J. exp. Biol. ***180****, 195-207 (1993)*
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THE EFFECTS OF BODY SIZE ON THE ACID–BASE AND METABOLITE STATUS IN THE WHITE MUSCLE OF RAINBOW TROUT BEFORE AND AFTER EXHAUSTIVE EXERCISE

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Accepted 18 March 1993

Summary

The effect of body size on the white muscle acid–base and metabolite status was examined in rainbow trout (*Oncorhynchus mykiss*) ranging in length from 8 to 54cm. Following 5min of exhaustive exercise, white muscle lactate concentration was approximately doubled (approximately 32 μmol g^{-1}) in larger fish than in smaller fish (approximately 16 μmol g^{-1}). Associated with this post-exercise increase in lactate was a nearly parallel increase in the number of metabolic protons produced by larger fish. Larger fish did not possess a greater non-bicarbonate buffering capacity or soluble protein concentration, so their mean muscle intracellular pH (pHi) decreased by approximately 0.70 units compared with a change in mean pHi of about 0.40 units in the smallest fish. The relationship between resting pHi and length was independent of size (mean pHi7.31). Concentrations of muscle energy metabolites were also determined in trout white muscle before and after exercise. Under resting conditions, larger fish possessed a twofold greater concentration of ATP (approximately 7 μmol g^{-1}) than did smaller fish (approximately 3μmol g^{-1}). Similarly, resting values of muscle glycogen range from about 6 μmol g^{-1} in the smallest fish to as high as 15 μmol g^{-1} in the largest fish. However, the smaller fish had higher levels (approximately 35 μmol g^{-1}) of phosphocreatine (PCr) than the larger fish (approximately 25 μmol g^{-1}). Following exercise, however, both ATP and glycogen concentrations remained size-dependent and increased with increases in fish length. Levels of PCr were size-independent following exercise. These results demonstrate that body size has an important influence on the acid–base and metabolic status of fish before and after exercise.

Introduction

Studies on the relationship between the size of an organism and various aspects of its biology are numerous (Peters, 1983; Schmidt-Nielsen, 1984; Bennett *et al*. 1985; Childress and Somero, 1990; Goolish, 1991). Earlier investigations have demonstrated that body size has an important influence on aerobic processes (Schmidt-Nielsen, 1984). More recently, a limited number of studies have also provided evidence of a relationship

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Key words: metabolism, pH, fish, rainbow trout, body size, scaling, exhaustive exercise, acid–base, activity, *Oncorhynchus mykiss.*

between body size and anaerobic processes in certain species of lower vertebrates. For example, Goolish (1989) demonstrated a size-dependent increase in the production of lactate in the white muscle of rainbow trout, *Oncorhynchus mykiss*, following exhaustive exercise. In addition, Somero and Childress (1990) have shown that levels of lactate dehydrogenase and creatine phosphokinase in white muscle are size-related in both the kelp bass, *Paralabrax clanthratus*, and the rainbow trout. The buffering capacity of white muscle in kelp bass also increases with body size (Somero and Childress, 1990). Taken together, these results suggest that body size may have an important influence on (i) the storage and/or utilization of the metabolic fuels (i.e. phosphocreatine, ATP and glycogen) required for anaerobic exercise and (ii) the acid–base status of white muscle following periods of burst activity. Indeed, several investigators have suggested that body size may be an important physiological constraint in exercising fish (Dalla Via *et al*. 1989; Pagnotta and Milligan, 1991). It is therefore likely that a considerable amount of the variability among previous studies examining the acid–base and metabolite status of white muscle following exercise may be size-related. However, there has been no detailed investigation of the relationship between body size and the metabolic and acid–base status of fish before and after periods of anaerobic activity. The purpose of the present investigation was to examine the acid–base and metabolic status of white muscle in a broad size range of rainbow trout at rest and immediately following exhaustive exercise.

Materials and methods

Rainbow trout [*Oncorhynchus mykiss* (Walbaum)], ranging in size from 8 to 54cm, were obtained from a local supplier (Linwood Acres, Ontario) and were maintained for at least 2 weeks prior to experimentation in dechlorinated Kingston water (15±1˚C). The fish were fed a commercial trout food every second day. Seven days prior to experimentation feeding was suspended.

Experimental protocol

Individual fish were removed by net from a common tank and exercised to exhaustion by manual chasing in a circular tank for 5min. This method produced sequential bursts of swimming rather than continuous swimming (see review by Wood, 1991). A period of 5 min provided an adequate time to exhaust the fish; this was shown by the fact that the fish no longer responded to chasing after this time. Following exercise, the fish were immediately placed into a well-aerated Perspex box containing a concentrated buffered ($NaHCO_3$) anaesthetic solution (MS-222 at a final concentration of $0.25\,g\,l^{-1}$). The animals lost balance after about 30s and were fully anaesthetized after a period of no more than 2min. Following anaesthetization, a sample of white muscle (2–10g) was taken from the epaxial musculature behind the operculum, well above the lateral red muscle band. Samples were immediately freeze-clamped in pre-cooled tongs and stored in a Dewar flask containing liquid nitrogen. The time between removing the fish from the anaesthetic and freeze-clamping the tissue was less then 10s. Following this, the fork length (to the nearest centimetre: from the tip of the nose to the fork in the caudal fin) and

the mass (to the nearest gram) of the fish were measured. The mass of the smaller fish was measured prior to muscle sampling, a step which did not take more than 5s.

Control (resting values) were obtained in a manner similar to that described above. Individual fish were isolated in separate Perspex boxes for at least 24h prior to sampling. Before sampling, the water flow to the boxes was reduced and the $NaHCO_3$/MS-222 mixture was added (see above). Once the fish had been fully anaesthetized, a sample of white epaxial muscle was subsequently removed in the manner described for the exercised animals (see above).

Frozen muscle tissues were analyzed for intracellular pH, total CO_2, [lactate], [glycogen], [glucose], [ATP], [phosphocreatine] (PCr), non-bicarbonate buffer capacity (β), soluble and insoluble protein concentration and total water content.

Analytical techniques and calculations

Acid–base status

The acid–base status of white muscle was determined using the technique of Pörtner *et al.* (1990) and Tang and Boutilier (1991). About 100mg of muscle was ground (behind a Plexiglas shield to reduce contamination by CO_2 from one's breath) under liquid nitrogen using a pre-cooled porcelain mortar and pestle. The fine powder was kept under liquid nitrogen at all times. The ground muscle was added to a tared Eppendorf tube containing 200 μl of ice-cold metabolic inhibitor (150 mmol l^{-1} potassium fluoride, 6 mmol l^{-1} nitrilotriacetic acid). Following this, another 200 μl of ice-chilled metabolic inhibitor was added to the Eppendorf tube, and the slurry was stirred with a needle for 5s and vortexed for 10s. The tube was weighed and then centrifuged for 8s. Samples of the supernatant were immediately taken for measurements of pH and total CO_2 content (C_{CO_2}). The pH of 80 μl of the supernatant was determined using a PHM 73 pH meter and associated micro-pH unit (Radiometer, Copenhagen, Denmark) thermostatted to 15°C. C_{CO_2} content of 200 μl of the supernatant was measured with a Corning model 965 CO_2 analyzer (CIBA Corning Canada Inc.). C_{CO_2} and pH measurements were carried out in duplicate and the mean was calculated. Total CO_2 of the tissue water (C_{CO_2}) was calculated using the equation derived by Pörtner *et al.* (1990). Measured values of muscle pH and C_{CO_2} were used to calculate P_{CO_2} and [HCO_3^-] using a rearrangement of the Henderson–Hasselbach equation. The values for αCO_2 were determined according to the formulae provided by Heisler (1986*a*,*b*). The values for pK were obtained from an experimental analysis of non-bicarbonate buffering capacity of tissue homogenates under metabolic control (Pörtner, 1990). The metabolic acid load (ΔH^+) after exercise in the muscle tissue was calculated according to Milligan and Wood (1986) using the following equation:

$$\Delta H^+ = [HCO_3^-]_r - [HCO_3^-]_{ex} - \beta(pHr - pHex),$$

where β is the non-bicarbonate buffer value, r is the resting value and ex is the value following exercise (see below).

Metabolite levels

Approximately 1–2g of tissue powder was transferred to a tared 15ml vial to which 4

vols of ice-cold 8% perchloric acid (PCA) solution containing 1mmol l^{-1} of EDTA had been added. This mixture was vortexed for 10s to form a slurry. A 5min extraction period followed, during which the slurry was slowly inverted and rotated at 5°C. 1ml of the acid slurry was removed and stored at 5°C for subsequent glycogen determination. This analysis was performed within 3 days of processing the tissue. The remaining acid slurry was divided into 1.5ml Eppendorf tubes and centrifuged for 2min at 5°C. A known volume of supernatant was immediately neutralized to pH7.0 (2mol l^{-1} KOH, 0.4mol l^{-1} KCl and 0.4mol l^{-1} imidazole) and stored at −80°C.

All assays, except that for glycogen, were performed on neutralized PCA extracts by the method of Lowry and Passonneau (1972). Muscle glycogen concentrations were determined on PCA slurries by the method of Keppler and Decker (1974).

Water content

Approximately 300–500mg of muscle tissue was transferred to a dried and tared Eppendorf tube, and the tube was weighed and placed into an oven at 80°C for several days. The samples were monitored until a constant weight was obtained. The water content of the samples was calculated from these values.

Non-bicarbonate buffering capacity (β)

Non-bicarbonate buffering capacity was determined by the method of Heisler and Piiper (1971) and Pörtner (1990). 1g of ground muscle tissue (under liquid nitrogen) was placed into a pre-weighed 15ml polypropylene tube containing 4 vols of ice-cold metabolic inhibitor (540mmol l^{-1} potassium fluoride, 10mmol l^{-1} nitrilotriacetic acid). This inhibitor has been shown to reduce homogenate metabolism and therefore to minimize accumulation of inorganic phosphate (for details, see Pörtner, 1990; Pörtner *et al*. 1990). This slurry was vortexed for 15s and placed into one of two pre-cooled tonometer vessels. The muscle tissues of large and small fish were always tested simultaneously (i.e. the tissue of a small fish was placed in one tonometer and the tissue of a larger fish in the other tonometer). The homogenates were then equilibrated for 30min at 15°C to various levels of P_{CO_2} using a gas-mixing pump (Wösthoff, Bochum, Germany). Following each equilibration period, a 1ml sample was withdrawn from each of the two tonometers and immediately centrifuged for 2min. The supernatant was analyzed for pH and C_{CO_2} as described above. From the C_{CO_2} measurements we determined the $[HCO_3^-]$ from the following formula:

$$[HCO_3^-] = C_{CO_2} - (\alpha CO_2 \times P_{CO_2}),$$

where αCO_2 is the solubility of CO_2 (determined according to Heisler, 1986*a*,*b*) and P_{CO_2} is the CO_2 partial pressure delivered by the Wösthoff pump. For the calculation of muscle αCO_2, we assumed $[M]$=0.21mol l^{-1} as molarity of the dissolved species (Heisler, 1986*a*). $[HCO_3^-]$ was plotted against pH (pH range 6.7–7.2), and β was calculated as the slope of the straight line (see Heisler and Piiper, 1971, or Pörtner, 1990, for more details).

Soluble and insoluble protein levels

Muscle protein concentration was determined by the method of Bates and Millward

(1983) as described by Somero and Childress (1990). White muscle was ground under liquid nitrogen. The powder was added to 10 vols of low-salt buffer (LSB, 50mmol l^{-1} K_2HPO_4, pH7.0) containing 1% Triton X-100. The homogenate was vortexed and allowed to sit for 3h at 4˚C. 300 μl of the homogenate was removed and 4 vols of 10mol l^{-1} urea and 40 μl glacial acetic acid was added. The mixture was stored at 4˚C overnight for later determination of the total protein content. The remaining homogenate was centrifuged in two 1.5ml Eppendorf tubes for 4min, and the supernatant was removed. The pellets were washed twice with 1ml of LSB, and the supernatants (the soluble protein fraction) from these washes were pooled and stored at 4˚C overnight.

To solubilize the fraction containing the insoluble proteins, the pellets were resuspended (with 300 μl of LSB, 1ml of urea and 80 μl of acetic acid) and vortexed. Another sample of 1.2ml of urea was added to each sample, and they were vortexed and centrifuged for 2–3s. This step was performed to remove all remaining insoluble proteins. The insoluble and total protein fractions were kept in a rotary shaker for 1 day at 4˚C. Protein concentrations were determined by the method of Bradford (1976) using reagents and a bovine gamma globulin standard obtained from BioRad.

Statistical analysis

Linear regressions of non-transformed data were performed (Statview SE+ program). The level of significance was taken as $P \leq 0.05$.

Results

The effects of body size on the acid–base and metabolite status of rainbow trout were computed on the basis of length, as length is most relevant in hydrodynamic theory (see Somero and Childress, 1990). The relationship between length and mass of the fish used in this study is described by the following equation: log(mass) = 2.91log(length) −1.85 ($P<0.0001$, $N=50$), where length is in cm and mass is in g.

Lactate production

The resting values for lactate increased slightly with increases in fish length (Fig. 1A); however, these values ranged from 0 μmol g^{-1} to only 1.17 μmol g^{-1}. Thus, there was actually minimal variation between the fish (mean 0.122 μmol g^{-1}, $N=18$). After exercise, however, there was a strong and positive relationship between fish size and the accumulation of white muscle lactate. In the largest fish, white muscle lactate concentrations were approximately twice those in the smallest fish (Fig. 1A). Associated with the size-dependent increases in lactate concentration, there was a positive increase in the production of metabolic protons with increases in size following exercise (Fig. 1B).

Buffering capacity

Non-bicarbonate buffering capacity (β) of white muscle tissue in rainbow trout showed no significant size-dependence (Fig. 1C). Consistent with this finding, there was no significant relationship between fish size and the percentage of soluble proteins. The

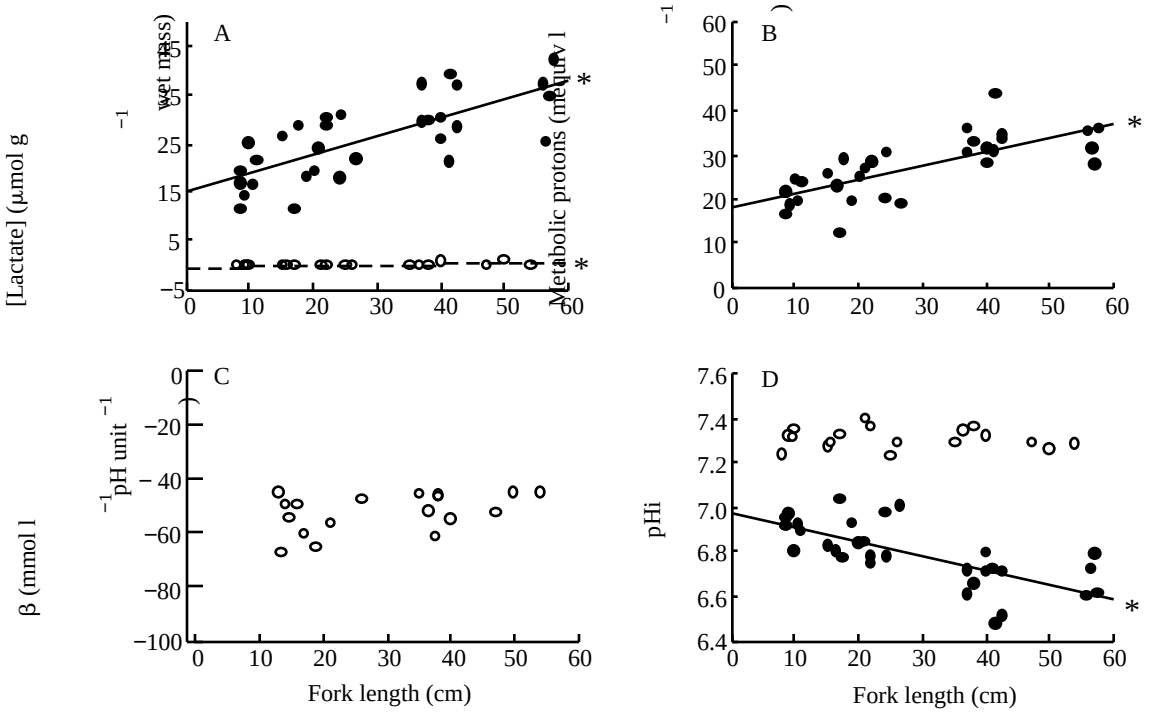

Fig. 1. The relationships between fish length and the white muscle lactate concentrations (A), metabolic protons (B), non-bicarbonate buffering capacity (β) (C) and intracellular pH (pHi) (D). Filled and open circles represent 5-min exercise and resting values, respectively. An asterisk denotes that the slope differs significantly from zero. Equations describing the regressions are given in Table 1. Note that the slope for β is non-significant and its mean value is $-51.5\,\mathrm{mmol\,l^{-1}\,pHunit^{-1}}$.

percentage of insoluble proteins (i.e. the myofibrillar component) was also size-independent.

Acid–base status

Resting values of white muscle pH (pHi) were size-independent and averaged 7.31±0.01 (Fig. 1D). Exhaustive exercise, however, resulted in a pronounced intracellular acidosis of the muscle in all fish, with larger fish exhibiting a greater acidosis than smaller fish (Fig. 1D). Following exhaustive exercise, there was a decrease in the mean muscle pHi of approximately 0.70 units in the largest fish (approximately 50–60cm) compared with a change in the mean pHi of about 0.40 units in the smallest fish (approximately 8–12 cm). The size-dependent acidosis was probably of mixed respiratory and metabolic origin, because P_{CO_2} increased significantly with size following exercise, along with a large increase in the production of metabolic protons (Fig. 1B; Table 1).

Carbohydrate, ATP and phosphocreatine status

Glycogen concentrations in trout muscle were size-dependent prior to and following

Table 1. *Linear regression equations relating fork length to acid–base and metabolic variables in white muscle of rainbow trout at 15°C*

Variable	Equation	*r*	*N*	*P*
Resting condition				
pHi	NS, 7.31±0.01		18	
P_{CO_2}	NS, 0.234±0.032kPa		18	
[HCO_3^-]	NS, 4.37±0.52mmol l^{-1}		18	
[Lactate]	y=0.01(±0.004)FL−0.17	0.50	18	0.04
[Glycogen]	y=0.18(±0.040)FL+5.16	0.78	13	0.0018
[Glucose]	NS, 0.39±0.04 μmol g^{-1}		17	
[PCr]	y=−0.26(±0.100)FL+35.67	0.55	18	0.019
[ATP]	y=0.09(±0.030)FL+2.55	0.57	18	0.013
%H_2O	NS, 77.5±1.00		18	
Exercise condition				
pHi	y=−0.01(±0.001)FL+6.97	0.71	32	0.0001
P_{CO_2}	y=0.014(±0.006)FL+0.309	0.38	32	0.03
[HCO_3^-]	NS, 3.67±0.35mmol l^{-1}		32	
[Lactate]	y=0.37(±0.060)FL+15.35	0.73	31	0.0001
[Glycogen]	y=0.10(±0.030)FL−0.26	0.60	27	0.0009
[Glucose]	y=0.01(±0.003)FL+0.83	0.67	31	0.0001
[PCr]	NS, 3.09±0.34 μmol g^{-1}		31	
[ATP]	y=0.05(±0.010)FL+0.29	0.66	31	0.0001
ΔH^+	y=0.31(±0.050)FL+18.50	0.73	32	0.0001
%H_2O	y=−0.11(±0.040)FL+79.72	0.43	32	0.014

Where slopes of equations are not significantly different from zero (NS), mean values are given. Further details are given in Figs 1 and 2.
y represents the physiological variable; FL, fork length (cm); PCr, phosphocreatine.

exercise (Fig. 2A). Resting values of muscle glycogen ranged from about 6 μmol g^{-1} in the smallest fish (approximately 8cm) to as high as 15 μmol g^{-1} in the largest fish (approximately 50–60cm). Following exercise, glycogen concentrations fell to approximately 12% and 30% of the resting values in small and large fish, respectively, and remained significantly greater in larger fish than in smaller fish. Similarly, muscle glucose levels increased following exercise in a size-dependent manner (Fig. 2B).

Resting concentrations of phosphocreatine (PCr) were size-dependent and decreased with increases in fish length (Fig. 2C). Smaller fish typically had resting values of about 30–35 μmol g^{-1}, whereas larger fish exhibited values of about 20–25 μmol g^{-1}. Post-exercise PCr levels were size-independent. Resting concentrations of ATP scaled positively with body size (Fig. 2D). ATP concentrations following exercise declined to about 20% and 30% of resting values in small and large fish, respectively, and were therefore also size-dependent.

Discussion

The acid–base and metabolic consequences of exhaustive exercise in rainbow trout are

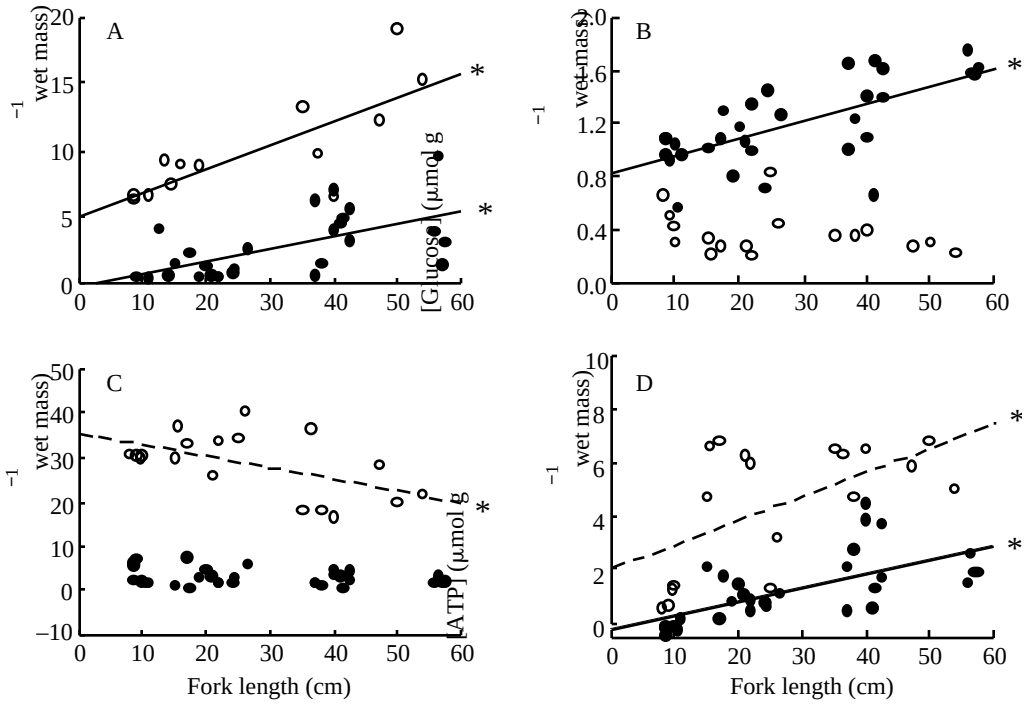

Fig. 2. The effects of fish length on the white muscle concentrations of (A) glycogen, (B) glucose, (C) phosphocreatine (PCr) and (D) ATP of rainbow trout following 5min of exhaustive exercise. Filled and open circles represent exercise and resting values, respectively. An asterisk denotes that the slope differs significantly from zero. Equations describing the regressions are given in Table 1.

clearly influenced by body size (Figs 1, 2; Table 1). In addition, it appears that the storage and utilization of the intracellular substrates which support this anaerobic swimming activity (i.e. glycogen, ATP, PCr) are also size-dependent. This study therefore demonstrates the physiological significance of the positive allometric scaling of glycolytic enzyme activities (i.e. lactate dehydrogenase, LDH) and/or power requirements for burst-type swimming in fish (Childress and Somero, 1990).

White muscle lactate and acid–base status

Our data indicate that the lactate accumulation (i.e. anaerobic capacity) of rainbow trout is significantly related to fish size (Fig. 1A), the largest fish having about twice the observed anaerobic capacity of the smallest fish. This demonstrates that there is a functional link between the positive allometric scaling of glycolytic potential (measured as maximal mass-specific LDH activity; Childress and Somero, 1990) and size-specific glycolytic throughput (Fig. 1A). Goolish (1989) also demonstrated a positive relationship between anaerobic capacity and size following 6min of exhaustive exercise in the rainbow trout. However, Goolish's results differ from our data mainly in the results for anaesthetized fish; in particular, his values for anaesthetized fish were of the same order

of magnitude as those for the experimental animals that had been subjected to 6min of exhaustive exercise. These data suggest that the animals possessed little or no anaerobic scope. The physiological validity of the values for our resting (control) fish is supported by the low lactate (Fig. 1A) and the high resting muscle PCr levels that we found (Fig. 2C). PCr levels in trout muscle are severely depressed by even the smallest disturbance (Dobson and Hochachka, 1987).

Given the 1:1 relationship between lactate and proton production during anaerobic glycolysis (Hochachka and Mommsen, 1983), one would expect greater metabolic proton (H^+) loads to be produced in the muscle of larger trout immediately following exhaustive exercise. As expected, metabolic proton levels in white muscle parallel the changes observed in lactate levels (Fig. 1B). Our data show, however, that the accumulation of protons is somewhat greater than the lactate accumulation observed. Since the vast majority of glycolytic end-products remain in the intracellular compartment immediately after exercise (Milligan and Wood, 1986; Tang and Boutilier, 1991), this discrepancy is probably due to the proton production associated with the uncoupling of ATP hydrolysis from ATP production (Hochachka and Mommsen, 1983; Milligan and Wood, 1986). The extent of ATP depletion (Fig. 2D) mirrors quite closely the degree of proton excess observed following exhaustive exercise.

As shown in previous studies, there was a marked intracellular acidosis following exhaustive exercise: the pHi decrease was greater in larger fish, mainly as a result of the greater number of metabolic protons produced (Fig. 1B). Our data also indicate that there is a greater increase in P_{CO_2} in larger fish following exhaustive exercise (Table 1). These findings are somewhat surprising given the previous evidence that intracellular buffering capacities may be adapted to compensate for differences in anaerobic glycolytic potential of white muscle in certain species (Somero and Childress, 1990).

White muscle buffering capacity

The intracellular bicarbonate concentrations decreased following exercise (Table 1), but remained independent of size before and after exercise. In addition, the non-bicarbonate buffer value of the white muscle of trout did not differ among fish of different sizes (Fig. 1C). This would explain, in part, why there were greater decreases in pHi after exercise as fish length increased (Fig. 1D). These results are in contrast to those of Somero and Childress (1990), who showed that in the white muscle of the kelp bass (*Paralabrax clathratus*) the buffering capacity increased with body size. These authors suggest that the higher buffer capacity in larger fish may compensate for the increase in the anaerobic capacity for work. Nelson and Magnuson (1987) also found that the buffer value in the white muscle of yellow perch (*Perca flavenscens*) was influenced by body size, but that smaller fish generally had higher buffer values than larger fish. However, as they point out, their results may have been influenced by seasonal and reproductive effects. Although our results do not show an increased buffering capacity with increases in body size, the values agree well with other published buffer values for rainbow trout of approximately 180–420g (Milligan and Wood, 1986). Thus, the relationship between the non-bicarbonate buffer value and body size in fish is not entirely clear and probably warrants further study.

Protein levels play an important role in intramuscular proton buffering by non-bicarbonate species (e.g. Woodbury, 1965). Somero and Childress (1990) showed that soluble (and consequently total) protein in the white muscle of kelp bass scales in parallel with non-bicarbonate buffering capacity. In our study, insoluble (myofibrillar) protein concentration did not scale with fish size. This result is in agreement with the results of Somero and Childress (1990) for kelp bass and suggests that the positive glycolytic scaling may not be related to the acceleration from rest to burst speeds. We also found that the size of the soluble protein fraction does not show any correlation with trout size. This finding is consistent with the absence of any relationship between fish size and non-bicarbonate buffering capacity. Thus, several pieces of evidence in the present study indicate that the higher anaerobic capacity of the larger trout is not accompanied by greater muscular non-bicarbonate buffering capacity. It has also been demonstrated that inorganic phosphates play an important role in muscle buffering capacity (Pörtner, 1990); however, the levels of free phosphates were not measured in our study. Nevertheless, our results appear to indicate that there is no intracellular mechanism, insofar as non-bicarbonate buffering is concerned, to protect the white muscle from the possibly deleterious effects of the greater lactacidosis in larger trout. One can speculate that this may be detrimental for larger fish. Indeed, according to Walsh and Milligan (1989), intracellular acidosis inhibits glyconeogenesis in trout muscle. It has been established, however, that both lactate and protons are important substrates for glycogen replenishment following exercise (Turner *et al.* 1983; Milligan and Wood, 1986). Moreover, according to Ferguson and Storey (1992), intracellular acidosis appears to be an important condition for gluconeogenic flux of lactate to glycogen. One could also speculate therefore, that, in the face of an increased intracellular lactate burden, a similarly enhanced proton availability would be compatible with lactate-utilizing processes such as gluconeogenesis. This issue was not the focus of the present investigation, however, and clearly requires further study.

White muscle metabolites

To our knowledge, this is the first study to examine whether the metabolic fuel reserves of white muscle scale with fish size in a manner paralleling anaerobic capacity. Our results indicate that the level of glycogen, the primary fuel for anaerobic glycolysis (Dobson *et al*. 1987) scales positively with fish length (Fig. 2A). Indeed, glycogen levels at rest reflect very well the levels of lactate which accumulate in the muscle following exercise as predicted by the 2:1 stoichiometry between glycogen utilization and lactate accumulation seen in fish muscle (Wardle, 1978; Figs 1A, 2A). In addition, glucose concentration increases in white muscle following exhaustive exercise (Fig. 2B). Thus, the substrate availability is apparently matched to its size-dependent demand. The source of this glucose, however, cannot be determined from the present experiment.

During exhaustive exercise, PCr and ATP provide the energy required to support muscular activity lasting of the order of seconds (Dobson *et al*. 1987). On a longer time scale (i.e. minutes), glycogenolysis provides the ATP required to sustain muscular exertion. Our results show that there is a near depletion of the PCr stores following 5min of exhaustive exercise in all sizes of trout (Fig. 2C). Resting levels of PCr, however, are

negatively correlated with fish size. The reason for this relationship is not clear. One can speculate, however, that it may be related to the locomotory behaviour of these animals: smaller fish often rely on short burst-like movements to acquire food and/or to avoid being preyed upon. They may, therefore, have a greater resting PCr reserve because they are more dependent on short-term and fast-acting energy reserves.

Unlike phosphocreatine, ATP and glycogen stores were not depleted following exercise in larger fish. In smaller fish, however, glycogen and ATP were depleted. It should be pointed out that, for glycogen, this could also be influenced by the feeding regime used in this experiment. As noted in the Materials and methods section, we terminated feeding 7 days before exercise and muscle sampling. It has been shown that glycogen stores are quite refractory to starvation in adult fish, but very sensitive to starvation in juveniles (Scarabello *et al.* 1991). Thus, the impact of nutritional status on the present results should be investigated further.

It has been argued that fatigue and exhaustion arise primarily as a response to glycogen depletion in the white muscle of fish (Dobson *et al.* 1987). The observation that ATP and glycogen levels were not depleted in larger fish may, therefore, indicate that these animals were not completely exhausted by the exercise protocol. However, exhaustion can also arise from the arrest of glycolytic flux. For instance, Ferguson and Storey (1992) showed that the activity of trout white muscle phosphofructokinase (PFK) is very sensitive to low pH; purified preparations of the enzyme show essentially zero activity at pH6.5 (close to the pH found after exercise in larger fish, Fig. 1D). It is possible that inactivation of PFK at this low pH could reduce the rate of glycogenolysis, causing a functional state of exhaustion. It is also important to point out that we measured total (i.e. free and bound) muscular ATP level. Schulte *et al.* (1992) suggest that this measurement will provide useful information, but the free concentrations of these metabolites must be determined to evaluate fully the metabolic status of an animal.

In conclusion, the present results clearly demonstrate that fish size has an important influence on the storage and utilization of the metabolic fuels required for anaerobic exercise and on the acid–base status of white muscle following burst activity in the rainbow trout. They also demonstrate the functional significance of the previously described relationships between body size and the activity of glycolytic enzymes (i.e. LDH). Fish size may account for much of the variability among previous studies that have examined the acid–base and metabolite status of fish muscle following exhaustive exercise. In addition to their biological significance, these results may also have important implications for fisheries managers. It has been shown that severe exercise may result in considerable mortality in fish under certain conditions (Wood *et al.* 1983). During netting or recreational angling, fish often struggle to exhaustion, but many individuals are then released back into the wild. Under these conditions, it may be important to consider that, in some species, larger individuals may experience a greater physiological disturbance that smaller ones following a similar period of exhaustive exercise.

It is a pleasure to acknowledge the many people who have contributed to this study. We thank S. Currie, B. Cameron, T. Holitzner and Dr G. P. Morris for support and

discussions. The research was supported by an NSERC/DFO Science Subvention Grant to B.L.T., an NSERC Post-doctoral Fellowship to R.A.F. and an E. G. Baumann Scholarship to J.D.K.

References

BATES, P. C. AND MILLWARD, D. J. (1983). Myofibrillar protein turnover: synthesis rates of myofibrillar and sarcoplasmic protein fractions in different muscles and the changes observed during postnatal development and in response to feeding and starvation. *Biochem.J.* **214**, 587–592.

BENNETT, A. F., SEYMOUR, R. S., BRADFORD, D. F. AND WEBB, G. J. W. (1985). Mass-dependence of anaerobic metabolism and acid–base disturbance during activity in the salt-water crocodile, *Crocodylusporosus*. *J.exp.Biol*. **118**, 161–171.

BRADFORD, M. (1976). A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein–dye binding. *Analyt. Biochem*. **72**, 248–254.

CHILDRESS, J. J. AND SOMERO, G. N. (1990). Metabolic scaling: a new perspective based on scaling of glycolytic enzyme activities. *Am. Zool*. **30**, 161–173.

DALLA VIA, J., HUBER, M., WIESER, W. AND LACKNER, R. (1989). Temperature-related responses of intermediary metabolism to forced exercise and recovery in juvenile *Rutilus rutilis* (L.) (Cyprinidae: Teleostei). *Physiol.Zool*. **62**, 964–976.

DOBSON, G. P. AND HOCHACHKA, P. W. (1987). Role of glycolysis in adenylate depletion and repletion during work and recovery in teleost white muscle. *J.exp. Biol.* **129**, 125–140.

DOBSON, G. P., PARKHOUSE, W. S. AND HOCHACHKA, P. W. (1987). Regulation of anaerobic ATP-generating pathways in trout fast-twitch muscle. *Am.J. Physiol.* **253**, R186–R194.

FERGUSON, R. A. AND STOREY, K. (1992). Gluconeogenesis in trout (*Oncorhynchus mykiss*) white muscle: purification and characterization of fructose-1,6-bisphospatase activity *invitro*. *FishPhysiol. Biochem.* **10**, 201–212.

GOOLISH, E. M. (1989). The scaling of aerobic and anaerobic muscle power in rainbow trout (*Salmo gairdneri*). *J. exp.Biol.* **147**, 493–505.

GOOLISH, E. M.(1991). Aerobic and anaerobic scaling in fish. *Biol. Rev*. **66**, 33–56.

HEISLER, N. (1986*a*). Buffering and transmembrane ion transfer processes. In *Acid–Base Regulation in Animals* (ed. N. Heisler), pp. 3–47. New York: Elsevier Science Publishers.

HEISLER, N. (1986*b*). Acid-base regulation in fishes. In *Acid–Base regulation in Animals* (ed. N. Heisler), pp. 309–356. New York: Elsevier Science Publishers.

HEISLER, N. AND PIIPER, J. (1971). The buffer value of rat diaphragm muscle tissue determined by P_{CO_2} equilibration of homogenates. *Respir.Physiol.* **12**, 169–178.

HOCHACHKA, P. W. AND MOMMSEN, T. P.(1983). Protons and anaerobiosis. *Science* **219**, 1392–1395.

KEPPLER, D. AND DECKER, K. (1974). Glycogen: determination with amyloglucosidase. In *Methods of Enzymatic Analysis* (ed. H. U. Bergmeyer), pp. 1127–1131. New York: Academic Press.

LOWRY, O. H. AND PASSONNEAU, J. V. (1972). *A Flexible System of Enzymatic Analysis.* New York: Academic Press.

MILLIGAN, C. L. AND WOOD, C. M. (1986). Tissue intracellular acid–base status and the fate of lactate after exhaustive exercise in the rainbow trout. *J. exp. Biol.* **123**, 123–144.

NELSON, J. A. AND MAGNUSON, J. J. (1987). Seasonal, reproductive and nutritional influences on muscle 'buffering capacity' in yellow perch (*Perca flavenscens*). *Fish Physiol. Biochem.* **3**, 7–16.

PAGNOTTA, A. AND MILLIGAN, C. L. (1991). The role of blood glucose in the restoration of muscle glycogen during recovery from exhaustive exercise in rainbow trout (*Oncorhynchus mykiss*) and winter flounder (*Pseudopleuronectesamericanus*). *J.exp. Biol.* **161**, 489–508.

PETERS, R. H. (1983). *The Ecological Implications of Body Size.* Cambridge: Cambridge University Press. 329pp.

PÖRTNER, H. O. (1990). Measurement of intracellular buffer values after metabolic inhibition by fluoride and nitrilotriacetic acid. *Respir.Physiol.* **81**, 275–288.

PÖRTNER, H. O., BOUTILIER, R. G., TANG, Y. AND TOEWS, D. P. (1990). Determination of intracellular pH and P_{CO_2} after metabolic inhibition by fluoride and nitrilotriacetic acid. *Respir. Physiol.* **81**, 255–274.

SCARABELLO, M., WOOD, C. M. and HEIGENHAUSER, G. J. F. (1991). Glycogen depletion in juvenile rainbow trout as an experimental test of the oxygen debt hypothesis. *Can. J. Zool.* **69**, 2562–2568.

SCHMIDT-NIELSEN, K. (1984). *Scaling: Why is Animal Size so Important?* Cambridge, New York: Cambridge University Press. 241pp.

SCHULTE, P. M., MOYES, C. D. AND HOCHACHKA, P. W. (1992). Integrating metabolic pathways in post-exercise recovery of white muscle. *J. exp.Biol.* **166**, 181–195.

SOMERO, G. N. AND CHILDRESS, J. J. (1990). Scaling of ATP-supplying enzymes, myofibrillar proteins and buffering capacity in fish muscle: relationship to locomotory habit. *J.exp.Biol.* **149**, 319–333.

TANG, Y. AND BOUTILIER, R. G.(1991). White muscle intracellular acid–base and lactate status following exhaustive exercise: a comparison between freshwater- and seawater-adapted rainbow trout. *J. exp. Biol.* **156**, 153–171.

TURNER, J. D., WOOD, C. M. AND CLARKE, D. (1983). Lactate and proton dynamics in the rainbow trout (*Salmogairdneri*). *J.exp.Biol.* **104**, 247–268.

WALSH, P. J. AND MILLIGAN, C. L.(1989). Coordination of metabolism and intracelluar acid–base status: ionic regulation and metabolic consequences. *Can.J.Zool.* **67**, 2994–3004.

WARDLE, C. S. (1978). Non-release of lactic acid from anaerobic swimming muscle of plaice *Pleuronectesplatessa* L.: a stress reaction. *J. exp. Biol.* **77**, 141–155.

WOOD, C. M. (1991). Acid–base and ion balance, metabolism and their interactions, after exhaustive exercise in fish. *J.exp.Biol.* **160**, 285–308.

WOOD, C. M., TURNER, J. D. AND GRAHAM, M. S. (1983). Why do fish die after severe exercise? *J. Fish Biol.* **22**, 189–201.

WOODBURY, J. W. (1965). Regulation of pH. In *Physiology and Biophysics* (ed. T. C. Ruch and H. D. Patton), pp. 899–934. Philadelphia, London: W. B. Saunders Company.