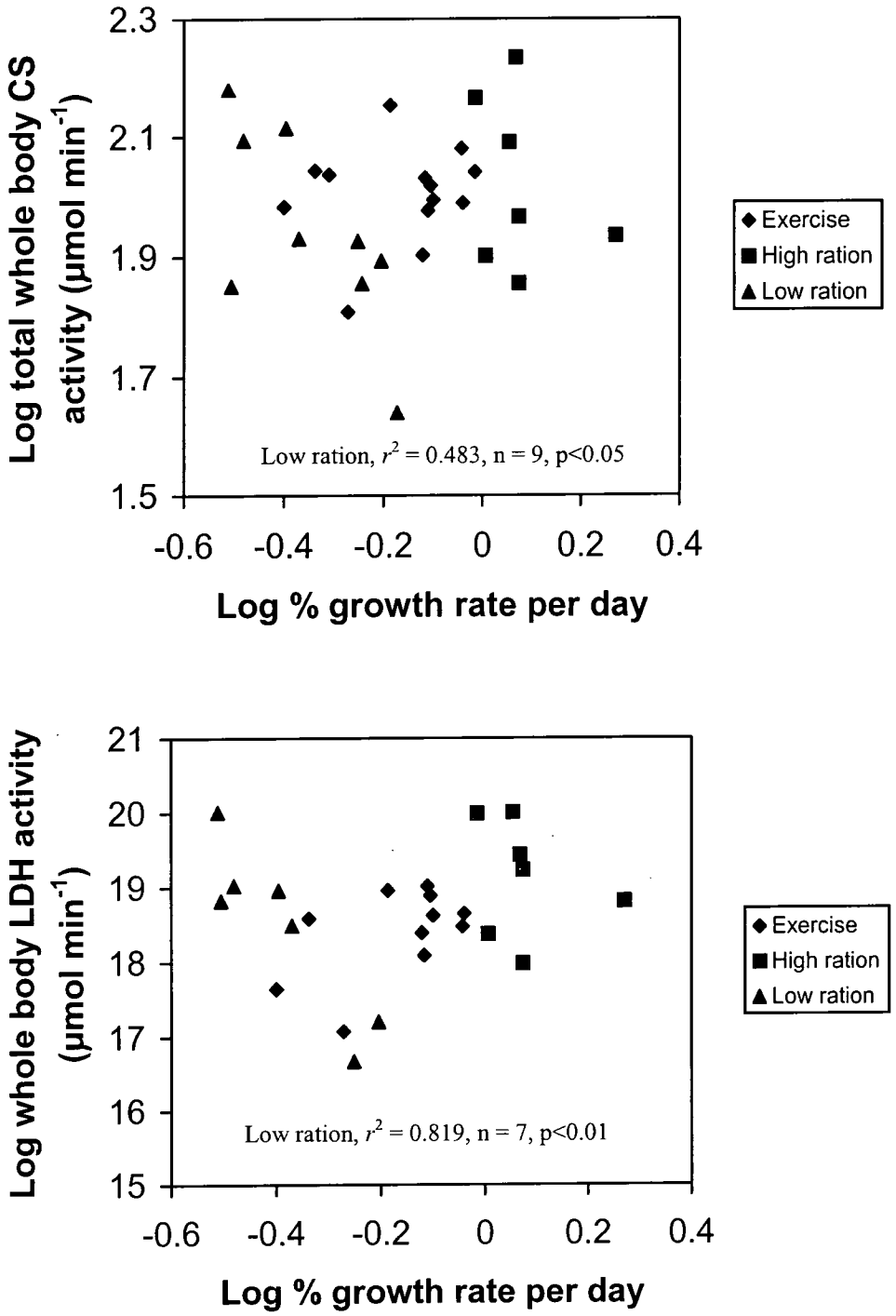


Figure 4.8 A correlation of log whole body citrate synthase (above) and log whole body LDH activity (below) vs percentage growth rate per day for pike.

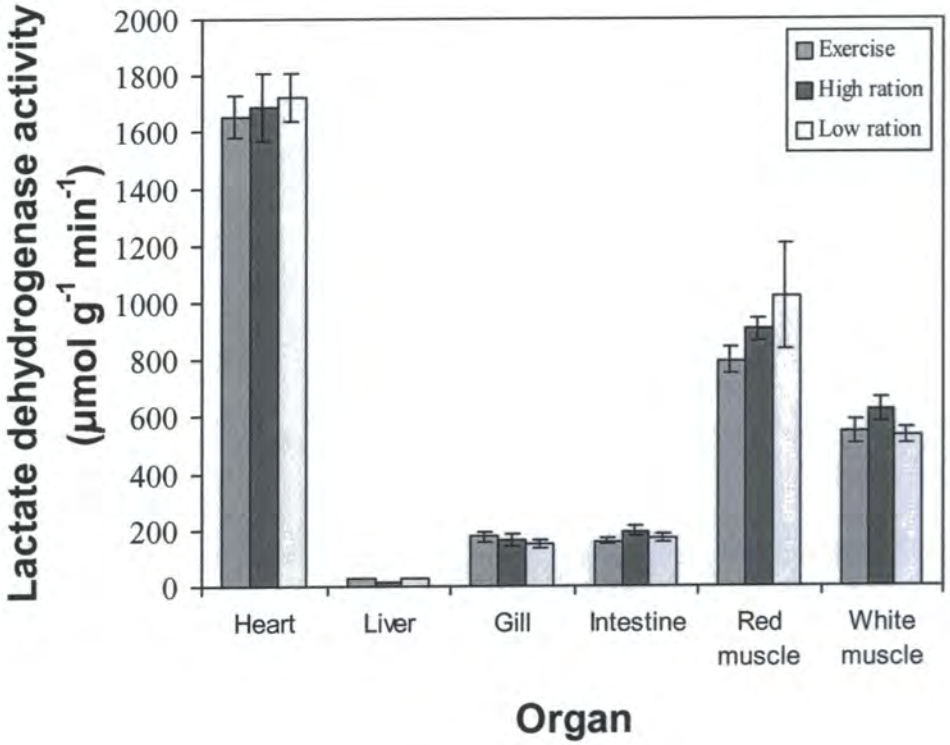


Figure 4.9 Mean ± SE of lactate dehydrogenase in the lactate oxidation mode per gram in different organs of mass standardised pike (n = 30) held at one of three treatment regimes for approximately 11 weeks. Pike were either exercised continually, or held in individual static water tanks at a high or low food ration (n = 30).

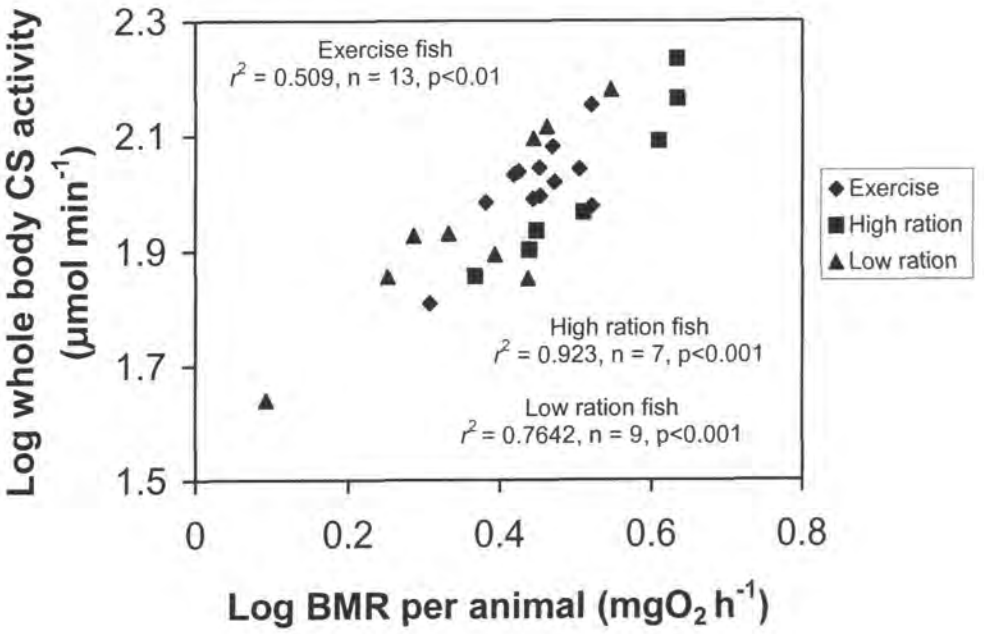
Bonferroni corrections revealed highly statistically significant differences between both the high ration and exercise fish ($p<0.001$) and the high ration and low ration fish ($p<0.001$).

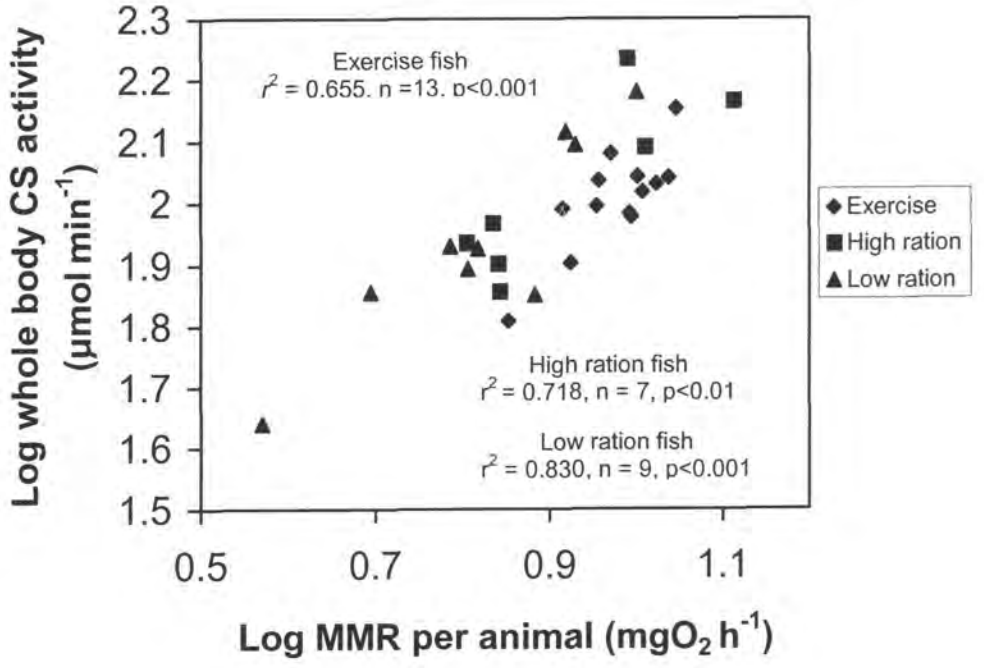
For the majority of organs there was no statistically significant correlation between activity of LDH per gram and body mass. The only organs that showed any statistically significant trend were those seen were for low ration fish with a positive correlation seen for red muscle and body mass ($F_8 = 23.4$, $r^2 = 0.80$, $p < 0.01$). A significant negative correlation between LDH activity per gram and body mass was seen for high ration fish and the liver ($F_7 = 6.49$, $r^2 = 0.52$, $p < 0.05$). Also as with CS there were very good correlations between log total organ LDH activity and log total organ mass for most tissues except the intestine (Table 4.7). These relationships were similar to those for CS and hence are not displayed graphically.

A comparison of the log LDH activity per organ vs. log organ mass, for each individual organ was performed using MANOVA. As with the CS the only organ to show any treatment difference of total organ LDH activity in relation to body mass was the liver (slope, $F_{2,29} = 4.46$, $p < 0.05$; elevation $F_{2,29} = 3.46$, $p < 0.05$). After Bonferroni correction there was found to be a significant difference in the slopes between both high ration and exercise fish ($p<0.05$) and high and low ration fish ($p < 0.05$). There was found to be a highly statistically significant correlation between LDH activity and percentage growth rate per day for low ration fish only ($F = 22.68$, $n = 6$, $r^2 = 82$, $p < 0.01$) (Figure 4.8b).

4.3.7 The relationship between BMR, MMR and enzyme levels

To assess the effects of enzyme levels on BMR and MMR, the total enzyme activity values were summed for each animal. This was done by summing the total log activities per organ (μmol of product min^{-1}) for each of the six organs measured, and correlating it to the log BMR and MMR values per animal taken at time 3, from the pike just prior to sacrifice. Only those animals with enzyme activity measurements for all six organs were used in the analysis. The mass of these summed tissues including the carcass (which was considered to be enzymatically inert) was (mean ± SE) 96.7 % ± 0.19 of the whole animal mass. On comparing the log summed whole body activity of





Figures 4.10a and b. Correlations of log whole body summated (as the total activity for all the tissues measured) citrate synthase activity per pike, vs. log total BMR oxygen consumption per pike (above) and log total MMR oxygen consumption per pike (below) for individual pike (n=29).

CS with BMR and MMR there were found to be highly significant correlations for both, with slightly higher correlations between whole body CS and MMR.

In both cases there was no differences between slopes for any of the treatment groups, only in the elevations between the groups for BMR (slope, $F_{2,28} = 0.75, p>0.05$; elevation, $F_{2,28} = 3.46, p < 0.05$), and MMR (slope, $F_{2,28} = 0.75, p>0.05$; elevation, $F_{2,28} = 6.11, p < 0.01$) (Figure 4.10). These differences in elevation probably reflect the differences in the group mean BMR and MMR values between the different treatment groups since tissue CS activities varied between treatments for liver only. On comparing the whole body LDH activity in relation to BMR and MMR, there was found to be a much weaker correlation of whole body LDH with MMR than BMR. There was found to be no statistical difference between either the slopes or elevations for whole body LDH and BMR values. For MMR there was a significant difference in elevation only between the treatment groups (elevation, $F_{2,26,} = 7.51, p < 0.01$) (Figure 4.11).

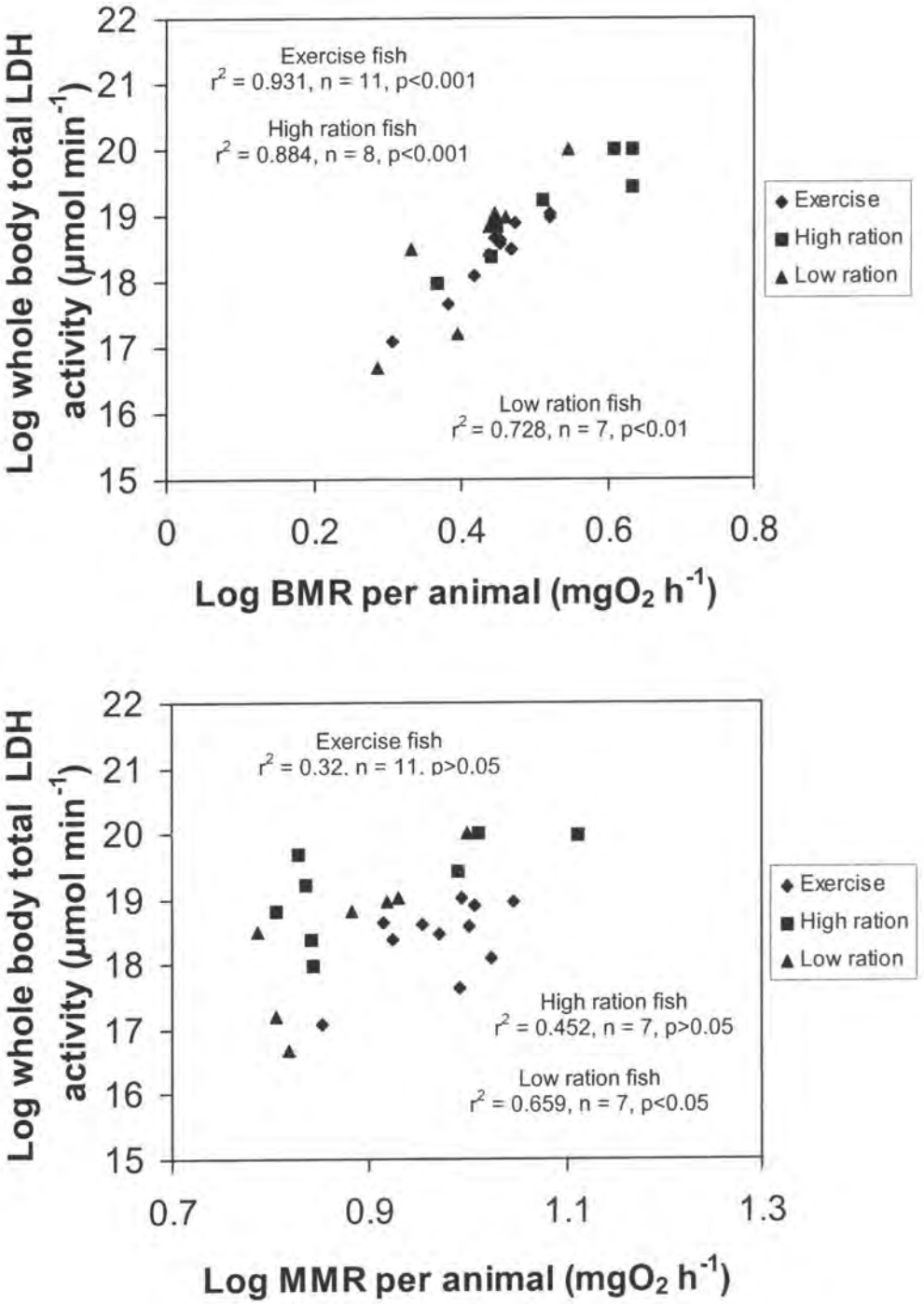


Figure 4.11 Correlations of whole body summed activity (as the total activity for all the tissues measured) total of the log lactate dehydrogenase activity per pike, vs. log total BMR oxygen consumption per pike (above) and log total MMR oxygen consumption per pike (below) for individual pike (n=29). Lactate dehydrogenase activity was measured in the direction of lactate oxidation.

4.4 Discussion

4.4.1 Organ masses

The major body constituents of pike were white muscle and carcass, together these constituted approximately 91-93 % of the total wet body mass. Both white muscle and carcass have been reported to have low mass specific metabolic rates (oxygen consumption per gram wet mass) in carp (Itazawa & Oikawa 1986). The remaining mass of the pike (approximately 7-9%) was composed of the highly metabolically active visceral organs. The rank orders of tissues with the highest rates of oxygen consumption in carp are brain, kidney, intestine and red muscle (Itazawa & Oikawa 1986), closely matching the organs found to be of greatest importance for predicting BMR and MMR of pike in this thesis.

Different organs had different allometric relationships with body mass. The greatest rate of negative decline of an organ with increasing body size was the mass of the brain, followed by those for most of the visceral organs all of which are highly metabolically active. Similar allometric scaling relationships for organ masses have been reported by Schultz *et al.* (1999) for channel catfish *Ictalurus punctatus*. The notable exceptions in the pattern of negative allometry of visceral organs were the liver and spleen, and the kidney for the low ration group only. Those organs with low metabolic activities per gram that exhibited positive allometry in this study were carcass and white muscle, as has been reported previously in carp (Itazawa & Oikawa 1986). From previous studies on vertebrates including fish (see above) it is known that the mass specific oxygen consumption rates of different tissues vary. Studies examining the effects of body scaling of organs size with increasing body mass, have reported a relative decrease in the mass of highly metabolically active visceral organs with increasing size for endothermic vertebrates (e.g. Field *et al.* 1939; Krebs 1950; Holliday *et al.* 1968; and in ectothermic vertebrates (Hulbert & Else 1981; Garland 1984; Oikawa & Itazawa 1984, 1985; Itazawa & Oikawa 1986; Weatherley 1990; Goolish 1991; Schultz *et al.* 1999).

The brain is a very highly metabolically active organ in both endothermic and ectothermic vertebrates (Field *et al.* 1939; Krebs 1950; Schmidt-Nielsen 1984; Itazawa & Oikawa 1986; Schultz *et al.*, 1999) and exhibits the greatest relative decline in size with increasing body mass of any organ. This is not that surprising as the brain is limited by the size of the skull and ultimately the rate of growth of the head is much less

than that of the rest of the body. For this reason and the expected finding that exercise and growth rates would have little effect on brain size, this organ was not used for enzyme analysis. Lactate was also found not to increase in concentration in the brain of rainbow trout following exhaustive exercise (Storey 1991).

On comparison of the scaled organ masses (adjusted to those of a 45 g pike, Appendix 5) for each organ between each treatment group, significant differences were noted for at least one of the treatment groups for brain, heart, kidney, red muscle, white muscle and carcass. Different relative masses reflect the differences in the body composition of the fish in the different treatment groups. Those fish on the high ration when scaled to 45 g had a larger percentage of white muscle than low ration fish, with low ration fish tending to have a larger carcass mass. Exercise fish had significantly higher amounts of red muscle than either high or low ration fish. Increases in the mass of red muscle following training has been reported previously for brown trout and goldfish, being linked to an increase in red muscle cell size (Davison & Goldspink 1977, 1978) and red muscle cell diameter in pollack *Pollachius virens* and brook trout (Johnston & Moon 1980a, 1980b). More recently increase in the mass of red muscle with exercise has been reported in nase, chub *Leuciscus aphalus*, Danube bleak and striped bass (Sanger 1992; Hinterleitner *et al.* 1992; Young & Cech 1993, 1994). A significant difference in liver mass between the high ration and low ration fish was not surprising as it represents an important energy storage organ. The reason for significant differences in the brain masses between exercise and high ration and exercise and low ration fish is unclear.

Principal components analysis revealed that the largest differences in body composition between individuals were due primarily to individual differences in non-visceral organs (red muscle, white muscle and carcass) and highly metabolically active tissues (heart and liver), which together explained 31.5 % of the variance. The second principal axis was loaded with the kidney and intestine, both of which have a very high mass specific metabolic rate (Itazawa & Oikawa 1986) and explained 16.6 % of the total variation. The third principal component was loaded with the spleen and stomach (11.8% of total variation) and the final component was related to brain mass (9.4% of the total variation). The first principal component is usually dependent upon size (Wiley 1981), but in this study organ masses were standardised relative to those of a 45 g pike prior to analysis, to remove this component. The components identified in this study included

the carcass and white muscle, which are relatively metabolically inactive, as well as several organs found to be highly metabolically active in other vertebrates (Field *et al.* 1939; Krebs 1950, Hulbert & Else 1985).

Using a stepwise discriminant analysis, individual pike could be allocated to their individual treatments groups in terms of their liver mass, which was located on the primary canonical discriminant functional axis. Brain and red muscle were both positively correlated on the secondary canonical discriminant functional axis. The fact that liver appeared on both the primary discriminant function (stepwise discriminant analysis) and loaded on the primary factor axis (PCA) was not unexpected due to the large differences in liver masses between the high ration fish and the low ration and exercised pike. Schmelzing & Claus (1990) reported that in farmed rainbow trout 70-84% of the total variance in the carcass mass for similar sized fish was explained by differences in organ masses, with 86-99% of this difference being due to variation in liver mass. The liver is often used as a fat store in non-fatty fish species such as cod (Love 1970, Lambert & Dutil 1997) and coalfish, which do not have discrete subcutaneous/visceral fat deposits. (Jensen 1979). In cod the liver may weigh up to 2-11% of wbm and contain 15-65 % lipid, depending upon the age of the fish, stage of maturity of the gonads and time of year (Jangaard *et al.* 1968). The use of the liver for energy storage has been reported in various fish species (Love 1979, 1999). However, the fat content of the liver in pike of 300-660g has been reported to be low, approximately 2% wbm (Kluytmans & Zandee 1973). There is an absence of large lipid stores in pike, with low levels of lipid stored in the muscle, and low lipid levels in the liver compared to those found in well fed cod (Medford & McKay 1978). On sampling pike of approximately 50 cm in length throughout the year Medford & McKay (1978) reported that the fat bodies found on the stomach and intestine of pike in March and June averaged only 0.2 % of the somatic body mass. The quantity of visceral fat was not significantly different between pre-spawning and post-spawning fish.

The heart was also loaded on the primary factor in the PCA analysis, this being a very metabolically active organ and varying considerably amongst individual fish. A larger relative heart mass is known to improve the circulatory performance of the fish (Farrell 1991). The kidney and intestine were found to be loaded on to the second PCA factor. The observed differences in intestinal mass between individuals with a tendency for

higher masses in the exercised and high ration fish may reflect the increased costs of food processing. Differences in growth performance among fish species may be related to the differences in their capacity to assimilate nutrients from the gut (Weatherley & Gill 1987). The length of the gut in fish is thought to be determined genetically and for a given species does not seem to vary between fish of a similar size (Weatherley 1990). Weatherley & Gill (1983) have shown that in rainbow trout fed a range of different rations, the length of the gut was remarkably constant. Gut length was not measured in this study, as it was believed that gut mass would better reflect any differences between fish.

4.4.2 Enzyme activity

A comparison between the CS activity measured for chain pickerel *Esox niger* at 15°C (Kleckner & Sidell 1984) and those levels measured in this study at 24°C for pike gave similar results (Table 4.9) in terms of the rank order of the activities of tissues per gram. Moreover, in considering the 10°C difference in experimental assay temperatures the CS activities between the study and that of Kleckner & Sidell (1984) were broadly similar. The highest levels of activity of CS was found in the heart, followed by red muscle, intestine, gill, white muscle and liver. The high level of variability in the CS activity per gram of liver appeared to be associated with very different liver masses between the three treatment groups. High ration fish had the highest liver masses and the lowest activity per gram. This suggests that the liver was possibly being used in a storage capacity, as the total amounts of CS activity per organ were similar between the three groups. The liver was possibly being used to store glycogen (Medford & McKay 1978) and as a result had a lower mass specific CS activity. The mass specific activity of both CS and LDH concentrations in the liver were found to be significantly different between the individual treatment groups, with the lowest levels occurring in the high ration fish.

The protein content of the liver of high ration fish was significantly higher than that of the low ration fish but not the exercise group. The protein content of the white muscle was significantly higher for high ration fish compared to that of low ration fish, with no difference between either group and exercised fish. The protein content of the white muscle and liver was found to vary during the year in adult pike, with the levels of protein in all other organs remained fairly constant (Medford & McKay 1978). The variable accumulation of protein with ration has previously been reported for pike

Table 4.8 A comparison of the rates of activity of citrate synthase as measured in the chain pickerel *Esox niger* (Klecker & Sidell 1985) at 15°C and pike at 24°C (this study). Chain pickerel were acclimated to 25°C and the enzymes assays performed at 15°C. In this study pike were acclimated at 15°C and the enzymes assayed at 24°C. Units were expressed in terms of μmols of product g^{-1} min^{-1}. The numbers of animals used are expressed in parentheses.

Organ	Kleckner & Sidell	This study
Brain	4.82 ± 0.39 (12)	Not measured
Heart	14.40 ± 1.00 (12)	25.65 1±.06 (28)
Gill	Not measured	3.85 ± 0.11 (29)
Liver	1.12 ± 0.13 (12)	Exercise 3.26 ± 0.13 (13)* High 1.33 ± 0.12 (8)* Low 2.57 ± 0.21 (9)*
Intestine	Not measured	11.71 ± 0.56 (30)
Red muscle	10.52 ± 1.22 (13)	25.16 ± 1.01 (30)
White muscle	1.18 ± 0.19 (13)	2.63 ± 0.07 (30)

* Groups statistically significant different from one another.

(Diana & McKay 1979; Diana 1982). There was found to be no variation in the water content of the white muscle of pike growing at different rates. In contrast, Courture *et al.* (1998) reported lower water content of both the liver and white muscle of fish with the highest growth rates in Atlantic cod.

In the current study there was clear evidence of allometric scaling of enzyme CS and LDH concentration in relation to organ mass despite the very limited size range of pike used (14.5-22.5 cm). Kleckner & Sidell (1985) found no evidence of scaling of aerobic enzymes in the chain pickerel ranging from 35-55 cm in length. They postulated that the size range of the pike used was possibly not large enough to detect the effects of size or that the effects of their different temperature regimes masked any scaling effects. Pelletier *et al.* (1993) reported a negative correlation between CS activity per gram in white muscle and body mass for wild Atlantic cod over the size range of 115-17,350g in mass. They also reported a positive weak correlation between CS activity and white muscle growth rate. Neither of those relationships was apparent in this study.

The apparently high values of LDH measured in this study are due mainly to the maximal conditions used to assay for the enzyme. To push the reaction to the conversion of lactate to pyruvate requires a high lactate concentration, pH and NAD^+ content (Hulbert & Moon 1978). The high rates of LDH activity measured for eel *Anguilla anguilla*, in the heart (500μmol g^{-1} min^{-1}) were measured at pH 9.0, 1.0 mM NAD, 25 mM lactate and at a reaction temperature of 15°C (Hulbert & Moon 1978). The assay conditions used in this study were pH 10.0, 0.9 mM NAD, 135 mM lactate assayed at 24°C. The lactate levels, assay temperature and pH used by Hulbert & Moon (1978) are below those used in this study. Since it is known that all these factors affect the rate of reaction (Hulbert & Moon 1978; Wieser *et al.* 1987), this partially explains the higher results reported in this study. Also Hulbert & Moon (1978) did not use a pyruvate-trapping agent, which was used in this study. Possible inhibition of the different LDH isoforms by pyruvate accumulation may affect the measured LDH activity (Everse & Kaplan 1973; Wieser *et al.* 1987); the accumulation of pyruvate is prevented under normal cellular conditions (Wieser *et al.* 1987). The rank orders of the activities of the tissues in the study almost exactly follow the same order for lactate oxidation in the eel (Hulbert & Moon 1978). The heart had by far the highest activity levels, followed by red and white muscle, gill, intestine, and liver. The latter had a very

low oxidative capacity compared to the other organs representing the abundance of the H type isoforms. The low lactate oxidative values for all tissues reported by Lind (1992) in the crucian carp *Carassius carassius* in the heart, red, white, liver and gill may possibly be partially explained by the lower pH used. The enzyme oxidative activities were lower than all those reported for pyruvate reduction including the heart, which would appear to be in error as the majority of this isoenzyme in the heart would be expected to be of the H_4 type, which is geared to lactate oxidation. However, Lind (1992) does not mention the use of a pyruvate trapping agent, and goldfish muscle LDH has been reported by Hochachka (1965) to be inhibited by the accumulation of low levels of pyruvate (3 mM). Davie *et al.* (1986) reported an increase in the aerobic capacity of rainbow trout white muscle, with a shift in the LDH isoforms to aerobic metabolism in fish that swam continuously.

Fish blood normally shows high levels of glucose but following exhaustive exercise higher concentrations of lactate prevail (Black *et al.* 1966). The heart is able to catabolise both glucose and lactate to CO_2 (Bilinski & Jonas 1972). Under aerobic conditions and after exercise the heart will preferentially utilise lactate over glucose as an energy source (Lanctin *et al.* 1980). The high rates of lactate oxidation reported in this study for pike heart have previously been reported for hearts of other fish species (Lim *et al.* 1975; Hulbert & Moon 1978; Lanctin *et al.* 1980; Driedzic *et al.* 1985). The rate of lactate oxidation in eel and brook trout hearts was found to be dependent on the lactate concentration (Hulbert & Moon 1978; Lanctin *et al.* 1980).

In the study high rates of lactate oxidation were also found in red and white muscle of pike. As pointed out by Lanctin *et al.* (1980), the very high rates of lactate oxidation by the heart are somewhat irrelevant in lactate clearance from the blood when considering the relative mass of the heart (approximately 0.09-0.14% wbm in this study). As approximately 80-85% of the lactate produced remains within in the white muscle of fish (Turner *et al.* 1983; Pagnotta & Milligan 1991), the most important organ in terms of lactate clearance is white muscle. The very high levels of lactate oxidation found in the tissues of these juvenile pike probably reflect the need to recovery quickly from oxygen debt. Pike are morphometrically designed as a 'sprinter' fish using bursts of acceleration to capture prey (Webb 1978) and spend much of the remaining time motionless or using the pectoral fins to 'scull about' slowly (Lucas *et al.* 1991).

Many recent studies have demonstrated that the glycolytic enzyme activities of different tissues of fish are correlated with whole body and white muscle growth rates. However, the correlations between aerobic mitochondrial enzymes and growth rates have been much weaker or not correlated at all in some studies (Goolish & Adelman 1987; Kiessling *et al.* 1991; Mathers *et al.* 1992; Pelletier *et al.* 1993b, 1994; Blier *et al.* 1997). Variation in growth rate has been reported to bring about differences in the chemical composition of fish tissues (Houlihan *et al.*, 1993, Guderley *et al.* 1996). With increased growth rates in cod, increased levels of aerobic and anaerobic enzyme capacities of the white muscle, liver and intestine have been reported (Houlihan *et al.* 1993; Pelletier *et al.* 1993a, 1993b, 1995; Couture *et al.* 1998). In this study there was found to be a highly negative correlation between whole body LDH and CS activities and growth rates for low ration fish only, the reasons for which are unclear. Pelletier *et al.* (1994) looked at the effects of differing growth rates on key aerobic and glycolytic enzymes of cod held individually. They found that glycolytic enzymes (including LDH) scaled well with growth rates, being up to four times higher in fish having higher growth rates than those that did not grow. Mitochondrial enzymes on the other hand, including CS, were unchanged in fish with positive growth.

In juvenile largemouth bass, high feeding rates and growth rates increased the contribution of whole body aerobic cytochrome co oxidase (CCO) activity of the intestine from 13 to 30% of whole body activity (Goolish & Adelman, 1987). In small fish much of the aerobic activity occurs in the visceral tissues associated with food processing and metabolism, with the visceral CCO percentage of whole body activity being approximately four fold that of the red muscle in small fish (Goolish 1991). In the study there was found to be a significantly higher activity per gram of intestinal CS with increasing size for exercised fish. This possibly indicates an increased aerobic capacity relating to the improved absorption of metabolites in larger fish, perhaps compensating for the relatively decreased intestine mass with increasing body mass. Schultz *et al.* (1999) suggested that from the limited experimental data on blood flow to date, blood flow to the visceral organs decreases with increasing body size. Exercised fish in this study must have had improved absorption of energy from the food as they had the same growth efficiency as pike held in static water tanks even though they were under a constant sustained swimming regime. Another possible explanation may be that

the exercised fish, which must accommodate both the demands of swimming and processing food at once, may possibly try to compensate for reduced blood flow to the intestines with an increased aerobic capacity of the intestine.

Axelsson & Fritsche (1993) reported a decreased blood flow to the intestines of cod following the commencement of exercise. In resting channel catfish, blood flow to the gut at rest was very low, with less than 0.5% of the total cardiac output passing to the stomach and intestines of fish that were not fed for 48 hours (Schultz *et al.* 1999). For this species at rest the majority of the cardiac blood flow was to the white muscle, representing about 72% of the blood flow. Values of blood flow at rest to the red muscle are variable between species. Rainbow trout, which is a good endurance swimmer with a red muscle mass of approximately 2.5 % of wbm, had a blood flow to the red muscle of approximately 15% of the total blood flow at rest (Barron *et al.* 1987). For the largescale sucker *Castostomus macrocheilus*, which is a relatively poor swimmer with red muscle content approximately 2% of wbm, red muscle received 1% of the blood flow at rest (Kolok *et al.* 1993). When largescale suckers were swimming near U_{crit} there was a 60-fold increase in blood flow to the red muscle whilst the blood flow to other tissues remained similar to that seen at rest (Kolok *et al.* 1993).

The intestine has been reported to respond variably with increased growth rates between fish species. In the largemouth bass increased growth rates were found to correlate with an increase in the relative mass of the intestine (Weatherly & Gill 1983; Goolish & Adelman, 1987). In Atlantic cod (Pelletier *et al.* 1994; Couture *et al.* 1998) the relative mass of the intestine was found to remain similar in mass and length for fish growing at high and low growth rates. There was a change, however, in the aerobic capacity of the gut. Pelletier *et al.* (1994) reported that large fast growing cod increased the CCO content of the intestine and suggested that the aerobic capacity of the gut may limit the rate of digestion and growth in Atlantic cod. A similar hypothesis has been reported for other species the so called 'central limitation hypothesis' suggested by Weiner (1989), which suggests that bottlenecks in shared metabolic machinery may limit maximal metabolic rates. For example, there may be a bottleneck in the gut's capacity to digest and absorb food, in the liver's capacity to process absorbed food, or the heart's capacity to pump blood and so on (Hammond & Diamond, 1997).

Comparisons of whole body LDH and CS activities calculated by summing enzyme activities in proportion to the to observed individual body composition for all the tissues measured were plotted against the BMR and MMR values recorded for individual animals immediately prior to death. For CS there was a slightly higher correlation for exercise and low ration fish with MMR, high ration fish being more closely correlated with BMR values. It would be expected that CS activity was correlated with MMR as CS activity is indicative of oxidative metabolism of the mitochondria, which is likely to be limiting to MMR (Weibel *et al.* 1996) and only fully utilised under maximal oxygen debt repayment following exhaustive exercise in pike (Armstrong *et al.* 1992). The reason that total whole body CS activity of high ration fish was better correlated to BMR is unclear, and may possibly be related to a more variable maximal performance between individual fish or that BMR was a better indicator of the differences in body composition between fish in the group and enzyme activity than MMR.

Summed whole body LDH activity was highly correlated with BMR, with no statistical differences between the group mean values. A significant positive correlation of LDH with BMR was seen only for low ration fish. White muscle essentially had the highest aerobic capacity of all the organs measured by virtue of the sheer mass of tissue. The LDH total activity may be best correlated with the BMR values as the BMR values represent the total metabolic costs of the tissues at rest. The majority of BMR is the metabolic costs of supporting the large white muscle mass (ranging from 52-62% of the wet body mass in the study). The large increase in oxygen consumption following exhaustive exercise has been associated with a large increase in whole body lactate, and near depletion in whole body glycogen, ATP and creatinine phosphate in trout (Scarbello *et al.* 1991). The reason for a poor correlation between LDH and MMR is probably due to the large and more variable part played by ionic and metabolic disturbances in the total increased oxygen consumption.

In higher endothermic vertebrates (predominantly mammals and birds) there have been reports of plasticity in organ mass to cope with varying metabolic energy demands such as increase food processing during cold conditions and lactation (Konarzewski & Diamond 1994; Speakman & McQueenie 1996) and during migratory flight (Piersma & Lindstrom, 1997; Piersma & Gill 1998; Battley *et al.*, 2000). In ectothermic vertebrates some species such as the Burmese python, show great flexibility of organ sizes

following consumption of a large meal, following a large fast (Secor *et al.* 1994; Secor & Diamond 1995). In fish there generally appears to be less organ flexibility (Weatherley & Gill 1983; Davison 1989; Weatherley 1990). On comparing the body composition in juvenile rainbow trout grown at a range of different rates (by the use of different rations and the use of bovine growth hormone on one group) Weatherley & Gill (1983) noted the overall trend of tissue growth was remarkably similar between treatments. There was a large degree of constancy reported in the allometric slope values for various tissues, indicating a relative constancy of the body proportions between fish growing at different rates.

4.4.3 Conclusions

In conclusion body composition of the fish did vary with the individual treatments and could relatively accurately be used to predict the group membership on the basis of discriminant analysis. There was found to be little variation in the relationships between total tissue mass and total enzyme activity within and between different treatment groups for all tissues except the liver (see Figure 4.7c). Therefore for a given organ mass enzymatic activity was relatively constant and whole body enzyme activity was determined by relative organ size. The lack of variation between treatment groups was surprising and supports the comments suggested by Lackner *et al.* (1988) and Davison (1989) that the changes in fish in response to exercise training tend to be systemic rather than at the enzymatic level of organisation. There were close relationships between the estimated whole body CS activity and both BMR and MMR for all the treatment groups. Lactate dehydrogenase (assayed in the lactate oxidation mode) was correlated to BMR with no significant difference between any of the treatment groups. However, LDH was poorly correlated with MMR. Estimated whole body LDH was negatively correlated with percentage growth rate per day for low ration fish only, with a similar weaker negative correlation noted for the relationship between CS activity and low ration fish only.

Chapter Five

General discussion

This chapter inter-relates the major findings of the thesis, which explored the extent of metabolic flexibility in BMR of juvenile Atlantic salmon parr and juvenile pike and also the MMR and metabolic scope of pike. A link between BMR and SMMR has been postulated as a possible driving system for the evolution of endothermy in mammals and birds (Hayes & Garland 1995; Ruben 1995), however there is still debate in this area as to whether there is a proven link (Koteja 2000). The question of whether or not BMR and MMR or SMMR are linked by some mechanism has received relatively little attention in fish species to date both interspecifically and, more interestingly, at the individual species levels. The final chapter explores the extent to which noted changes in BMR, MMR and metabolic scope of juvenile pike could be explained by corresponding changes in organ masses and/ or activity levels of enzymes.

The work contained in the thesis is some of the first to explore plasticity in metabolic rate within fish species. Only relatively recently has the significance of individual variation in physiology and behaviour become truly recognised. Some of the first clear evidence for intraspecific variation in metabolic capacity of ectothermic vertebrates was noted from observations of repeatable individual variation when measuring maximal locomotor performance of lizards (Bennett 1987). Prior to 1990, studies measuring the maximal locomotor performance of fish (critical swimming speed) tended to concentrate solely upon the group mean value as a comparison between groups of fish, with individual variation about the mean being attributed to statistical noise or experimental error (Kolok 1999). More recent studies of fish have started to examine repeatable individual maximum performance in relation to both physiological and morphological traits (Farrell *et al.* 1991; Kolok 1992b, 1994; Nelson *et al.* 1996; Gregory & Wood 1998, 1999; Reidy *et al.* 2000). These studies have predominantly explored maximal swimming performance in a range of species with moderate to high swimming ability rather than in more sluggish fish species. The thesis has examined the effects of sustained swimming exercise on metabolic characteristics of pike, a species regarded as a poor sustained swimmer with a very low critical swimming speed (Jones *et al.*1974; Diana 1996).

5.1 Variation in BMR

The results of several experiments revealed that there was a significant amount of plasticity in the BMR of individuals of the two fish species studied. These fish species have very different lifestyles, Atlantic salmon representing an active fish with high-sustained swimming performance and pike as a sedentary species, exhibiting sprinting behaviour. The substantial amount of metabolic flexibility in BMR within individuals observed for both fish species, with contrasting life styles, suggests that this flexibility in BMR with varying environmental conditions is likely to be widespread rather than reflecting two isolated examples. Salmon parr showed more dramatic changes in BMR in the second experiment when fish were assigned to different groups associated with positive and negative residuals from the allometric body mass-BMR relationship (Experiment 2 , 1997, section 2.2.9.2). Those fish with initially positive residuals of the BMR-mass relationship decreased in BMR significantly more than those fish with initial negative residuals when BMR was re-measured. This indicates that fish with above average BMR fish may have more capacity to reduce BMR than those with below average BMR. A multiple linear mixed model revealed that at the population level, the effects of within fish variation between the different experimental phases were larger than and swamped the differences in BMR between individual salmon parr that are known to exist (section 1.15; Metcalfe *et al.* 1995). The current generally accepted paradigm of a fixed BMR for an individual salmon but varying between individuals at a constant temperature during the freshwater phase of growth (Metcalfe *et al.* 1995) is somewhat surprising. Its is known that BMR is dependent upon the masses of highly metabolically active tissues (section 1.9.2) and that these may alter with different conditions such as individual ration (e.g. Weatherley & Gill 1983, 1990).

5.2 MMR and metabolic scope

The metabolic scopes in 33 juvenile pike were compared before and after the implementation of different treatments, comprising two feeding regimes and a group with sustained exercise. A statistically significant increase in MMR measured during oxygen debt repayment after exhaustive exercise was noted for exercised fish between the initial measurement and measurements after approximately 3 and 11 weeks of sustained exercise. Similar findings were noted by Hochachka (1961) for exercise-trained rainbow trout after

exhaustive exercise, all had larger maximal oxygen consumptions compared to trout held in static water. In contrast, Thorarensen *et al.* (1993) found no such increase in maximal oxygen consumption of chinook salmon following exercise training.

Recently Kolok (1999) reported an absence in the literature of studies on fish that examined the relationship between U_{crit} and maximal oxygen consumption or metabolic scope. This seems surprising as there may be expected to be a correlation between maximal oxygen consumption and U_{crit} in fishes. However, individual endurance has been linked with maximal oxygen consumption in the lizard *Ctenosaura similis* (Garland 1984), but not with *Amphibolurus nuchalis* (Garland & Else 1987).

5.3 Is there a genuine relationship between BMR and MMR?

A relationship between BMR and SMMR in both endothermic and ectothermic vertebrates has been suggested by various authors (Bennet & Ruben 1979; Taylor *et al.* 1981; Priede 1985; Ruben 1995). Ricklefs *et al.* (1996) recently concluded that the BMR of mammals was correlated to the daily energy expenditure rate (DEE), measured as the energy rate of a free-living organism, but that there was no relationship in birds. The DEE is, however, clearly different to SMMR, and varying levels of SMMR (metabolic ceilings) may be produced under different physiological conditions for the same species e.g. lactation in mice versus heat production at cold ambient temperature (Hammond & Diamond 1997). A key assumption of the aerobic capacity model for the evolution of endothermy in vertebrates as proposed by Bennett & Rubin (1979) is that BMR and SMMR are somehow linked and cannot occur independently and that selection pressures on organisms for increased activity levels lead to the evolution of a higher BMR.

On comparing the BMR and MMR between 18 different mammal species Koteja (1987) failed to find any correlation between mass-independent values of BMR and MMR, although the allometric regression equation slope for MMR was found to be significantly higher than that for BMR. This indicated that larger animals would be expected to have larger relative scopes than smaller animals, as found for several fish species including pike (Schmidt-Nielsen 1984; Armstrong *et al.* 1992). The opposite has been reported for the relative metabolic scope in nine species of lizards, with a decrease in the metabolic scope with larger species of lizard (Thompson & Withers 1996). A more important perspective

on the possible relationship between BMR and MMR may actually be found by examining intraspecific relationships rather than interspecific ones. Direct evidence for a causal link between BMR and SMMR for mass has yet to be found for any animal species (Hayes & Garland 1995). In the present study there were significant correlations between mass standardised BMR and MMR for juvenile pike at time 1 (prior to the start of the experiments). However, after approximately 3 weeks on each experimental regime (time 2) there was no correlation found. After approximately 11 weeks on each experimental regime (time 3), a significant correlation between BMR and MMR was again apparent, the range of values comprising several distinct groups of points associated with the treatments. These circumstances are strongly suggestive of a clear link between BMR and MMR with the lack of correlation after approximately 3 weeks (time 2) being associated with a period of physiological flux.

In this study, the initial increase in MMR for exercised pike occurred without a resultant increase in BMR, leading to an increase in the relative metabolic scope for all fish after 3 weeks of sustained exercise. After 11 weeks MMR of the exercise group was similar to that at 3 weeks, but BMR had increased. This suggests that there may be a differential response in the time taken for increases in MMR and BMR.

Metabolic ceilings have been reported to be set by structural limitations of the oxygen transport system to the mitochondria (Weibel *et al.* 1996). It has also been suggested that performance limits to MMR may also be set by, for example, the absorption rate of the alimentary tract and energy supplying machinery (Daan *et al.* 1989; Peterson *et al.* 1990), which has been termed the central limitation hypothesis. Alternatively limits to the system as a whole may lie at the sites of energy utilisation, at the cellular level, the peripheral limitation hypothesis (Weiner 1989). Other hypotheses include the concept of metabolic bottlenecks, where different processes are using the same-shared metabolic machinery (Hammond & Diamond 1997).

Basal metabolic rate has recently been suggested to scale with the fractal geometry of the circulatory system (West *et al.* 1997). However, as pointed out by Bishop (1999), the circulatory system is at minimal capacity whilst at rest and more likely to limit maximal performance (Di Prampero 1985). Bishop (1999) argued that once the differences in

relative heart size and haemoglobin concentration between endotherm species have been standardised for, that MMR scaled at the same proportion as maximal heart rate. Although a similar relationship between maximal heart rate and maximal oxygen consumption has been reported in fish (Steffensen 1987), Kolok & Farrell (1994) were unable to correlate differences in cardiac output in northern squawfish to differences in individual performance.

Interspecific variation in the exact relationship between BMR and MMR might be expected to be due to adaptations of species that enable a higher MMR. On comparing the MMR of goats to that of the similar sized pronghorn antelope *Antilocarpa americana* Linstedt *et al.* (1991) found that the maximal aerobic uptake of the antelope was about five times that of a similar sized goat, with the pronghorn having larger respiratory surface area, a greater capacity to deliver oxygen to the muscle and much larger skeletal muscle mitochondrial volume. In one of a series of papers, Weibel *et al.* (1996) compared the MMR of goats and dogs, dogs having an MMR in excess of twice that measured for similar-sized goats. They concluded that the differences in MMR between the two species were associated with physiological traits including a much higher total mitochondrial surface area, greater capillary network volume, and greater red cell volume in the dog together with larger intracellular stores of glycogen and lipid.

5.4 Is there a genetic component to BMR and MMR?

Thorpe *et al.* (1998) commented that the developmental paths used during the various life history strategies of Atlantic salmon parr are based on a comparison of the current physiological status of the fish and the rate of change of state, in particular growth rates or accumulation of lipid with a genetic threshold. This approach therefore assumes a genetic and environmental interaction. A genetic basis to competitive ability is not in doubt, however, just how much of an individual trait is attributable to it is uncertain. Dunbrack *et al.* (1996) and Rosenau & McPhail (1987) have demonstrated a genetic component to differing levels of inherited aggression between wild individual populations of brook charr and coho salmon. It has also been shown with coho salmon that hatchery reared individuals that have been cultured in the hatchery for five consecutive generations were more aggressive than fish obtained from wild parents but raised under hatchery conditions (Swain & Riddell 1990). Genetic maximal performance has been studied more often in

thoroughbred race horses (Gaffney & Cunningham 1988) and humans, and it is known that there is a heritable genetic component in maximal human aerobic performance from comparison of top athletes to the rest of the population (Bouchard *et al.* 1992). There are also known to be multiple heritable errors in human metabolism (Bowman & Rand 1990).

Maximal aerobic capacity has been artificially selected for in terms of levels of voluntary wheel running in mice, with selected lines running faster than others, thus demonstrating the selection of a genetic trait for increased MMR (Swallow *et al.* 1998; Koteja *et al.* 1999). Different genetic strains of mice have been noted to have different BMR values, those strains with exceptionally high or low BMR tending to have disproportionately high and low organ masses (Konarzewski & Diamond 1995). On comparing the levels of genetic variation between mussels *Mytilus edulis* Hawkins & Day (1996) found that a major component to the selected differences was in terms of improved growth rates, with reduced whole-body protein turnover underlying lower energy expenditure and higher growth efficiencies. In a recent comparison between the growth rates of upper modal and lower modal Atlantic salmon parr, Morgan *et al.* (2000) noted that upper modal fish had significantly higher rates of protein growth during February and lower protein degradation rates in May, indicating that early migrant fish minimise their rate of protein turnover.

Faster growing Atlantic cod have been shown to have higher protein synthesis rates in all the tissues examined (Houlihan *et al.* 1988). Individual Atlantic salmon parr have different protein growth efficiencies, which are related to individual differences in protein retention efficiencies (Carter *et al.* 1993). Recently Kelley A.; Schmitz M.; Cutts C.; Adams C. & Brynes L. (unpublished data) explored a possible molecular basis for variation in metabolic rate of Arctic charr. They compared fish with high and low metabolic rates and looked for differential gene expression between the two groups of fish. Of all the different genes expressed there was no conclusive difference in expression between those fish categorised as either high or low BMR.

5.5 Organ plasticity

A major component of BMR is the maintenance of masses of highly metabolic organs whilst at rest. The higher BMR values noted for exercise and high ration pike at time 3 compared to those for low ration fish are probably related to the higher group mean masses

of visceral organs noted for both groups. The overall variations in organ composition appeared to be relatively slight compared to the large changes that are seen in birds and mammals during metabolically expensive activities such as preparation for migration, during migration or during lactation (Speakman & McQueenie 1996; Piersma & Lindstrom 1997; Piersma & Gill 1998; Battley *et al.* 2000), the general consensus being that peak performances in birds and mammals are sustained by temporarily enlarging the supporting organs such as the alimentary tract. The increased metabolic activity is associated with increases in the BMR of the animal. Usually the gut is maintained a sub maximal size to save energy (Piersma & Lindstrom 1997).

The overall limited plasticity in organ size that was observed between the three pike treatment groups, despite low and high food rations and a high level of sustained exercise, may reflect limited plasticity in body composition of pike as a whole. The major effect of a high ration was an increase in the mass of white muscle. Exercise fish although expending a lot more energy daily than either the low or high ration fish that were kept in static tanks, appeared to have body composition in between that of the other two groups. The comparable percentage liver mass values and white muscle protein concentrations indicate that exercised fish had a sustained energy supply and were not having to utilise internal energy stores (Greer-Walker & Pull 1973), as energy is predominantly stored as protein in the white muscle and the liver of pike (Medford & McKay 1978).

Weatherley & Gill (1990) noted in immature rainbow trout that were fed different ration levels to achieve different growth rates, and a group administered bovine growth hormone, only slight differences in the masses for the various organs (carcass, gut, skin and liver) as a percentage of body mass. They suggested that fish must have a level of control that conserves the relative dimensions of tissues and organs as fish grow, independent of the rate of growth. The tight control of organ growth allows tissues and organs to keep their proportional size as the fish grows. Lackner *et al.* (1988) commented that most of the changes seen in endurance exercised fish tend to be systemic rather than at the cellular and molecular level (section 4.4.3). Increased exercise in rainbow trout lead to an increase in the rate of protein turnover for most tissues along with increased aerobic enzymes, although only as long as the animal was swimming (Houlihan & Laurent 1987). Aerobic enzyme levels have been shown to increase in exercised salmonids (e.g. Farrell *et al.* 1990, 1991).

In the present study, juvenile pike displayed a general lack of difference in aerobic and anaerobic enzyme levels between the three treatment groups. Similar findings were reported by Hinterleitner *et al.* (1992) for exercised and unexercised Danube bleak and nase, which showed little change to either aerobic or anaerobic enzyme levels. The overall tightness of control in organ sizes and general lack of differences in enzyme activities between the groups may indicate low plasticity by comparison to homeotherms. Nevertheless, the changes do seem to be responsible for the observed changes in BMR and MMR of pike. Conversely the conditions imposed on the pike were within the normal environmental conditions, compared to more seasonal changes such as in food availability and temperature differences which are know to affect enzyme levels in the chain pickerel (Kleckner & Sidell 1985).

Davison (1997) concluded that in general the central and peripheral changes seen during exercise training in mammals and fish are essentially similar but not as great in fish, with mammals having more flexibility. This is perhaps not that surprising given the substantially smaller visceral organ masses of ectothermic lizards compared to similar-sized endothermic mammals (Else & Hulbert 1991). These much smaller organ masses may simply offer less flexibility in overall mass in the first place.

5.6 Within species individual variation

It has long been known that individual fish species have different swimming abilities (Webb 1978, 1994). Also, as mentioned the maximal swimming performance (U_{crit}) of individual fish of several species has been shown to repeatable under constant biotic and abiotic conditions (Kolok 1992, 1994; Nelson *et al.* 1994, 1996; Gregory & Wood 1998, 1999; Reidy *et al.* 2000), indicating that individual fish may have a consistent, repeatable performance, which is larger than the intra-individual variation in measurements. All fish probably do not have the same ability to change, as there are known to be good swimming performers and fish with poor swimming performance within a population (see above).

The importance of studies at the 'individual' level depends upon the nature of the study. Recently Schulman & Love (1999) have argued for study at the population or species level for migrating fish species. They argued that studies at the population levels are more appropriate as individuals do not reveal the overall pattern of a population. They use the

example that when fish and birds migrate, only a relatively small proportion of the animals have time to accumulate the maximal levels of lipids. These animals are termed leaders (Schulman 1974) and decide the time of migration, dragging the rest of the population with them, the majority of which are less well prepared. Migrations may inevitably lead to the mortality of the less well-prepared individuals. Schulman & Love (1999) argue that the strategy of individuals is subordinate to that of the population as a whole, which in migratory populations is driven by leaders. However, it seems clear that study of individuals is paramount to elucidating the nature of physiological mechanisms and their significance to individual fitness within the natural environment. For these reasons, further study of energetic strategies and their underlying mechanisms at the individual level seems important to developing further understanding in this field.

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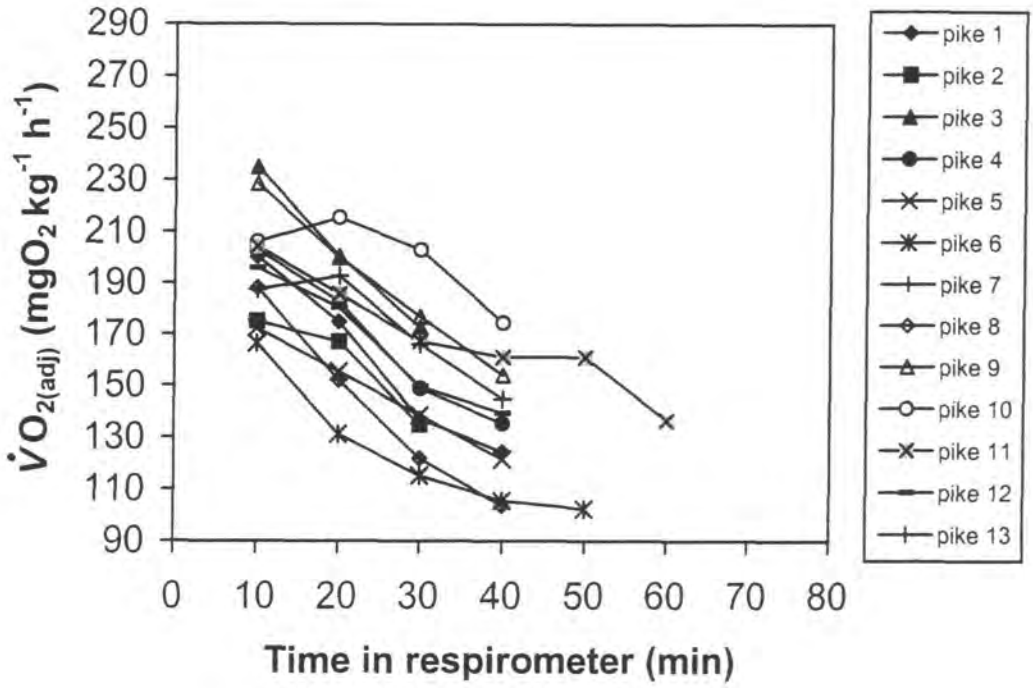
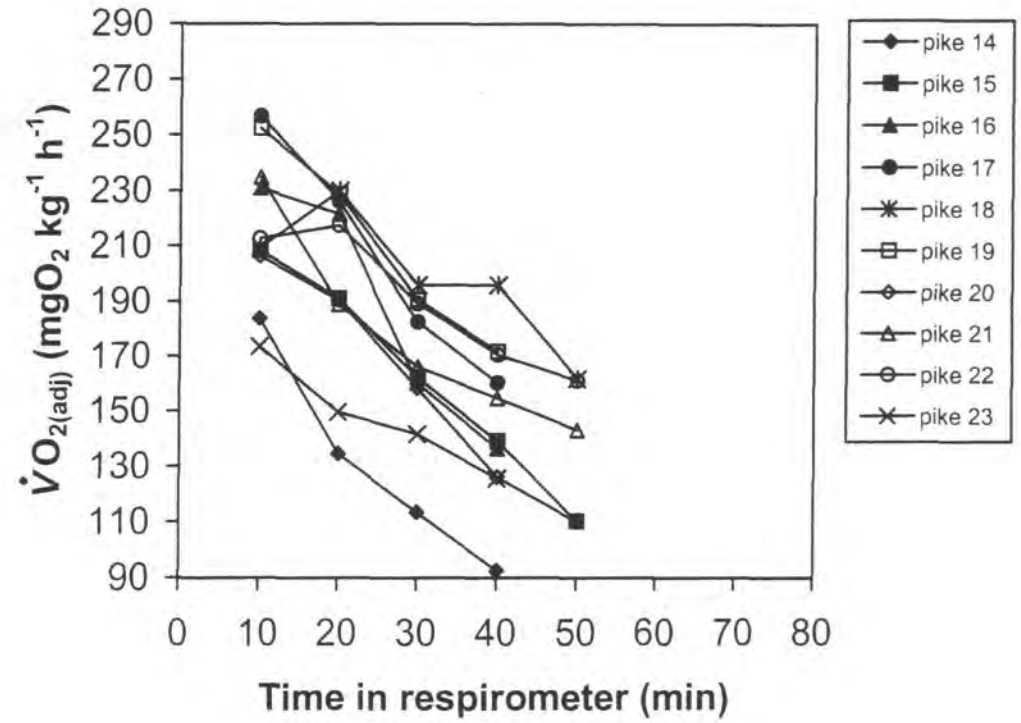
Appendix 1 Total food consumption for the individual pike between different time periods, conversion efficiency of food in to increased body mass and overall % mean wet body mass consumed per day over the experimental period (using the mean weight per pike between the two time periods to calculate the individual consumption as a % of wet body mass per day.).

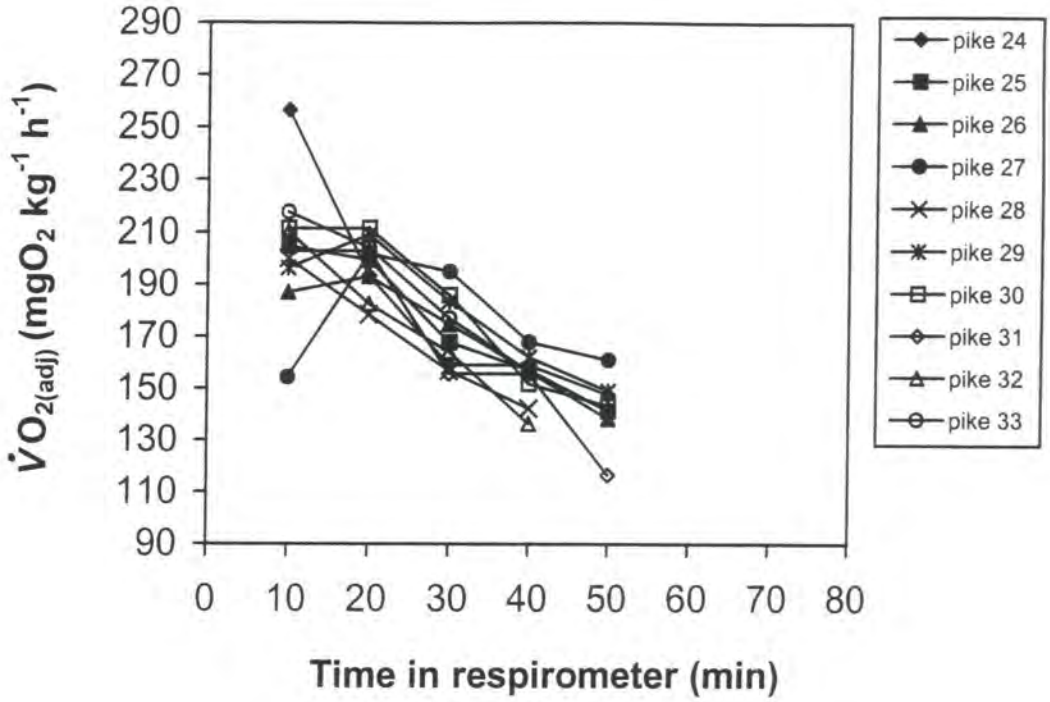
	Time 1 – Time 2			Time 2 –Time 3		
Pike number	Weight of food consumed (g)	Conversion %	% wbm day	Weight of food consumed (g)	Conversion %	% wbm day
1	5.09	-8.25	0.57	44.09	31.37	2.04
2	6.74	-39.32	1.03	33.07	22.71	2.03
3	8.36	7.42	0.94	35.03	31.43	2.43
4	8.96	17.75	0.88	32.29	23.75	1.73
5	8.14	51.11	0.82	32.08	28.37	1.51
6	5.55	-20.36	0.71	36.98	32.83	1.92
7	8.05	-31.43	0.88	42.78	29.87	2.20
8	3.99	-44.36	0.51	30.42	36.79	1.70
9	5.58	-43.37	0.58	39.46	33.96	2.38
10	8.59	-13.50	1.08	36.01	28.99	2.65
11	0.00	-	0.00	36.04	32.27	2.08
12	6.25	-19.68	0.65	41.30	30.07	1.86
13	5.26	-83.08	0.60	34.91	22.11	1.63
Mean	6.20	-18.92	0.71	36.50	29.58	2.01
14	22.40	38.57	3.03	82.74	43.24	1.83
15	26.99	35.64	2.97	91.88	39.27	1.77
16	17.69	31.60	3.69	69.10	29.25	2.46
17	13.36	30.16	2.38	Dead	Dead	-
18	12.29	38.65	1.83	82.26	38.27	2.05
19	10.91	24.47	3.26	66.96	27.48	3.04
20	13.12	2.36	2.79	68.65	25.00	2.81
21	10.32	35.95	3.45	68.47	37.64	2.98
22	8.72	-5.39	3.26	43.99	32.53	2.01
23	10.39	-29.45	3.04	Dead	Dead	Dead
Mean	14.62	20.26	2.97	7.17	34.08	2.37
24	8.69	24.97	1.26	28.48	29.74	1.00
25	7.03	-7.40	0.86	34.27	36.59	0.95
26	6.66	33.78	1.08	29.77	27.41	1.03
27	5.50	15.45	0.97	33.08	31.71	1.16
28	Dead	Dead		Dead	Dead	-
29	10.99	36.76	1.14	47.20	27.86	0.99
30	2.49	-7.63	0.86	28.66	33.88	1.53
31	4.24	8.49	1.24	28.20	34.26	1.38
32	2.20	0.45	0.77	24.47	32.24	1.50
33	2.23	9.42	1.42	23.66	29.29	1.82
Mean	5.56	12.70	1.07	30.87	31.44	1.26

Appendix 2 (a) A comparison of the rate of recovery from exercise induced MMR for individual exercise pike at time 1 (above) and individual high ration pike at time 1 (below) prior to the commencement of the experiment.

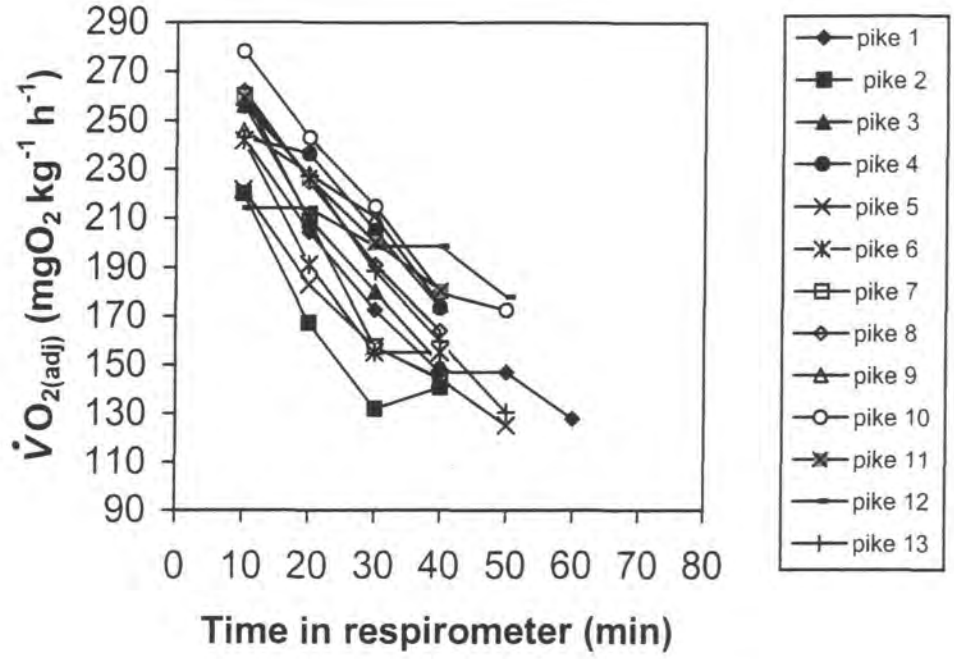


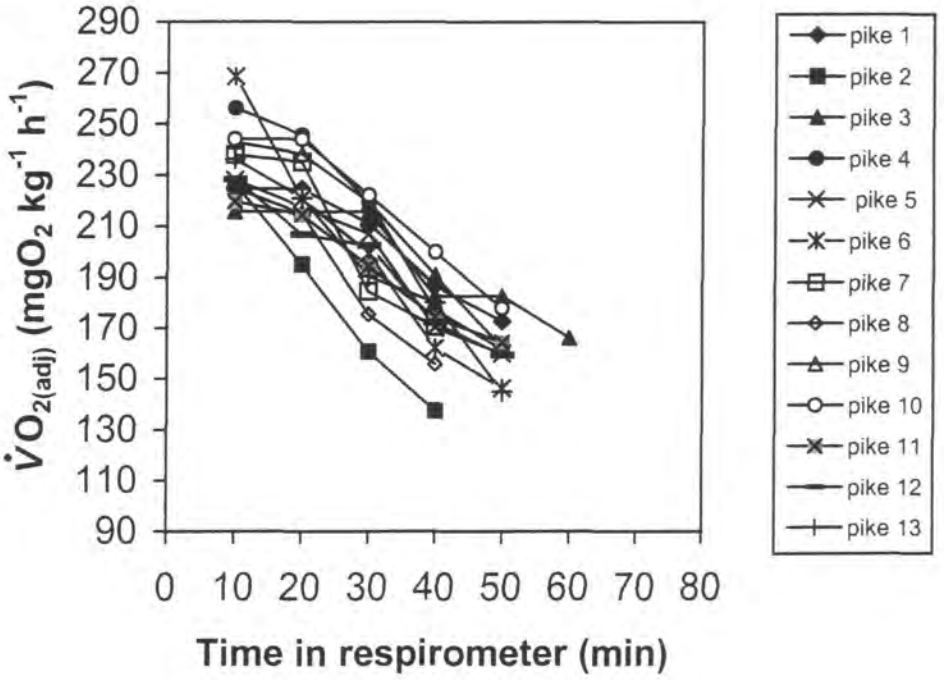


Appendix 2 (b) A comparison of the rate of recovery from exercise induced MMR for individual low ration pike at time 1 prior to the commencement of the experiment.

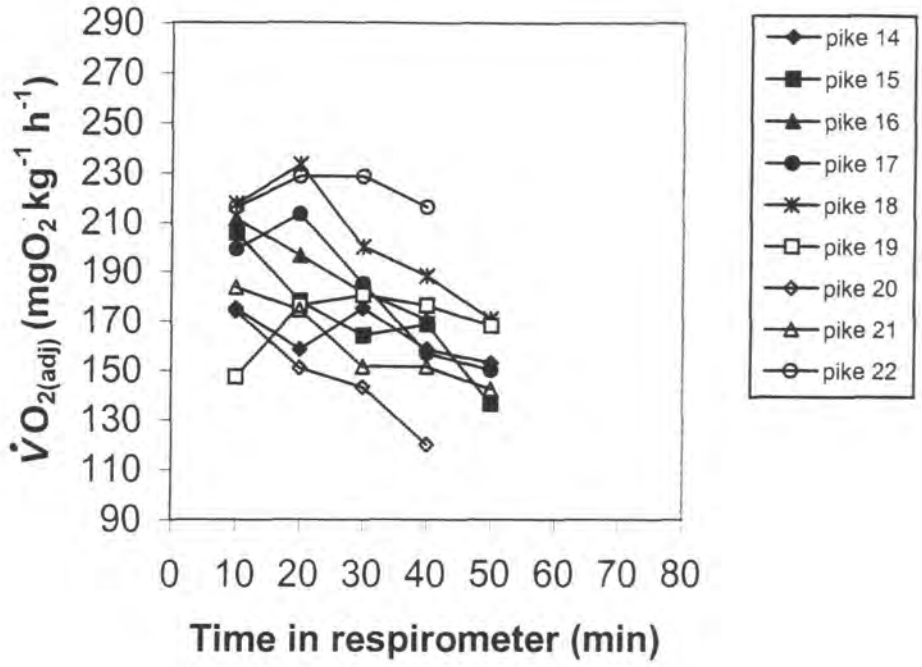


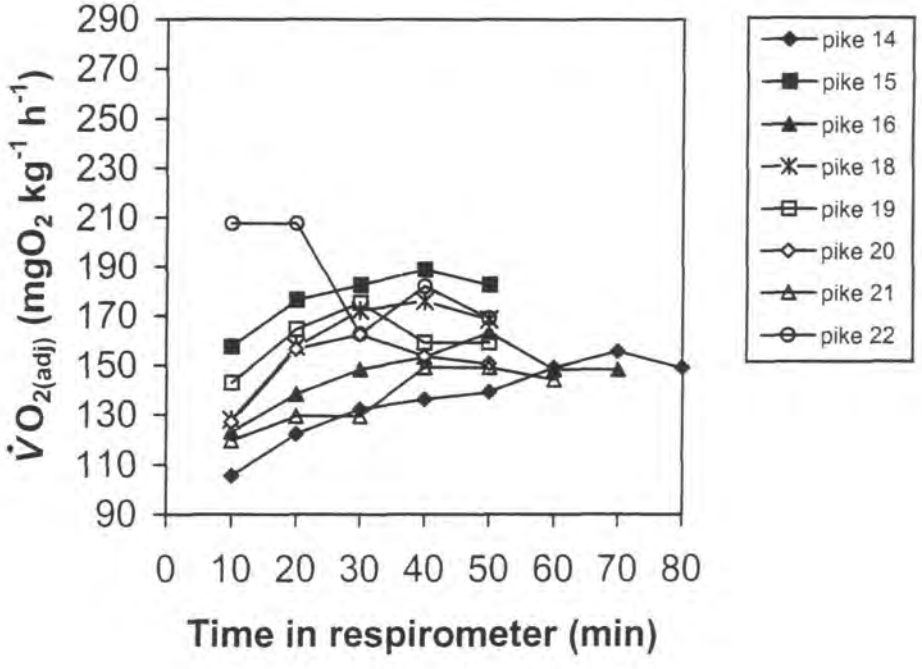
Appendix 2 (c) A comparison of the rate of recovery from exercise induced MMR for for individual exercised pike at time 2 (after c. 3 weeks, above) and time 3 (after c.11 weeks, below) of the experiment.



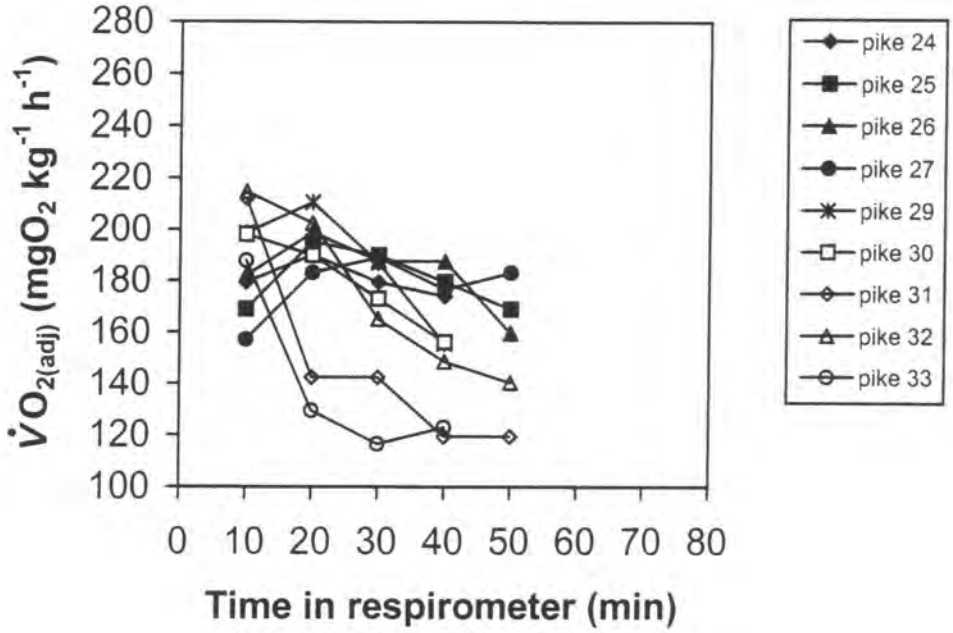


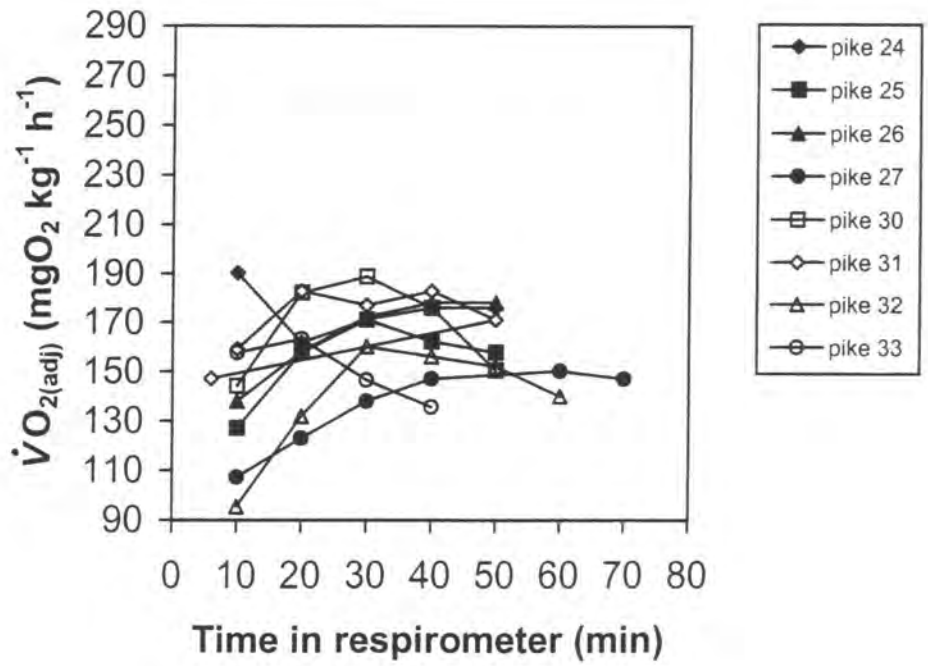
Appendix 2 (d) A comparison of the rate of recovery from exercise induced MMR for individual high ration pike at time 2 (after c. 3 weeks, above) and time 3 (after c. 11 weeks below) of the experiment.



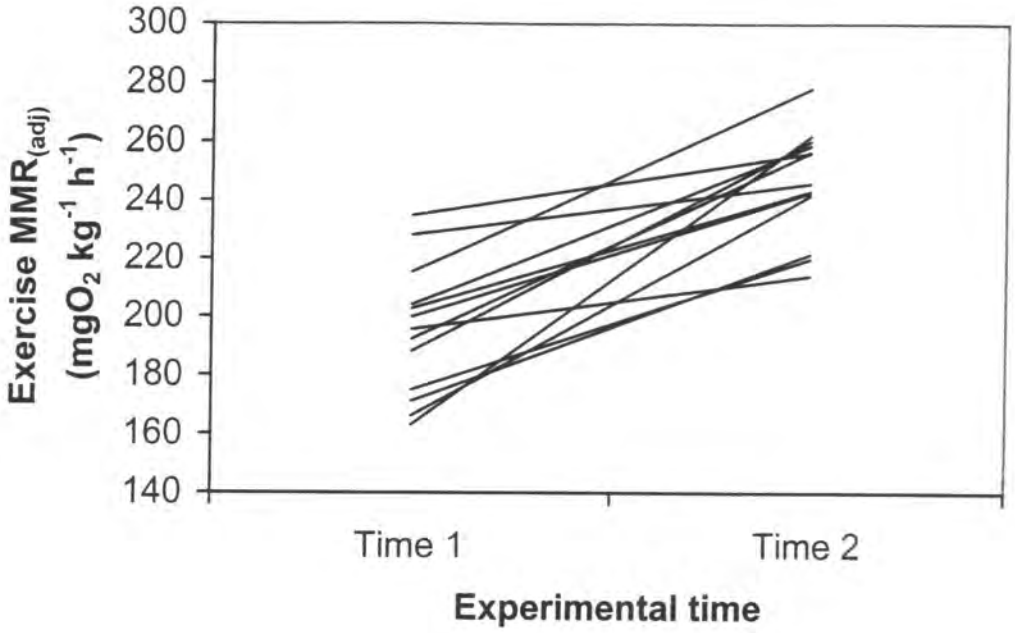


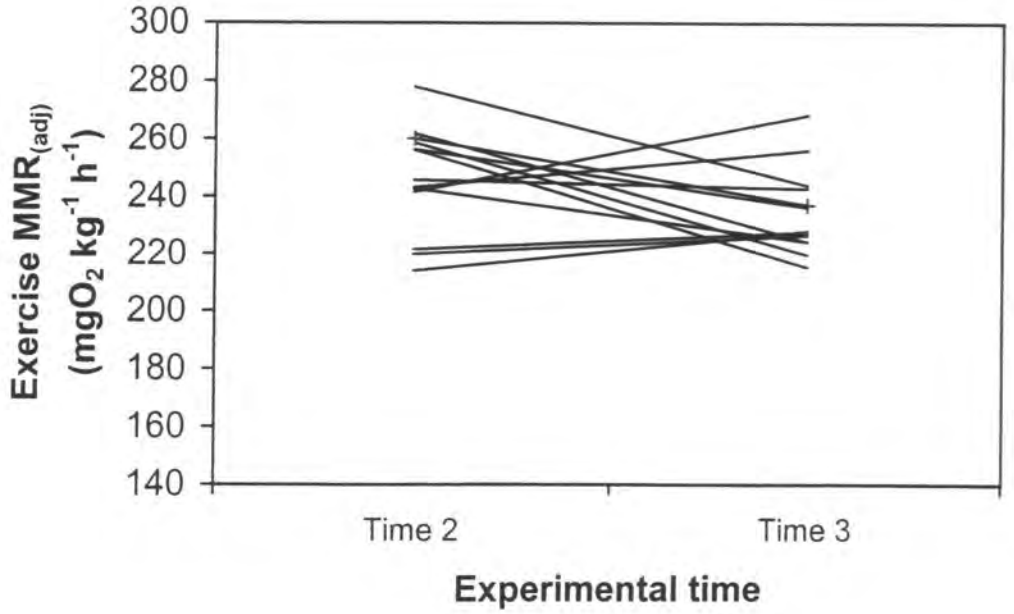
Appendix 2 (e) A comparison of the rate of recovery from exercise induced MMR for individual low ration pike at time 2 (after c. 3 weeks, above) and time 3 (after c. 11 weeks, below) of the experiment.



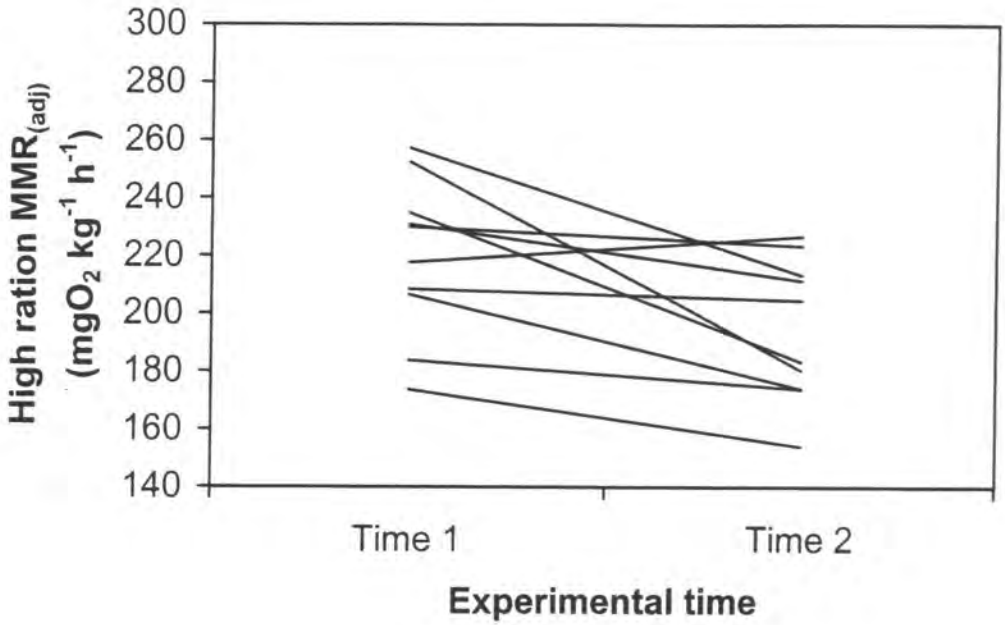


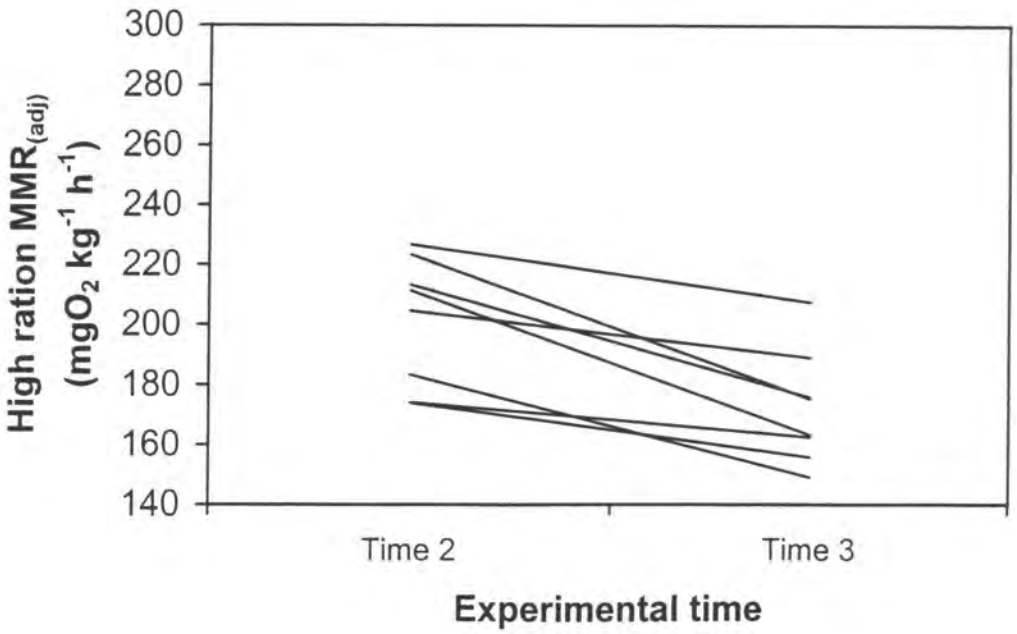
Appendix 3. A and B, the change in $MMR_{(adj)}$ following the commencement of sustained swimming in pike (time 1 prior to the start of the experiment, time 2 after c. 3 weeks and time 3 after c. 11 weeks). A lack of parallel lines indicate a change in the rank order of individual pike between times.



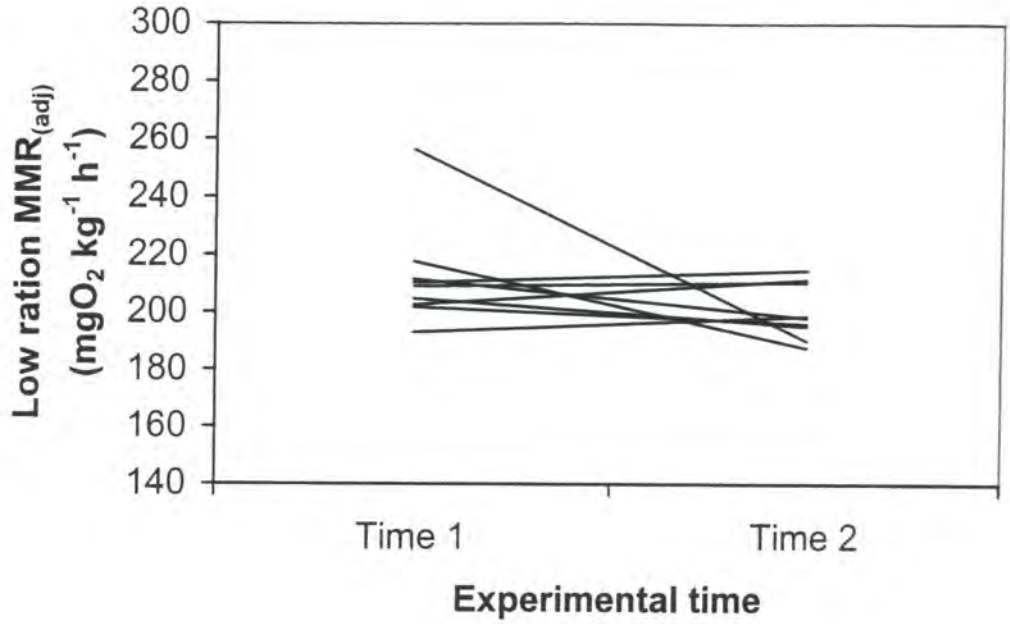


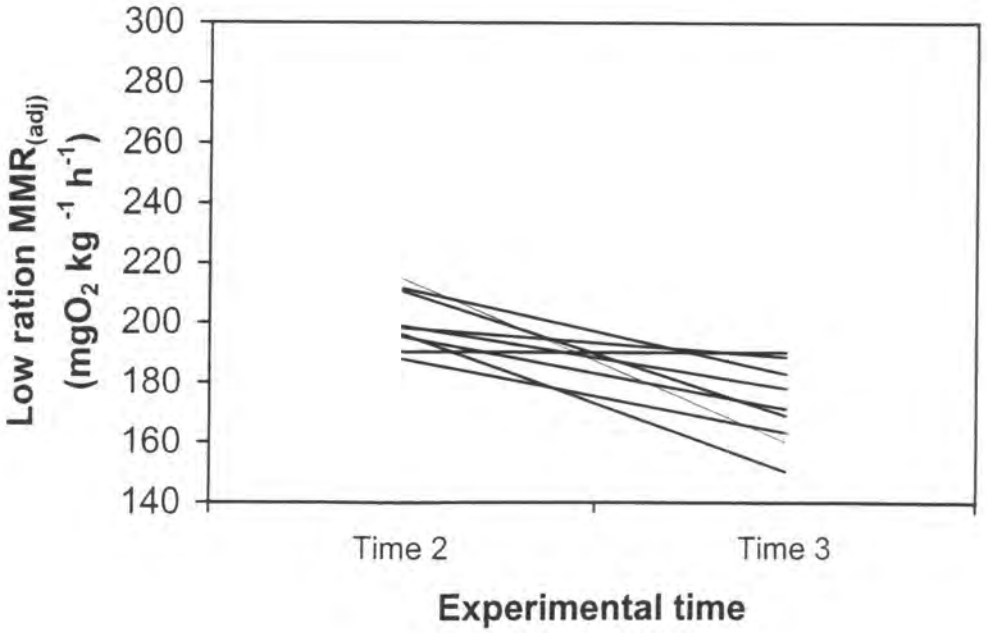
Appendix 3. C and D, the change in $MMR_{(adj)}$ following the commencement of a high ration diet in pike (time 1 prior to the start of the experiment, time 2 after c. 3 weeks and time 3 after c. 11 weeks). A lack of parallel lines indicate a change in the rank order of individual pike between times.





Appendix 3.E and F, the change in $MMR_{(adj)}$ following the commencement of a low ration diet (times 2 and 3) in pike,(time 1 prior to the start of the experiment, time 2 after c. 3 weeks and time 3 after c. 11 weeks). A lack of parallel lines indicate a change in the rank order of individual pike between times.





Appendix 4 wet organ weights for dissected pike (g), after c. 11 weeks at either sustained exercise, high ration or low ration treatment

Pike No.	Treatment	Source	Wet weight	Brain	Heart	Gills	Kidneys	Liver	Spleen	Stomach	Intestine	Red muscle	White muscle	Carcass	Total	% Wet weight
1	Exercise	C	46.87	0.157	0.081	0.388	0.158	0.470	0.026	0.452	0.400	1.262	24.764	16.149	44.307	94.531
2	Exercise	C	30.49	0.148	0.037	0.329	0.135	0.238	0.034	0.402	0.244	0.699	15.826	11.273	29.365	96.311
3	Exercise	C	39.07	0.115	0.066	0.441	0.135	0.261	0.029	0.403	0.326	1.096	20.080	15.035	37.987	97.227
4	Exercise	C	40.38	0.148	0.070	0.257	0.159	0.237	0.045	0.457	0.315	1.340	22.290	14.232	39.550	97.945
5	Exercise	C	41.09	0.147	0.023	0.308	0.157	0.282	0.048	0.446	0.282	1.217	22.420	14.043	39.373	95.821
6	Exercise	C	40.53	0.168	0.051	0.114	0.120	0.306	0.037	0.422	0.309	1.135	22.409	14.203	39.273	96.899
7	Exercise	C	45.33	0.160	0.060	0.263	0.149	0.443	0.013	0.460	0.311	1.264	22.644	17.757	43.524	96.017
8	Exercise	C	38.09	0.120	0.050	0.414	0.123	0.346	0.012	0.756	0.328	1.077	21.890	11.893	37.009	97.162
9	Exercise	C	48.07	0.141	0.113	0.540	0.182	0.442	0.023	0.649	0.502	1.156	24.764	17.537	46.049	95.796
10	Exercise	C	39.24	0.122	0.051	0.401	0.154	0.343	0.000	0.373	0.433	1.273	22.316	13.054	38.521	98.166
11	Exercise	C	42.75	0.137	0.052	0.385	0.167	0.409	0.046	0.519	0.327	1.041	24.078	14.011	41.172	96.309
12	Exercise	C	53.4	0.180	0.056	0.578	0.168	0.518	0.040	0.452	0.413	1.546	29.149	18.451	51.550	96.536
13	Exercise	C	43.58	0.150	0.070	0.471	nr	0.253	0.047	0.488	0.292	0.914	22.957	16.998	42.640	97.843
14	High ration	C	73.67	0.201	0.092	0.802	0.280	1.705	0.045	0.639	0.519	1.377	44.491	22.207	72.157	97.947
15	High ration	C	82.23	0.189	0.076	0.681	0.182	1.697	0.086	0.953	0.579	1.444	48.561	25.242	79.690	96.911
16	High ration	C	44.81	0.093	0.050	0.455	0.198	0.816	0.042	0.358	0.335	1.036	25.158	14.214	42.755	95.413
18	High ration	C	67.1	0.133	0.063	0.940	0.195	1.344	0.030	0.536	0.538	1.250	40.355	19.592	64.976	96.835
19	High ration	C	40.25	0.127	0.000	0.550	0.156	0.642	0.029	0.548	0.422	0.935	22.682	12.382	38.473	95.585
20	High ration	C	43.44	0.128	0.060	0.387	0.149	0.796	0.019	0.487	0.357	0.711	26.380	12.751	42.225	97.202
21	High ration	C	45.24	0.122	0.046	0.506	0.196	0.959	0.034	0.611	0.342	0.623	27.343	12.618	43.400	95.933
22	High ration	C	33.17	0.121	0.032	0.263	0.137	0.506	0.027	0.410	0.304	0.580	18.582	10.672	31.634	95.368
24	Low ration	C	47.87	0.156	0.069	0.497	0.133	0.292	0.040	0.591	0.381	1.204	25.493	16.781	45.637	95.335
25	Low ration	C	52.98	0.133	0.056	0.574	0.144	0.430	0.035	0.622	0.294	0.961	32.950	15.248	51.446	97.105
26	Low ration	C	45.44	0.127	0.060	0.453	0.152	0.346	0.011	0.500	0.331	1.233	23.613	16.496	43.323	95.340
27	Low ration	C	42.39	0.111	0.049	0.350	0.068	0.331	0.027	0.432	0.226	0.904	24.130	14.307	40.935	96.567
29	Low ration	C	68.53	0.177	0.085	0.579	0.183	0.801	0.042	0.579	0.463	1.648	40.531	21.537	66.625	97.221
30	Low ration	C	33.62	0.113	0.034	0.248	0.134	0.231	0.029	0.322	0.311	0.695	20.627	9.970	32.714	97.304
31	Low ration	C	36.17	0.127	0.053	0.421	0.077	0.282	0.029	0.378	0.296	0.896	19.402	12.899	34.860	96.377
32	Low ration	C	29.83	0.102	0.043	0.225	0.088	0.273	0.023	0.340	0.221	0.814	17.258	8.961	28.349	95.034
33	Low ration	C	20.15	0.074	0.023	0.203	0.074	0.208	0.015	0.304	0.167	0.374	11.419	6.030	18.892	93.756
a	Low ration	F	160.45	0.243	0.117	1.322	0.816	2.443	0.348	0.943	1.434	3.338	85.867	60.212	157.083	97.901
b	Low ration	F	104.37	0.230	0.125	0.705	0.506	0.875	0.244	0.543	0.990	2.721	50.216	44.215	101.370	97.126
c	Low ration	C	93.6	0.185	0.092	0.590	0.427	1.031	0.044	0.485	0.811	3.473	48.728	36.117	91.983	98.272
d	Low ration	C	118.32	0.226	0.149	0.623	0.462	1.426	0.087	0.995	0.712	2.506	66.852	41.650	115.688	97.775
e	Low ration	F	159.84	0.149	0.159	1.335	0.800	1.974	0.164	1.258	1.242	3.481	84.254	61.974	156.790	98.092
f	Low ration	F	199.3	0.276	0.198	1.514	0.791	1.860	0.273	1.721	0.967	4.025	105.826	78.525	195.976	98.332
g	Low ration	C	64.55	0.158	0.077	0.526	0.225	0.465	0.056	0.616	0.311	1.390	36.090	22.785	62.699	97.132
h	Low ration	C	74.16	0.144	0.066	0.588	0.285	1.014	0.023	0.920	0.492	1.981	38.411	29.590	73.514	99.129
I	Low ration	F	70.96	0.153	0.130	0.594	0.238	0.669	0.173	0.569	0.414	2.065	40.528	23.279	68.812	96.973

Source of pike, C = from the River Conan near Inverness, F = Framlington Fisheries, Ipswich.

Appendix 5. Adjusted organ mass for dissected pike, adjusted to those of a pike of 45g. After c.11 weeks treatment.

New No.	Wet weight	Treatment	Brain	Heart	Gills	kidney	Liver	Spleen	Stomach	Intestines	Red muscle	White muscle	Carcass	Total adj weight	% of Adj weight
1	46.87	Exercise	0.154	0.078	0.374	0.154	0.447	0.025	0.440	0.385	1.210	23.779	16.711	43.757	97.238
2	30.49	Exercise	0.182	0.052	0.470	0.168	0.383	0.054	0.521	0.349	1.043	23.330	18.324	44.876	99.724
3	39.07	Exercise	0.124	0.075	0.502	0.146	0.310	0.034	0.443	0.371	1.268	23.118	18.203	44.593	99.096
4	40.38	Exercise	0.157	0.077	0.284	0.169	0.270	0.051	0.491	0.348	1.498	24.832	17.001	45.179	100.398
5	41.09	Exercise	0.154	nr	0.335	0.165	0.315	0.053	0.474	0.307	1.336	24.547	16.771	44.482	98.850
6	40.53	Exercise	0.178	0.056	0.125	0.127	0.348	0.042	0.452	0.340	1.264	24.872	17.053	44.857	99.683
7	45.33	Exercise	0.159	0.060	0.261	0.148	0.439	0.013	0.458	0.309	1.255	22.480	18.564	44.146	98.101
8	38.09	Exercise	0.131	0.058	0.482	0.135	0.424	0.015	0.844	0.382	1.279	25.848	15.192	44.790	99.533
9	48.07	Exercise	0.136	0.107	0.508	0.175	0.408	0.021	0.621	0.472	1.080	23.187	17.501	44.217	98.261
10	39.24	Exercise	0.131	0.058	0.455	0.166	0.405	nr	0.409	0.491	1.466	25.582	16.016	45.178	100.395
11	42.75	Exercise	0.141	0.054	0.404	0.172	0.435	0.049	0.537	0.343	1.097	25.342	15.522	44.096	97.991
12	53.4	Exercise	0.164	0.048	0.494	0.153	0.420	0.033	0.403	0.353	1.296	24.576	16.213	44.154	98.121
13	43.58	Exercise	0.153	0.072	0.485	nr	0.263	0.049	0.499	0.301	0.945	23.702	18.384	44.851	99.670
14	73.67	High ration	0.155	0.060	0.510	0.222	0.934	0.025	0.461	0.330	0.829	27.217	13.669	44.412	98.694
15	82.23	High ration	0.137	0.045	0.392	0.137	0.813	0.043	0.639	0.333	0.776	26.623	13.702	43.639	96.975
16	44.81	High ration	0.093	0.050	0.457	0.198	0.820	0.042	0.359	0.336	1.041	25.264	15.227	43.888	97.529
18	67.1	High ration	0.108	0.044	0.652	0.161	0.825	0.019	0.411	0.373	0.829	27.096	13.408	43.926	97.613
19	40.25	High ration	0.135	nr	0.609	0.164	0.736	0.033	0.590	0.468	1.049	25.350	15.282	44.415	98.700
20	43.44	High ration	0.130	0.062	0.400	0.152	0.831	0.020	0.499	0.369	0.737	27.325	13.986	44.509	98.910
21	45.24	High ration	0.122	0.046	0.504	0.196	0.953	0.034	0.609	0.340	0.620	27.198	13.343	43.963	97.695
22	33.17	High ration	0.142	0.042	0.348	0.158	0.734	0.039	0.502	0.402	0.794	25.186	16.097	44.444	98.764
24	47.87	Low ration	0.151	0.065	0.470	0.123	0.271	0.037	0.567	0.360	1.130	23.969	16.655	43.798	97.328
25	52.98	Low ration	0.122	0.049	0.494	0.117	0.352	0.029	0.558	0.253	0.812	28.000	13.638	44.425	98.723
26	45.44	Low ration	0.126	0.059	0.449	0.150	0.342	0.011	0.497	0.328	1.221	23.385	17.335	43.904	97.564
27	42.39	Low ration	0.115	0.052	0.370	0.073	0.356	0.029	0.449	0.239	0.961	25.611	15.926	44.181	98.180
29	68.53	Low ration	0.142	0.059	0.394	0.107	0.479	0.026	0.438	0.315	1.069	26.648	14.245	43.921	97.602
30	33.62	Low ration	0.132	0.044	0.324	0.194	0.330	0.041	0.391	0.407	0.938	27.584	14.993	45.377	100.838
31	36.17	Low ration	0.143	0.064	0.514	0.102	0.368	0.037	0.437	0.362	1.122	24.123	17.440	44.712	99.359
32	29.83	Low ration	0.127	0.062	0.328	0.149	0.451	0.037	0.447	0.322	1.243	26.003	15.269	44.437	98.748
33	20.15	Low ration	0.113	0.047	0.424	0.206	0.554	0.038	0.518	0.349	0.855	25.441	15.245	43.792	97.315
a	160.45	Low ration	0.124	0.038	0.412	0.162	0.518	0.079	0.405	0.446	0.902	24.174	15.683	42.943	95.430
b	104.37	Low ration	0.147	0.060	0.326	0.173	0.314	0.092	0.311	0.457	1.145	21.706	17.991	42.720	94.934
c	93.6	Low ration	0.125	0.048	0.301	0.168	0.422	0.019	0.298	0.414	1.634	23.478	16.848	43.756	97.237
d	118.32	Low ration	0.135	0.064	0.257	0.135	0.438	0.028	0.524	0.293	0.926	25.499	15.275	43.574	96.832
e	159.84	Low ration	0.076	0.052	0.418	0.159	0.421	0.037	0.542	0.387	0.944	23.810	16.107	42.954	95.454
f	199.3	Low ration	0.125	0.054	0.387	0.119	0.303	0.048	0.641	0.246	0.870	24.001	15.902	42.696	94.879
g	64.55	Low ration	0.131	0.056	0.378	0.142	0.299	0.037	0.485	0.223	0.959	25.187	16.560	44.456	98.792
h	74.16	Low ration	0.111	0.043	0.372	0.151	0.551	0.013	0.660	0.311	1.185	23.343	17.761	44.500	98.888
I	70.96	Low ration	0.120	0.087	0.391	0.133	0.384	0.102	0.421	0.272	1.292	25.736	15.170	44.109	98.019

nr = not recorded.

Appendix 6 Adjusted organ mass standardised to that of a 45 g pike using the appropriate organ mass scaling relationship. Results are expressed as a realtive percentage of the standardised bodymass, for individual pike after c. 11 weeks of treatment.

Pike No.	Treatment	Brain	Heart	Gills	kidney	Liver	Spleen	Stomach	Intestine	Red muscle	White muscle	Carcass
1	Exercise	0.341	0.174	0.831	0.343	0.994	0.055	0.978	0.856	2.689	52.842	37.135
2	Exercise	0.404	0.116	1.045	0.374	0.850	0.119	1.157	0.775	2.319	51.844	40.721
3	Exercise	0.275	0.166	1.116	0.325	0.689	0.076	0.984	0.825	2.817	51.373	40.450
4	Exercise	0.348	0.171	0.631	0.376	0.601	0.113	1.091	0.773	3.329	55.183	37.781
5	Exercise	0.343	nr	0.744	0.367	0.700	0.119	1.053	0.681	2.970	54.549	37.269
6	Exercise	0.395	0.124	0.279	0.283	0.773	0.093	1.005	0.756	2.809	55.272	37.895
7	Exercise	0.354	0.132	0.581	0.330	0.976	0.029	1.017	0.686	2.788	49.955	41.254
8	Exercise	0.291	0.129	1.072	0.300	0.942	0.032	1.877	0.850	2.841	57.440	33.759
9	Exercise	0.303	0.237	1.130	0.390	0.906	0.047	1.380	1.050	2.400	51.527	38.891
10	Exercise	0.292	0.128	1.010	0.370	0.901	nr	0.908	1.091	3.257	56.848	35.591
11	Exercise	0.313	0.121	0.897	0.382	0.968	0.109	1.193	0.762	2.439	56.315	34.494
12	Exercise	0.365	0.107	1.098	0.339	0.934	0.073	0.897	0.784	2.881	54.614	36.030
13	Exercise	0.339	0.160	1.078	nr	0.585	0.108	1.108	0.668	2.099	52.672	40.853
14	High ration	0.344	0.133	1.134	0.492	2.076	0.056	1.024	0.733	1.842	60.482	30.376
15	High ration	0.305	0.099	0.871	0.304	1.807	0.095	1.419	0.739	1.725	59.162	30.448
16	High ration	0.207	0.112	1.015	0.441	1.823	0.094	0.798	0.747	2.312	56.143	33.837
18	High ration	0.239	0.099	1.448	0.359	1.834	0.042	0.914	0.828	1.841	60.213	29.795
19	High ration	0.299	nr	1.354	0.365	1.635	0.073	1.311	1.039	2.331	56.333	33.959
20	High ration	0.290	0.138	0.888	0.337	1.847	0.044	1.108	0.819	1.638	60.721	31.080
21	High ration	0.270	0.102	1.119	0.434	2.117	0.075	1.353	0.756	1.377	60.440	29.651
22	High ration	0.316	0.093	0.773	0.352	1.631	0.086	1.116	0.894	1.764	55.969	35.770
24	Low ration	0.335	0.145	1.044	0.273	0.602	0.083	1.261	0.800	2.511	53.264	37.011
25	Low ration	0.271	0.108	1.098	0.260	0.783	0.064	1.240	0.562	1.805	62.223	30.308
26	Low ration	0.281	0.132	0.998	0.334	0.760	0.024	1.104	0.729	2.713	51.967	38.523
27	Low ration	0.255	0.115	0.822	0.163	0.791	0.064	0.999	0.531	2.136	56.914	35.391
29	Low ration	0.315	0.131	0.875	0.238	1.066	0.057	0.973	0.699	2.375	59.218	31.656
30	Low ration	0.293	0.098	0.720	0.432	0.733	0.091	0.868	0.903	2.085	61.298	33.318
31	Low ration	0.317	0.143	1.143	0.226	0.818	0.083	0.971	0.804	2.493	53.606	38.756
32	Low ration	0.282	0.137	0.729	0.330	1.002	0.083	0.993	0.717	2.762	57.784	33.930
33	Low ration	0.252	0.104	0.942	0.457	1.232	0.085	1.152	0.777	1.900	56.536	33.879
a	Low ration	0.275	0.085	0.916	0.359	1.151	0.176	0.901	0.991	2.004	53.721	34.851
b	Low ration	0.327	0.133	0.724	0.385	0.697	0.203	0.690	1.015	2.544	48.235	39.980
c	Low ration	0.279	0.107	0.670	0.374	0.938	0.042	0.663	0.919	3.632	52.174	37.440
d	Low ration	0.301	0.142	0.571	0.300	0.974	0.063	1.164	0.651	2.059	56.665	33.944
e	Low ration	0.169	0.116	0.928	0.354	0.934	0.083	1.205	0.861	2.098	52.912	35.792
f	Low ration	0.279	0.119	0.860	0.264	0.673	0.107	1.424	0.547	1.933	53.336	35.337
g	Low ration	0.290	0.125	0.840	0.316	0.665	0.082	1.077	0.496	2.131	55.971	36.800
h	Low ration	0.246	0.095	0.826	0.335	1.225	0.029	1.467	0.691	2.632	51.873	39.469
I	Low ration	0.267	0.194	0.869	0.296	0.853	0.226	0.934	0.605	2.872	57.192	33.710

nr = Not recorded.

Appendix 7 White muscle and liver mean water content as a percentage of wet weight and mean protein content expressed as mg of protein per gram of tissue, after c. 11 weeks of treatment.

Pike number	White muscle		Liver	
	Water content	Protein (mg/g)	Water content	Protein (mg/g)
1	78.6	169.7		
2	80.5	173.8		171.6
3	81.2	188.5		
4	79.8	202.6		
5	78.9	203.6		198.8
6	79.2	158.8	67.2	194.4
7	79.6	147.4		
8	78.8	160.0		
9	78.4	184.2		210.1
10	79.1	191.3		
11	77.3	166.1		
12	81.7	158.9		
13	80.3	170.1		
14	81.4	198.5	62.3	202.5
15	78.6	213.4	63.3	216.9
16	78.5	194.8	58.6	214.7
18	78.3	188.7	61.9	176.3
19	79.2	162.2	65.9	214.7
20	80.0			
21	79.0	159.7	65.8	223.5
22	78.0	152.5	66.8	212.3
24	79.1	165.0	65.8	205.7
25	78.7	158.8	71.8	158.9
26	79.0	138.4	66.0	151.3
27	79.2	160.3	61.9	214.8
29	79.3	150.1	66.3	202.0
30	79.4	181.8	64.9	129.0
31	79.0	157.3	65.3	172.4
32	80.0	147.3	69.7	183.3
33	79.3	145.6		160.4

Appendix 8. Activity of citrate synthase per gram of tissue for all pike analysed, after c. 11 weeks of treatment ($\mu mol\ g^{-1}\ min^{-1}$).

Pike no	Treatment	Heart	Gill	Liver	Intestine	Red muscle	White muscle
1	Exercise	21.93	3.82	2.63	13.08	18.77	2.37
2	Exercise	27.90	3.70	3.80	5.45	26.76	2.61
3	Exercise	24.95	4.49	3.21	17.53	23.58	3.07
4	Exercise	19.76	4.68	3.64	10.49	24.48	2.74
5	Exercise	27.83	3.37	3.48	13.08	28.30	2.98
6	Exercise	23.26	4.30	3.24	11.66	30.19	2.99
7	Exercise	29.41	3.73	3.11	11.66	27.90	2.71
8	Exercise	30.28	3.05	2.70	9.96	22.89	2.21
9	Exercise	nr	3.41	2.91	13.48	28.19	2.72
10	Exercise	28.45	4.84	3.51	17.19	28.83	3.21
11	Exercise	28.37	4.13	2.70	12.75	23.61	2.74
12	Exercise	29.93	4.38	3.31	16.76	26.28	3.05
13	Exercise	35.09	4.42	4.17	13.66	36.93	2.31
14	High ration	20.00	3.88	1.29	10.93	25.14	2.83
15	High ration	17.56	3.46	1.06	10.76	11.72	2.42
16	High ration	34.89	3.43	1.41	13.54	17.14	2.11
18	High ration	28.58	2.76	1.10	13.52	17.23	2.11
19	High ration	41.79	3.17	1.71	8.67	30.11	2.47
20	High ration	26.06	4.42	0.98	13.84	29.55	2.79
21	High ration	19.34	4.33	1.18	12.18	19.97	2.39
22	High ration	22.36	4.04	1.92	13.44	28.33	2.62
24	Low ration	23.44	3.96	1.89	12.98	34.86	2.87
25	Low ration	24.22	3.53	2.37	4.07	21.58	3.15
26	Low ration	26.39	nr	2.64	9.86	28.47	1.28
27	Low ration	20.32	3.61	1.83	9.24	27.47	2.29
29	Low ration	23.94	2.75	2.09	9.72	30.87	2.23
30	Low ration	nr	4.12	3.31	12.45	23.80	2.72
31	Low ration	21.96	3.25	3.61	10.02	24.27	2.96
32	Low ration	21.28	3.43	2.43	12.79	18.44	2.99
33	Low ration	18.93	5.07	2.97	6.41	19.24	2.91

nr = Not recorded

Appendix 9 Lactate oxidation activity per gram of tissue for all pike analysed, after c. 11 weeks of treatment (μmol g^{-1} min^{-1}).

Pike no.	Treatment	Heart	Gill	Liver	Intestine	Red muscle	white muscle
1	Exercise	1821.9	163.3	22.5	208.7	682.6	564.7
2	Exercise	1591.1	115.4	29.5	130.1	648.2	333.9
3	Exercise	1797.3	238.2	23.6	191.5	888.8	476.3
4	Exercise	1826.8	164.5	25.0	127.7	805.4	952.7
5	Exercise	1630.4	103.1	22.3	117.9	1208.0	638.4
6	Exercise	1355.4	191.5	31.4	174.3	962.5	648.2
7	Exercise	2092.0	341.3	26.5	221.0	677.7	442.0
8	Exercise	1542.0	137.5	24.2	186.6	834.8	559.8
9	Exercise	nr	248.0	23.8	124.7	687.5	520.5
10	Exercise	1890.6	148.5	29.1	130.1	662.9	496.0
11	Exercise	1198.2	179.2	26.5	216.1	815.2	628.6
12	Exercise	1777.7	132.6	24.6	122.8	893.8	461.6
13	Exercise	1335.7	115.4	24.1	108.0	579.5	373.2
14	High ration	1895.5	144.9	10.3	167.0	756.3	471.4
15	High ration	1384.8	179.2	9.6	191.5	874.1	687.5
16	High ration	2229.5	51.6	13.5	201.3	746.4	628.6
18	High ration	1679.5	208.7	13.5	104.6	947.8	672.8
19	High ration	1866.1	144.9	26.3	225.9	942.9	726.8
20	High ration	1605.8	234.5	10.2	248.0	1006.7	407.6
21	High ration	1767.9	175.6	16.3	169.4	933.0	717.0
22	High ration	1090.2	176.8	21.5	235.7	1031.3	687.5
25	Low ration	1826.8	181.7	27.5	201.3	864.3	569.6
25	Low ration	1669.6	126.2	32.3	216.1	913.4	530.4
26	Low ration	1640.2	179.2	32.9	154.7	844.6	579.5
27	Low ration	1900.4	178.0	25.2	208.7	952.7	658.0
29	Low ration	1746.6	136.3	22.8	228.3	1046.0	648.2
30	Low ration	1453.6	135.0	29.7	108.0	746.4	392.9
31	Low ration	1433.9	103.1	23.8	147.3	677.7	412.5
32	Low ration	1591.1	nr	29.5	117.9	687.5	500.9
33	Low ration	2258.9	nr	33.6	167.0	2455.4	500.9