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**FACTORS GOVERNING PROLONGED SWIMMING PERFORMANCE  
OF JUVENILE RAINBOW TROUT (*Oncorhynchus mykiss*)**

**By  
WENDY J. McFARLANE, B.Sc.**

**A Thesis  
Submitted to the School of Graduate Studies  
in Partial Fulfilment of the Requirements  
for the Degree  
Doctor of Philosophy**

**McMaster University**

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## **FACTORS GOVERNING SWIMMING PERFORMANCE OF RAINBOW TROUT**

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## **ABSTRACT**

This study investigated the factors governing prolonged swimming performance in juvenile hatchery-reared rainbow trout. The primary exercise test used was a fixed velocity sprint test in which fish were rapidly accelerated to a speed of 6-7 body lengths  $s^{-1}$ . Time-to-fatigue was recorded as the measure of endurance. The ultimate goal of this research was to develop a model describing the limitations of sprint performance in juvenile trout under conditions of imposed velocity. This was accomplished through the completion of five studies. The specific objectives of the first study were to examine the effects of varying the rate of acceleration and the influence of experience (i.e. training) on sprint endurance. The second study examined the relationship between endurance and fuel depletion in white muscle [i.e. glycogen, phosphocreatine (PCr) and ATP], to determine whether a specific metabolic change within the muscle evoked fatigue. The dependence of endurance on muscle glycogen levels was also assessed by manipulating glycogen in two ways, through elevated ration and through sprint training. The third study examined the effect of creatine supplementation on muscle total creatine levels and endurance to determine the importance of creatine and PCr stores. The fourth study exploited individual variability in sprint performance to correlate other physiological performance measures [e.g. growth, critical velocity ( $U_{crit}$ ) and response to induced stress] with endurance. The fifth and final study examined the effect of oxygen supply on swim performance. Together, these five studies have produced the following results: slowing the rate of acceleration and periodic sprint training both increase endurance; chronic stress reduces the ability to train; the reduction of ATP appears to evoke fatigue in both  $U_{crit}$  and sprint tests;



glycogen levels do not determine endurance; creatine loading enhances endurance *via* a mechanism independent of muscle free creatine and PCr levels; fish with high sprint performance have a higher  $U_{crit}$ , but most other physiological performance measures do not correlate with swim performance;  $U_{crit}$  performance is oxygen diffusion limited in high performers, but is more convectively limited in low performers; convection limitations are more important following a rapid acceleration in both groups; and neither sprint nor  $U_{crit}$  performance is influenced by blood oxygen carrying capacity. This study has led to the conclusion that endurance is determined by cardiovascular response, specifically the ability to perfuse white muscle, rather than by the amount of fuel in the muscle to power swimming.

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## CHAPTER 1

### GENERAL INTRODUCTION AND OBJECTIVES

#### **Introduction**

Swimming is perhaps the most commonly observed activity in fish. Swimming activities have been broadly characterized into three main types: i) sustained swimming, which by definition, lasts for > 200 min, ii) prolonged swimming which lasts from 20 seconds to 200 min, and ends in fatigue, and iii) burst swimming, which is of shorter duration (< 20 seconds), but also ends in fatigue (Brett, 1967; Beamish, 1978). Prolonged swimming activities are probably the most ecologically relevant for stream-dwelling salmonids. This is likely the type of swimming involved in such routine activities as food acquisition, predator avoidance and habitat selection. The main goal of this study was to investigate the factors that limit prolonged swimming performance. The general approach was to use hatchery-reared juvenile rainbow trout, with little to no high speed swimming experience, to study the physiological response to swim tests involving imposed velocities. The following sections describe the development and use of swim performance tests and provide the rationale for the specific objectives of this study.

#### *Development of swim tests*

For decades, researchers in the laboratory have mimicked the challenges that fish face when swimming in current through the use of swim flumes or swimming respirometers, where water velocity and rate of acceleration can be precisely controlled. To enable the quantitative assessment of swim performance J.R. Brett, in 1964, developed

two methodologies, the critical velocity, or  $U_{crit}$  test, and the fixed velocity sprint test. The former is a stepwise, incremental velocity swimming test designed to measure the critical swimming velocity (i.e., a close approximation of the maximum sustainable swimming speed, a speed that can be maintained for >200 min; Brett, 1967) and is presumed to be a reasonable estimate of the aerobic capacity of a fish swimming at steady-state (Hammer, 1995). The latter measures endurance (in min) following a rapid acceleration to a prolonged (i.e., non-sustainable) swimming speed, where fish are swimming at non-steady state.

#### *Historical use of $U_{crit}$ and sprint tests*

The  $U_{crit}$  test is the most widely used measure of swimming performance. This is likely because it is considered a surrogate measure for maximum oxygen consumption ( $VO_2max$ ; Beamish, 1978; Thorarensen et al., 1993; Hammer, 1995). In addition,  $U_{crit}$  tests are commonly employed to examine species differences (e.g. Beamish, 1978), test the effect of training (e.g. Farlinger and Beamish, 1978; Farrell et al., 1990; Thorarensen et al., 1993), investigate the effect of individual differences in growth rate on performance (e.g. Kolok and Oris, 1995; Gregory and Wood, 1998), and measure the effect of toxicant exposure (e.g. Waiwood and Beamish, 1978; Alsop et al., 1999; Hollis et al., 1999). Fixed velocity sprint tests have also been used to examine species differences (e.g. McDonald et al., 1998a,b), the effect of training (e.g. Nahhas et al., 1982; Houlihan and Laurent, 1987), the relationship between growth rate and performance (e.g. Gregory and Wood, 1998) and the influence of toxicant exposure (e.g. Alsop et al., 1999; Taylor et al., 2000). However, in general, sprint performance has been examined much less often than maximum sustainable swimming performance (Hammer, 1995).



### *Importance of studying sprint performance*

Although the  $U_{crit}$  test has proven to be a useful tool for determining the maximum sustainable swimming speed, the situation where a fish is gradually accelerated to a fatiguing velocity over a period of hours is unlikely to occur in nature. In contrast, a sprint is a more likely activity. However, the factors that cause fish to stop swimming during a sprint are not well understood. The lack of information on sprint performance has led to a number of general questions including the following: what is the effect of acceleration rate on endurance? Acceleration rate is a major determinant of  $U_{crit}$  (Farlinger and Beamish, 1977), however the effect of acceleration rate on sprint endurance is unknown. How does experience influence endurance? Previous studies have shown only modest effects of training on such endpoints as  $U_{crit}$ , acceleration speed and distance swum in a given time period (see Davison, 1997 for review), however, training effects on endurance at fixed velocity have not been established. What metabolic changes coincide with fatigue? The metabolic changes associated with exhaustion have been well documented (see Milligan, 1996 and Kieffer, 2000 for reviews), however the factors leading to fatigue, which sets in before the point of exhaustion (McDonald et al., 1998a), are less well documented. Does the amount of muscle glycogen determine endurance? Previous studies have linked higher muscle glycogen with elevated lactate production during exercise (Pearson et al., 1990; Schulte et al., 1992), faster recovery (Scarabello et al., 1991) and greater survivability upon stream release (Hochachka and Sinclair, 1962). However, whether the amount of on-board fuel actually determines endurance is not clear. Similarly, does the amount of muscle total creatine (i.e., free creatine and PCr) influence endurance? Human studies show that dietary creatine supplementation leads to enhanced muscle creatine and PCr stores and a corresponding increase in high intensity exercise performance (see Terjeung et al., 2000 for review). However, it is unknown whether fish possess an ability to

accumulate creatine and if there are resulting ergogenic effects on muscle performance. Does sprint performance correlate with other performance measures? A number of fairly recent studies have recognized and exploited the large degree of individual variability in swim performance to correlate it to other physiological measures such as body shape, growth rate, cardiac output, and hematocrit (see Kolok, 1999 for review). However, the majority of studies have focused on  $U_{crit}$  performance, and less is known of the measures that correlate with sprint performance. How does oxygen supply affect endurance? The effects of hypoxia and hyperoxia on  $U_{crit}$  performance have been studied (e.g. Davis et al., 1963; Dahlberg et al., 1968), yet, the effect of oxygen supply on sprint performance has never been investigated.

Together, these observations will further our understanding of prolonged swimming performance and allow for the development of a model that describes factors limiting endurance when fish are accelerated to non-sustainable swimming speeds.

## **Objectives**

The specific goals of this thesis were:

1. To examine the effects of acceleration rate on sprint endurance; whether experience acquired through repeated sprint exercise leads to changes in endurance; and if the specific type of training regime (either repeated sprint exercise, continuous aerobic exercise at  $1.5 \text{ BL s}^{-1}$  or manual chasing to exhaustion once every 2 days) influences sprint endurance (Chapter 2).
2. To examine the relationship between white muscle fuel depletion and fatigue in a sprint test; whether a specific metabolic change within muscle coincided with fatigue; if metabolic changes at fatigue were similar regardless of the type of swim test (i.e., sprint test vs.  $U_{crit}$  test); the dependence of endurance on muscle glycogen levels;

and to what degree fuel use patterns are altered as a result of various training regimes (Chapter 3).

3. To determine whether creatine and PCr stores can be altered in fish through supplementing with exogenous creatine *via* the diet or intraperitoneal injection, and to determine whether creatine supplementation leads to greater endurance (Chapter 4).
4. To exploit individual variability in sprint performance to examine physiological correlates of performance, such as growth,  $U_{crit}$ , response to induced stress (epinephrine injection or toxicant exposure), resting muscle glycogen, lactate, PCr and ATP, glycolytic and oxidative enzyme activities, oxygen carrying capacity (i.e., hemoglobin levels), and plasma cortisol (Chapter 5).
5. To examine the dependence of  $U_{crit}$  and sprint performance on oxygen supply by sprinting fish under hypoxic, normoxic and hyperoxic conditions; to determine whether differences in resting oxygen consumption reflect performance ability, and whether swim performance can be predicted by oxygen carrying capacity of the blood, by measuring blood hemoglobin and methemoglobin (Chapter 6).

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## CHAPTER 2

### RATE OF ACCELERATION AND EXPERIENCE AFFECTS PROLONGED SWIM PERFORMANCE OF JUVENILE RAINBOW TROUT

#### Summary

This study examined the endurance, measured as time to fatigue, of juvenile trout swimming at velocities above the threshold for white muscle recruitment. Fish were accelerated to a speed approximately equal to their critical swimming velocity ( $U_{crit}$ , a close approximation of a speed sustained for >200 min; Brett, 1967) and sprinted until they could no longer maintain station in current. Several factors influenced endurance, the most prominent of which were the rate of acceleration and experience. Fish rapidly accelerated to  $U_{crit}$  fatigued in less than 5 min once the final velocity was reached. Slowing the acceleration rate by 30-fold improved endurance at the final velocity by as much as 12-fold. Similarly, repeated sprints once daily for as few as 5-7 days produced up to a 10-fold increase in endurance. Slowing the acceleration rate produced a uniform improvement in endurance, whereas the effects of experience on endurance were more variable. The amplitude of the training effect depended on temperature, body size, interval between sprints and the type of experience (e.g. sprinting vs. continuous swimming at low speed vs. manual chasing to exhaustion). Periodic fatigue was not necessary to elicit a training effect. We conclude that training is largely the result of fish 'learning' to more rapidly 'tune-up' white muscle to peak aerobic efficiency, a conclusion based on previous studies

in trout that reported slow cardiovascular responses to rapid increases in imposed swimming velocity. Stress and incomplete recovery from anaerobic debt (and a possible interaction between the two) appear to be the main reasons for a lack of training. This study suggests that performance under conditions of imposed velocity may be very different from voluntary performance and raises doubts concerning the ecological relevance of swim flume-derived performance measures. At the very least, the dramatic and variable results of experience must be considered in any future measures of swimming performance.

## **Introduction**

Much of the effort to characterize swimming performance of fish has been devoted to essentially two extremes: the maximum achievable or 'burst' velocity and the maximum sustainable or 'critical' velocity. The former is typically an escape response which is very brief in duration (<20 sec; Beamish, 1978), while the latter is the result of gradual stepwise acceleration until a velocity is reached at which the animal fatigues. A time-weighted average of the last two velocities is then calculated and termed the critical velocity ( $U_{crit}$ ). The test is designed so that a velocity at this value should closely approximate the maximum sustainable swimming speed; sustainable for at least 200 min (Brett, 1967). Both critical and burst velocities have been measured on a wide variety of fish species using well established protocols, and considerable data comparing species and examining effects of such variables as season, temperature and body size have been reported (e.g. reviews by Beamish, 1978; Hammer, 1995, Domenici and Blake, 1997).

Relatively less attention has been paid to swimming performance in that broad region between the two extremes, the region where most of the swimming behaviour of salmonids lies. One approach for assessing intermediate (i.e., prolonged) swimming



performance, pioneered by Brett (1964), is the fixed velocity test. In this test, fish are rapidly accelerated to a constant velocity and the time to fatigue recorded. This test is a surrogate for one common type of activity, the circumstance where a fish darts into current and holds station until falling back or exiting the current. Recently, McDonald et al (1998) further developed the fixed velocity test as a tool for assessing prolonged swim performance in fish. In their study, the authors measured the fixed velocity sprint performance of three species of stream dwelling salmonids, and showed that fatigue time (FT) was reproducible in repeat trials and scaled with body size ( $\text{Length}^4$ - $\text{Length}^5$ ). Moreover, they showed that when fish were rapidly accelerated to  $U_{\text{crit}}$  (6-7 BL  $\text{s}^{-1}$  for fingerlings) they would fatigue in a few minutes instead of sustaining swimming for >200 min.

Previous studies have, indeed, shown that the rate of acceleration affects the outcome of a  $U_{\text{crit}}$  test (e.g. Farlinger and Beamish, 1977; Jain et al., 1997). These findings have led to specific recommendations concerning both the duration and magnitude of the increments in velocity (c.f. Hammer, 1995). Jain et al (1997) further showed that the duration of the  $U_{\text{crit}}$  test could be shortened by rapidly accelerating fish to 75% of  $U_{\text{crit}}$  (approximately the white muscle recruitment threshold) then completing the test with the usual small, stepwise accelerations. This modification had no effect on the outcome of the test. However, at velocities >75%  $U_{\text{crit}}$  little is known about the effects of acceleration rate on performance.

The role of experience in modifying swim performance in flumes is also not well established. However, some studies have shown that conditioning fish to both the flume and the testing protocol is necessary to achieve reproducible results (e.g. Farlinger and Beamish, 1977). In any case, those studies on teleosts that have examined the effects of training on swim performance have typically reported rather modest effects. For example, repeated high speed sprints (every other day for 9 weeks) had no effect on either maximum

burst speed, acceleration rate or  $U_{crit}$  (Gamperl et al., 1991), and only a minor effect on total distance covered (14% increase, Pearson et al., 1990). Also, continuous low speed aerobic exercise for weeks to months had either minor effects on  $U_{crit}$  (15% increase after 30 days at 3 body lengths  $s^{-1}$ ; Farlinger and Beamish, 1978) or no effect (no increase after 8 months at 1.5 BL  $s^{-1}$ , Thorarensen et al., 1993). Indeed, these and other related studies led Davison (1989) to conclude that teleost fishes show little plasticity in response to exercise training compared to mammals. However, it could be argued that the modest outcomes resulted either because the training protocol was highly stressful (e.g. Gamperl et al., 1991) or simply because the training regimes were not well matched to the testing regime.

Thus the main objectives of this study were to examine in detail the effects of acceleration and experience on sprint performance in rainbow trout. Our main approach with the latter was to match the experience to the test, so the primary training regime was the fixed velocity sprint administered repetitively. The effect of body size, temperature, the interval between sprints, and the presence/absence of periodic fatigue on the training outcome were examined. Training effects associated with two other training regimes used in previous studies; continuous aerobic exercise (e.g. Thorarensen et al., 1993) and periodic high speed burst exercise (e.g. Pearson et al., 1990; Gamperl et al 1991) were also examined. To further characterize factors influencing endurance, the effects of sprinting fish in groups *vs.* individually and the influence of acclimation temperature on sprint performance were investigated. Behavioural changes occurring with training were also monitored.

One key concern in this study was to reduce individual variability that might be associated with prior experience. Consequently, the choice of experimental animal was very young rainbow trout (<5 months post-hatch in most instances) of hatchery origin,

where the nature of the rearing conditions are such that they would have had little or no high speed swimming experience prior to experimentation.

## **Materials and methods**

### *Experimental animals*

All but one of the swim trials were performed at McMaster University using fingerling rainbow trout (*Oncorhynchus mykiss* Walbaum) obtained from Rainbow Springs Trout Hatchery (Thamesford, Ontario) and ranging in weight from 1 to 20 g. In one trial, larger fish from the same source were used (52-144 g; mean  $97 \pm 8$  g,  $N = 15$ ), and experiments were conducted at the University of Waterloo (Waterloo, Ontario). Fish at both locations were held in circular tanks with a continuous flow of aerated, de-chlorinated water. Temperature fluctuated seasonally, so that experimental temperatures ranged from 7 - 18° C. Fish were fed a ration of approximately 4% body weight per day (estimated by the weight of all fish within a tank) of commercial trout chow (Corey Feed Mills, Ltd.), fed over 2 meals, except for the acceleration trial in which they were fed a 2% ration. Fish were starved for approximately 12 h prior to swim testing, regardless of holding temperature, to minimize post-prandial effects.

### *Exercise apparatus*

Exercise tests at McMaster University were performed using an open, recirculating swim flume (102 L volume) designed according to the recommendations of Vogel (1978). Fish were tested in groups (typically 10 per trial) in most swim procedures. The fish were placed in the rectangular working section of the flume. The dimensions were 73 x 11 x 20 cm (L x W x H), with a curved bottom to eliminate eddy effects. Flow was generated by a propeller connected to a 373-W DC motor (Dart Controls, Inc., Zionsville, IN), and flow

was calibrated using an electromagnetic flow meter (Marsh-McBirney Model 2000). The realistic upper limit of velocity was  $70 \text{ cm s}^{-1}$ . When higher speeds were required ( $80\text{-}100 \text{ cm s}^{-1}$  to fatigue larger fish), a closed Blazka-type respirometer (c.f. Beamish, 1978) was used at the University of Waterloo. This respirometer had a total volume of 112 L and the working section was a cylinder 123 x 24 cm (L x D).

### *Swim test procedures*

Two types of swim tests were used: a fixed velocity sprint test and a critical velocity ( $U_{\text{crit}}$ ) test.

i) Sprint: The sprint procedure was as described by McDonald et al (1998). Fish were sprinted in groups or individually. The group size was typically ten for fish of 1-5 g mass, five for fish of 15-26 g and one for fish of 52-144 g. Group size was adjusted to ensure that blocking effects and altered flow dynamics were minimal (c.f. Jones et al., 1974). For the first 5 min in the flume an orienting velocity of  $\sim 1$  body length (BL)  $\text{s}^{-1}$  was provided. Over the next 2 min, velocity was steadily increased to a test velocity of 30 to  $100 \text{ cm s}^{-1}$  (depending on body size), or a relative speed of 5-7 BL  $\text{s}^{-1}$ . In general, test velocities were chosen that were equal to or slightly less than measured  $U_{\text{crit}}$  values for fish of the same size. Fish were removed from the flume as they fatigued (see below for fatigue criteria), time to fatigue, length and weight were recorded and the fish returned to the holding tank. Mean time to fatigue (in min) in each trial was calculated as the geometric mean (i.e., mean of the log sprint time) as recommended by Brett (1964). Such a calculation produces an asymmetric standard error of the mean, but since the upper and lower errors were rarely very different, they have been averaged and expressed as a single error.

ii) Critical velocity: The 30-minute, incrementally stepped velocity test was performed as outlined by Brett (1967) using fish of 1-5 g. Fish were transferred to the

flume in groups of 10 and oriented for 30 min at a starting velocity of  $1 \text{ BL s}^{-1}$ . Velocity was rapidly increased by  $1 \text{ BL s}^{-1}$  ( $5\text{-}8 \text{ cm s}^{-1}$  in most trials) every 30 minutes until fatigue. Each velocity increase was completed within 2-3 seconds, i.e., a faster acceleration than in sprint trials.  $U_{\text{crit}}$  was calculated according to the following expression from Brett (1964):

$$U_{\text{crit}} = U_p + \left( \left( \frac{t_f}{t_i} \right) + U_i \right)$$

where  $U_i$  is the velocity increment ( $\text{cm s}^{-1}$ ),  $U_p$  is the penultimate velocity at which the fish swam prior to fatigue,  $t_f$  is the elapsed time from the velocity increase to fatigue, and  $t_i$  is the time between velocity increments.

#### *Fatigue endpoint*

Previous exercise studies using salmonids (Dobson and Hochachka, 1987; Parkhouse et al., 1988) have made a distinction between fatigue and exhaustion which we have employed here. Fatigue is the point at which fish can no longer maintain the required velocity, but can resume swimming if exposed to a lower velocity. Exhaustion, on the other hand, is the point at which the fish loses equilibrium and cannot maintain swimming at any speed. In the present study, the first sign of fatigue was when the fish lost its position in the flume and drifted to the back screen. Upon contact with the back screen the fish was encouraged to resume swimming by manual prods to the tail and would usually do so two to three times. Once it became unresponsive it was immediately removed from the flume and time recorded. The same fatigue criterion was used for both sprint and  $U_{\text{crit}}$  tests.

### *Monitoring performance of individual fish*

In one sprint training trial (trial #3, Table 2-1) performance of individuals on successive days was monitored using Passive Integrated Transponder (PIT) tags (2 x 12 mm, 134.2 kHz, Destron Fearing, Inc.). To implant the tag, fish were lightly anaesthetized with tricaine methane sulphonate (MS-222, Syndel Laboratories, 0.8 g L<sup>-1</sup>, neutralized to pH 7.5) and the tag injected into the body cavity. The wound was then sealed with a drop of Vet Bond tissue adhesive (Minnesota Mining and Manufacturing (3M)), eliminating the need for sutures and preventing loss of the tag. Tag numbers were read using a hand-held scanner (Pocket Reader EX; Destron Fearing, Inc.). Fish were recovered for one week prior to experimentation. Initial trials showed that the tag did not impair either swim performance or survival of fish as small as 1 g.

### *Experimental series*

#### 1. Effects of swimming fish in groups

This experiment tested whether swim performance was altered by swimming fish in a group as opposed to individually. From an initial trial on 30 fish ( $1.6 \pm 0.5$  g), swum in 3 groups of 10 each, the 10 fish that exhibited the lowest performance were selected. One day (24 h) later, this group was sprinted as a group to assess whether their performance was any different. One day after that, each fish was then sprinted alone in the flume.

#### 2. Effect of acceleration rate and temperature on sprint performance

In this experiment, the time taken to accelerate fish to the test velocity ('ramp-up' time) was increased from 2 min to either 16, 30 or 60 min. Thirty fish were sprinted (in 3 groups of 10) at each acceleration rate (i.e., total N=120; weight= $1.5 \pm 0.04$  g) and fatigue times recorded. This procedure was carried out at 7° C and 15° C on the same fish. Fish

originally tested at 7° C were warmed from 7° C to 15° C over a period of one week (at ~1° C d<sup>-1</sup>) and held for an additional 3 days at 15° C prior to testing.

### 3. Effects of experience on sprint performance

Three different types of experience were tested for their effects on sprint performance: multiple repeat sprints at the same test velocity (approximately equal to  $U_{crit}$ ), continuous low level (i.e., aerobic) swimming, and repeated manual chasing for a fixed period (8 min). The effects of all three regimes were evaluated on the basis of their effect on fatigue time and specific growth rate, relative to inexperienced (i.e., control) fish.

#### 3. i) Repeated sprints

In total, 10 sprint training trials (9-30 fish per trial) were completed. Trials were 5 to 14 days in length and were subdivided into four different groups to test the variables listed below. Within each group, the trials were matched as closely as possible for the variables not being tested (e.g. size, temperature, sprint velocity, and sprint frequency). Each regime continued until at least 5 sprints were completed.

- a) Temperature. Two trials; one at 7° C (trial 1, Table 2-1) and one at 16° C (trial 2, Table 2-1). Fish were sprinted once daily for at least 6 sprints.
- b) Body size. Three different size classes,  $2.1 \pm 0.2$  g (trial 3, Table 2-1);  $20.4 \pm 0.8$  g (trial 4, Table 2-1) and  $97.1 \pm 7.8$  g (trial 5; Table 2-1). Fish in each size class were sprinted once daily for at least five days.
- c) Interval between sprints. Three sprint regimes were compared: every other day (trial 6, Table 2-1), once a day (trial 7, Table 2-1) and twice a day (trial 8, Table 2-1). For the latter, the second daily trial started 30 min after the completion of the first trial.
- d) Effect of fatigue. Two trials. In one trial (trial 9, Table 2-1) fish were accelerated daily to a velocity of  $45 \text{ cm s}^{-1}$  ( $\sim 7 \text{ BL s}^{-1}$ ) and swum to fatigue

for 5 successive sprints. In the second trial (trial 10, Table 2-1) fish were treated identically except the daily sprint was terminated as soon as the 2 min acceleration period was complete.

### 3. ii) Continuous aerobic exercise

In this trial (trial 2, Table 2-2), 10 fish ( $1.2 \pm 0.2$  g) were swum continuously at  $5 \text{ cm s}^{-1}$  ( $1.5 \text{ BL s}^{-1}$ ) for 14 days at  $7^\circ \text{ C}$ , with speed reduced twice daily to  $0.5 \text{ BL s}^{-1}$  for 20 min to enable two feedings ( $2\%$  body mass  $\text{d}^{-1}$  each). Controls for this trial were taken from the same cohort and were maintained on an identical ration and temperature but remained in the holding tanks. Specific growth rate ( $\%$  body weight  $\text{d}^{-1}$ ) was assessed in both groups by recording total tank weights at the beginning and end of the 14 day period. Growth rate was calculated according to the following equation:

$$\text{SGR} = \frac{\ln\left(\frac{w_f}{w_i}\right)}{t_d} \times 100$$

where SGR is specific growth rate as a percentage of body weight per day,  $w_i$  is initial tank bulk weight,  $w_f$  is final tank bulk weight, and  $t_d$  is the experimental period in days. At the end of the trial, both aerobically-trained fish and untrained controls were sprint tested at  $7 \text{ BL s}^{-1}$ . This trial was carried out at the same time as sprint training trial # 1 (Table 2-1), and variables such as temperature and body size were closely matched.

### 3. iii) Repeated chasing

In this trial (trial 3, Table 2-2), 10 fish ( $1.1 \pm 0.3$  g) were chased to exhaustion for 8 min once every 2 days for 14 days (a similar protocol to Pearson et al (1990)), and returned to holding tanks for feeding and recovery. Experimental temperature, and fish body size were closely matched to both 3i (trial 1; Table 2-1) and 3ii (trial 2; Table 2-2).



### *Statistical Analysis*

Reproducibility of sprint performance was assessed through Spearman rank correlation analysis (Zar, 1996). Comparisons of treatment groups with respective controls were tested by one-way ANOVA. If the result of the ANOVA was significant, a Tukey-Kramer HSD test for multiple comparisons was applied to test for significant differences among groups. When a comparison between only two groups was required, a Dunnett's t-test was performed. Differences at  $P < 0.05$  were considered significant. All statistical tests were performed using JMP 2.0.5 software (SAS Institute, Inc.).

## **Results**

### *Behaviour in the swim flume*

When fish were transferred to the swim flume for the first time, most responded by darting back and forth, both at zero flow and for most of the orientation period (5 min at  $1 \text{ BL s}^{-1}$ ). However, each fish had assumed a stationary position by the end of this period and the group had distributed themselves uniformly throughout the working section of the flume. In sprint tests, some fish accelerated smoothly once the ramp-up began, maintaining a constant position, while others exhibited more erratic burst-and-glide swimming throughout the acceleration, periodically reversing (i.e., moving backwards with the current) and contacting the back screen. The latter tended to be the first to fatigue. In the  $U_{\text{crit}}$  trials, the velocity increments while small, were virtually instantaneous. All fish showed a very brief period of erratic swimming movements in response to each velocity increment, yet settled into a steady-state mode of swimming in less than 2 min. At the final velocity increment, fish that exhibited prolonged erratic behaviour were typically the first to fatigue.

### *Individual variability in sprint performance*

Individual fatigue times (FT) varied considerably in each trial, typically from 30 s to 3 min (e.g., Fig. 2-1A). Scaling to body size explained a significant fraction (~30%) of this variability (e.g., Fig. 2-1A) and an exponential relationship provided the best fit (i.e.,  $FT = a \text{Weight}^b$ ). The scaling exponent (b) ranged from 1.2 to 1.7 (based on nine trials with 22-60 fish per trial,  $\Sigma N = 353$ ) but the  $r^2$  was rarely greater than 0.3, meaning that approximately 70% of the variability in FT was not explained by body size variations.

Individual performance was reproducible. This was illustrated by separating the 10 lowest performers from a group of 30 fish, and then sprinting them again the following day (Fig. 2-1B). This group exhibited essentially the same average performance (i.e., no significant improvement in FT even though the better performers were no longer present; Fig. 2-1B). When the same fish were sprinted individually one day later (Fig. 2-1C), again, overall performance did not change significantly, and fish fatigued in a similar rank order to the previous day (Spearman rank coefficient;  $R_s=0.67$ ;  $p<0.05$ , with individuals identified based on body weight). This again confirmed the reproducibility of individual performance and also illustrated that swimming fish in groups, as opposed to singly, does not affect individual performance.

### *$U_{crit}$ performance vs. sprint performance*

The  $U_{crit}$  for fingerling trout ( $2.4 \pm 0.1$  g;  $5.8 \pm 0.1$  cm) at  $14^\circ$  C was  $38 \pm 0.8$  cm  $s^{-1}$  or  $6.8 \pm 0.1$  BL  $s^{-1}$  ( $N = 20$ ). In this measurement, acceleration to the final velocity ( $42$  cm  $s^{-1}$ , a velocity higher than the critical velocity) took approximately 210 min from the initial orientation period at  $1$  BL  $s^{-1}$ , and the average duration at this final velocity was  $8.9 \pm 1.5$  min. At the critical velocity, fish should be able to sustain swimming for at least 200

min (not tested in this study). In contrast, when fish of comparable size and at the same temperature were rapidly accelerated (over 2 min) to  $35 \text{ cm s}^{-1}$ , a velocity slightly lower than the critical velocity, they fatigued in an average time of  $2.3 \pm 0.6 \text{ min}$  ( $N = 20$ ).

#### *Effect of acceleration rate and temperature on sprint performance*

At  $7^\circ \text{ C}$ , increasing the acceleration period (slowing the acceleration rate) from 2 min to 60 min progressively increased endurance (Fig. 2-2, open symbols). Fish that were accelerated to a sprint velocity of  $35 \text{ cm s}^{-1}$  over 60 min had an 8-fold greater endurance than fish accelerated to the same velocity over 2 min ( $36 \pm 5$  vs.  $3.3 \pm 0.5 \text{ min}$ ). Warming these same fish to  $15^\circ \text{ C}$  over 2 weeks led to a significant increase in sprint performance when the fish were tested using the same acceleration rate regime. With a two min acceleration period, the improvement in performance was 2-fold greater than that measured at  $7^\circ \text{ C}$  (Fig. 2-2). At slower rates of acceleration the improvement in performance was similar regardless of temperature.

#### *Effects of experience on swim behaviour*

The swimming behaviour of most fish changed in response to repeat sprinting. Fish were observed to orient more quickly in low flow. This response typically occurred within 10 seconds by the third or fourth sprint. Furthermore, this behavioural change occurred irrespective of whether fish showed any improvement in endurance. With experience, erratic swimming and burst-and-coast movements also became less common during the 2 min ramp-up period, and were replaced by smooth acceleration to match the increase in water velocity.

### *Variability in the effects of experience*

In most of the repeat sprint trials (7 of 10), training did occur, with significant improvements in mean fatigue time by the fifth sprint or earlier. However, marked individual variability in the training effect was apparent in each sprint trial. An example is shown in Fig. 3 (same fish as trial 3, Table 2-1) where some fish exhibited dramatic improvement in performance while others showed no improvement at all. Two individuals with virtually identical fatigue times on day one (29 s in fish 'a' vs. 39 s in fish 'b') exhibited the full range of response; i.e., an 18-fold increase in performance in 'a' vs. essentially no change in 'b' by day seven (and on day 8, this fish died; Fig. 2-3). In this trial, the persistence of the training effect was also tested. After 8 days of training, fish were allowed 1 week of rest and then re-tested on day 14. On the final sprint there was no decrement in performance, with the average performance being essentially identical to that on day 7 (Fig. 2-3).

### *Factors influencing the training effect*

In those repeat-sprint trials where training occurred, the mean amplitude of the improvement in endurance ranged from 1.6- to 10.6-fold. The variability in training effect amongst all ten trials can be attributed to the following factors (in decreasing order of importance): temperature, body size, interval between sprints, and whether or not the sprint was terminated at fatigue.

#### a) Temperature

After six once daily sprints, the average improvement in fatigue time of fish acclimated to 7° C was 1.6-fold (trial 1, Table 2-1), whereas it was 10.6-fold for fish at 16° C (trial 2, Table 2-1). Furthermore, when fish at the lower temperature were trained for an

additional 8 days, their mean fatigue had only improved by 2.6-fold over initial values (trial 1, Table 2-1).

**b) Body size**

Only fish in the smallest size class ( $2.1 \pm 0.2$  g) showed a significant improvement in mean fatigue time following repeat sprinting (2.5 fold at 5 days, 3.5 fold at 7 days, trial 3, Table 2-1). Neither of the two larger size classes, ~20 g (range of 15-26 g, trial 4, Table 2-1) and ~97 g (range of 52-144 g, trial 5, Table 2-1) showed any significant improvement after 5 sprints, when the trials were terminated. The slightly colder temperature for the ~20 g group, compared to the ~2 g group ( $10^\circ$  vs.  $13^\circ$  C), is unlikely to explain the lack of improvement. In any case, the ~97 g group also showed no improvement, and these fish were tested at an intermediate temperature ( $12^\circ$  C; Table 2-1). In the ~20 g group, the daily sprints were clearly stressful, as indicated by the development of fin erosion (a bacterial disease usually promoted by a stressor such as overcrowding; Soderberg and Meade, 1987) that was not seen in untrained controls. Furthermore, these fish did not eat offered food and lost weight ( $-1.67\%$  BW  $d^{-1}$ ) while their respective controls gained weight ( $2.05\%$  BW  $d^{-1}$ ) on the same offered ration. In the ~97 g fish, there were no obvious external signs of stress, and growth rates were identical for both sprinted fish and non-sprinted controls ( $0.41\%$  BW  $d^{-1}$ ). In this trial, fish were sprinted alone and individuals could be reliably tracked through time by their body weight. However, reproducibility of performance was low, overall individual variability in performance was high, and no improvement in endurance was seen. In this group, fatigue time scaled to body size ( $L^{1.9}$ ;  $r^2=0.39$ ), similar to that of the smallest (2 g) size group ( $L^{1.6}$ ;  $r^2=0.28$ ). In contrast, there was no correlation between size and fatigue time in the intermediate (~20 g) group, however that could simply be explained by the more limited size range (15-26 g).

### c) Interval between sprints

Fish that were sprinted once daily for six days at 12° C showed an average improvement in fatigue time of 5.1 fold (trial 6; Table 2-1). Similarly, fish sprinted on alternate days at 15° C showed a 5.6 fold increase in performance after five sprints or 10 days training (trial 7, Table 2-1). In contrast, fish sprinted twice daily (with at least 30 minutes separating the end of the first sprint to the start of the second sprint) showed no significant improvement in fatigue time after 6 days of training or 12 sprints (trial 8, Table 2-1). Furthermore, performance in the second daily sprint was generally lower than the first sprint, and became progressively worse over time (29% lower on day 1, and 38% lower by day 6).

### d) Presence/absence of fatigue

When fish were accelerated to sprint velocity, but not fatigued, there was a 2.4 fold increase in endurance following only 5 daily sprints (trial 9; Table 2-1). In a comparable trial (i.e., fish were matched according to body size and acclimation temperature) where fatigue was the endpoint, a 2.9 fold increase in fatigue time occurred following 5 sprints (trial 10, Table 2-1). In the latter trial, the total time spent training was approximately 4 times longer than the acceleration only trial (trial 9: 10 min accelerating (2 min x 5 days) plus  $32.3 \pm 4.1$  min sprinting at the test velocity vs. trial 10: only 10 min accelerating).

### *Comparison of sprint training to other training regimes*

Neither the continuously-exercised or the chase-trained fish exhibited any improvement in sprint performance after 14 days of training at 7° C, and in fact, performed no differently than untrained, control fish (Table 2-2). In contrast, the sprint-trained group showed a 2.6 fold improvement in sprint performance (trial 1, Table 2-1). Growth rates were highest for the un-exercised controls (2.9 % day<sup>-1</sup>) and were identical for the chased and sprint-trained fish (2.5 % day<sup>-1</sup>; Table 2-2). There were no outward signs of stress

(such as fin erosion) in either of the groups that would indicate chronic stress. Lower growth in the continuously exercised group ( $1.6\% \text{ day}^{-1}$ ) probably reflects the increased metabolic demands of the continuous exercise.

#### *Effect of sprint training on critical velocity*

Critical velocity was not significantly improved in sprint-trained fish compared to untrained controls (Fig. 2-4 inset) even though they exhibited a 3.5 fold improvement in sprint performance after 7 daily sprints (trial 3, Table 2-1) relative to controls. Furthermore, when ramp-up time was extended to 30 min, the difference in endurance between the control and trained groups disappeared (Fig. 2-4). If a linear relationship is assumed between ramp-up time and sprint performance (see Fig. 2-2), an untrained fish would need a 16 min ramp-up to achieve the same performance as a trained fish achieved after 2 min (Fig. 2-4; dotted line).

## **Discussion**

This study describes behaviour and performance of juvenile trout in a swim flume where the rate of acceleration to, and swimming at, a fixed velocity were imposed, not voluntary. Trout rapidly accelerated to a swim velocity at, or just below, critical velocity (a velocity that should be sustainable for >200 min) fatigued in a few minutes. Endurance was improved by slowing the acceleration rate (Fig. 2-2) and by increasing the fish's experience with rapid acceleration (Fig. 2-3). Nonetheless, the effects of acceleration rate and of training, while substantial, did not appreciably close the gap between endurance in the  $U_{\text{crit}}$  trial and endurance in the sprint trial. Increasing the acceleration period from 2 to 60 min (a 30-fold reduction of the acceleration rate) led to an increase in endurance of 33 min (from 3 min to 36 min; Fig. 2-2). To achieve >200 min endurance (sustained

performance), we estimate (by linear extrapolation from Fig. 2-2) that the acceleration period would have to be increased to over 3 hours. Similarly, the maximum effect of training was an endurance of 24 minutes (a nearly 11-fold improvement after 6 daily sprints; trial 2, Table 2-1), still far from 200 min.

When a fish is swimming at its critical velocity, a significant fraction of its propulsion must be powered by white (or mosaic) muscle since white muscle is recruited at sub- $U_{crit}$  velocities (70-94% of  $U_{crit}$  in salmonids; Webb, 1971b; Hudson, 1973; Wilson and Egginton, 1994). Thus, for a fish to sustain swimming at  $U_{crit}$ , white muscle must be functioning aerobically, i.e., oxygen demand and supply must be closely matched. If, instead, a fish fatigues when swimming at  $U_{crit}$ , there must be a mismatch between oxygen demand and supply, and it follows that the larger the mismatch the more rapidly fatigue will occur. It further follows that the general effects of slowing the acceleration rate and of training are the same, and that is to reduce the mismatch, assuming that muscle fuels are not a limiting factor.

#### *Effects of acceleration rate*

There are a number of observations of transient effects of rapid acceleration that could explain the mismatch. These include an initial period of unsettled burst-and-coast swimming (Webb, 1971a,b), hyperactivity of white muscle (Hudson, 1973), a 'spike' in oxygen consumption interpreted as a metabolic response to the shock of the velocity change (Brett 1964; Brett and Glass, 1973), and a lag in the response of the cardiovascular system (Stevens and Randall, 1967; Kiceniuk and Jones, 1977). The first two (inefficient or metabolically costly swim behaviours and hyperactivity) will contribute to a transient elevation in the oxygen demand of white muscle above the steady-state demand while the lag in cardiovascular response would undoubtedly compromise oxygen supply to white



muscle, all of which would provoke anaerobic metabolism, even at sub-maximal swimming speeds (Wokoma and Johnston, 1981; Puckett and Dill, 1984).

Although each of these factors would amplify the mismatch, their relative importance is likely to change with time. In fish that are forced, for the first time, to rapidly accelerate, hyperactivity and inefficient swimming behaviours are probably of primary importance in determining fatigue. However, even inexperienced trout settled quickly into a more economical mode of swimming and with experience that adjustment was even more rapid, irrespective of whether that individual showed an improvement in sprint endurance.

In contrast, previous studies suggest that cardiovascular adjustments may take much longer. For example, Kiceniuk and Jones (1977) showed, in rainbow trout accelerated to 75% of  $U_{crit}$ , that heart rate took from 3-15 min to reach a maximum value following an increase in swim velocity, and blood pressure took over 30 minutes to stabilize. As far as we are aware, the transient delays in cardiovascular adjustments with imposed velocity increments have not been systematically investigated in fish, nor are the specific effects on white muscle blood flow known. Nonetheless, it is worth noting that Thorarensen et al (1996) reported that it took about 10 min for cardiac output and arterial pressure to stabilize in rainbow trout in response to relatively small increments in velocity ( $\sim 0.25 \text{ BL s}^{-1}$ ).

In any case, in mammals the above cardiovascular adjustments occur rapidly at the onset of exercise; cardiac output and blood pressure typically stabilize after about 1-2 min at a moderate workload (e.g.  $\sim 75\text{-}80\% \text{ VO}_2\text{max}$ ; Smith et al., 1976; Guyton, 1981). That cardiovascular adjustments do not occur rapidly in fish, at least under circumstances of imposed swimming, suggests that they may be centrally suppressed and that such suppression has adaptive value. As to what the adaptive value may be is unclear, but it could be related to preventing high transmural pressure at the gills (i.e., a mechanism to

protect the integrity of gill lamellae). In any case, the delay in cardiovascular adjustments seems the most likely explanation for the long time it takes a fish to adjust to swimming at high velocity.

#### *Effect of experience on sprint performance*

Following the above line of logic, it can be argued that the main effect of training was to speed up cardiovascular adjustments. Although highly speculative, it is a conclusion that is at least consistent with the observations. First, the training effect appears to be centered primarily on adjustments occurring in the ramp-up period. Fish that were simply repeatedly accelerated and not sprinted to fatigue exhibited a comparable improvement in endurance (trial 9, Table 2-1) to those fish that were similarly accelerated and then sprinted (trial 10, Table 2-1). Second, the main effect of sprint training was to shorten the adjustment period. Trained fish reached the same level of endurance with only 2 min of ramp-up, while inexperienced fish needed 16 min of ramp-up to reach the same performance (Fig. 2-4). Third, there was no increase in  $U_{crit}$  resulting from sprint training (Fig. 2-4, inset) which suggests that there was no increase in aerobic capacity ( $U_{crit}$  measurements can be regarded as a surrogate for  $\dot{V}O_{2max}$ ; Hammer, 1995). In fact, an increase in aerobic capacity would be highly unlikely in the short time frame of the training regimes.

Temperature was also important to the training effect. An increase in temperature not only increased endurance in untrained fish (with a  $Q_{10}$  of around 2), but it also amplified the training effect (from 2 fold at 7° to 10 fold at 16° C, trials 1 and 2, Table 2-1). The effect of temperature on sprint performance is relatively easy to explain. Temperature has direct effects on both muscle and cardiac performance in fish, and essentially acts to increase the efficiency of muscle contraction, allowing more rapid velocity of shortening in swimming muscle (Sidell et al., 1989; Johnson et al., 1996) and higher maximum cardiac

output (Farrell, 1997). It is therefore possible that training results in improved muscle efficiency, i.e., a lower energy requirement for similar power output. However, the effect of temperature on the amplitude of the training effect is much harder to explain. The difficulty lies with our lack of understanding of the specific effect of training. If, as we believe, sprint training is essentially teaching fish to speed up adjustments to cardiovascular performance that are normally made at a slower rate, then we have to conclude that the higher temperature is facilitating learning. We are unaware of any evidence that learning in salmonids is influenced by temperature but it is worth noting that 16° C is near the physiological optimal temperature range for trout (11-16° C; Scott and Crossman, 1973).

#### *Variability in the training effect*

There were three trials that produced no training effect. Fish that were sprinted twice daily (with a 30 min separation), fish sprinted daily that were greater than 15 g in size, and fish that were manually chased to exhaustion every other day, all exhibited no improvement in sprint endurance. The one thing these trials had in common was that each would have evoked a greater degree of anaerobic metabolism in white muscle, relative to those trials exhibiting the training effect. Repeated bouts with only a brief separation can produce cumulative metabolic effects (Stevens and Black, 1966), anaerobic metabolism increases exponentially with body size (Goolish, 1989; Ferguson et al., 1993; McDonald et al., 1998), and anaerobic metabolism is greater in fish swum to exhaustion than sprinted to fatigue (McDonald et al., 1998).

The link between greater anaerobic metabolism in white muscle and a lack of a training is not certain. It could simply be that fish were not fully recovered from the previous swim. Hence, when they were tested again there was a residual debt, which inhibited performance and masked the training effect. However, we believe that the interaction is actually more complex than that. Recently, Milligan et al (2000) showed, in

exhaustively exercised rainbow trout, that post-exercise cortisol elevation inhibited recovery from anaerobiosis and that the oxygen debt, itself, evoked the stress response. Fish, injected with a cortisol synthesis inhibitor, or allowed to swim slowly post-exercise, a procedure that lowers circulating cortisol levels, showed much more rapid recovery (2 h vs. 8 h). In the present study, one of the trials (trial 4; Table 2-1) where no improvement in endurance occurred produced visible signs of stress (i.e., weight loss and fin erosion) that was directly attributable to the training regime. Taken together these observations suggest that there may, in fact, be positive feedback between anaerobic metabolism and stress, each promoting the other. Furthermore, a link between stress and learning was made by Olla and Davis (1989), who found that handling stress inhibited the learning of predator avoidance in juvenile coho salmon.

### *Conclusions and implications*

This study demonstrates that both rate of acceleration and experience are important determinants of prolonged swimming performance in juvenile rainbow trout, and should be taken into consideration in performance assessment. We conclude that these two factors could be acting at the same level by affecting the ability of fish to rapidly 'tune-up' white muscle to peak aerobic efficiency. The complex interaction between stress and recovery from previous exercise serves to limit both subsequent performance and the ability to train.

Although these results have shed new light on some of the factors that govern prolonged swim performance in juvenile trout, they also raise three key questions. Why does it take trout so long to adjust to swimming at their critical velocity, a velocity they should be able to sustain indefinitely if sufficiently well prepared? What adaptive value is there in delaying those adjustments? Are these phenomena seen only when high velocity swimming is imposed? In other words, do fish that *choose* to swim at high velocity show substantially greater endurance than fish forcibly accelerated to that same velocity? If the

latter is true, then it raises serious doubt about the ecological relevance of swimming capacity estimates derived through the use of swim flumes.

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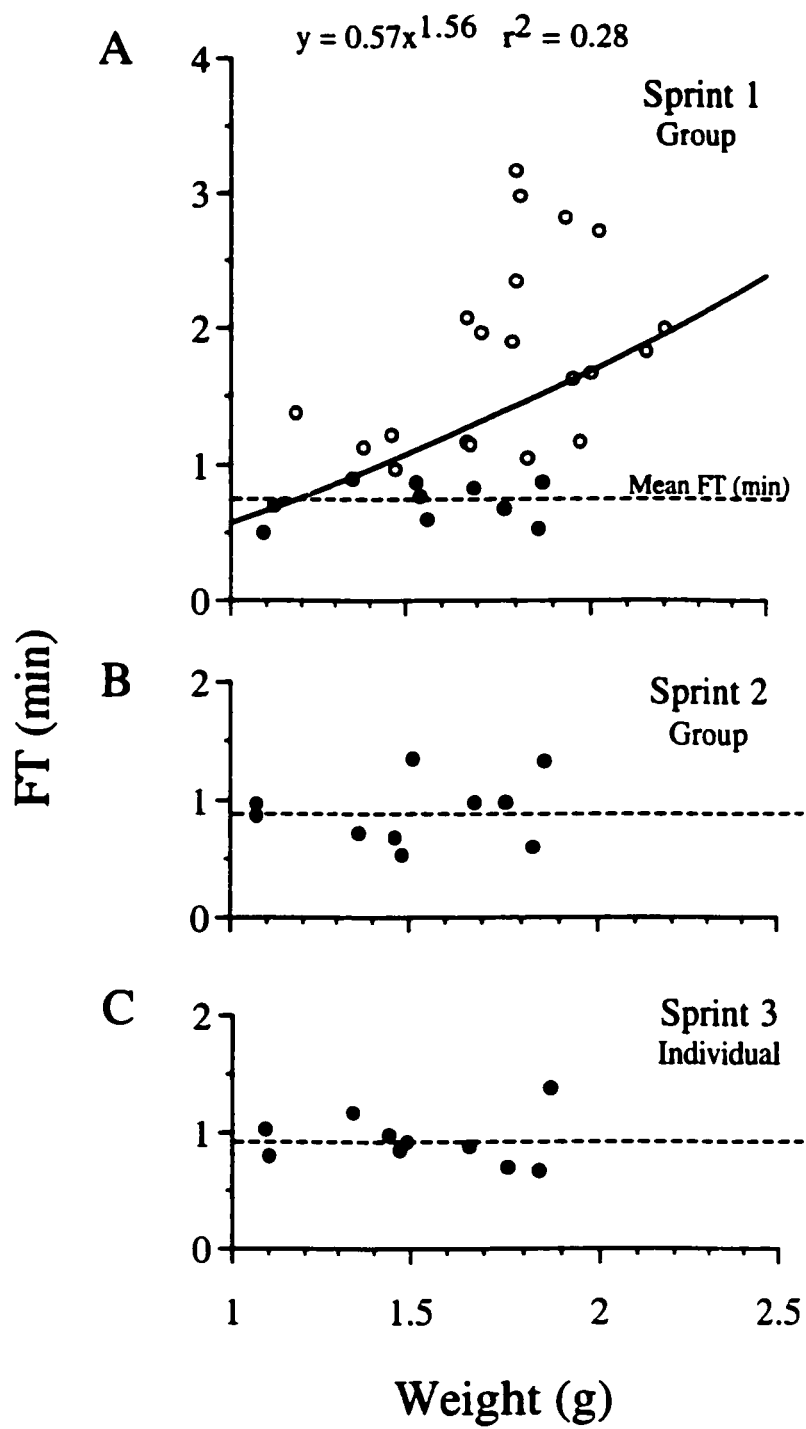
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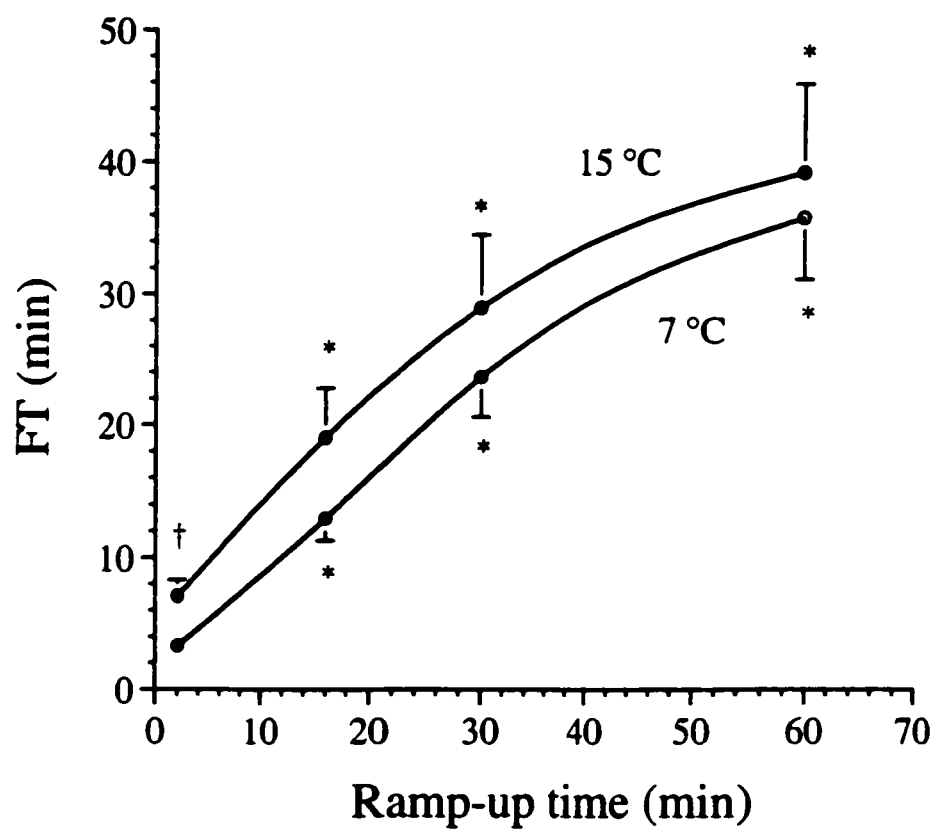
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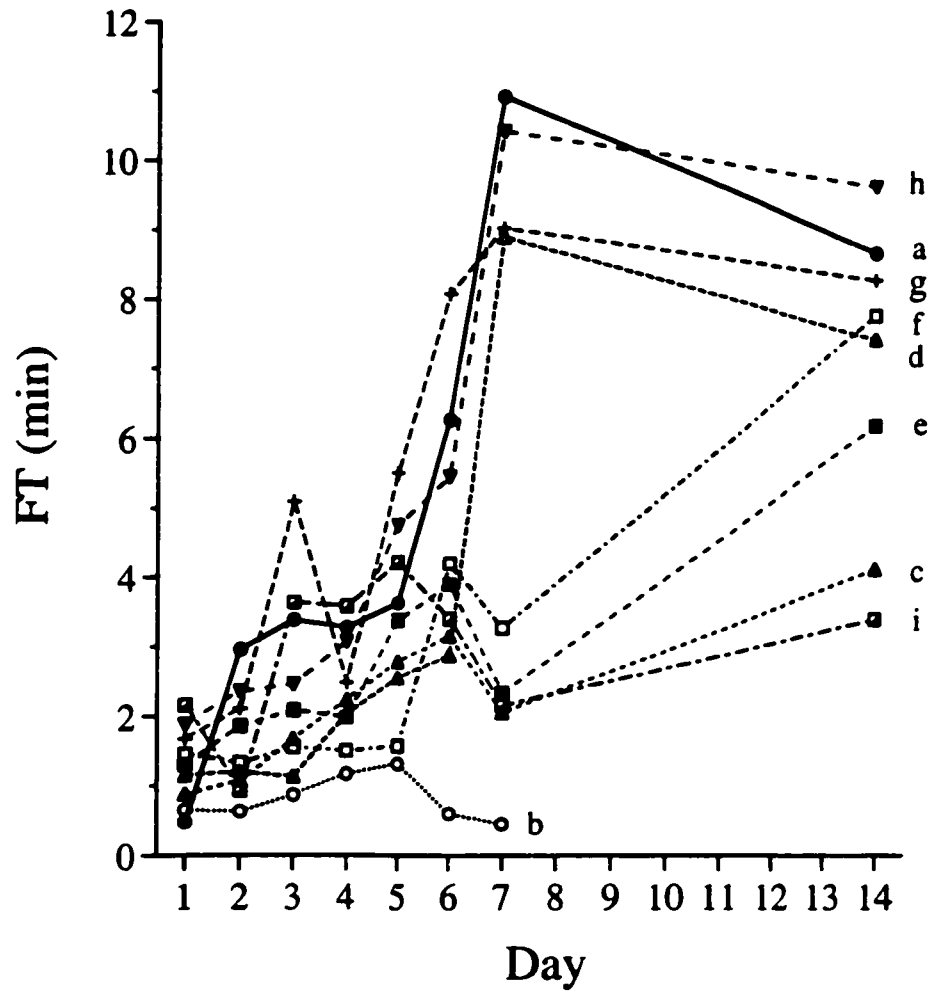
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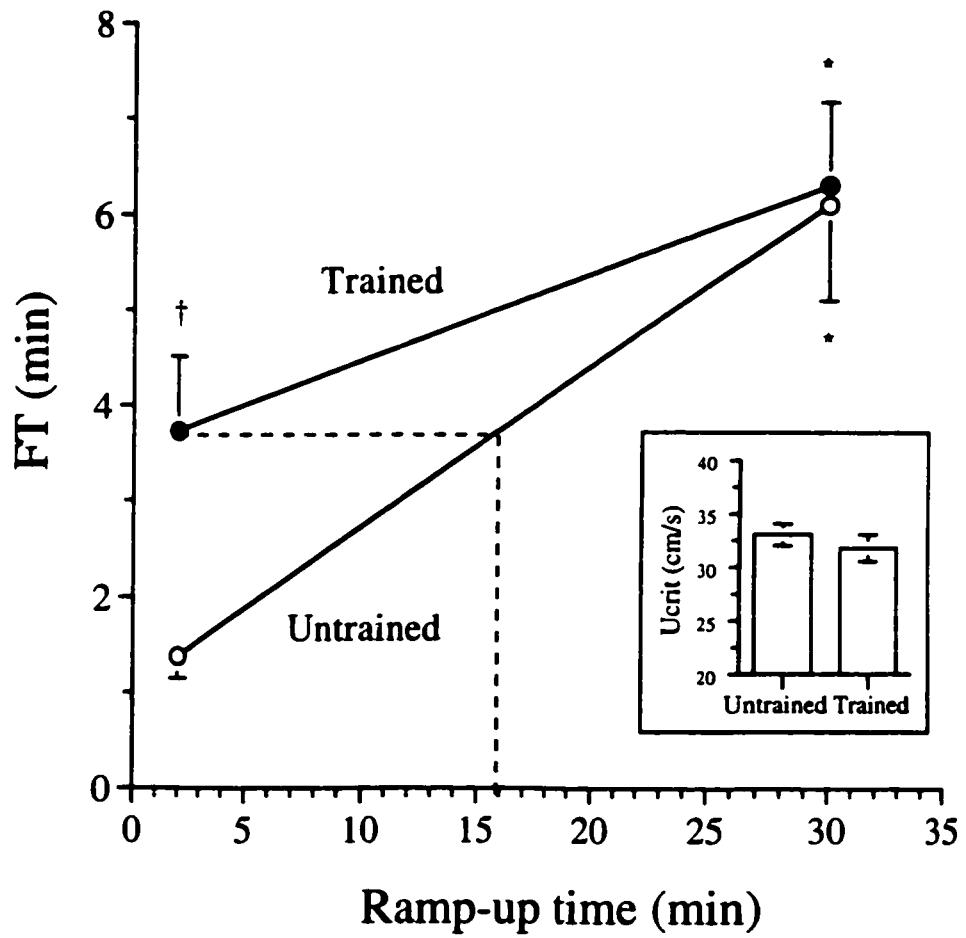
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**Table 2-1.** The effects of temperature, body size, interval between sprints and the presence of fatigue on sprint performance (i.e. fatigue time in min) improvement in juvenile rainbow trout (total N=197).

Variable	Trial number	Temperature (°C)	N	Number of sprints	Days of training	Initial weight (g)	Relative sprint speed (BL s <sup>-1</sup> )	Initial fatigue time (min)	Final fatigue time (min)	Performance improvement
Temperature	1	7	24	6	6	1.1 ± 0.1	7.0	2.2 ± 0.3	3.5 ± 0.4	1.6 x
				14	14				5.6 ± 0.8	2.6 x
Body size	2	16	20	6	6	0.7 ± 0.1	7.4	2.2 ± 0.7	23.6 ± 7.8	10.6 x
	3	13	9	5	7	2.1 ± 0.2	7.0	1.3 ± 0.2	3.3 ± 0.5	2.5 x
	4	10	20	5	5	20.4 ± 0.8	6.7	1.5 ± 0.4	4.5 ± 0.9	3.5 x
	5	12	15	5	5	97.1 ± 7.8	5.0	1.4 ± 0.2	1.9 ± 0.6	NS
Interval between sprints	6	12	30	6	6	3.1 ± 0.2	6.0	4.7 ± 0.9	23.7 ± 6.0	5.1 x
	7	15	19	5	10	4.8 ± 0.2	6.6	2.1 ± 0.4	11.5 ± 2.8	5.6 x
	8	11	20	12	6	2.8 ± 0.1	6.0	3.4 ± 0.7	4.5 ± 0.9	NS
Fatigue	9 (no)	18	20	5	5	3.3 ± 0.1	7.0	3.3 ± 0.5	7.8 ± 1.6	2.4 x
	10 (yes)	18	20	5	5	2.8 ± 0.1	7.0	3.3 ± 0.5	9.5 ± 2.0	2.9 x

**Note:** The table includes temperature, sample size (N), total number of sprints, number of days of training, initial weight (g), relative sprint speed (BL s<sup>-1</sup>), average initial fatigue time (min), average final fatigue time (min), and performance improvement. Statistical differences between initial and final fatigue times were tested by a Dunnett's t-test (P<0.05) and expressed as performance improvement (e.g. 5.6 x = 5.6 fold difference) or as NS (non-significant). Where calculated values are shown, they are means ± S.E.M. (N).

**Table 2-2. The effects of continuous low speed exercise and manual chasing training regimes on sprint performance (relative to untrained controls) in juvenile rainbow trout (total N=24).**

Training regime	Trial number	Temperature (°C)	N	Length of trial (days)	Initial weight (g)	Specific growth rate (% body wt. d <sup>-1</sup> )	Relative sprint speed (BL s <sup>-1</sup> )	Initial fatigue time (min)	Final fatigue time (min)	Performance improvement
Untrained	1	7	8	14	1.0 ± 0.2	2.9	7.0	1.6 ± 0.5	1.6 ± 0.4	NS
Continuous low speed (1.5 BL s <sup>-1</sup> )	2	7	8	14	1.2 ± 0.2	1.6	7.0	1.5 ± 0.5	2.1 ± 0.7	NS
Manual chasing	3	7	8	14	1.1 ± 0.3	2.5	7.0	1.5 ± 0.4	1.8 ± 0.4	NS

**Note:** The table includes temperature, sample size (N), length of trial, specific growth rate, relative sprint speed, initial fatigue time, final fatigue time, and performance improvement. Statistical differences between initial and final fatigue times were tested by a Dunnett's t-test (P<0.05), with NS indicating a non-significant difference. Where calculated values are shown, they are means ± S.E.M. (N).

### CHAPTER 3

#### DOES MUSCLE FUEL DEPLETION DETERMINE ENDURANCE DURING PROLONGED EXERCISE IN JUVENILE RAINBOW TROUT?

##### Summary

This study examined fuel depletion in white muscle in juvenile rainbow trout sprinted to fatigue. Two questions were asked: i) Can the point of fatigue be associated with a measurable metabolic change within the muscle? and ii) Do muscle glycogen levels influence endurance? Accumulation of lactate, and reduction of phosphocreatine (PCr) and ATP within white muscle were used to calculate, in ATP equivalents, the anaerobic energy expenditure (AEE, in  $\mu\text{mol g}^{-1}$ ). Fuel depletion following forced exercise (chasing) to exhaustion was used to estimate maximum AEE (i.e., anaerobic capacity). The progression of fuel use was examined by sampling fish during sprinting (i.e., prior to showing signs of fatigue), and following fatigue. Most of the AEE prior to fatigue was due to PCr depletion. However, at the first signs of fatigue there was a 32% drop in ATP. Similarly, when fish were slowly accelerated to a velocity above the maximum sustainable or critical velocity, the *only* significant change at fatigue was a 30% drop in ATP levels. Muscle glycogen levels were manipulated by altering ration (1% vs. 4% body weight ration per day) combined with either daily or no exercise. A higher ration alone led to significantly greater muscle glycogen but had no effect on sprint performance. Sprint training led to even higher glycogen levels and an average 3 fold improvement in sprint performance. Chase-training produced a similar increase in glycogen but had no effect on

sprint performance. Taken together these observations suggest that the reduction in ATP may be the proximate signal for fatigue in fish and that availability of glycogen correlates with but does not directly determine endurance.

## **Introduction**

The behaviour and swim capacity of salmonids swimming at imposed velocities in swim flumes has been fairly well described (Brett, 1964; Webb, 1971b; Beamish, 1978; Hammer, 1995; McDonald et al., 1998a,b; McFarlane and McDonald, 2001). When trout are accelerated to high speed, white muscle fibres are recruited at 80-94% of the maximum sustainable velocity (or  $U_{crit}$ ; Webb 1971b; Hudson, 1973; Kiceniuk and Jones, 1977; Wilson and Egginton, 1994) and the onset of fatigue is highly dependent on how rapidly the fish is accelerated to the test velocity (McFarlane and McDonald, 2001).

Regardless of rate of acceleration, trout typically settle fairly quickly into a steady-state swim behaviour in the flume once they reach the test velocity. They maintain a stationary position in the flume, free of any surface, and that continues until fish begin to drift back. Once they contact the back screen of the flume they will usually spontaneously resume swimming or will do so with manual prodding. However, they will eventually reach a point at which they will cease swimming and will not resume even with mechanical stimulation. At this point, the fish is considered fatigued, and the test is terminated. Parkhouse et al (1988) showed that by lowering the velocity, fatigued fish would resume swimming. By cycling velocity up and down, they showed that fish eventually reached a point at which they became entirely refractory to stimulation, and did not resume swimming at any speed. At this stage, fish were flaccid and unresponsive to stimulation, and considered exhausted. In other words, the authors made a clear distinction between fatigue and exhaustion, two terms that are often used interchangeably.



A simple, reproducible and commonly employed method for reaching the exhaustion endpoint is manual chasing for a fixed period of time (usually 5-10 min; Stevens and Black, 1966; Milligan and Wood, 1986; McDonald et al., 1998a; Kieffer, 2000). Previous studies (e.g. McDonald et al 1998a) have shown that the depletion of glycogen, PCr and ATP as well as the accumulation of white muscle lactate reach maximal values before the end of a manual chasing protocol, and hence this is a reliable method for estimating the realized or effective anaerobic capacity. Based on the assumption that exhaustion sets in when depletion of anaerobic resources reaches maximal values, this suggests that another, sub-maximal threshold exists that triggers fatigue.

Fatigue has been defined as the inability to maintain a given level of performance or work (Green, 1995), and is thought to be invoked by a combination of central and peripheral factors. Little is known about central (neural) factors in humans, let alone fish, however, a relatively large body of work on fish has been devoted to looking at the role of peripheral (intramuscular) factors such as intracellular acidification (Nelson, 1989; Wood, 1991; Butler and Day, 1993) and fuel depletion (Black et al., 1962; Brett, 1964; Jones, 1982) as potential causes for fatigue. In a preliminary examination of this issue, McDonald et al (1998a) suggested that fatigue might set in when a certain fraction of the anaerobic capacity was utilized (e.g., 60-70%), since trout and salmon typically had lower anaerobic energy expenditures following a sprint test than after forced exercise.

There is no doubt that fuel depletion coincides with fatigue, but studies have yet to establish that fuel depletion *causes* fatigue. Therefore, the overall goal of this study was to further investigate the relationship between fuel depletion and endurance. The first objective was to evaluate whether there was a specific metabolic change in muscle (i.e., a metabolic trigger) that evoked fatigue during a sprint test. This was assessed by monitoring the progression of fuel utilization (glycogen depletion, as assessed by lactate accumulation, and ATP and PCr depletion) throughout a sprint, with the index of muscle

performance being endurance at a fixed velocity. Fuel depletion at fatigue following a  $U_{crit}$  test was also examined to assess the effects of acceleration rate on fuel utilization.

The second objective was to investigate how important muscle fuel sources (such as glycogen) are to endurance. Muscle glycogen levels of juvenile fish show great variability in response to ration, increasing by up to 9 fold on a high ration (Hochachka and Sinclair, 1962) and decreasing by 50% during 5-7 days of fasting (Scarabello et al., 1991; Kieffer and Tufts, 1998). Therefore, the dependence of endurance on muscle glycogen levels was examined through dietary manipulation of resting glycogen levels. The final objective was to determine whether exercise training, which in some circumstances greatly improves endurance (McFarlane and McDonald, 2001), alters fuel deposition and pattern of utilization during a sprint. Three training regimes were employed: continuous exercise at  $1.5 \text{ BL s}^{-1}$ , manual chasing every second day, and once daily sprints.

## **Materials And Methods**

### *Experimental animals*

All experiments were performed at McMaster University using hatchery reared 0+ rainbow trout (*Oncorhynchus mykiss* Walbaum) from Rainbow Springs Trout Farm (Thamesford, Ontario). Fish ranged in size from 1 to 15 g, but for the majority of trials were less than 5 g. Fish were held in 40 L circular tanks, with partial replacement of aerated, dechlorinated water, and were acclimated for at least 2 weeks prior to experimentation. Temperature fluctuated seasonally, creating a range of experimental temperatures from 7 to 16° C, which were controlled for within experiments. Fish were offered a ration of approximately 4% body weight per day of commercial trout chow (Corey Feed Mills, Ltd.), over 2 meals, except where noted (see below). In general,

feeding took place at least 12 hours prior to swim testing to allow for complete digestion of the meal.

### *Exercise apparatus*

Exercise tests were performed using an open, recirculating swim flume (102 L volume) designed according to the recommendations of Vogel (1978). The dimensions of the swimming section were 73x11x20 cm. Flow was generated by a propeller with a 373-W DC motor (Dart Controls, Inc., Zionsville, IN), and calibrated using an electromagnetic flow meter (Marsh-McBirney Model 2000). Fish were always swum in groups (typically 10 fish per trial for fish less than 5 g in weight, and 5 fish per trial for fish 10-15 g), which has previously been shown to have no influence on individual endurance (McFarlane and McDonald, 2001).

### *Exercise protocols*

i) Sprint: The fixed velocity sprint test was performed as described by McDonald et al (1998a). Fish were transferred to the tunnel and acclimated for 5 min in an orienting current of  $\sim 1 \text{ BL s}^{-1}$ . They were then ramped up over 2 min to a test velocity of between 30 and 50  $\text{cm s}^{-1}$  (depending on size) that produced a relative speed close to the critical (i.e., maximum sustainable) velocity of approximately 5 to 7  $\text{BL s}^{-1}$  (McFarlane and McDonald, 2001). Fish exhibiting the initial stages of fatigue typically drifted backwards in the current, and lay motionless against the back screen of the flume. They often resumed swimming either on their own, or when gently prodded on the tail. The criterion for fatigue was when fish fell against the back screen and no longer resumed swimming after three manual prods to the tail. At this time, they were removed from the flume, and in some cases terminally anaesthetized prior to the sampling of muscle tissue (see below).

Time to fatigue (in min) was recorded for each fish as a measure of endurance. From the log sprint times, the mean time to fatigue was calculated for all fish, and the geometric mean calculated as in Brett (1964). Such a calculation produces an asymmetric standard error of the mean, but since the two errors were rarely very different, they have been averaged and expressed as a single error.

ii) Critical velocity ( $U_{crit}$ ) test: A modified incremental velocity test similar to that outlined by Brett (1967) was performed using stepwise velocity intervals of 30 min rather than 45 min. The orientation velocity was  $1 \text{ BL s}^{-1}$ , with subsequent velocity increments of  $1 \text{ BL s}^{-1}$  every 30 minutes until fatigue. The criterion for fatigue was identical to that in a sprint test (i.e., when fish fell back and did not resume swimming following three manual prods). Following fatigue, the critical velocity, a speed that should be sustainable for at least 200 min (c.f. Beamish, 1978), was calculated according to the following expression from Brett (1964):

$$U_{crit} = U_p + \left( \left( \frac{t_f}{t_i} \right) + U_i \right)$$

where  $U_i$  is the velocity increment ( $\text{cm s}^{-1}$ ),  $U_p$  is the penultimate velocity at which the fish swam prior to fatigue,  $t_f$  is the elapsed time from the velocity increase to fatigue, and  $t_i$  is the time between velocity increments. Relative  $U_{crit}$  was calculated according to individual fork lengths, and expressed as  $\text{BL s}^{-1}$ .

iii) Forced exercise: Fish were transferred, in groups of 10, to 40 L tanks (filled with 20 L of water) and immediately chased for 8 minutes through continuous manual stimulation. Following 8 min of forced exercise, fish were typically unresponsive and exhibited a loss of equilibrium, both indicators of exhaustion (Parkhouse et al., 1988).

They were then removed, anaesthetized, and white muscle samples taken as described below.

### *Tissue sampling and analysis*

Fish were killed with a high concentration ( $1 \text{ g l}^{-1}$ ; neutralized to pH 7.5) of MS-222 (tricaine methane sulphonate, Syndel Laboratories), typically within 3 to 5 seconds. Two methods of sampling fish were performed, depending on fish size. For fish  $< 5 \text{ g}$ , the entire fish was freeze-clamped between two aluminum blocks, pre-cooled with liquid nitrogen. White muscle samples were excised from the frozen fish, and ground to a fine powder under liquid nitrogen. For larger fish, white muscle was rapidly excised from posterior to the dorsal fin, and dorsal to the lateral line, and freeze-clamped. The entire sampling procedure generally took less than 10 seconds for each fish. All tissues were stored at  $-70 \text{ }^\circ\text{C}$  for later analysis.

Aliquots of ground white muscle were analyzed for glycogen, lactate, PCr and ATP. For glycogen analysis, 100 mg of frozen wet tissue was digested according to the methods of Hassid and Abraham (1957), and glucose was enzymatically analyzed following the protocol outlined in Bergmeyer (1983). For analysis of lactate, PCr and ATP, 100 mg of tissue was homogenized with 1 ml of 8% perchloric acid, and enzymatic analysis of supernatant was carried out based on procedures in Bergmeyer (1983).

### *Experimental series*

#### i) Fuel use at fatigue vs. exhaustion

Fish ranging in size from 4-12 cm length ( $< 15 \text{ g}$ ) were either sprinted to fatigue at a fixed velocity or forced to exercise by manual chasing for 8 min. White muscle was sampled for analysis of lactate, PCr and ATP, and the sum of changes in each metabolite from resting to post-exercise levels expressed as the anaerobic energy expenditure (AEE) in

ATP equivalents (see *Calculations* section). To maintain similar levels of exertion for fish of different sizes, sprint speed was scaled according to fish length. Fish of 4-5 cm length were sprinted at  $30 \text{ cm s}^{-1}$  ( $6-7.5 \text{ BL s}^{-1}$ ), fish 6-7 cm were sprinted at  $40 \text{ cm s}^{-1}$  ( $6-7 \text{ BL s}^{-1}$ ), fish 8-10 cm were sprinted at  $50 \text{ cm s}^{-1}$  ( $5-6 \text{ BL s}^{-1}$ ), and fish >10-12 cm were sprinted at  $60 \text{ cm s}^{-1}$  ( $5-6 \text{ BL s}^{-1}$ ). The forced exercise protocol was identical regardless of fish size.

### ii) Progression of fuel use during a sprint

To assess fuel depletion both prior to fatigue (i.e., during steady swimming), and after showing signs of fatigue (i.e., at the first, second and third fall-back) during a fixed velocity sprint test, fish were sampled at different time intervals throughout the test. Fish were removed from the flume at each interval, and muscle samples were taken for analysis of lactate accumulation, and depletion of PCr and ATP (see below).

### iii) Relationship of muscle glycogen content to endurance

White muscle glycogen content was altered by manipulating dietary ration (feeding either 1 or 4% BW  $\text{d}^{-1}$ ) for 2 weeks. Fish from both groups were then sprinted to fatigue at a speed of  $\sim 7 \text{ BL s}^{-1}$ . Following fatigue, they were immediately anaesthetized, and white muscle was removed and rapidly frozen for subsequent glycogen determination (as above). Initial glycogen levels were back-calculated based on post-exercise lactate accumulation (see *Calculations* section). In addition to being fed a 4% ration, one group of fish were sprinted at a fixed velocity ( $7 \text{ BL s}^{-1}$ ) once daily for 7 days.

iv) Training regimes

*a) Repeated sprints:* The daily regime involved performing sprint trials once daily for 14 days, and returning fish to holding tanks (for 24 h) to allow complete recovery between sprints. Fish muscle from trained fish was sampled either following the last exercise bout on day 14, or on day 15, following recovery, to obtain post-training resting levels of lactate, ATP and PCr (as compared to untrained controls). To examine the effects of repeated sprints on performance when fish were not allowed to fully recover between sprints (i.e., the standard 24 hour recovery period), a second cohort of fish were sprinted 8 times with a 30 min recovery period between sprints.

*b) Continuous aerobic exercise:* Fish were maintained in a flume at a speed of 8 cm s<sup>-1</sup> (1.5 BL s<sup>-1</sup>; i.e., below the white muscle recruitment threshold), with speed reduced to 0.5 BL s<sup>-1</sup> during two daily feedings (approximately 15 min each). At the end of the 14-day training period, fish were sprinted at 7 BL s<sup>-1</sup>. White muscle was sampled either following fatigue or 24 h following the final training sprint.

*c) Forced exercise:* Fish were manually chased to exhaustion for 8 min once every two days, and returned to holding tanks between trials for feeding and recovery. Following 14 days, fish were sprinted, endurance was evaluated as compared to control (i.e., untrained) fish, and resting and post-exercise white muscle sampled for analysis of fuel depletion.

*Calculations*

Anaerobic energy expenditure: The anaerobic energy expenditure (AEE or anaerobic ATP turnover) in ATP equivalents was calculated, as described by Pearson et al (1990), according to the following formula:

$$\text{AEE} = (\Delta \text{Lactate}) \times 1.5 + \Delta \text{ATP} + \Delta \text{PCr}$$

where  $\Delta$  represents the difference between resting (control) and fatigued/exhausted animals, 1.5 ATP are produced per lactate, and 1 PCr = 1 ATP. During anaerobic metabolism, ATP is also provided through ADP hydrolysis, however, this was ignored due to the very small overall contribution (less than 10% of AEE; Pearson et al., 1990).

**Initial glycogen:** In order to approximate initial levels of muscle glycogen in fatigued fish, glycogen was back-calculated based on muscle lactate accumulation during the exercise period. By assuming that all muscle lactate had originated as glycogen (due to the relatively small contribution by endogenous or circulating glucose; Pearson et al., 1990), and two lactate are produced from every glucose molecule, initial (pre-exercise) glycogen was calculated according to the following equation:

$$\text{Initial glycogen} = \text{post-exercise glycogen} + (\Delta \text{ lactate} / 2)$$

where  $\Delta$  lactate is calculated as the difference between resting and post-exercise levels (in  $\mu\text{mol g}^{-1}$  wet weight). The assumption associated with this calculation is that all glucose comes from glycogen, and there is no glucose stored in the form of glycolytic intermediates. Therefore, if anything, the error involved in this calculation would lead to an underestimate of initial glycogen levels.

### *Statistical Analysis*

Data are expressed either as individual values, or as means  $\pm$  one S.E.M. To establish individual variation around a mean value, the coefficient of variation (C.V.) was calculated according to Zar (1996), and comparisons between two C.V.'s made using a Z-test (Zar, 1996). Where necessary, comparisons of treatment groups with respective



controls were tested by one-way analysis of variance (ANOVA). If the result from the ANOVA was significant, a Tukey-Kramer HSD test for multiple comparisons was applied to test for significant differences among treatments (Zar, 1996). Differences at  $P < 0.05$  were considered significant. Tests were performed using JMP 2.0.5 software (SAS Institute, Inc.).

## Results

### *Anaerobic energy utilization: fatigue vs. exhaustion*

When fish were chased to exhaustion, the anaerobic energy expenditure (a measure of anaerobic capacity; expressed as ATP equivalents) was dependent on body size (Fig. 3-1, closed symbols, solid line,  $r^2 = 0.731$ ). This confirms previous observations by McDonald et al (1998a). In contrast, the anaerobic energy expenditure (AEE) following a sprint to fatigue was not correlated with body size (Fig. 3-1, open symbols), although it was generally lower at fatigue than at exhaustion. More importantly, the AEE at fatigue was significantly more variable than at exhaustion (coefficient of variation of 0.50 vs. 0.18) suggesting that this measure is not a good predictor of fatigue. However, the upper limit of AEE was the similar to that in chased fish (Fig. 3-1), and exhibited the same relationship to body size, suggesting that some fish that fatigued during a sprint were near exhaustion.

The components of AEE (i.e., changes in ATP, PCr and lactate from resting to post-exercise conditions) reveal a different pattern of metabolic response to the two exercise protocols. Not surprisingly, changes in ATP, PCr and lactate all scaled with body size in fish chased to exhaustion, with an  $r^2$  ranging from 0.28 for PCr to 0.47 for lactate (closed symbols, solid lines; Fig. 3-2A, B and C). However, in fish sprinted to fatigue, there was

a negative relationship between the drop in ATP and body size (Fig. 3-2A; open symbols, dashed line), with an  $r^2$  of 0.29, a slightly positive correlation between the drop in PCr and size (Fig. 3-2B;  $r^2$  0.13), and no correlation between lactate accumulation and body size (Fig. 3-2C).

#### *Progression of fuel utilization during a sprint*

Depletion of ATP equivalents began before fatigue (Fig. 3-3). Fish that were removed from the flume and sampled before exhibiting signs of fatigue showed a progressive depletion of PCr with sprint time (Fig. 3-3A). The depletion of PCr was linear at a rate of  $0.98 \pm 0.52 \mu\text{mol g}^{-1} \text{min}^{-1}$ . The regression line, extrapolated to a  $\Delta$  PCr of zero, transected the x-axis at approximately -2 min, which corresponds to the beginning of the ramp-up (acceleration) period of the sprint test (Fig. 3-3A).

In all fish sampled prior to fatigue, PCr depletion made the greatest contribution to AEE (nearly 70%, Fig. 3-3B, column 1). ATP stores were not significantly reduced, and glycolysis (lactate accumulation) made up the remainder. However, once fish began to exhibit signs of fatigue (i.e., the first time they fell back to the rear screen of the flume) there was a significant, 32% drop in ATP (a seven fold higher  $\Delta$  ATP compared to non-fatigued fish), but no further change in PCr (Fig 3-3B; compare columns 1 and 2). After the initial fall back, fish could typically be stimulated to resume swimming, yet this led to no significant change in ATP use, but rather, an increase in lactate accumulation (Fig. 3-3B; columns 3 and 4). Indeed, the longer a fish swam before it fatigued, the greater was the contribution of lactate to the anaerobic energy expenditure (Fig. 3-3B, columns 2 to 4). This trend continued in fish chased to exhaustion (Fig. 3-3B, column 5). The exhausted fish showed similar amounts of ATP and PCr depletion as fish sprinted to fatigue (Fig. 3-3B, columns 4 vs. 5), but a 40% greater accumulation of lactate. In contrast, when fish were swum to fatigue in a critical velocity test, the *only* significant change in muscle was a

30% drop in ATP (Fig. 3-3B, column 6). The change in ATP levels was not significantly different from that seen in fish falling back for the first time in a sprint test (Fig. 3-3B, column 2). The critical velocity was  $38 \pm 0.8 \text{ cm s}^{-1}$  or  $6.8 \pm 0.1 \text{ BL s}^{-1}$ , which was similar to the sprint speed used for fish of similar size. The difference was that it took approximately 180 min to reach fatigue in the  $U_{\text{crit}}$  test and only 3 min in the sprint test.

#### *Effect of muscle glycogen on performance*

White muscle glycogen levels were highly dependent on both ration and experience. By reducing ration from 4% to 1% BW  $\text{d}^{-1}$  for 14 days, glycogen levels were decreased by more than 75% (from  $20.3 \pm 1.2$  to  $4.7 \pm 0.7 \mu\text{mol g}^{-1}$ , open circles and open triangles, respectively; Fig. 3-4). In contrast, when fish were fed a 4% ration and repeatedly sprinted once daily for 7 days, glycogen levels were elevated a further 50% above those in fish that were fed a 4% BW  $\text{d}^{-1}$  ration but not exercised ( $31.4 \pm 1.6 \mu\text{mol g}^{-1}$  vs.  $20.3 \pm 1.2 \mu\text{mol g}^{-1}$ , closed circles, Fig. 3-4).

Despite the differences in glycogen, sprint endurance was virtually identical in fish fed a 1% ration compared to fish fed a 4% ration ( $1.2 \pm 0.1 \text{ min}$  vs.  $1.3 \pm 0.1 \text{ min}$ , respectively; Fig. 3-4) suggesting no effect of glycogen on performance. In contrast, the higher glycogen in sprint-trained fish was accompanied by a 3 fold greater endurance compared to controls fed the same ration ( $4.7 \pm 0.7$  vs.  $1.3 \pm 0.1 \text{ min}$ , Fig. 3-4). Similarly, in a separate trial where there was naturally occurring variability in muscle glycogen in untrained fish, fatigue time increased in relation to muscle glycogen levels ( $r^2 = 0.320$ ; Fig. 3-5).

#### *Effects of training on performance and muscle metabolism*

Daily exercise for a period of 14 days led to significant metabolic and performance changes. Chase-trained and sprint-trained fish had significantly lower resting muscle

lactate and a tendency towards higher resting levels of muscle glycogen (only significant in sprint-trained fish) compared to continuously exercised fish and controls (Table 3-1). In contrast, muscle ATP and PCr were not affected by any of the three swimming protocols (Table 3-1). Nonetheless, sprint-trained fish showed much better sprint endurance than chase-trained fish ( $5.6 \pm 0.8$  vs.  $1.8 \pm 0.4$  min). Furthermore, the performance of chase-trained fish was not significantly different from either control fish ( $1.5 \pm 0.5$  min) or aerobically trained fish ( $2.1 \pm 0.7$  min).

The two high intensity training regimes led to similar anaerobic energy expenditures in sprints, and these were nearly 50% greater compared to untrained controls and continuously exercised fish (Fig. 3-6). However, the pattern of anaerobic metabolite use was different between the sprint- and chase-trained fish. Chase-trained fish exhibited a greater use of high energy phosphates (~10% of total anaerobic energy expenditure) compared to controls, while the sprint-trained fish exhibited negligible ATP and PCr depletion, relying instead almost entirely on glycolysis for ATP production.

#### *Multiple sprints in one day*

When fish were sprinted 8 times in one day, with only a 30 min recovery period between sprints (as compared to previous sprint training when fish were given 24 h to recover between sprints), there was no significant improvement in performance after 8 sprints (Fig. 3-7). There was a slight drop in endurance in the second sprint, but by the third sprint, performance was increased to a level similar to the initial sprint. Furthermore, fish were able to maintain performance for up to 7 repeat sprints, without a significant decrement until the eighth sprint (Fig. 3-7). Although fish from this trial were not sampled for muscle metabolite analysis, the recovery time between sprints was too short to allow for full metabolic recovery, as the total elapsed time, including both the sprint and recovery periods was approximately 4.5 h.

## **Discussion**

### *The cause of fatigue*

Although fatigue in this study was a well defined and unambiguous behavioural endpoint, it had highly variable metabolic consequences to white muscle (Fig. 3-1). Therefore, it is unlikely that the total amount of fuel depletion *per se* evokes fatigue. The only significant metabolic change coinciding with the onset of fatigue in the sprint test was the depletion of ATP. PCr was maximally depleted prior to fatigue, and lactate accumulation continued after the initial onset of fatigue, but did not correlate with fatigue time. This pattern of fuel depletion (i.e., PCr, ATP and then glycogen) is comparable to that seen in exhaustively exercised fish (Parkhouse et al., 1988; Scarabello et al., 1992; Wang et al., 1994), and is likely the universal pattern of fuel depletion regardless of the type of exercise protocol. However, since ATP is the only significant metabolic change occurring at fatigue, depletion of this energy source may trigger fatigue. Following fatigue at the final (i.e., non-sustainable) velocity in the  $U_{crit}$  trial, a drop in ATP was the *only* metabolic change detected in muscle. Here the behavioural endpoint was ostensibly the same as in a sprint test, with fatigue determined when fish drifted back against the rear screen and did not re-enter current following 3 manual prods. Therefore, the only difference was that the time taken to reach the endpoint was much longer.

### *Adaptive value of the ATP trigger*

Fatigue induced by ATP depletion has adaptive value in protecting anaerobic resources because ATP recovery is generally more rapid (1-4 h; Milligan and Wood, 1986; Wang et al., 1994; McDonald et al., 1998a) than glycogen recovery (8-24 h; Stevens and Black, 1966; Milligan and Wood, 1986; Kieffer et al., 1994; Wang et al., 1994; McDonald

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In addition to physiological changes, there is without a doubt a psychological component to performance. Brett (1995) found that following rapid acceleration, there was a spike in oxygen consumption measured, that was attributed to 'excitability' of the fish. This same phenomenon has been alluded to by others (Beamish, 1978; Scarabello et al., 1992), prompting the idea of performing 'practice swims' prior to testing to minimize psychological disturbance (Peake et al., 1997; Farrell et al., 1998). Given the volitional nature of the sprint test (in comparison to forced exercise), psychological input governing the cessation of swimming would explain the lack of scaling of AEE during a sprint. A metabolic trigger would act as a physiological protective mechanism signaling the fish to stop swimming, however, psychological or motivational factors may be more influential, leading some fish to continue swimming despite the drop in ATP. The complicating interaction between these two signals would contribute to both the performance and AEE variability seen in a seemingly homogeneous population. The influence of psychological factors may also prove to change with age, since larger trout were found to fatigue with less ATP depletion, suggesting a larger psychological, and perhaps experience-based influence.

#### *Relationship between muscle glycogen and endurance*

Glycogen manipulated by diet and training or due to intrinsic variability within a population often correlates with endurance, however absolute glycogen levels do not determine endurance. For example, when fish with an extremely large (10-fold) range in resting glycogen were sprinted, the correlation between endurance and fatigue time was present. In contrast, a change in ration alone did not have a significant impact on endurance, even though there were considerable effects on muscle glycogen content. Previous studies have shown that altering ration influences glycogen levels, but has no impact on PCr or ATP stores (Scarabello et al., 1991; Kieffer and Tufts, 1998).

Therefore, if ATP depletion provokes fatigue prior to glycogen depletion, initial muscle glycogen levels will have no bearing on endurance. Nonetheless, Hochachka and Sinclair (1962) found that fish with a similar magnitude (8 fold) of variation in muscle glycogen had lower mortality upon release into stream environments, suggesting that high glycogen has an adaptive value for survival. It is possible that high glycogen is the result of another adaptation or trait that directly impacts endurance.

Endurance training results in both an enhancement of glycogen stores and sprint performance. Previous studies imposing training regimes, and measuring metabolic changes have shown similar increases in glycogen in trained fish, whether the training be at maximal speeds (Pearson et al., 1990) or sub-maximal speeds (Davison and Goldspink, 1977). However, chase-trained fish had a similar elevation of glycogen at the end of the regime, yet no performance improvement. Furthermore, higher glycogen generally led to a greater AEE in both trained and untrained fish, a similar result seen by both Pearson et al (1990) and Schulte et al (1992) in fish with  $>20 \mu\text{mol g}^{-1}$  glycogen. Yet, these studies utilized exercise protocols in which fish were either chased to exhaustion, or exhausted in a swim flume by oscillating the speed up and down. In this study, when fish were sprinted to fatigue, a higher AEE was not necessarily reflected by higher performance. Thus, in this type of exercise, metabolic changes are not directly linked to endurance. Furthermore, an increase in endurance while maintaining the same AEE, can only be explained if there is a dramatic change in the energy efficiency of working muscle, or if ATP is being provided *via* oxidative metabolism.

### *Conclusions*

During sprint exercise, the energy management strategy of fingerling trout appears to be avoidance of anaerobic metabolism. This is apparent from the comparison of AC to AEE, with the lower AEE during a sprint due to conservation of anaerobic resources. An



early-warning fatigue trigger, such as ATP depletion, is adaptive for stream dwelling fish, as it would prevent them from accumulating a considerable anaerobic debt, which becomes important for fish that need to preserve intermittent swimming ability. In this study, we have shown that dietary ration and training can alter glycogen levels but not necessarily endurance. Based on these findings, it can be concluded that in very small juveniles, fuel availability is not the sole determinant of fatigue. We also believe that white muscle cannot possibly be fueling sprint exercise solely via anaerobic metabolism (i.e., breakdown of muscle glycogen with end production of lactate), but there is a significant oxidative contribution that is amplified in experienced fish. Although we were able to deduce that fuel depletion does not lead to fatigue, we are far from fully understanding the exact mechanism that provokes fish to stop swimming. It is undoubtedly the complex interaction of a suite of factors, both physiological and perhaps even psychological that leads to this easily quantifiable endpoint.

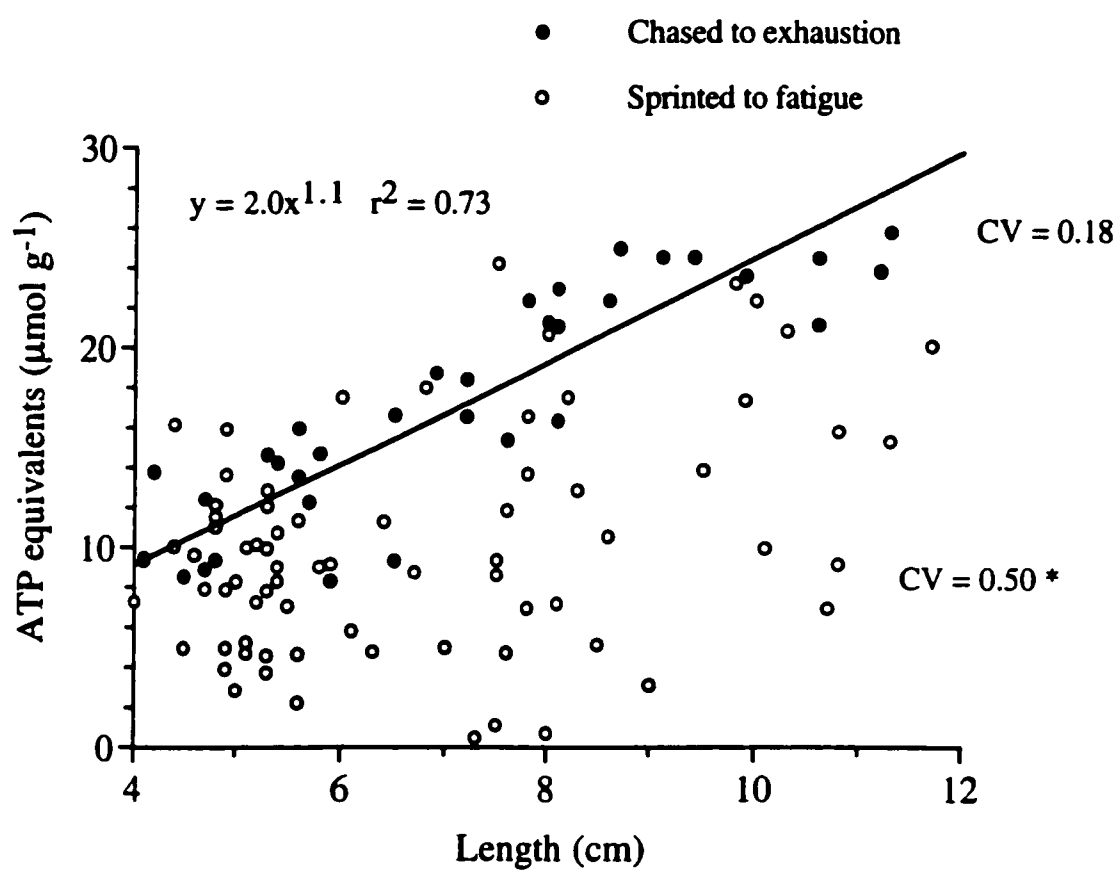
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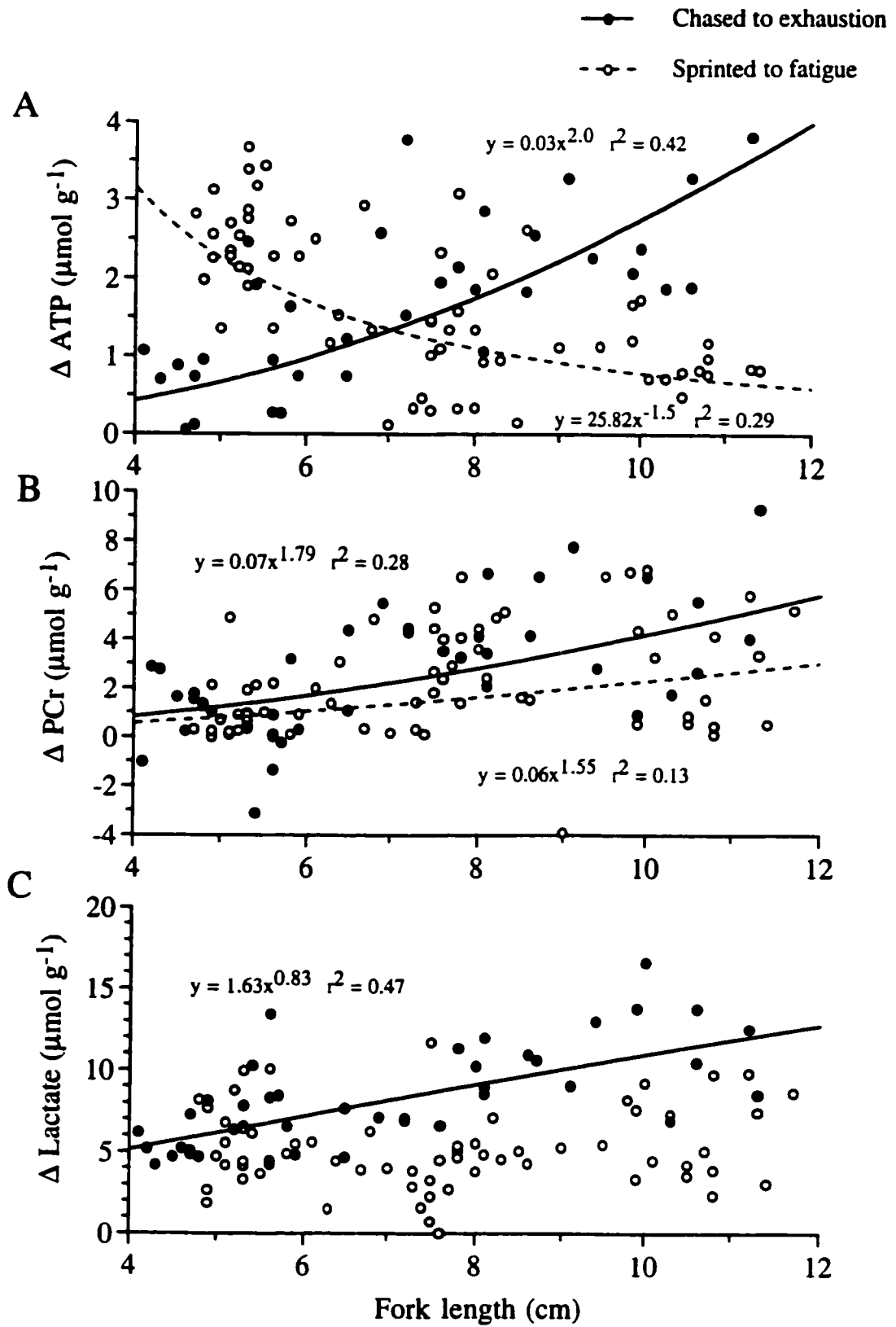
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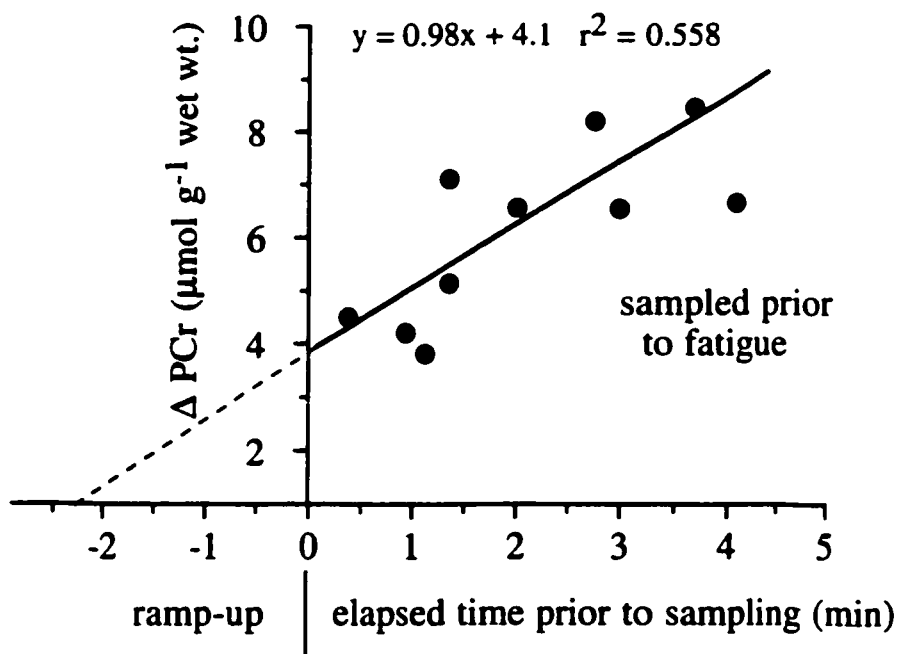
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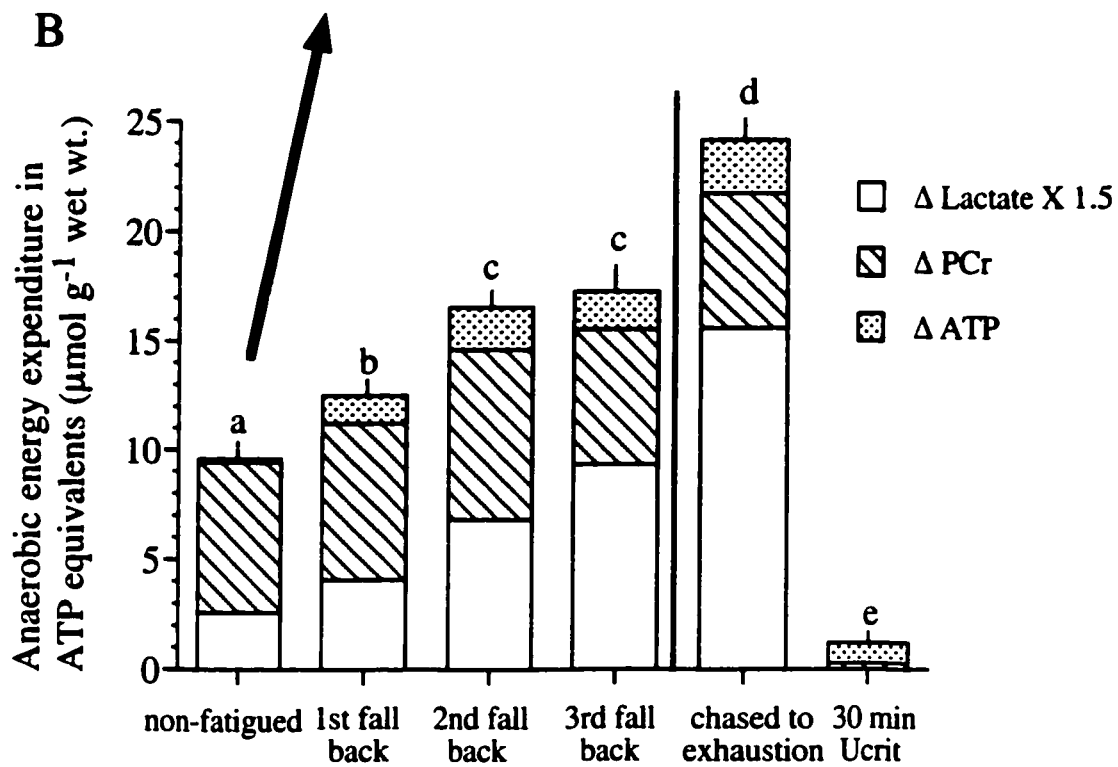


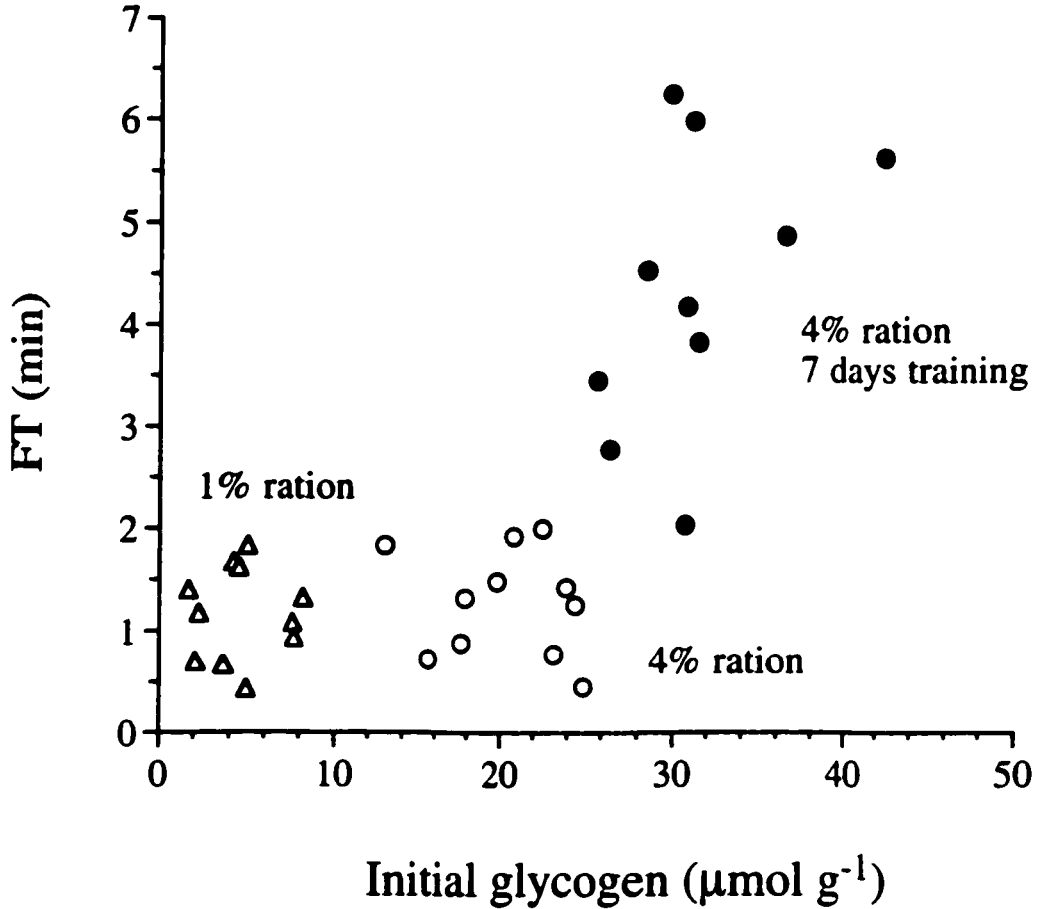


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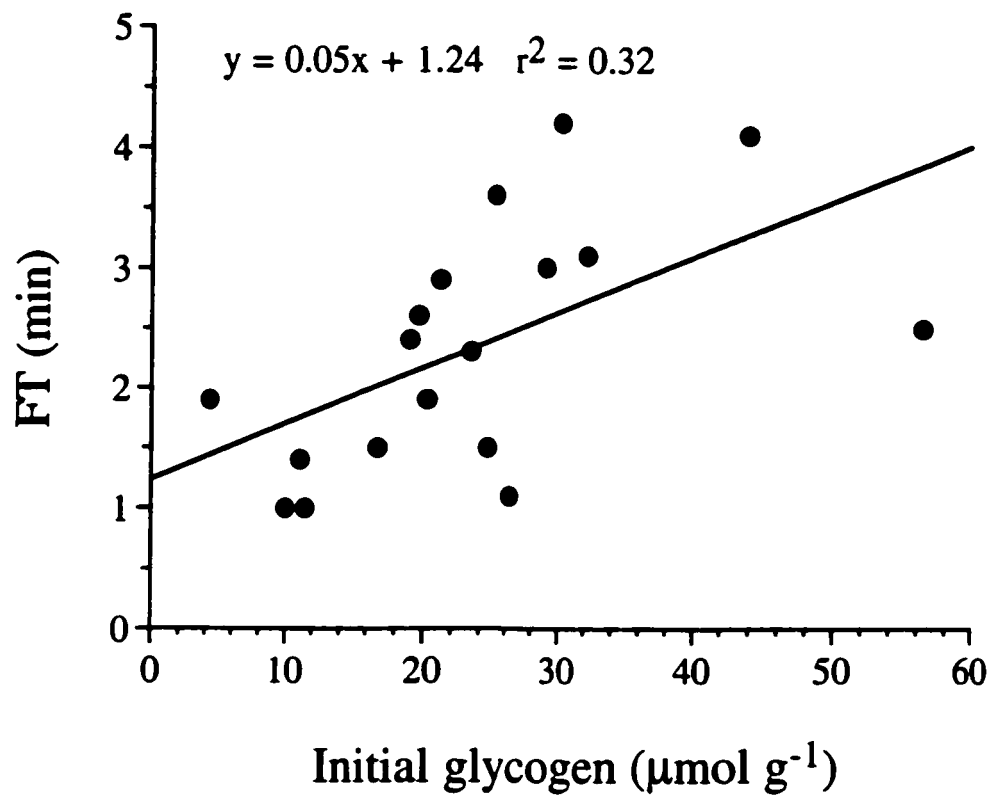


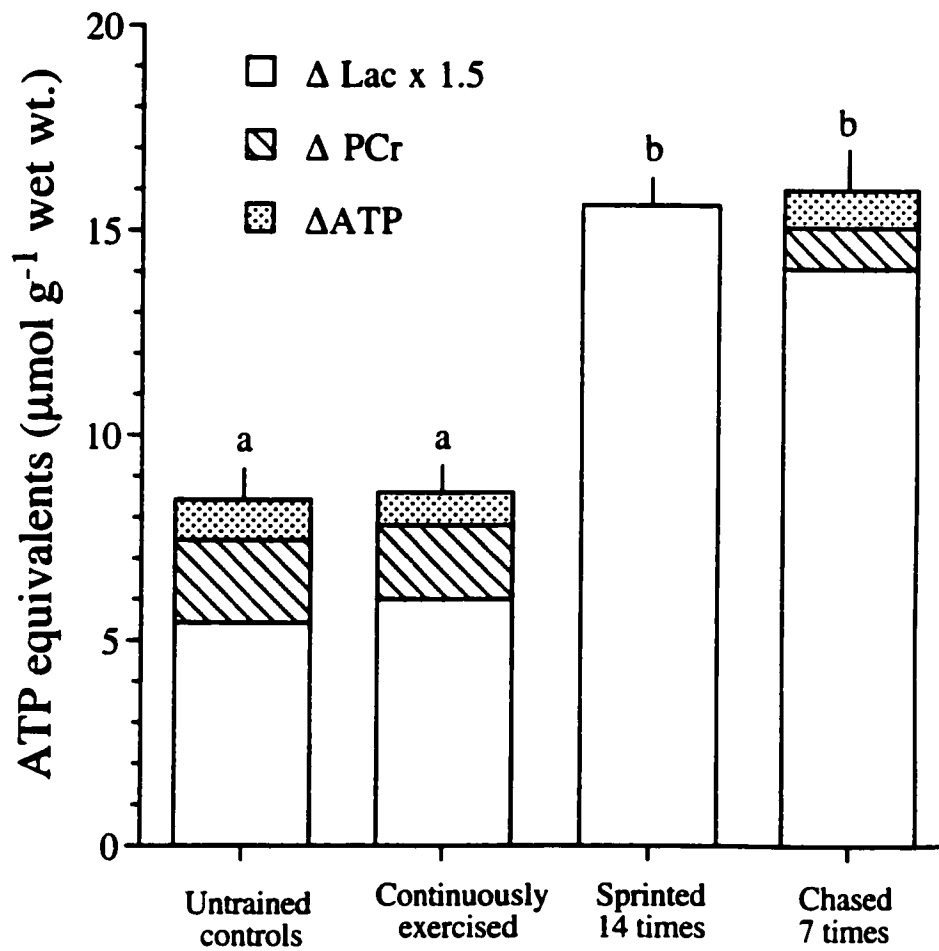
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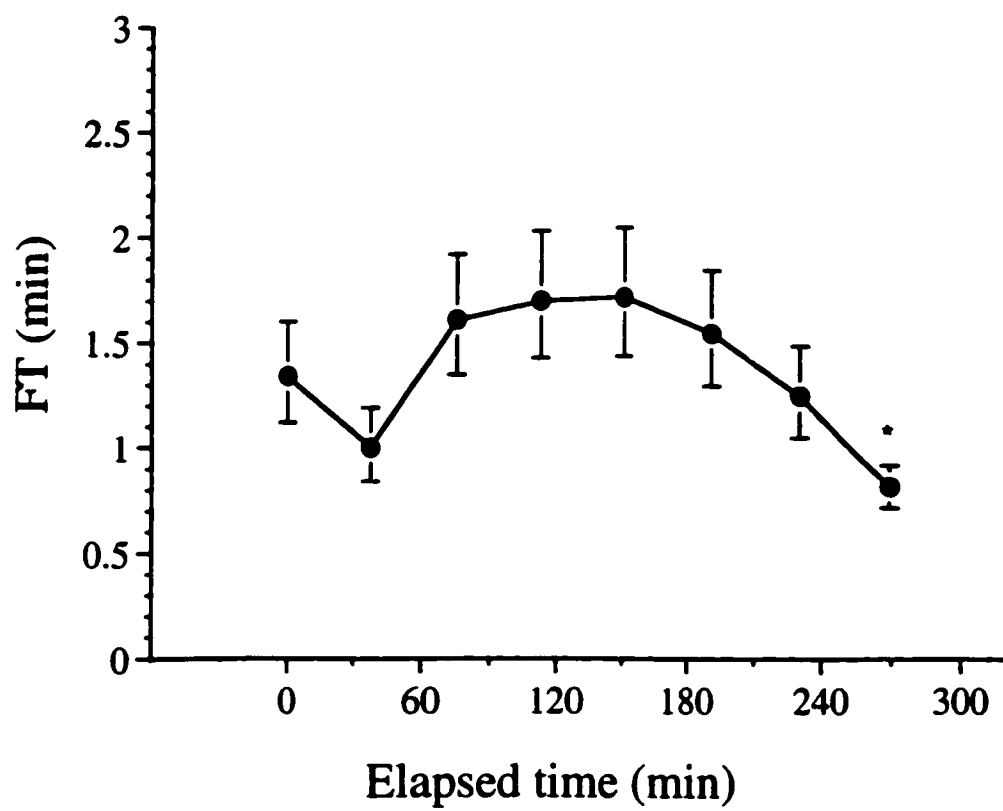












**Table 3-1.**

The effect of training regime on white muscle resting levels of ATP, PCr, lactate and glycogen (in  $\mu\text{mol g}^{-1}$ ) in juvenile rainbow trout. Resting levels were measured in untrained controls, and following 14 days of training with one of three training regimes: continuous exercise at 1.5 BL  $\text{s}^{-1}$ , manual chasing for 8 min every second day or sprinted once daily at 7 BL  $\text{s}^{-1}$ .

Training Regime	White muscle levels at rest ( $\mu\text{mol g}^{-1}$ wet wt.)			
	ATP	PCr	Lactate	Glycogen
Untrained controls	5.0 $\pm$ 0.4 a	13.0 $\pm$ 3.0 a	5.3 $\pm$ 0.9 a	24.6 $\pm$ 2.7 a
Low speed continuous exercise	5.1 $\pm$ 0.8 a	13.9 $\pm$ 2.4 a	5.6 $\pm$ 0.9 a	25.3 $\pm$ 2.5 a
Chase-trained	4.8 $\pm$ 0.3 a	13.6 $\pm$ 1.7 a	2.2 $\pm$ 0.1 b	36.0 $\pm$ 5.3 ab
Sprint-trained	5.0 $\pm$ 0.5 a	12.8 $\pm$ 3.6 a	2.5 $\pm$ 0.1 b	37.3 $\pm$ 3.0 b

**Note:** Values are means  $\pm$  S.E.M. (N=8). Average body size was 1.1  $\pm$  0.03 g; 4.7  $\pm$  0.04 cm. Significant differences between groups were tested by a one-way ANOVA followed by a Tukey-Kramer HSD test ( $P < 0.05$ ). Different letters denote significant differences.

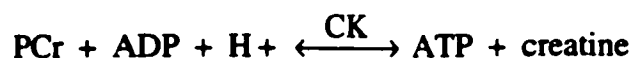
**CHAPTER 4**  
**CREATINE SUPPLEMENTATION AFFECTS PROLONGED EXERCISE**  
**PERFORMANCE IN FINGERLING RAINBOW TROUT**

**Summary**

Fingerling rainbow trout were supplemented with equal amounts of creatine (Cr) by two routes: dietary (12.5 mg Cr per g food), or intraperitoneal injection (0.5 mg Cr per g fish). Endurance in a fixed velocity sprint test (at a speed of 7 BL s<sup>-1</sup>), and resting levels of white muscle metabolites (total creatine (a measure of free creatine plus phosphocreatine (PCr), ATP, lactate and glycogen) were assessed following 7 days of supplementation, and compared to controls. None of the treatments had a significant effect on growth, muscle total creatine, percent phosphorylation of creatine, ATP or lactate. However, resting muscle glycogen was elevated in creatine-supplemented fish. Higher muscle glycogen corresponded to significantly greater endurance in creatine-supplemented fish. Although fish do not actively transport additional creatine into the muscle, a mechanism whereby circulating creatine acts to enhance muscle glycogen is present. These results suggest that the improved endurance may be due to an insulin-dependent mechanism (similar to that elucidated in mammalian studies) that allows fish to supercompensate muscle glycogen stores, thus extending endurance through enhanced glycolytic flux.

## Introduction

Creatine is a non-essential amino acid derivative that is naturally found in the highest abundance in vertebrate skeletal muscle (Walker, 1979). Although it is not synthesized in muscle, creatine is actively taken up *via* a sodium dependent transporter within the muscle membrane (Loike et al., 1986). Upon phosphorylation by the enzyme creatine kinase (CK), phosphocreatine (PCr) is formed from creatine, providing a rapid source of ATP by re-phosphorylating ADP under “anaerobic” conditions (i.e., when ATP demand exceeds ATP production) according to the following equation:



PCr hydrolysis becomes particularly important during the transition between rest and exercise, contributing a substantial amount to the ATP pool during short-term, high intensity exercise in both fish and humans (Parkhouse et al., 1988; Schulte et al., 1992; Meyer and Foley, 1996). In addition to this role, the creatine kinase reaction consumes  $\text{H}^+$  ions, and therefore contributes to the buffering of intracellular acidosis resulting from exercise. Furthermore, the PCr shuttle provides for energy transport between sites of oxidative ATP synthesis (mitochondria), and ATP utilization (myofibrils) (Meyer et al., 1984).

The relative importance of PCr during exercise greatly depends on the type and duration of exercise. Under conditions of high intensity exercise, power output by working muscles demands greater energy supply than is made available by oxidative phosphorylation, and this increased rate of ATP breakdown results in a decrease in the concentration of ATP, and a corresponding increase in ADP and inorganic phosphate ( $\text{P}_i$ ; Wilson, 1994). To provide the necessary ATP, the majority of energy is therefore produced by substrate phosphorylation from both PCr and glycolysis (Jones et al., 1985),

activated by a shift in the [ATP]:[ADP][P<sub>i</sub>] ratio (Wilson, 1994). Under these conditions, the size of the PCr pool would undeniably influence short-term, high intensity exercise performance. It is the great plasticity of this energy store within muscle that has been the focus of an abundance of human exercise performance studies, and subsequently public interest, within the last decade.

Based on a number of studies (reviewed by Terjung et al., 2000), elevating dietary creatine intake by 20 g per day for as little as 5 days leads to an increase in muscle total creatine (the sum of PCr and creatine) by more than 20% (of which approximately 20% is in the form of PCr; Harris et al., 1992). The process of orally dosing creatine is referred to as creatine 'loading', and the outcome shows a great deal of inter-individual variability in loading potential (from 0-40% change in total creatine; Harris et al., 1992; Greenhaff et al., 1994), which is partially dependent on initial creatine levels. However, despite the variability in response to short-term creatine supplementation, most studies report a resulting increase in the production of muscle force or power output during short bouts of high intensity exercise (Greenhaff et al., 1993; Balsom et al., 1995). Given the ease with which total creatine stores can be manipulated, and the profound effects on muscle performance, application of this methodology to the study of exercise in lower vertebrates, such as fish, could provide a relatively simple means for enhancing fish locomotory ability.

Relative to human studies, less is known of the importance of total creatine levels on high intensity performance in fish. PCr levels in fish white muscle are commonly made metabolic measurements, particularly in studies where the effects of high intensity (exhaustive) exercise regimes on substrate level phosphorylation are being examined, necessitating the comparison of resting and post-exercise PCr levels (Dobson and Hochachka, 1987; Dobson et al., 1987; Parkhouse et al., 1988; Pearson et al., 1990; Scarabello et al., 1991; Wang et al., 1994). Muscle creatine levels are measured less often, and then typically to provide an estimate of percent phosphorylation of creatine to validate

PCr estimates. To our knowledge, the only study on fish that looked specifically at dynamics of the total creatine pool was that of Danulat and Hochachka (1989), who reported a time course for creatine transport from plasma into tissues of starry flounder. By injecting a creatine radioisotope ( $^{14}\text{C}$ -creatine), they concluded that overall creatine turnover was relatively slow in fish muscle (as compared to mammals) and total creatine levels were attenuated in starved fish.

Based on previous work focusing on the exercise metabolism of trout, PCr is the first source of high energy phosphates to be utilized during high intensity exercise (i.e., prior to stored ATP), due to the high activity of creatine kinase and its high affinity for ADP (Dobson and Hochachka, 1987; Mommsen and Hochachka, 1988; Parkhouse et al., 1988). Thus, this metabolite will be crucial for fueling the initial stages of the transition to exercise and possibly even more important than in higher vertebrates, given the evidence that cardiovascular changes are slow to occur in fish (i.e., 5-15 min for cardiac output to increase and blood pressure to stabilize; Kiceniuk and Jones, 1982). This suggests that oxidative phosphorylation during rapid accelerations may not be able to match ATP demand, rendering fish reliant on substrate level phosphorylation (i.e., anaerobic metabolism) in the initial stages of a high intensity bout of exercise. Furthermore, small juvenile fish are susceptible to frequent bouts of this type of exercise during prey capture and predator avoidance, therefore any metabolic adaptation that would enhance energy provision during a rapid acceleration would prove beneficial prior to the up-regulation of oxidative metabolism.

A previous study from this lab has shown that during a fixed velocity sprint test, PCr is depleted to maximal levels (up to 60% depletion) prior to fatigue (Chapter 3). Therefore, altering this storage form of high energy phosphate could provide a greater source of ATP during the initial moments of rapid acceleration (i.e., prior to the up-regulation of both the glycolytic and oxidative pathways), leading to enhanced endurance.



The specific objectives of this study were 1) to establish whether creatine and PCr stores could be altered in fish and 2) to determine whether creatine supplementation could postpone fatigue during a fixed velocity sprint test.

## **Materials and Methods**

### *Experimental animals*

Juvenile rainbow trout (*Oncorhynchus mykiss* Walbaum) were purchased from a local hatchery (Rainbow Springs Trout Farm, Thamesford, ON) and held in a 40 L circular tank, aerated and supplied with a continuous flow of dechlorinated Hamilton tap water for 2 weeks prior to the experiment. During holding, fish were fed a 4% body weight  $d^{-1}$  ration of commercial trout pellets (50% protein content, Corey Feed Mills, Ltd.) fed over 2 meals. In the case of injected fish, they were fed immediately following the injection procedure, upon return to the holding tanks. Thus, fish from all groups were fed within 30 min of one another every day. Fish were acclimated to the experimental temperature, which was  $13 \pm 1$  °C over the entire acclimation and experimental period (approximately 4 weeks).

Following the initial 2 week acclimation period, fish were individually implanted with passive integrated transponder (PIT) tags (2 x 12 mm, 134.2 kHz, Destron Fearing, Inc.). To implant the tag, fish were lightly anaesthetized with tricaine methane sulphonate (MS-222, Syndel Laboratories,  $0.8 \text{ g L}^{-1}$ , neutralized to pH 7.5) and the tag injected into the body cavity. The wound was then sealed with a drop of VetBond tissue adhesive (Minnesota Mining and Manufacturing (3M)), eliminating the need for stitches and preventing loss of the tag. Tag numbers were read using a hand-held scanner (Pocket Reader EX; Destron Fearing, Inc.). Previous trials in our lab have shown that tagging does not impair either sprint performance or survival of fish as small as 1 g. Fish were

randomly separated into 6 tanks (with either 10 or 11 fish per tank; total N=62) and recovered for one week prior to experimentation. After recovering from the tagging procedure, two tanks of fish were fed a creatine supplemented diet, while the remaining 4 tanks were fed a control (creatine-free) diet. Of the 4 tanks fed the control diet, fish from 2 of the tanks were periodically injected with either creatine in saline, or saline alone (sham; see below for details).

#### *Exercise apparatus and sprint protocol*

Sprint tests were performed at McMaster University using an open, recirculating swim flume (102 L volume) designed according to the recommendations of Vogel (1978). The fish were placed in the rectangular working section of the flume which had dimensions of 73 x 11 x 20 cm (L x W x H) and a curved bottom to eliminate eddy effects. Flow was generated by a propeller connected to 373-W DC motor (Dart Controls, Inc., Zionsville, IN), and calibrated using an electromagnetic flow meter (Marsh-McBirney Model 2000).

The sprint test was as described by McDonald et al (1998). Fish were tested in groups (10 or 11 fish per trial) in most swim procedures, for both practical reasons (to swim all fish in a shorter period of time), and because we have previously shown no difference in performance when fish were swum in groups vs. individually (McFarlane and McDonald, 2001). For the first 5 min in the flume an orienting velocity of  $\sim 1$  body length (BL)  $s^{-1}$  was provided. Over the next 2 min, velocity was steadily increased to the test velocity of  $40 \text{ cm s}^{-1}$ , or a relative speed of  $7 \text{ BL s}^{-1}$ . Fish were removed from the flume as they fatigued. The criteria for fatigue was when fish became impinged on the back screen of the flume, and did not resume swimming after three manual prods to the tail. Fatigue time, tag number, length and weight was recorded for each fish, and they were returned to the appropriate holding tank. When the last fish was returned to the holding tank, food was offered. Mean time to fatigue (in min) in each trial was calculated as the

geometric mean (i.e., mean of the log sprint time) as recommended by Brett (1964). Such a calculation produces an asymmetric standard error of the mean, but since the upper and lower errors were rarely very different, they have been averaged and expressed as a single error.

### *Experimental protocols*

Growth rates, fatigue times, and resting muscle creatine, PCr, ATP, lactate and glycogen levels were compared in fish from each treatment group: control, creatine diet, creatine injected, and saline injected (sham).

The dietary creatine (Cr) dose was based on the human dose of 20 g Cr d<sup>-1</sup> for a 70 kg male (equal to 0.29 g Cr kg<sup>-1</sup> d<sup>-1</sup>, Harris et al., 1992). The dose was increased to 0.5 g Cr kg<sup>-1</sup> d<sup>-1</sup>, administered for 7 days. Assuming that a 1 g fish needs a dose of 0.0005 g creatine, and fish are all feeding at a 4% BW d<sup>-1</sup> ration, the amount of creatine added per gram of food was 12.5 mg. Commercial trout pellets (200 g) were ground to a powder, mixed with the appropriate amount of creatine dissolved into 20 ml of water (to allow for even mixing with the ground diet), re-extruded and dried at room temperature for 24 hours. The control diet was formulated in the same manner (but with no added creatine).

The injected creatine dose was adjusted to provide approximately the same amount of creatine on a per gram basis (0.0005 g creatine g<sup>-1</sup> fish). The injected volume was 20 µl g<sup>-1</sup> fish. When fish were removed from the tank, tag number was verified to identify which treatment the fish was to receive (i.e., saline, or saline with creatine), as all injected fish were mixed within the 2 tanks. Fish were weighed, and injected with the weight-scaled volume of the appropriate solution on days 1, 3, 5 and 7.

Following the 7 day experiment (i.e., on day 8), all fish were sprinted at 40 cm s<sup>-1</sup> (7 BL s<sup>-1</sup>), and performance was recorded. Fish were returned to holding tanks and allowed to recover for 24 h, after which muscle samples were taken (as described below).

Based on previous studies on trout, this amount of recovery time is adequate for complete recovery of exercise-induced metabolic changes (for a review, see Kieffer, 2000). Individual growth rates (as % body weight change  $d^{-1}$ ) were calculated based on individual weights on day 1 and day 8.

#### *Tissue sampling and analysis*

Fish were killed by adding a high concentration ( $2\text{ g l}^{-1}$ ; neutralized to pH 7.5) of MS-222 (tricaine methane sulphonate, Syndel Laboratories) to water in the holding tank. Fish generally lost equilibrium within 1 minute, after which they were removed from the tank, and within 90 s, all fish were processed. Sampling involved making an incision to remove the tag, then freeze-clamping the entire fish between two aluminum blocks, pre-cooled with liquid nitrogen. White muscle samples were excised from the frozen fish, and ground to a fine powder under liquid nitrogen. The entire sampling procedure generally took less than 10 seconds. All tissues were stored at  $-70^{\circ}\text{ C}$  for later analysis of metabolites.

Aliquots of ground white muscle were analyzed for creatine, PCr, ATP, glycogen and lactate. For analysis of creatine, PCr, ATP and lactate, 100 mg of tissue was homogenized with 1 ml of 8% perchloric acid, and enzymatic analysis of supernatant was carried out based on procedures in Bergmeyer (1983). For glycogen analysis, 100 mg of frozen wet tissue was digested according to the methods of Hassid and Abraham (1957), and glucose was enzymatically analyzed following the protocol outlined in Bergmeyer (1983).

### *Statistical analysis*

Values are reported as means  $\pm$  1 S.E.M. (N) or as individual values. Creatine supplemented fish were compared to their respective controls (i.e., Control vs. creatine diet, sham vs. creatine injected) using a Dunnett's t-test ( $P < 0.05$ ). The tests were performed using JMP 2.0.5 software (SAS Institute, Inc.).

## **Results**

### *Growth*

Average initial weights for fish from the four treatment groups were not significantly different (Table 4-1). Furthermore, specific growth rate (% body weight change  $d^{-1}$ ), a measure of food conversion efficiency, was not different between the control and the creatine-fed groups or between the sham and creatine-injected groups, although there was a trend (non-significant;  $P=0.10$ ) towards lower growth for the creatine-injected fish, despite the identical daily feeding ration.

### *Endurance*

Creatine supplementation did influence endurance during a fixed velocity test. At the end of the 7 day experimental period, fish fed the control diet swam for  $2.96 \pm 0.34$  min, whereas fish fed the creatine diet swam an average of 30% longer ( $4.40 \pm 0.62$  min, Fig. 4-1). Sham injected fish swam an average of  $2.43 \pm 0.48$  min, which was significantly lower than fish that were given 4 injections of creatine dissolved in saline ( $4.76 \pm 0.54$ , Fig. 4-1). Fatigue times for creatine fed and creatine injected fish were not significantly different.

### *Resting muscle metabolites*

#### Total creatine, ATP and lactate

After 7 days of treatment, there was no difference in total creatine (either free creatine or PCr) levels between creatine supplemented groups and controls (Fig. 4-2). Percent phosphorylation of creatine was relatively high (average range from 73-80%), indicating that the methodology used in sampling muscle tissues adequately preserved PCr. There were also no significant differences in resting levels of ATP (control,  $6.12 \pm 0.26 \mu\text{mol g}^{-1}$ ; creatine diet,  $5.85 \pm 0.25 \mu\text{mol g}^{-1}$ ; sham,  $6.05 \pm 0.32 \mu\text{mol g}^{-1}$ ; creatine injected,  $6.03 \pm 0.20 \mu\text{mol g}^{-1}$ ) or lactate (control,  $5.16 \pm 0.45 \mu\text{mol g}^{-1}$ ; creatine diet,  $5.33 \pm 0.31 \mu\text{mol g}^{-1}$ ; sham,  $4.42 \pm 0.26 \mu\text{mol g}^{-1}$ ; creatine injected,  $4.43 \pm 0.25 \mu\text{mol g}^{-1}$ ).

#### Glycogen

Resting muscle glycogen was significantly higher in creatine-fed and creatine-injected fish, than in control and sham-injected fish (Fig. 4-3). Within group glycogen levels exhibited relatively low variability, whereas between group variability was considerably higher. Furthermore, average glycogen levels corresponded to average fatigue times for each group (compare Fig. 4-3 to Fig. 4-1), meaning that fish with higher glycogen had greater endurance. When individual resting glycogen levels were plotted against individual fatigue times, there was a positive relationship for both creatine-injected and creatine-fed groups ( $r^2$  of 0.193 and 0.111, Fig. 4-4). Nonetheless, the slope of the regression line was almost identical for both treatments (0.137 vs. 0.139, Fig. 4).

## Discussion

This study has shown that supplementation with exogenous creatine (dietary or injected) does not alter muscle creatine levels. However, coincident with creatine intake there is an increase in muscle glycogen and an increase in endurance during a fixed velocity sprint test. Muscle total creatine levels in fingerling trout of  $24.5 \pm 0.5 \mu\text{mol g}^{-1}$  wet weight (a range of 19-35; N=62) are similar to previously reported values in fish (23-50  $\mu\text{mol g}^{-1}$ , Dunn et al., 1983; Dobson and Hochachka, 1987; Wang et al., 1994), and in humans (90-160  $\mu\text{mol g}^{-1}$  dry weight, approximately equal to 18-32  $\mu\text{mol g}^{-1}$  wet weight; Harris et al 1992; Hultman et al., 1996; Greenhaff et al., 1996). This is the first study to examine the effects of creatine loading on fish muscle total creatine levels, and illustrates that fish muscle creatine and PCr levels are less susceptible to manipulation than human muscle stores.

A potential reason for the lack of creatine loading is diet. Trout are carnivores, with a protein content in the diet typically ranging from 40-60% (Cho and Kaushik, 1985). Because the greatest source of creatine is from muscle tissue and fish are reliant on the diet to obtain creatine (due to a low capacity to endogenously synthesize creatine; Danulat and Hochachka, 1989), the amount of creatine fish are ingesting on a daily basis is already far higher than would be seen in a typical omnivore. Human studies have shown that initial creatine levels determine the extent of accumulation of creatine; subjects with high initial creatine show very little loading capacity in comparison with subjects having low endogenous creatine levels (such as vegetarians; Harris et al., 1992). The lack of effect of supplemented creatine on muscle creatine content suggests that fish may already be at the upper range of their creatine capacity. Given the fact that creatine is an osmotically active molecule (Ziegenfuss et al., 1998), it could be disadvantageous to take up greater amounts, as this would induce higher water retention within the muscle, potentially affecting contractile properties. Since at least 60% of the body mass of fish is comprised of muscle

(Johnston, 1981), this represents a potentially large amount of fluid that may significantly affect muscle function. Nonetheless, the lack of creatine uptake does explain the absence of growth differences between treatment groups, since creatine uptake leads to weight increase *via* water retention (Hultman et al., 1996).

Fish muscle appears to have adapted to possess maximum total creatine stores, and creatine loading in a manner proven to be effective in elevating total creatine in human studies is of minor consequence, at least in fish maintained on an adequate ration. However, the possibility does exist that the time frame was too short (7 days) and dose too low to detect similar changes as seen in humans, given the lower metabolic rates of these poikilotherms. There may also be differences in the ability to transport creatine, or in the up-regulation of creatine transport capacity of the muscle in response to high circulating creatine levels.

Despite the lack of creatine uptake in supplemented fish, there was a significant (32-48%) increase in endurance during a sprint test. This is different than human studies, where changes in performance are solely due to the elevation of muscle creatine levels. The enhancement of performance in trout appears not due to changes in the available adenylate pool (PCr or ATP), or lower resting lactate levels, but can only be explained by alterations in muscle glycogen. During this type of prolonged exercise, the contribution of glycogen is not only significant, but resting glycogen has previously been shown to be positively correlated with endurance ( $r^2=0.32$ ; Chapter 3) indicating that greater glycogen must lead to higher glycolytic flux and thus an increase in substrate level phosphorylation of ADP. Elevated PCr stores could contribute during the initial bursts of activity (i.e., during the acceleration period) but glycogen will be a more important contributor to sprint endurance. In human studies, it was found that in longer-term high intensity exercise (lasting minutes), the effect of enhanced PCr stores was 'diluted' by the greater contribution by glycolysis, which is why no effect of creatine loading was detected (Stroud et al., 1994). However,



we do not know whether this same logic would apply to a fish system, as an elevation of PCr stores was not achieved, and therefore the relative contribution not tested. Nonetheless, given the fact that higher glycogen levels were only seen in creatine fed and creatine injected fish, by what mechanism is creatine responsible for elevating muscle glycogen?

Human studies show that creatine and carbohydrate (glucose) taken together lead to high uptake of creatine into muscle (Green et al., 1996). This is due to a glucose-induced surge in insulin secretion that increases the sodium-dependent transport capacity of muscle cells for creatine, and therefore leads to greater flux of creatine, against the concentration gradient, into skeletal muscle (Steenge et al., 1998). Recently, it has been determined that the insulin response is greater when creatine is ingested in conjunction with carbohydrate as opposed to just creatine alone (Steenge et al., 2000). This implies that circulating creatine levels have an effect on insulin secretion, resulting in a greater transport capacity of creatine and also a greater ability to transport glucose into skeletal muscle. The response to circulating levels of creatine in trout, over and above amounts of creatine provided through the diet, may exert an insulin-mediated effect on the glycogen accumulating ability of white muscle. Since fish were always fed in conjunction with the creatine dose, this provided a significant source of metabolites (i.e., amino acids and glucose) to channel into glycogen production.

Historically, insulin was thought to be of little importance in metabolic regulation in fish, however, this was due to analytical problems revolving around the lack of homology between fish and mammalian insulin antibodies for RIA determination of circulating levels (Tilzey et al., 1985). In general, insulin concentrations in the systemic circulation of fish tend to be higher than in mammals (Mommensen and Plisetskaya, 1991), however, a similar insulin response is noted in response to feeding as seen in mammals. In addition, a decrease in hepatic glycogen and resulting hyperglycemia is noted upon insulin infusion

(Ince and Thorpe, 1976; Lewander et al., 1976; Carneiro and Amaral, 1983), however, one major difference is that amino acids are stronger secretagogues than glucose in fish (Mommsen and Plisetskaya, 1991). Trout, being fed a high protein diet, possess fairly high levels of circulating amino acids following feeding, and if a similar mechanism exists as in mammals, in conjunction with higher than normal levels of circulating creatine, this could lead to a greater overall insulin secretion in creatine supplemented groups. From human studies, it is known that maximum circulating levels of creatine occur within an hour (Harris et al., 1992) and are back to resting levels within 4 hrs, this being paralleled by the insulin response (Steenge, 2000). However, in fish it takes days to see a response from injected insulin (Brinn, 1973), which suggests that insulin has a much slower time course of action in fish. Future studies should involve investigating the time course of creatine and insulin appearance in plasma, and the effect of dose and length of supplementation on muscle creatine levels.

Most research involving the metabolic regulation by insulin focuses on hepatic glucose uptake in adult fish, with less emphasis placed on skeletal muscle effects (Mommsen and Plisetskaya, 1991). Insulin has variable effects on skeletal muscle glucose uptake and resulting glycogen content, with some studies showing an increase in glycogen (e.g. Tashima and Cahill, 1968), and others showing no change (i.e., apparent insulin resistance; e.g. Carneiro and Amaral, 1983). It is possible that both amino acids and glucose are taken up by skeletal muscle, with amino acids being directed into protein synthesis (Ablett et al., 1981) and glucose directed into glycogen storage, given the lack of gluconeogenic capacity of skeletal muscle (Moon, 1988). Regardless of the mode of administration of creatine (dietary or injected), the effect on muscle glycogen levels and on subsequent fatigue time was identical, which suggests that a similar performance enhancing mechanism is responsible in both cases. However, the process by which creatine amplifies

the insulin response is not clear, and in fact is barely even understood in humans, where this area of research originated.

It is possible that we are investigating a phenomenon only present in juvenile fish. At this life stage, the uptake of amino acids and glucose into muscle is likely different than for adult fish, given the fact that they are in a period of rapid growth. Insulin may possess a more pivotal role in juveniles, as it has been alluded to that insulin may have growth promoting properties in fish (Mommensen and Plisetskaya, 1991). In addition, muscle glycogen levels of juvenile salmonids are extremely sensitive to dietary changes (i.e., changes in ration; Hochachka and Sinclair, 1962; Scarabello et al., 1991; Chapter 3, this thesis). Thus the requirement of fish of this size for either dietary or hepatically provided glucose, may be quite high, making the secretion of insulin crucial in the determination of muscle glycogen content, and muscle glycogen crucial in the determination of swimming endurance.

### *Conclusions*

Creatine supplementation leads to enhanced endurance, and elevated white muscle glycogen stores, irrespective of whether it is supplemented through the diet or injected. The effect of creatine on endurance and glycogen may be linked, with a possible mechanism for the elevation in glycogen being insulin-mediated, due to the high levels of circulating amino acids and creatine, and higher glycogen leading to better sprint endurance. Although this may be a response only present in juvenile fish, given the significant impact of the ergogenic effects of creatine on human exercise, the application of creatine as a dietary supplement in fish feeds will not only aid in our understanding of the importance of this metabolite, but could also be applied in the study of survivability and fitness of hatchery raised fish upon stocking.

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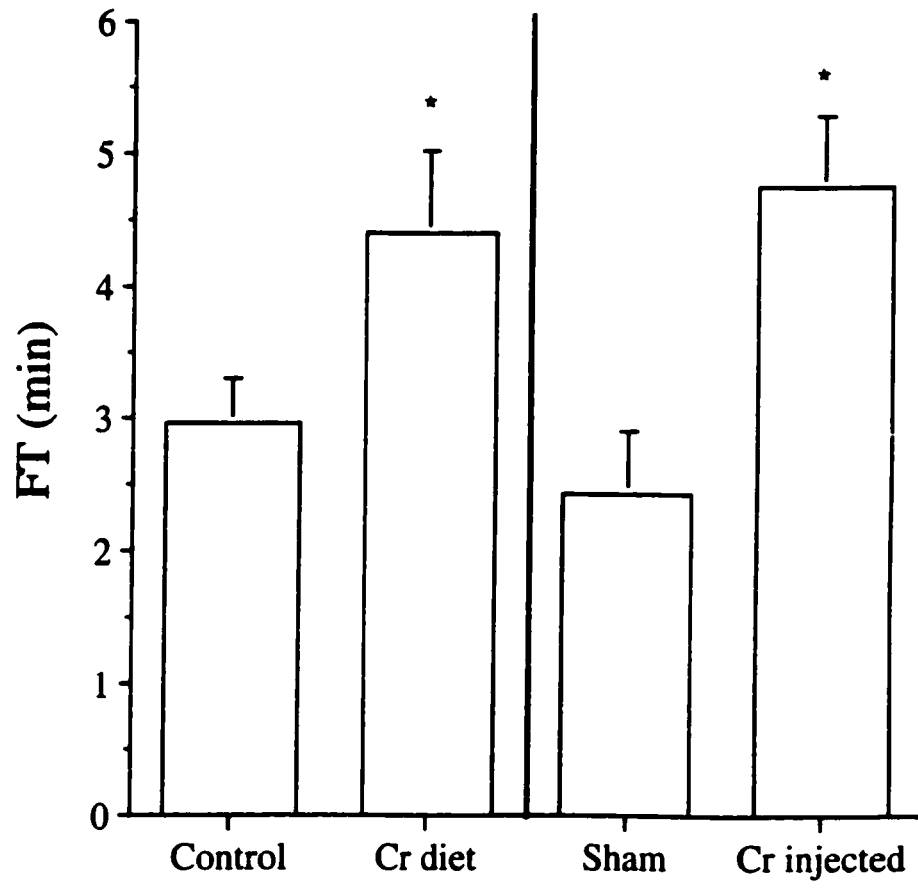
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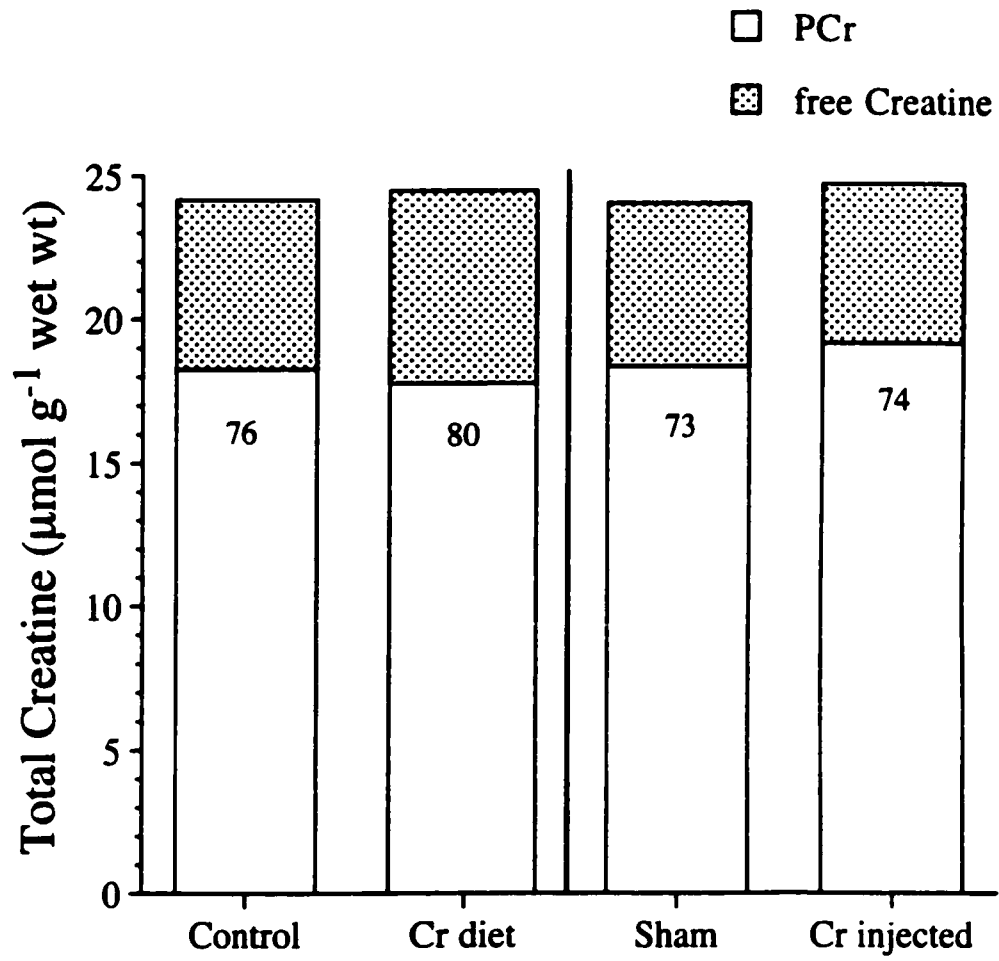
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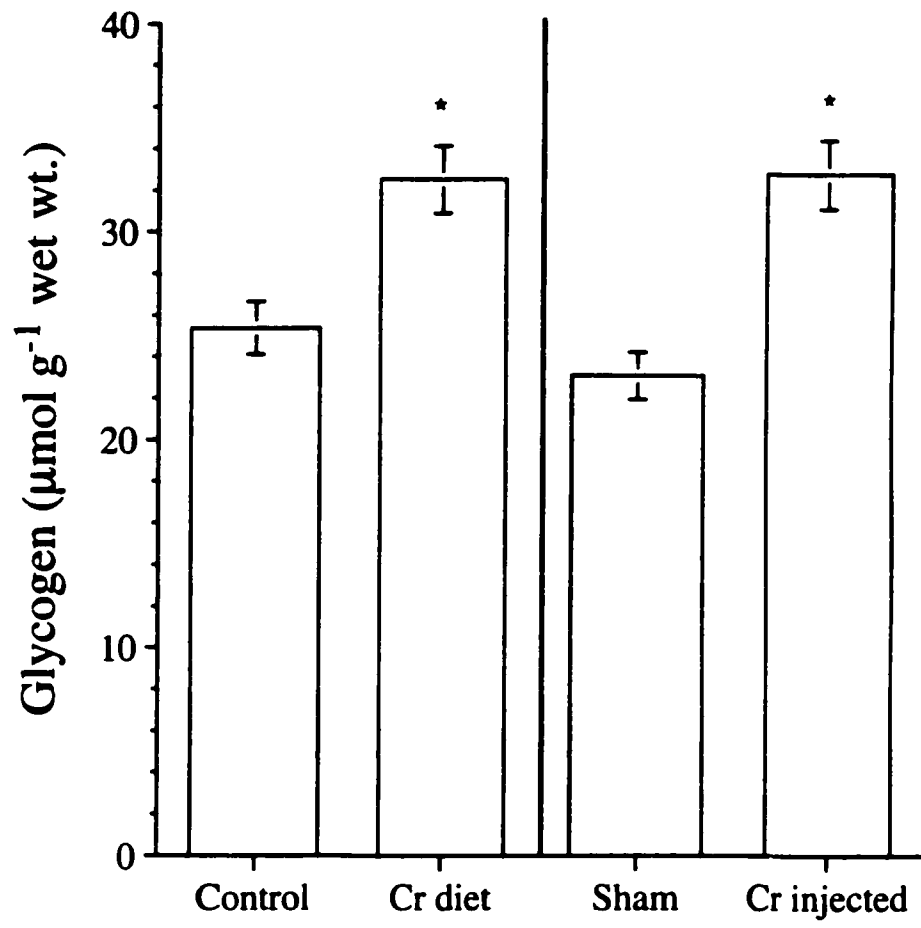
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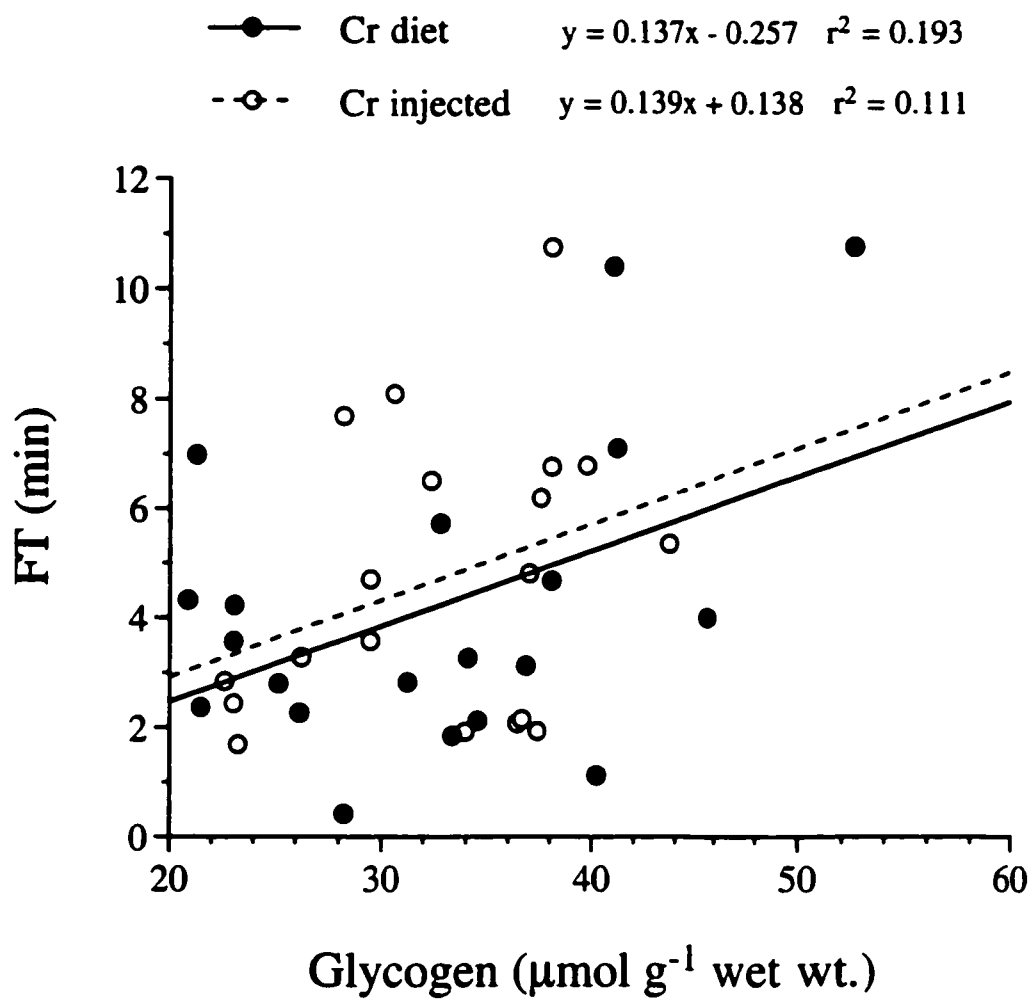
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**Table 4-1.**

Initial and final weights and calculated specific growth rates for juvenile rainbow trout from each of 4 treatment groups: control diet, creatine diet, control diet/sham (saline injected), and control diet/creatine injected. Values are means  $\pm$  S.E.M. (N).

Treatment Group	Initial weight (g)	Final weight (g)	Specific Growth Rate (% body weight d <sup>-1</sup> )
Control	1.82 $\pm$ 0.09 (11)	2.42 $\pm$ 0.14	4.01 $\pm$ 0.37
Creatine diet	1.74 $\pm$ 0.08 (20)	2.35 $\pm$ 0.12	3.97 $\pm$ 0.29
Sham	1.65 $\pm$ 0.10 (10)	2.19 $\pm$ 0.16	4.17 $\pm$ 0.33
Creatine injected	1.70 $\pm$ 0.09 (21)	2.16 $\pm$ 0.13	3.21 $\pm$ 0.14

## **CHAPTER 5**

### **EXPLOITING INTER-INDIVIDUAL VARIABILITY TO EXAMINE PROLONGED SWIM PERFORMANCE IN FINGERLING RAINBOW TROUT**

#### **Summary**

In this study, individual sprint performance was examined in a large, homogeneous group of juvenile rainbow trout (1-2 g) in order to address the following questions: What is the scope of inter-individual variability? How long do individual differences persist? What metabolic factors limit sprint performance? and How does endurance correlate with other performance measures (such as growth or stress response)? Fish were divided into high and low performance groups based on endurance in a fixed velocity sprint test (at  $\sim 7$  BL  $s^{-1}$ ), and a number of correlates of sprint performance were examined. Changes in the accumulation of lactate, and depletion of phosphocreatine (PCr) and ATP within white muscle were used to calculate, in ATP equivalents, the anaerobic energy expenditure (AEE, in  $\mu\text{mol g}^{-1}$ ). Fish with poor sprint endurance displayed higher anaerobic energy expenditure during a fixed velocity sprint, whereas fuel depletion during a manual chasing protocol (an estimate of anaerobic capacity) was not correlated to sprint performance. Differences in the ability to train were seen between high and low performers, with only high performers showing an improvement in endurance after 7 daily sprints. Other measurements such as blood hemoglobin (Hb) level or activity of key enzymes of glycolysis, oxidative metabolism or lipid metabolism (lactate dehydrogenase, LDH; citrate synthase, CS;  $\beta$ -hydroxyacyl CoA dehydrogenase, HOAD) were not significantly different

between high and low performers. Furthermore, when high and low performing fish were exposed to challenges unrelated to swimming, their performance was no different, suggesting that sprint performance cannot be used as a predictor of overall fitness. Nonetheless, endurance in a fixed velocity sprint test was correlated with performance in a more aerobic test, the 30 min critical velocity ( $U_{crit}$ ) test, indicating a link between sprint performance and aerobic capacity. Furthermore, the difference in training ability was probably related to differences in cardiovascular performance. There was a negative influence of circulating stress hormones as plasma cortisol levels were inversely related to both performance and trainability. This study provides insight into the basis of inherent sprint performance, and also introduces a sorting methodology that facilitates the examination of this performance variability in juvenile trout.

## **Introduction**

Previous studies have demonstrated that individual performance during a fixed velocity sprint is both highly variable and reproducible (Gregory and Wood, 1998; McDonald et al., 1998a; McFarlane and McDonald, 2001). However, the physiological basis of this great variability has not been thoroughly investigated. Similar variability in swim performance has also been demonstrated in critical velocity (Kolok, 1992a; Kolok and Farrell, 1994a, Gregory and Wood, 1998, 1999a; Reidy et al., 2000) and high intensity performance (Kolok, 1992b; Reidy et al., 2000) in a number of different species of fish.

Within the last decade, studies examining the correlation between locomotor performance and other traits have become more common (for review Kolok, 1999). In an attempt to determine the physiological basis for variability in performance, correlations between performance (most commonly assessed by  $U_{crit}$ ) and other morphological,

physiological or genetically-linked traits such as fin size (Plaut, 2000), body shape (Taylor and McPhail, 1985), growth rate (Kolok and Oris, 1995; Gregory and Wood, 1999a), metabolic rate (Reidy et al., 2000), cardiovascular parameters (i.e., cardiac output, heart rate and stroke volume; Kolok and Farrell, 1994a,b), blood oxygen carrying capacity (i.e., hematocrit or hemoglobin; Jones, 1971; Gallagher et al., 1992; Brauner et al., 1993) aerobic and anaerobic enzyme activities (Farrell et al., 1991; McDonald et al., 1998a), and LDH phenotype (Dimichele and Powers, 1982) have been investigated. The logic behind these types of studies is that by determining correlations between different physiological traits and swim performance, it will lead to the understanding of the traits upon which swimming performance is dependent.

The purpose of this study was to exploit the individual variability in sprint performance to examine physiological mechanisms underlying the ability to sprint and determine how they relate to other measures of performance. A methodology by which to sort fish according to sprint performance was developed, in which fish were divided into high, medium and low performers, depending on their fatigue times during a sprint. This allowed for fish to be sorted into either high or low performance groups. The specific objectives of this study were to determine how long individual performance differences persisted, whether they correlated with growth,  $U_{crit}$  or response to an induced stress (epinephrine injection or toxicant exposure), and to determine the relative ability to improve performance through sprint training. Furthermore, a suite of muscle and blood measurements were compared between high and low performers: resting glycogen, lactate, PCr and ATP, AEE during a sprint, anaerobic capacity (AC), enzyme activities of key enzymes of both glycolytic and oxidative metabolism (LDH, CS and HOAD), oxygen carrying capacity (by blood hemoglobin, Hb) and as an indicator of relative stress level, plasma cortisol. Together, these physiological measures will provide insight into the

factors governing prolonged swim performance following a rapid acceleration in fish with diverse endurance abilities.

## **Materials and methods**

### *Experimental animals*

Fish used in this study were juvenile rainbow trout (*Oncorhynchus mykiss* Walbaum; N=650;  $1.33 \pm 0.01$  g;  $5.20 \pm 0.01$  cm) obtained in a single batch from Rainbow Springs Trout Hatchery (Thamesford, Ontario). Fish were held in a 300 L circular tank supplied with dechlorinated Hamilton tap water with a partial replacement of approximately 1 L per minute, and were acclimated to the holding conditions for at least 2 weeks prior to use. Fish were acclimated to a water temperature of  $13 \pm 1.5^\circ$  C over the experimental period, and were offered a 4% body weight per day ration of commercial trout feed (Corey Feed Mills, Ltd) fed over two meals. A minimum of 12 hours separated the feeding and experimental periods to minimize postprandial effects. Ration was adjusted and specific growth rate (% body weight change per day) calculated by measuring bulk tank weights (i.e., mass of all fish contained within the tank).

### *Exercise apparatus*

Exercise tests were performed at McMaster University using an open, recirculating swim flume (102 L volume) designed according to the recommendations of Vogel (1978), with a swimming section of dimensions 73 x 11 x 20 cm (L x W x H). Flow was generated by a propeller connected to 373-W DC motor (Dart Controls, Inc., Zionsville, IN), and calibrated using an electromagnetic flow meter (Marsh-McBirney Model 2000).



### *Swim procedures*

Two types of swim procedures were used: a fixed velocity sprint test, and a critical velocity ( $U_{crit}$ ) test.

i) Sprint: The sprint protocol was as described by McDonald et al (1998a). Fish were typically swum in groups of 10, as this has previously been shown to have no effect on individual performance (McFarlane and McDonald, 2001). For the first 5 min in the flume, an orienting velocity of approximately 1 body length (BL)  $s^{-1}$  was provided. Over the next 2 min, velocity was steadily increased to a test velocity of 35 or 40  $cm s^{-1}$ , adjusted to the average body length of the group, but always producing a relative speed of between 6 and 7 BL  $s^{-1}$ . These test velocities were chosen as they are approximately equal to previously measured  $U_{crit}$  values for fish of similar size (Chapters 2 and 3). Our criterion for fatigue was when fish fell against the back screen and no longer resumed swimming after three manual prods to the tail. At this time, they were removed from the flume, and in some cases terminally anaesthetized prior to the sampling of muscle tissue (see below for sampling procedures). Fish were removed as they fatigued, without interrupting the flow through the tunnel or disturbing the remaining fish. Individual fatigue times, lengths and weights were recorded from each fish, and mean time to fatigue (in min) in each trial was calculated as the geometric mean (i.e., mean of the log sprint time) according to the recommendations of Brett (1964). Such a calculation produces an asymmetric standard error of the mean, but since the upper and lower errors were rarely very different, they have been averaged and expressed as a single error.

ii) Critical velocity: The 30 minute incrementally stepped velocity test was a modified version of that outlined by Brett (1967), in which the velocity intervals were 30 min rather than 45 min. Fish were transferred to the flume in groups of 10 and oriented for 30 min at a starting velocity of 1 BL  $s^{-1}$ . Velocity was rapidly increased by 1 BL  $s^{-1}$

(typically 5-6 cm s<sup>-1</sup>, based on the average length of the group of fish) every 30 minutes until fatigue. Each step increase was completed within 2-3 seconds, i.e., an instantaneous acceleration.  $U_{crit}$  was calculated according to the following expression from Brett (1964):

$$U_{crit} = U_p + \left( \left( \frac{t_f}{t_i} \right) + U_i \right)$$

where  $U_i$  is the velocity increment (cm s<sup>-1</sup>),  $U_p$  is the penultimate velocity at which the fish swam prior to fatigue,  $t_f$  is the elapsed time from the velocity increase to fatigue, and  $t_i$  is the time between velocity increments. Relative  $U_{crit}$  was calculated according to individual fork lengths, and expressed as BL s<sup>-1</sup>.

#### *Sorting methodology*

In order to obtain groups of fish with diverse performance, the entire population of fish were sprinted to fatigue, and divided into high and low performance groups according to fatigue time. To avoid sorting fish according to body size, a scaling relation of fatigue time to body size was first determined to allow fatigue times to be corrected to a reference body weight (as outlined in McDonald et al., 1998). Fifty fish were sprinted at 35 cm s<sup>-1</sup> (in groups of 10), and fatigue times were plotted against weight. This relation produced a scaling coefficient of 1.5, and an  $r^2$  of 0.28. The scaling coefficient was used to size correct fatigue times to a reference weight of 1 g according to the following equation from McDonald et al (1998a):

$$FT_c = \frac{FT \text{ (min)}}{\left( \frac{\text{Body wt. (g)}}{\text{Reference wt. (g)}} \right)^b}$$

where  $FT_c$  is the size corrected fatigue time and  $b$  is the scaling coefficient from the relation of fatigue time to fish weight.

To determine the endurance criteria by which fish would be sorted, 30 fish were sprinted, their fatigue times were size corrected and the group was divided into thirds based on the corrected fatigue times. The resulting endurance limits were as follows: low performers fatigued in less than 100 s (1.67 min), high performers in greater than 150 s (2.5 min), and medium performers in between. However, only the two extremes, (i.e., high and low performers) were utilized in this study, to ensure no overlap in the fatigue times. The remaining 570 fish ( $1.34 \pm 0.02$  g;  $5.2 \pm 0.01$  cm) were subsequently sprinted at the same speed ( $35 \text{ cm s}^{-1}$ , an average relative speed of  $\sim 6.7 \text{ BL s}^{-1}$ ), fatigue times were size corrected as outlined above, and fish were sorted according to the above endurance ranges. Upon recording the fatigue time and weight of individual fish, the corrected fatigue time was immediately calculated, and each fish was allocated to the appropriate performance group. All fish were held in circular 40 L tanks at a density of  $\sim 50$  fish per tank.

#### *Comparisons between high and low sprint performers*

Following the initial sorting procedure, fish from both the high and low performance groups underwent a number of different protocols that were either swimming challenges (sprint tests,  $U_{crit}$  tests) or “stress” challenges (epinephrine injection, waterborne copper exposure, and waterborne phenol exposure). Separately, or in conjunction with the above tests, a number of physiological measurements were made on high and low performers: specific growth rate, resting glycogen content, resting and post-exercise levels of lactate, PCr and ATP (i.e., at rest or following either a sprint or a forced exercise/chasing protocol), plasma cortisol, white muscle enzyme activity, whole blood

hemoglobin, sodium loss, copper accumulation, and mortality. In order to preserve the inexperienced nature of each fish, and because sampling of fish (for blood or tissue) on more than one occasion was not possible, different fish were utilized for each measurement (with appropriate controls).

### *Swim performance*

To determine whether performance differences persisted over time, high and low performers (N=20 each) were sprinted at  $35 \text{ cm s}^{-1}$  on three occasions: 1, 15 and 30 days following the initial sorting procedure.

The relative ability to improve sprint performance was also examined in 20 fish from each of the high and low performance groups, by sprinting fish to fatigue once daily at  $35 \text{ cm s}^{-1}$  ( $7 \text{ BL s}^{-1}$ ) for a period of 7 days. Determination of their relative abilities to train was assessed by the amount of improvement in endurance at day 7 as compared to day 1.

The maximum sustainable (or critical) velocity was also estimated in 20 high and 20 low performers to correlate sprint performance with maximum aerobic performance.

### *Growth*

Specific growth rate was monitored on three occasions: over a 14 day period by measuring bulk tank weights of 4 tanks of 30 fish from both high and low performance groups, in conjunction with the 30 day period when fish were sprinted on days 1, 15 and 30, and during the 7 day sprint training period. Specific growth rate for high and low performers was calculated according to the following equation:

$$\text{SGR} = \frac{\ln\left(\frac{w_f}{w_i}\right)}{td} \times 100$$

where SGR is specific growth rate as a percentage of body weight per day,  $w_i$  is initial weight,  $w_f$  is final weight, and  $t_d$  is the experimental period in days.

#### *Muscle and blood measurements*

White muscle samples were taken from both trained fish and untrained fish (see below for sampling methodology) to measure resting levels of glycogen, lactate, PCr and ATP. Post-exercise sampling provided an estimate of anaerobic energy expenditure during a sprint and anaerobic capacity.

*i) Anaerobic energy expenditure during a sprint.* To assess anaerobic ATP utilization during a sprint test, the AEE (or anaerobic ATP turnover) in ATP equivalents was calculated, as described by Pearson et al (1990), according to the following equation:

$$\text{AEE} = (\Delta \text{Lactate}) \times 1.5 + \Delta \text{ATP} + \Delta \text{PCr}$$

where  $\Delta$  represents the difference between resting and fatigued/exhausted animals, 1.5 ATP are produced per lactate, and 1 PCr = 1 ATP. During anaerobic metabolism, ATP is also provided through ADP hydrolysis, yet this was ignored due to its very small overall contribution (less than 10% of AEE; Pearson et al., 1990).

*ii) Anaerobic capacity.* This measurement provides an indication of the maximum anaerobic energy utilization (i.e., fuel depletion) of a fish by using a forced exercise protocol (McDonald et al., 1998). In this exercise protocol, fish were transferred (in groups of 10) to 40 L tubs filled with 20 L of water and immediately chased for 8 minutes through continuous manual stimulation. Following 8 min of forced exercise, fish were typically unresponsive to manual stimulation and exhibited a loss of equilibrium, both indicators of exhaustion (Parkhouse et al., 1988). Fish were then removed, anaesthetized,

and sampled (as described below). ATP, PCr and lactate were measured to provide an estimate of the energy utilization in ATP equivalents, as above.

White muscle enzyme activities of a key enzyme of the glycolytic pathway (lactate dehydrogenase, LDH), the TCA cycle (citrate synthase, CS) and lipid oxidation (hydroxyacyl CoA dehydrogenase, HOAD) were examined in high and low performers to determine whether maximal enzyme activities correlated with sprint ability.

Plasma cortisol was measured in blood sampled in high and low performers (N=10 each) prior to and following sprint training (24 h following the last sprint) as an indicator of relative stress levels of each group and the effect of repeated exercise bouts (see *Blood sampling* section for details).

Blood hemoglobin levels were measured in 65 fish (both high and low performers) at rest, and in 22 fish following a sprint, to correlate both group and individual performance with oxygen carrying capacity (see *Blood sampling* section for details).

### *'Stress' challenges*

*i) Epinephrine injection:* Stress was imposed by injecting fish intraperitoneally with epinephrine dissolved in saline ( $1 \text{ mg ml}^{-1}$ ) at a dose of  $25 \mu\text{l g}^{-1}$  (adjusted according to individual weights; N=12 each for high and low), while respective controls were injected with saline alone (sham; N=12 each group). Fish were placed, 4 at a time, into 250 ml tubs containing 200 ml of aerated, sodium-free (soft) water. Water samples (5 ml volume) were removed after 10 min, 30 min and 60 min, and sodium content of the water was measured by atomic absorption spectroscopy (Varian AA-1275). Sodium loss from the fish (i.e., sodium appearance in the water at each interval) was expressed in both  $\mu\text{Eq g}^{-1}$ , and as a % of whole body sodium lost at the end of the 60 min period, as compared to control fish. Performance was assessed by the relative amount of sodium loss.

*ii) Waterborne copper exposure:* Fish from both high and low groups were placed in sodium-free (soft) water containing either 400  $\mu\text{g l}^{-1}$  copper, spiked with 200  $\mu\text{l}$  radioactive copper ( $^{64}\text{Cu}$ ; N=12 each for high and low), or no copper (controls; N=12 each). Water samples were taken at 0, 1, 2 and 4 hours following addition of copper. Sodium loss (expressed in  $\mu\text{Eq g}^{-1}$  and % whole body loss) and whole body copper burden ( $\mu\text{g g}^{-1}$ ) at the end of the 4 hour period were used as indicators of ionoregulatory disturbance and copper uptake, respectively. Samples were measured for  $^{64}\text{Cu}$  activity (with automatic decay correction) in a Canberra-Packard MINAXI g Auto-Gamma 5000 series Gamma counter for 5 min or until 2% accuracy had been obtained. Newly accumulated copper ( $\text{Cu}_{\text{new}}$ ,  $\mu\text{g g}^{-1}$  wet mass) was then calculated according to the following equation:

$$\text{Cu}_{\text{new}} = \frac{R}{M \times \text{SA}}$$

where R (counts  $\text{min}^{-1}$ ) is the radioactivity of the tissue corrected for background, M is body mass (g), and SA is specific activity (counts  $\text{min}^{-1} \mu\text{g}^{-1} \text{Cu}$ ) of the radioactive Cu solution (200  $\mu\text{l}$ ) counted at the same time as the whole bodies. Sodium content of water samples were measured in the same manner as following the epinephrine injection. Performance was assessed based on the relative amounts of sodium loss, and  $\text{Cu}_{\text{new}}$  between high and low performers.

*iii) Waterborne phenol exposure:* Fish from each group (N=16) were exposed to water containing phenol (15  $\text{mg l}^{-1}$ ), a reference toxicant known to produce mortality (Environment Canada, 1990). Fish were monitored hourly, and removed upon death. Times were converted to log times, and plotted against probit mortality. A linear regression

yielded a slope for the line and allowed for the calculation of time to 50% mortality. Performance was assessed based on relative time to 50% mortality for each group.

#### *Tissue sampling and analytical techniques*

Fish were terminally anaesthetized either prior to or following swim protocols (outlined above) in NaHCO<sub>3</sub> buffered tricaine methane sulphonate (MS-222) at a dose of 1 g L<sup>-1</sup>. Within a few seconds fish were flaccid, and the entire body was freeze-clamped between two aluminum blocks, pre-cooled with liquid nitrogen. White muscle samples were excised from the frozen fish, and ground to a fine powder under liquid nitrogen. The entire sampling procedure generally took less than 10 seconds. All tissues were stored at -70° C for later analysis.

Aliquots of ground white muscle were analyzed for glycogen, lactate, PCr and ATP. For glycogen analysis, 100 mg of frozen wet tissue was digested according to the methods of Hassid and Abraham (1957), and glucose was enzymatically analyzed following the protocol outlined in Bergmeyer (1983). For analysis of lactate, PCr and ATP, 100 mg of tissue was homogenized with 1 ml of 8% perchloric acid, and enzymatic analysis of supernatant was carried out based on procedures in Bergmeyer (1983).

For enzyme analysis, frozen tissue (100 mg) was placed in ice-cold 50 mM imidazole buffer (pH 7.4) and homogenized with a Polytron PT 10 unit for three 10 second bursts. The homogenate was centrifuged at 4° C for 10 min, and the supernatant used for analysis. Maximal enzyme activities were determined using a Hewlett Packard 8452A diode array spectrophotometer with a water-jacketed cuvette holder. Temperature was controlled with a Haake D8 circulating water bath at 15 ± 0.2° C. Reaction times of lactate dehydrogenase (LDH) and hydroxyacyl CoA dehydrogenase (HOAD) were determined by the change in absorbance from the oxidation of NADH to NAD at 340 nm ( $\epsilon_{340}$  6.22).



Citrate synthase (CS) activity was measured at 412 nm ( $\epsilon_{412}$  13.6) using 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB). Conditions were optimized with respect to substrate concentration as follows: CS (E.C. 4.1.3.7): 50 mM imidazole pH 8.0 at 20° C, 0.1 mM DTNB, 0.3 mM acetyl CoA, 0.5 mM oxaloacetate (omitted for control). HOAD (E.C. 1.1.1.35): 50 mM imidazole pH 7.4 at 20° C, 0.1 mM NADH, 0.1 mM acetoacetyl CoA (omitted for control). LDH (E.C. 1.1.1.27): 50 mM imidazole pH 7.4 at 20° C, 2.0 mM NADH, 10.0 mM pyruvate (omitted for control).

#### *Blood sampling and analytical techniques*

Either prior to or following a sprint test, fish from both the high and low performance groups were killed by a sharp blow to the head, and blood was carefully sampled from fish via caudal severance. Whole blood was kept on ice for subsequent determination of hemoglobin levels. Hemoglobin was measured (in g/100ml blood) using the cyanmethemoglobin method (Sigma diagnostics, 525-A assay kit). In some cases, blood was centrifuged for 3 min, and plasma extruded and frozen in liquid nitrogen prior to storage at -70° C, for subsequent cortisol analysis. Cortisol concentrations were measured using an ImmuChem Coated Tube Cortisol  $^{125}\text{I}$  Radioimmunoassay Kit (ICN Biomedicals).

#### *Statistical analyses*

Data are expressed as either individual values, or more commonly as means  $\pm$  1 S.E.M. Comparisons of treatment groups with respective controls were tested by one-way analysis of variance (ANOVA). If the result from the ANOVA was significant, a Tukey-Kramer HSD test for multiple comparisons was applied to test for significant differences among treatments ( $P < 0.05$ ; Zar, 1996). When a comparison of only one treatment with a

respective control was necessary, a Dunnett's t-test was applied ( $P < 0.05$ ). The tests were performed using JMP 2.0.5 software (SAS Institute, Inc.).

## **Results**

### *Results of the sorting procedure*

The range of fatigue times of 600 fish was highly variable, but nonetheless, did scale with body size (Fig. 5-1A). However, both the  $r^2$  and the scaling coefficient of the regression line was lower than that produced from the scaling relation for 50 fish (0.13 vs. 0.65 and 1.1 vs. 1.5, respectively). Nonetheless, following size correction of fatigue times, body size was removed as a factor determining endurance, yet significant variability in endurance within the population still existed (a nearly 40 fold range; Fig. 5-1B). When the sorting criteria were applied, this produced an approximately equal number of fish in each group, with 211 low performers, 195 high performers and 194 medium performers (Fig. 5-1B). Furthermore, there were no significant differences in initial size between high and low performers ( $1.27 \pm 0.07$  g vs.  $1.39 \pm 0.06$  g).

### *Persistence of performance*

One day following the sort, high performers exhibited significantly greater endurance than low performers, with an average 3 fold difference in fatigue times ( $N=20$  for each group; Fig. 5-2). This result confirmed the effectiveness of the sorting procedure. The diversity in performance continued through day 15, and became amplified by day 30, at which point there was a nearly 6 fold difference in endurance (Fig. 5-2). However, the greater difference was not explained by differential growth as specific growth rates over this 30 day period were not significantly different between low and high performers (2.33 vs.  $2.27 \% d^{-1}$ , respectively; Table 5-1).

*Comparisons in which there was no difference between high and low performers*

Most of the comparisons made between high and low performers resulted in very similar responses. Growth rate, measured by taking bulk tank weights of fish on 4 occasions revealed that high and low performers grew equally well (3.62 vs. 3.78 % d<sup>-1</sup>; Table 5-1). A number of metabolic indicators also proved to be nearly identical, including resting levels of lactate, PCr and ATP, and maximal enzyme activities of the key enzymes LDH, HOAD and CS (Table 5-1). Furthermore, the anaerobic capacity measured following a manual chase to exhaustion (i.e., all fish exercised at the same high level of exertion) was similar for high and low performers (Table 5-1). Individual levels of hemoglobin exhibited large variability, ranging from 2 to 14 g 100 ml<sup>-1</sup>, however, they did not correlate with fatigue time (Fig. 5-3), and overall were not significantly different between low and high sprint performers (7.01 vs. 6.86 g 100 ml<sup>-1</sup>; Table 5-1). Lastly, the response to different stressors did not produce any differences in performance between high and low sprint performers. Fish that were sham-injected showed no difference in whole body sodium loss between high performers (0.73 ± 0.02 µEq g<sup>-1</sup>; 1.9 % whole body sodium) and low performers (0.68 ± 0.02 µEq g<sup>-1</sup>; 1.8 % whole body sodium). Furthermore, 60 min after an epinephrine injection, sodium loss was elevated by nearly 2 fold in both groups, but there no difference between high and low performers was apparent (Table 5-1). Following waterborne copper exposure, sodium loss was not different for high than for low sprint performers, being approximately 5 µEq g<sup>-1</sup> (~11-12% whole body levels, Table 5-1) for both groups. In addition, levels of newly accumulated copper were also similar, indicating that along with a lack of effect on sodium loss, there was also no difference in copper uptake and retention over a 4 hour period between groups (Table 5-1). In response to waterborne phenol exposure, the calculated time to 50% mortality was not

significantly different in either high or low performers, being nearly 4 hours for both groups (Table 5-1).

#### *Comparisons producing differences between high and low performers*

The maximum sustainable swimming speed, assessed by a  $U_{crit}$  test, was significantly different between high and low performers, with the velocity for high performers being more than  $1 \text{ BL s}^{-1}$  ( $\sim 6 \text{ cm s}^{-1}$ ) higher (Table 5-2). Metabolic differences between the groups also existed with high performers exhibiting 20% greater resting muscle glycogen levels (Table 5-2). However, this difference in glycogen was not reflected in the AEE during a sprint, as high performers showed a significantly lower AEE ( $20.21 \pm 1.51$  vs.  $25.40 \pm 1.39 \mu\text{mol g}^{-1}$ ), yet a 3 fold greater endurance (Table 5-2). Resting levels of circulating cortisol were also significantly reduced in high performers as compared to low performers (Table 5-2).

#### *Effects of sprint training*

The most dramatic effect between the 2 groups was the result of training. After only 7 days of once daily sprints, high performers exhibited a 2.5 fold increase in average endurance, becoming significantly higher by day 5 (Fig. 5-4). In contrast, low performers showed no significant change in endurance over the 7 days. This performance improvement was not attributed to growth differences, as both groups grew equally well ( $3.08$  vs.  $2.94 \text{ \% d}^{-1}$  for low and high performers, respectively; Table 5-1).

Despite the different performance response elicited through repeated sprints, both high and low performers showed similar metabolic adaptations. In both groups there was a significant increase in resting muscle glycogen levels with training (from  $34.95 \pm 2.08$  to  $45.85 \pm 2.08 \mu\text{mol g}^{-1}$  in low performers, and from  $45.85 \pm 3.63$  to  $54.69 \pm 3.47 \mu\text{mol g}^{-1}$  in high performers; Table 5-2). However, the elevation in muscle glycogen was not

reflected by a change in anaerobic capacity in either group, as high and low performers exhibited post-training AC's of  $30.04 \pm 0.91$  and  $32.17 \pm 1.23 \mu\text{mol g}^{-1}$ , respectively. Nonetheless, a significant reduction in AEE during a sprint was noted in low performers following training, despite their lack of performance improvement (Table 5-2). In contrast, high performers exhibited no change in AEE after training, even though fish had significantly greater endurance.

Circulating plasma cortisol levels were also influenced by training, with a decrease in cortisol exhibited in high performers, yet no significant change in low performers (Table 5-2), as compared to pre-training levels.

## Discussion

This study has demonstrated that sprint performance of fish from a homogeneous population is highly variable, and this variability is persistent for up to 30 days. Other studies have also shown persistence of swim performance (fish re-tested from 2 to 14 days later; Kolok, 1992b; Gregory and Wood, 1998; Kolok et al., 1998) suggesting that prolonged swimming ability is indeed an inherent trait. New to this study is the occurrence of number of physiological traits that are *not* influenced by prolonged swimming ability. The lack of correlation suggests that these traits are unrelated, and do not contribute to endurance at high speeds. Nonetheless, some traits *are* related to sprint performance. Most striking is the correlation between sprint and  $U_{\text{crit}}$  performance, suggesting that a common phenotype is responsible for prolonged swim performance. Given the fact that  $U_{\text{crit}}$ , the maximum sustainable swimming speed, is an estimate of  $\text{VO}_{2\text{max}}$  in salmonids (Beamish, 1978; Hammer, 1995), and the fixed velocity sprint test is largely aerobic in nature (McFarlane and McDonald, 2001), sorting fish according to sprint performance is, in effect, also sorting them according to aerobic capacity.

*Correlates of sprint performance*

Although prolonged performance is known to scale with body size (Brett and Glass, 1973; Beamish, 1978), size differences do not explain the high variability seen in this population of fingerling trout. Fish from both groups were identical in size, and growth rate was unrelated to performance. Over time, fish growing at the same rate, and sprinted at the same absolute speed, became increasingly divergent in performance. Previous work has shown that reducing the sprint speed results in an exponential increase in endurance (Croke, 2001), suggesting that as relative swim speed decreases, a threshold is reached at which fish will be able to swim indefinitely. This threshold phenomenon was also noted by McDonald et al (1998b) in which small changes in size led to large changes in endurance. The great increase in performance at day 30 suggests that the high performers, who have better performance to begin with, are undergoing enough growth in 30 days to approach this threshold, resulting in greater endurance than would be expected by growth alone. Other studies have noted a negative relation between growth rate and  $U_{crit}$  performance (Kolok and Oris, 1995; Gregory and Wood, 1998), however in the latter study, the correlation was dependent on restricted ration, and disappeared when fish were fed to satiation. Similarly, only a low ration led to a positive correlation between endurance in a fixed velocity test and growth rate (Gregory and Wood, 1998). This suggests that competition for resources influences performance indirectly through alterations in growth rate. In the present study, fish were fed a 4% ration, which appears to have been adequate in limiting the establishment of social dominance hierarchies that lead to variable growth rates among fish (Abbott et al., 1985). A trait, other than growth, appears to be governing inherent endurance performance in juvenile trout and appears related to metabolic capacity.

The only difference in resting muscle energy sources between high and low performers was glycogen. On the surface, this implies that glycogen may predict

prolonged performance through greater glycolytic potential. However, both high and low performers had similar anaerobic capacities, meaning that irrespective of the amount of glycogen within muscle, all fish utilize a constant amount during forced exercise to exhaustion. A higher AEE during a sprint occurred in low performers, which implies that high performers are better able to produce ATP through oxidative phosphorylation. It is unlikely that the difference between groups can be explained by differential muscle recruitment patterns, i.e., high performers utilizing oxidative red muscle to power swimming at higher velocities, because fish are sprinted at  $\sim 100\%$   $U_{crit}$ , well above the  $\sim 80\%$  threshold for white muscle recruitment in salmonids (Webb, 1971). Therefore, high performers must have a greater oxygen supply to the muscle.

Differences in AEE were also not supported by activities of LDH, HOAD or CS. If maximal enzyme activities can be used as a measure of flux capacity through a pathway (Moyes et al., 1989; Farrell et al., 1991), and high performers are relying more on oxidative pathways, oxidative enzymes should have higher activity in high performers. However, this was not the case, as enzyme activities were identical for both groups. Other studies have implicitly correlated maximal oxidative enzyme activities of HOAD, CS (Farrell et al., 1991) and cytochrome oxidase (Kolok; 1992b) in red and cardiac muscle, with  $U_{crit}$  performance. However, Farrell et al (1991) also found a positive correlation between performance and CS in white muscle of aerobically trained trout that did not exhibit an elevated aerobic capacity (i.e., change in  $U_{crit}$ ). This study suggests that elevated oxidative enzyme activity does not necessarily reflect an elevated aerobic capacity. Along the same line of reasoning, both high and low performers had identical anaerobic capacities, and similar levels of LDH, an enzyme whose activity can be effectively used as a surrogate measure of anaerobic capacity (McDonald et al., 1998b). Therefore, since maximal enzyme activities appear to be sufficient in both high and low performers,

differences in oxygen supply to the muscle in high and low performers is again a possibility.

An increase in prolonged swim performance necessitates a corresponding increase in transport of oxygen to working muscles. Therefore, the two processes that will significantly impact performance are oxygen transfer across the gills, and oxygen transport to tissues by the blood. The gills serve a dual purpose, as they are the both the sites of oxygen transfer as well as ion loss (Wood and Perry, 1985). The stress challenges imposed were essentially indirect tests of gill function, as epinephrine injection leads to  $\text{Na}^+$  loss across the gills (McDonald and Milligan, 1997), and toxicants (such as copper) act at the gills, resulting in ion loss by the same route (Laurén and McDonald, 1987). Based on these tests, there were no differences in gill function between the two performance groups.

There was also no difference in average levels of blood hemoglobin between high and low performers, and therefore, the oxygen carrying capacity of the blood is presumed to be equal. The lack of correlation between prolonged performance and hemoglobin levels is supported by a number of studies (Jones, 1971; Gallagher et al., 1992, Brauner et al., 1993). It has been suggested that fish could compensate for a below average hemoglobin concentration through cardiovascular adjustments (i.e., higher cardiac outputs) to maintain  $\text{VO}_2\text{max}$  (Gallagher, 1995). Together with the lack of difference in both gill function and Hb, it can be deduced that the oxygen transfer across the gills as well as the oxygen transport capacity of the blood were essentially equal, and unrelated to prolonged swim performance. If these are two of the key features that determine prolonged performance, and they are not different, what feature is responsible for the consistent difference in endurance?

Since we are sorting for high aerobic capacity, i.e., fish with high sprint performance have higher  $U_{\text{crit}}$  performance and therefore higher  $\text{VO}_2\text{max}$ , the



physiological trait separating a high from a low performer must be linked to  $VO_2\text{max}$ . The two determinants of  $VO_2\text{max}$  are cardiac output and arterial-venous oxygen difference (Jones and Randall, 1978). Therefore, to meet an increase in oxygen demand, fish must increase one or more of these factors. With a lack of difference in Hb, the main determinant of arterial oxygen concentration, the difference seen in  $VO_2\text{max}$  must, *by a process of elimination*, be due to either differences in venous oxygen concentration or cardiac output. Along the same line of reasoning, sorting fish according sprint performance may be indirectly sorting them according to cardiac performance. Greater cardiac performance would improve white muscle perfusion (Neumann et al., 1983) thus facilitating oxidative metabolism. However, Kolok and Farrell (1994a) found no relation between cardiac output and  $U_{\text{crit}}$  in adult northern squawfish, which may be a species-specific response, or may be dependent on life-stage. In contrast, rainbow trout have been shown to alter cardiac output after only 28 days of aerobic (i.e., 60%  $U_{\text{crit}}$ ) training, resulting in an 18% improvement in cardiac output due to higher stroke volume (Farrell et al., 1991). This finding suggests that rainbow trout may have a relatively plastic cardiac response, and inherent differences in cardiac performance in juveniles is entirely plausible. Whether the greater  $VO_2\text{max}$  in fish with high sprint performance is due to faster cardiac output up-regulation, or a different maximum level of performance from the heart is not known, and would likely be difficult to resolve, particularly due to the small size of these juvenile fish. However, what it really comes down to is that sprint performance is dependent on oxygen delivery to working muscle. Thus, a greater oxidative contribution would not only provide for efficient energy metabolism, but would also prolong exercise bouts and reduce post-exercise recovery time.

### *The training effect*

Only high performers were found to enhance performance after only 7 daily sprints. It has previously been suggested that the effects of sprint experience on performance improvement is not to elevate aerobic capacity, but to enhance cardiovascular “tune-up” (McFarlane and McDonald, 2001). Therefore, high performers also have a greater ability to learn to tune-up blood flow to working muscle, whereas low performers (who already may exhibit compromised cardiac performance) do not exhibit the same response. The end result is that low performers are forced to rely on glycolytic metabolism for adequate ATP provision, with the end production of lactate. Metabolic differences exist both prior to and following training, with the most obvious difference being the increase in glycogen storage in low performers following training, with no change in performance, and furthermore, a reduction in AEE. I have previously suggested that fatigue during a fixed velocity test is due to ATP depletion (Chapter 3). Therefore, if the threshold for ATP depletion is reached prior to a significant depletion of glycogen, additional glycogen will be of no benefit. This would explain how increased glycogen is of no advantage in low performers, and is likely just a product of repeated fatiguing exercise (i.e., “glycogen rebound”). Yet, another factor is also involved, the role of perceived stress.

Circulating levels of cortisol were higher in low performers, and appear to be playing a role in impeding performance improvement. Furthermore, cortisol was higher in low performers both prior to and following training, suggesting that the training regime itself did not elevate cortisol. This is also supported by the similar growth rates in both groups, i.e., if the training was excessively stressful to only one group, it would manifest as reduced growth. McFarlane and McDonald (2001) noted that within a group of trained fish, some individuals did not train at all, and these were often the poorest swimmers in the group. But is low performance due to elevated cortisol, or is high cortisol the product of

an inability to swim efficiently? High cortisol is associated with slow recovery of acid-base status after exhaustive exercise (Pagnotta and Milligan, 1991; Eros and Milligan, 1996; Milligan et al., 2000). Therefore, low performers not only have a greater AEE (i.e., greater debt to repay during recovery), but also have higher resting cortisol which may affect complete recovery between sprints. However, low performers have good growth, and have lower circulating cortisol than fish from studies where cortisol levels have been artificially elevated (Barton et al., 1987; Gregory and Wood, 1999b), suggesting that the elevation in cortisol may not have been high enough to cause appetite suppression, and the threshold for performance effects is lower. A link between stress and trainability (i.e., ability to learn) following a once daily sprint regime was offered by McFarlane and McDonald (2001) with fish showing outward signs of chronic stress lacking the ability to improve performance. Although low performers did show metabolic adjustments to training (i.e., a lower AEE), the effect of higher circulating cortisol may have overshadowed any potential improvements in performance.

### *Conclusions and implications*

In this study, I have exploited variability in sprint performance to divide fish according to fatigue times. I believe that fish were actually sorted based on aerobic capacity. Furthermore, I conclude that the mechanism by which high performers achieve a greater aerobic capacity is through enhanced oxygen delivery to white muscle. Specifically, high performers must have higher maximal cardiac outputs or greater oxygen extraction efficiency, because gill transfer and arterial oxygen concentration were unchanged. This characteristic of high performers is undoubtedly linked to their ability to sprint train, whereas low performers have lower aerobic capacities and lack “trainability”. This study has provided a methodology by which fish can be divided based on a specific phenotype, but the obvious challenge is to link this phenotype with a specific genetic trait.

Such a finding would lead to selection studies similar to that for hatchery reared salmonids selected for low stress response (Fevolden et al., 1991; Pottinger et al., 1994), and ultimately could result in the development of a strain of high performance trout which would have implications in hatchery release programs.

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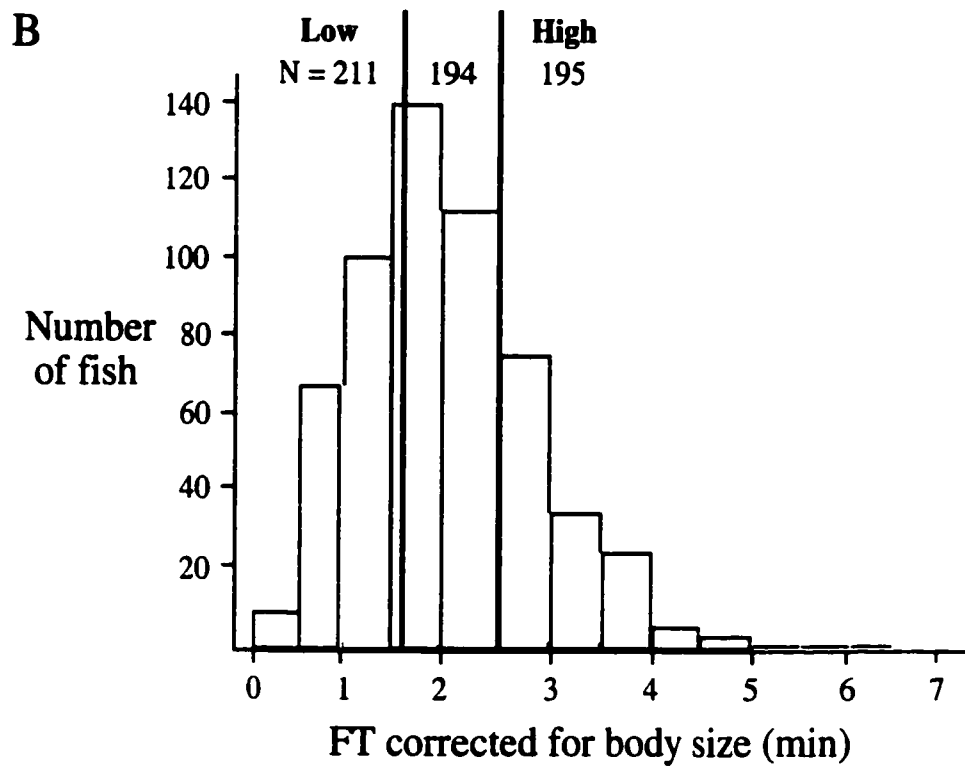
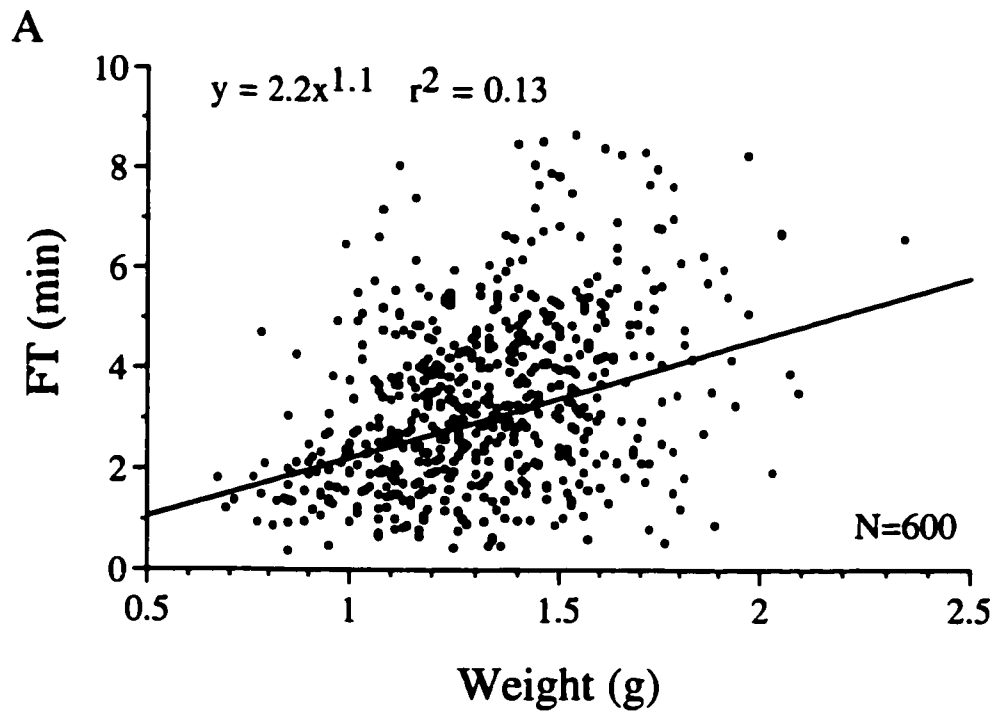
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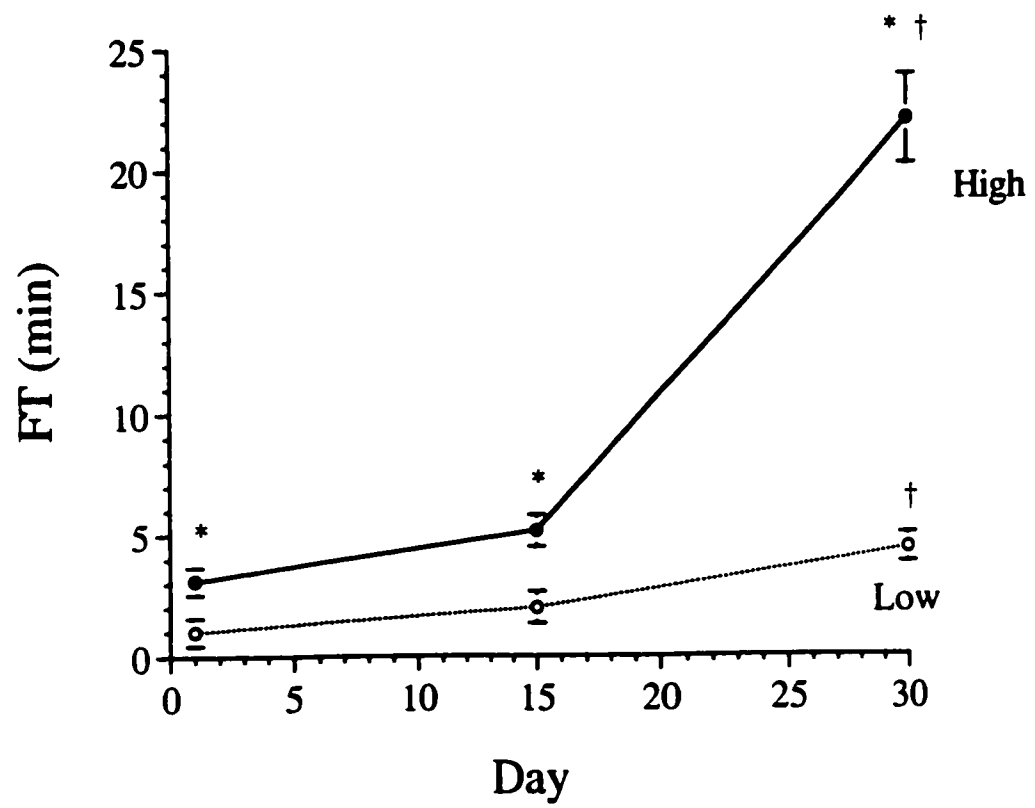
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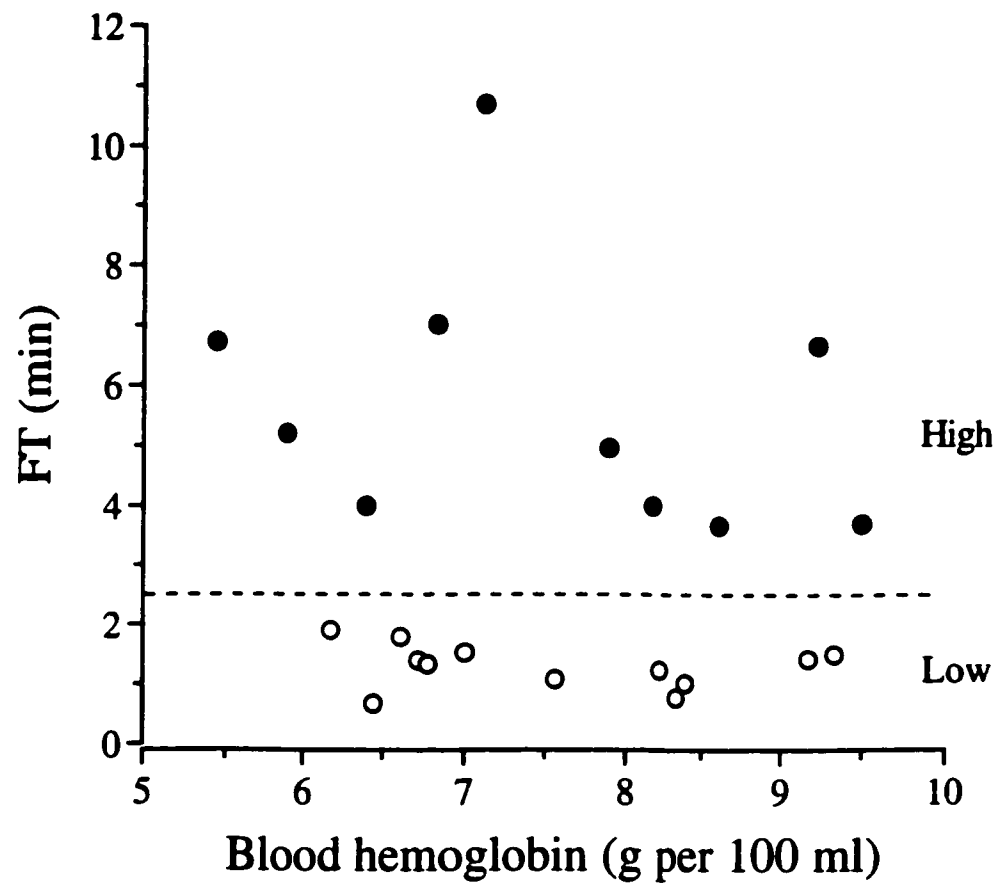
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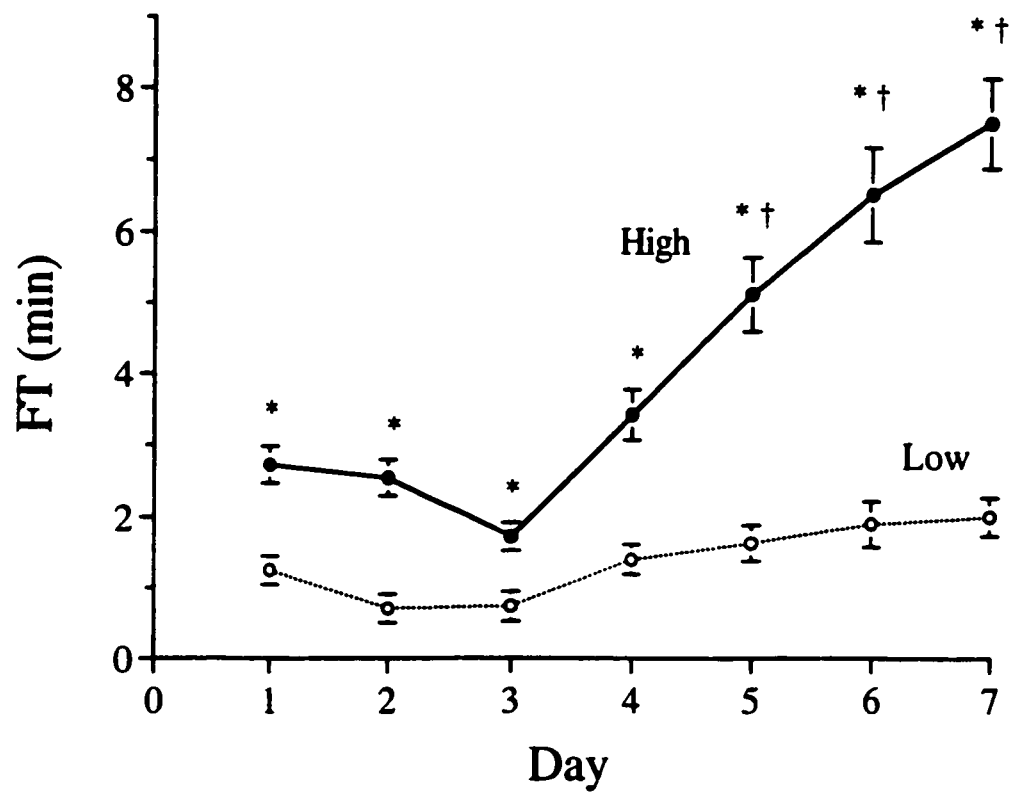
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**Table 5-1.**

Measurements that were not significantly different between fish sorted for low or high sprint performance. Values are means  $\pm$  S.E.M (except for bulk growth rates).

Measurement	N	Low	High	
<b>Initial mass (g)</b>	211, 195	1.39 $\pm$ 0.06	1.27 $\pm$ 0.07	
<b>Growth (% body weight d<sup>-1</sup>)</b>	individual weights: 30 d	20	2.33 $\pm$ 0.21	2.27 $\pm$ 0.19
	bulk tank weights: 7d	40	3.08	2.94
	bulk tank weights: 14 d	150?	3.62	3.78
<b>Resting muscle levels (<math>\mu\text{mol g}^{-1}</math>)</b>	lactate	10	5.26 $\pm$ 1.40	4.47 $\pm$ 1.11
	PCr	10	13.25 $\pm$ 0.49	13.97 $\pm$ 0.52
	ATP	10	4.02 $\pm$ 0.21	4.32 $\pm$ 0.30
<b>Enzyme activity (<math>\mu\text{mol substrate converted g}^{-1} \text{ min}^{-1}</math>)</b>	LDH	10	46.5 $\pm$ 0.20	48.27 $\pm$ 4.03
	HOAD	10	0.26 $\pm$ 0.02	0.25 $\pm$ 0.02
	CS	10	3.87 $\pm$ 0.12	3.85 $\pm$ 0.10
<b>Anaerobic capacity (<math>\mu\text{mol g}^{-1}</math>)</b>	$\Delta$ lactate	10	14.88 $\pm$ 0.64	12.54 $\pm$ 2.20
	$\Delta$ PCr	10	5.48 $\pm$ 0.62	6.26 $\pm$ 0.48
	$\Delta$ ATP	10	2.25 $\pm$ 0.15	2.61 $\pm$ 0.18
	Total	10	30.06 $\pm$ 1.65	27.68 $\pm$ 2.95
<b>Blood hemoglobin (g 100 ml<sup>-1</sup>)</b>	32, 33	7.01 $\pm$ 0.32	6.86 $\pm$ 0.32	
<b>Response to stress challenge</b>				
i) <i>Epinephrine injection</i>	sodium loss ( $\mu\text{Eq g}^{-1}$ )	12	1.22 $\pm$ 0.07	1.26 $\pm$ 0.15
	(% whole body)		3.18 $\pm$ 0.19	3.09 $\pm$ 0.37
ii) <i>Copper exposure</i>	sodium loss ( $\mu\text{Eq g}^{-1}$ )	12	4.98 $\pm$ 0.60	5.08 $\pm$ 1.70
	(% whole body)		12.19 $\pm$ 1.02	11.44 $\pm$ 0.83
	newly accumulated copper ( $\mu\text{g Cu g}^{-1}$ )		0.83 $\pm$ 0.09	0.90 $\pm$ 0.06
iii) <i>Phenol exposure</i>	time to 50% mortality (h)	12	3.82 $\pm$ 0.44	3.93 $\pm$ 0.51

**Table 5-2.**

Measurements that were significantly different between fish sorted for low or high sprint performance. Values are means  $\pm$  S.E.M.

Measurement	N	Low	High		
<b>Critical velocity (<math>U_{crit}</math>)</b> ( $cm\ s^{-1}$ ) ( $BL\ s^{-1}$ )	20	36.46 $\pm$ 0.74	42.77 $\pm$ 1.01 *		
		6.20 $\pm$ 0.08	7.43 $\pm$ 0.10 *		
<b>Resting muscle glycogen</b> ( $\mu mol\ g^{-1}\ wet\ wt.$ )	untrained	34.95 $\pm$ 2.08	44.07 $\pm$ 0.91 *		
	sprint trained	45.85 $\pm$ 3.63 †	54.69 $\pm$ 3.47 * †		
<b>Anaerobic energy expenditure during a sprint</b> ( $\mu mol\ g^{-1}\ wet\ wt.$ )					
untrained	$\Delta$ lactate	10	12.35 $\pm$ 0.75	10.15 $\pm$ 0.83 *	
	$\Delta$ PCr	10	4.71 $\pm$ 0.39	3.09 $\pm$ 1.53	
	$\Delta$ ATP	10	2.16 $\pm$ 0.11	1.89 $\pm$ 0.28	
	Total	10	25.40 $\pm$ 1.39	20.21 $\pm$ 1.51 *	
	trained	$\Delta$ lactate	10	8.49 $\pm$ 0.96	11.28 $\pm$ 0.54 *
		$\Delta$ PCr	10	5.53 $\pm$ 0.13	4.50 $\pm$ 0.43
		$\Delta$ ATP	10	2.49 $\pm$ 0.06	2.30 $\pm$ 0.05
		Total	10	20.76 $\pm$ 0.92 †	23.73 $\pm$ 0.86 *
<b>Plasma cortisol</b> ( $ng\ ml^{-1}$ )	untrained	10	87.83 $\pm$ 23.33	51.53 $\pm$ 12.3 *	
	sprint trained	10	51.53 $\pm$ 12.3	21.23 $\pm$ 6.2 * †	

**Note:** An asterisk (\*) denotes significant differences between high and low performers, and a dagger (†) denotes differences between trained and untrained fish. Differences were tested using a Dunnett's t-test ( $P < 0.05$ ).

**CHAPTER 6**  
**THE INFLUENCE OF OXYGEN SUPPLY ON PROLONGED SWIM PERFORMANCE**  
**IN FINGERLING RAINBOW TROUT**

**Summary**

This study examined the effects of varying the oxygen content of water on both critical velocity ( $U_{crit}$ ) and sprint performance (assessed as time to fatigue), in order to determine whether performance in each test was limited by convection (i.e., oxygen supply via the blood) or diffusion (i.e., oxygen supply into the muscle). The approach used was to determine  $U_{crit}$  under hyperoxia ( $PO_2 \sim 300$  mmHg) and normoxia ( $PO_2 \sim 155$  mmHg) in fish ( $\sim 2$  g) sorted according to sprint performance. Along with hyperoxia and normoxia, endurance in a fixed velocity sprint test was also determined under hypoxia ( $PO_2 \sim 100$  mmHg). Elevated  $PO_2$  led to an increase in  $U_{crit}$  in low performers, yet no change in high performers. These findings suggest that during a  $U_{crit}$  test, low performers are more convection limited than high performers. In contrast, hyperoxia improved sprint performance in both high and low performers. This suggests that during a sprint where fish are rapidly accelerated, convection limitation is more important than when fish are slowly accelerated, regardless of the inherent swimming abilities of fish. Hypoxia evoked a 40% decrease in endurance in high performers but had no effect on endurance in low performers. This suggests that low performers are more reliant on substrate level phosphorylation for ATP production during a sprint. Blood oxygen carrying capacity in both high and low performers was highly variable, yet was unrelated to sprint

performance. Similarly, active metabolic rates did not correlate with performance ability, even though routine metabolic rates were greater in high performers than in low performers.

## **Introduction**

For nearly as long as exercise has been studied in fish, researchers have sought to determine the extent to which oxygen level in the water influences swim performance. By varying the partial pressure of oxygen in the water, effects on maximum sustainable swimming speeds (i.e.,  $U_{crit}$ ; e.g. Davis et al., 1963; Brett, 1964; Bushnell, 1984) have been assessed.

The threshold at which hypoxia decreases maximum sustainable swimming speed was determined to be at a  $PO_2$  of 120 mmHg for coho salmon (Davis et al., 1963; Dahlberg et al., 1968) and 80 mmHg for largemouth bass (Dahlberg et al., 1968). Hypoxia also reduced maximum sustainable swimming speed ( $U_{crit}$ ) of rainbow trout when they were exposed to a  $PO_2$  of 40-70 mmHg (Jones, 1971; Bushnell et al., 1984).

In contrast, hyperoxia ( $PO_2$  levels of 300-400 mmHg) has no effect on maximum sustainable swimming speeds of juvenile salmon or largemouth bass (Davis et al., 1963; Brett, 1964; Dahlberg et al 1968). This independence suggests that performance is determined by the ability of muscle to utilize oxygen and produce energy, and is not limited by oxygen delivery (Davis et al., 1963). In other words, these studies provided convincing evidence that maximum sustainable swimming speed is not convection limited, but it is diffusion limited. The transfer of oxygen from the environment to the tissues occurs by both convection and diffusion, with the slowest conductance determining the rate of oxygen delivery to working muscle. In other words, oxygen supply to the muscle can only be limited by either convection or diffusion, but not both.



The first objective of this study was to confirm that  $U_{crit}$  is diffusion limited in juvenile rainbow trout sorted for high and for low sprint performance. Hyperoxia was the technique used to increase the oxygen supply to the muscle, with a  $PO_2$  of 300 mmHg chosen, as this level has been utilized in previous studies (Davis et al., 1963). The second objective was to determine if sprint performance (i.e., endurance at fixed velocity) is convection or diffusion limited. This would confirm the hypothesis from a previous study (McFarlane and McDonald, 2001) that sprint performance may be convection limited as a result of the cardiovascular system taking a long time to tune-up. The third objective was to test the effects of hypoxia on endurance to determine to what degree sprint performance is dependent on oxygen supply in high and low performers. A  $PO_2$  of 100 mmHg was chosen as the hypoxic partial pressure, because this was below the threshold for effects seen previously for coho salmon (Davis et al., 1963). To determine whether differences in blood oxygen carrying capacity influenced individual sprint performance, hemoglobin (Hb) and methemoglobin (metHb) were also measured in each of the performance groups. Finally, routine and post-exercise  $MO_2$  was measured in fish with high and low sprint performance, to determine whether differences existed between groups.

## **Materials and methods**

### *Experimental animals*

Fish used in this study were juvenile rainbow trout (*Oncorhynchus mykiss* Walbaum) obtained from Rainbow Springs Trout Hatchery (Thamesford, Ontario). Fish (N=280;  $2.21 \pm 0.04$  g;  $5.70 \pm 0.04$  cm) were held in 40 L circular tanks supplied with dechlorinated Hamilton tap water with a partial replacement of approximately 1 L per minute, and were acclimated to a water temperature of  $13 \pm 1^\circ$  C for at least 2 weeks prior to the sorting according to sprint performance. A 4% body weight per day ration of

commercial trout feed (Corey Feed Mills, Ltd.) was offered over two meals. A minimum of 12 hours separated the feeding and experimental periods to minimize postprandial effects.

#### *Exercise apparatus*

Exercise tests were performed at McMaster University using an open, recirculating swim flume (102 L volume) designed according to the recommendations of Vogel (1978), with a swimming section of dimensions 73 x 11 x 20 cm (L x W x H). Flow was generated by a propeller connected to a 373-W DC motor (Dart Controls, Inc., Zionsville, IN), and calibrated using an electromagnetic flow meter (Marsh-McBirney Model 2000).

#### *Swim procedures*

Two types of swim procedures were used: a critical velocity ( $U_{crit}$ ) test and a fixed velocity sprint test.

i) Critical velocity: The 30 minute incrementally stepped velocity test was a modified version of that outlined by Brett (1967), in which the velocity intervals were 30 min rather than 45 min. Fish were transferred to the flume in groups of 10 and oriented for 30 min at a starting velocity of 1 BL  $s^{-1}$ . Velocity was rapidly increased by 1 body length (BL)  $s^{-1}$  (6 cm  $s^{-1}$ ) every 30 minutes until fatigue. Each step increase was completed within 2-3 seconds, i.e., essentially an instantaneous acceleration.  $U_{crit}$  was calculated according to the following expression from Brett (1964):

$$U_{crit} = U_p + \left( \left( \frac{t_f}{t_i} \right) + U_i \right)$$

where  $U_i$  is the velocity increment ( $\text{cm s}^{-1}$ ),  $U_p$  is the penultimate velocity at which the fish swam prior to fatigue,  $t_f$  is the elapsed time from the velocity increase to fatigue, and  $t_i$  is the time between velocity increments. Critical velocity was calculated according to individual lengths, and expressed in  $\text{BL s}^{-1}$ .

ii) **Sprint**: The sprint protocol was as described by McDonald et al (1998). Fish were transferred to the flume in groups of 10, and for the first 5 min in the flume, an orienting velocity of approximately  $1 \text{ BL s}^{-1}$  was provided. Over the next 2 min, velocity was steadily increased to a test velocity of  $40 \text{ cm s}^{-1}$ , producing a relative speed of close to  $7 \text{ BL s}^{-1}$ . The test velocity was chosen as it was approximately equal to previously measured  $U_{\text{crit}}$  values for fish of similar size (Chapters 2 and 3). Our criterion for fatigue was when fish fell against the back screen and no longer resumed swimming after three manual prods to the tail. At this level of exertion, fish are not completely *exhausted*, i.e., they are still able to swim slowly (Dobson and Hochachka, 1987; Parkhouse et al., 1988), however, they are *fatigued*, meaning they can no longer sustain the sprint speed. Fish were removed as they fatigued. Individual fatigue times, lengths and weights were recorded from each fish, and mean time to fatigue (in min) in each trial was calculated as the geometric mean (i.e., mean of the log sprint time) according to the recommendations of Brett (1964). Such a calculation produces an asymmetric standard error of the mean, but since the upper and lower errors were rarely very different, they have been averaged and expressed as a single error.

#### *Initial sorting according to performance*

Prior to sorting, 30 fish ( $2.04 \pm 0.13 \text{ g}$ ;  $5.56 \pm 0.14 \text{ cm}$ ) were sprinted at  $40 \text{ cm s}^{-1}$  in three groups of 10, and fatigue times plotted against weight. This relation produced a scaling coefficient of 1.5, and an  $r^2 = 0.45$ , similar to previous scaling relations (e.g.

chapter 5). The remaining trout ( $N=250$ ;  $2.25 \pm 0.04$  g;  $5.76 \pm 0.04$  cm) were then sprinted using the above described sprint procedure. Depending on fatigue times, they were divided into high, medium and low performance groups (see below). To ensure that fish did not sort according to size alone, a scaling correction was applied to correct initial fatigue time to a reference weight of 2 g, according to the following equation (as outlined in McDonald et al., 1998):

$$FT_c = \frac{FT \text{ (min)}}{\left( \frac{\text{Body wt. (g)}}{\text{Reference wt. (g)}} \right)^b}$$

where  $FT_c$  is the fatigue time corrected to a reference weight (2 g) and  $b$  is the scaling coefficient. The sorting criteria chosen were size corrected fatigue times similar to those that have previously been shown to produce an even distribution of fish into performance groups: low performers  $<1.67$  min, high performers  $> 2.5$  min, and medium performers in between (as in chapter 5). To avoid any overlap in sprint performance, only the two extremes (i.e., high and low performers) were used for further comparisons.

#### *Maintenance of oxygen partial pressure in the swim flume*

Normoxic conditions were maintained by aeration with compressed air (i.e., 78%  $N_2$ , 21%  $O_2$ ), while hypoxic and hyperoxic conditions were achieved through aerating with a mixture of  $N_2$  gas and air, or  $O_2$  gas and air, through a gas mixing box (model 208-01; Instrumentation Laboratory, Inc.). Normoxic conditions in the flume could be maintained indefinitely, and levels were relatively constant between each swim test at  $155 \pm 2$  mmHg. It typically took 30 min to make the  $PO_2$  of the entire volume in the flume hypoxic ( $\sim 100$  mmHg). However it proved difficult to maintain for long periods, and due

to the gradual decrease in  $P_{O_2}$  to levels far below 100 mmHg, this treatment was only useful for the relatively short sprint tests, and was not used for  $U_{crit}$  tests (as they typically take 3-4 hours to complete). When the  $P_{O_2}$  was stable at  $100 \pm 2$  mmHg, sprint tests were completed, and  $P_{O_2}$  levels were monitored between tests. Hyperoxic conditions were easier to maintain, and in fact, were fairly constant when monitored for up to 4 hours, with the range of  $P_{O_2}$  values during this time being  $304 \pm 8$  mmHg. Between tests, the  $P_{O_2}$  was adjusted, so that from test to test, it did not drift more than 6 mmHg. Prior to adjusting  $P_{O_2}$  (i.e., between pairs of tests), water in the flume was replaced. As a result, water temperature remained fairly constant at  $13 \pm 0.5^\circ$  C throughout the entire experimental series.

### *Experimental series*

#### *i) Effect of $P_{O_2}$ on $U_{crit}$*

Fish from either the high or low performance groups (N=10 each) were placed into the flume once the  $P_{O_2}$  had stabilized at either normoxia ( $P_{O_2} \sim 155$  mmHg) or hyperoxia ( $P_{O_2} \sim 300$  mmHg) for at least 10 min. To maintain the inexperienced nature of the fish, individuals were only used for a single critical velocity test. In between each test, water in the flume was replaced, and the  $P_{O_2}$  was adjusted accordingly.

#### *ii) Effect of $P_{O_2}$ on sprint performance*

Fish from both high and low performance groups were sprinted at  $7 \text{ BL s}^{-1}$  ( $40 \text{ cm s}^{-1}$ ) at one of 3 different oxygen partial pressures: hypoxia ( $P_{O_2} \sim 100$  mmHg), normoxia or hyperoxia. Two groups of 10 fish from each group were sprinted, alternating between high and low performance groups.

*iii) Routine and post-exercise O<sub>2</sub> consumption*

Rates of routine oxygen consumption ( $M_{O_2}$ ;  $\mu\text{mol g}^{-1} \text{h}^{-1}$ ) were measured for individual fish held in small respirometers over a period of 2 hours (N=15 from each group). The respirometers were darkened, 20 ml syringe barrels fitted with three-way stopcocks to enable inflow and outflow water sampling. Fish were acclimated to the respirometers for 24 hours prior to measurements. Water flow was adjusted to provide each respirometer with exactly  $12 \text{ ml min}^{-1}$  ( $0.72 \text{ L h}^{-1}$ ), and inflow and outflow water samples were collected with 5 ml air-tight syringes for  $P_{O_2}$  measurement every 30 min for 2 hours.  $P_{O_2}$  was measured immediately with a Radiometer E5046  $P_{O_2}$  electrode connected to a Cameron Instruments OM-200 oxygen meter. The oxygen consumption ( $M_{O_2}$ ) at each time period was calculated by the Fick principle:

$$M_{O_2} = \frac{(\Delta P_{O_2} \times \alpha_{O_2} \times f)}{m}$$

where  $\Delta P_{O_2}$  is the difference in  $P_{O_2}$  between inflow and outflow water (mmHg; 1 mmHg = 1 torr),  $\alpha_{O_2}$  ( $\mu\text{mol torr}^{-1} \text{L}^{-1}$ ) is the solubility constant for  $O_2$  in water (Boutilier et al., 1984),  $f$  ( $\text{L h}^{-1}$ ) is flow rate of water through the respirometer and  $m$  is the wet weight (g) of the fish. Inflow  $P_{O_2}$  was typically 152-155 mmHg; outflow  $P_{O_2}$  was never lower than 120 mmHg; typically  $\Delta P_{O_2}$  was ~20-25 mmHg at rest.

For post-exercise  $M_{O_2}$  measurement, fish were manually chased to exhaustion for 8 min, then placed into the respirometers (similar to the protocol of Scarabello et al., 1991). Inflow and outflow water samples were taken at 5 min to determine the post-exercise (or active)  $M_{O_2}$ , and then at 10 min intervals until 90 min, and a final sample at 120 min. The post-exercise  $\Delta P_{O_2}$  was typically 30-45 mmHg.

### *Blood sampling and analysis*

Resting fish from both the high and low performance groups (N=15 each) were killed by a sharp blow to the head, and blood was carefully sampled from each fish *via* caudal severance. Whole blood was kept on ice for subsequent determination of hemoglobin (Hb) levels (in g per 100ml blood) using the cyanmethemoglobin method (Sigma diagnostics, 525-A assay kit). Methemoglobin (metHb) was also measured to estimate the % of Hb available for oxygen transport, according to the methods of Benesch et al (1973).

### *Statistical analysis*

Values are expressed as means  $\pm 1$  S.E.M (unless otherwise stated). Comparisons of treatment groups with respective controls were tested by one-way analysis of variance (ANOVA). If the result from the ANOVA was significant, a Tukey-Kramer HSD test for multiple comparisons was applied to test for significant differences among treatments ( $P < 0.05$ ; Zar, 1996). When a comparison of only one treatment with a respective control was necessary, a Dunnett's t-test was applied ( $P < 0.05$ ). The tests were performed using JMP 2.0.5 software (SAS Institute, Inc.).

## **Results**

### *Results of the sorting procedure*

Following size correction of fatigue times and sorting of fish according to performance, there was an equal number of fish in each group: 84 low, 82 high and 84 medium performers. However, there were significant differences in initial size between

fish in the high and low performing groups (high:  $2.13 \pm 0.08$  g; low:  $2.51 \pm 0.17$  g), which necessitated size correction of fatigue times for subsequent sprint trials.

#### *Effects of oxygen partial pressure on $U_{crit}$*

Under normoxic conditions, high performers were able to sustain a speed that was more than  $1 \text{ BL s}^{-1}$  greater than that of low performers ( $U_{crit}$  of 6.7 vs. 5.5  $\text{BL s}^{-1}$ ; Fig. 6-1). However, upon exposure to hyperoxia, only low performers exhibited an increase in  $U_{crit}$ , while the elevation in  $P_{O_2}$  had no significant effect on the  $U_{crit}$  for high performers. Furthermore, the increase in  $U_{crit}$  seen in low performers under hyperoxic conditions was not significantly different from that of high performers at normoxia (6.3 vs. 6.9  $\text{BL s}^{-1}$ ; Fig. 6-1).

#### *Effects of oxygen partial pressure on sprint performance*

Under normoxic (control) conditions ( $P_{O_2} \sim 155$  mmHg), there was a significant, 2.5 fold greater endurance in high performers than in low performers (4.7 vs. 1.8 min; Fig. 6-2). However, when fish were swum in hypoxic water ( $P_{O_2} \sim 100$  mmHg), there was a decrease in endurance in high performers to 2.8 min, yet low performers showed essentially no change, with an average endurance similar to that under normoxic conditions (2.1 min; Fig. 6-2). Under hyperoxic conditions ( $P_{O_2} \sim 300$  mmHg), both high and low performers enhanced their sprint performance, with an increase of 4.5 min for high performers and 2.2 min for low performers (Fig. 6-2). Furthermore, under hyperoxia, low performers exhibited similar performance to high performers at normoxia (fatigue times of 4.0 vs. 4.7 min, respectively; Fig. 6-2).



### *Comparison of routine and post-exercise oxygen consumption*

When routine oxygen consumption of individual high and low performing fish (N=15 each) in respirometers was monitored over a 2 hour period, high performers had consistently greater oxygen consumption than low performers ( $10.1 \pm 0.9 \mu\text{mol g}^{-1} \text{h}^{-1}$  vs.  $7.5 \pm 0.5 \mu\text{mol g}^{-1} \text{h}^{-1}$ ; Fig. 6-3). Furthermore, routine  $\dot{M}_{\text{O}_2}$  was consistent when monitored over a 2 hour period in fish from both groups. However, after manually chasing fish to exhaustion, there was a significant increase in  $\dot{M}_{\text{O}_2}$ , but no significant difference between the groups ( $18.1 \pm 0.9 \mu\text{mol g}^{-1} \text{h}^{-1}$  for high performers vs.  $19.5 \pm 0.8 \mu\text{mol g}^{-1} \text{h}^{-1}$  for low performers; Fig. 6-3).

### *Comparison of blood oxygen carrying capacity*

Total hemoglobin concentration in the blood was highly variable (ranging from 5.8 to 14.6 g per 100 ml whole blood), and was unrelated to sprint performance, with an average of  $9.7 \pm 0.7 \text{ g } 100 \text{ ml}^{-1}$  for low performers and  $9.0 \pm 0.6 \text{ g } 100 \text{ ml}^{-1}$  for high performers (Fig. 6-4). Furthermore, the percent of Hb present as metHb was not significantly different for high or low performers, with an average of 16% for low performers and 17% for high performers (Fig. 6-4).

## **Discussion**

### *The role of oxygen supply in $U_{\text{crit}}$ performance*

By making the water hyperoxic ( $\text{PO}_2 \sim 300 \text{ mmHg}$ ), a condition was created in which the partial pressure gradient of oxygen from blood to tissue was increased, and therefore, any oxygen supply limitation removed. Under these conditions, there was no improvement in  $U_{\text{crit}}$  in high performers suggesting that oxygen supply to muscle tissue

was not limiting. Rather, fish were diffusion limited, with the upper limit to performance being set by the amount of oxygen utilized by muscle cells to produce ATP oxidatively. This confirms findings of previous studies on coho salmon and largemouth bass (Davis et al., 1963; Dahlberg et al., 1968). In contrast, low performers exhibited an elevation in  $U_{crit}$ , with hyperoxia, suggesting that they are more convection limited than high performers. This is the first study to show two different responses to hyperoxia, related to inherent performance ability. Other studies have shown no effect of hyperoxia (Davis et al., 1963; Brett, 1964; Dahlberg et al., 1968) yet, in these studies, average responses of groups of fish may have masked any individual differences. By the same reasoning, if this study had been performed on an un-sorted group of fish, an effect of hyperoxia may not have been detected.

In low performers, variations in  $U_{crit}$  were not correlated to variations in blood functional hemoglobin levels. Therefore, any oxygen supply limitation exhibited by these fish must be due to inadequate flow rather than capacitance.

#### *The role of oxygen supply in sprint performance*

This is the first study to examine the effects of hypoxia and hyperoxia on sprint performance. During hyperoxia, both high and low performers exhibited an increase in endurance, which suggests that rapidly accelerated fish may be more convectively limited. It is unlikely that cardiac output has reached a maximum during the sprint test (which is generally completed in under 5 min), given the relatively slow up-regulation of cardiovascular performance at the onset of exercise (Kiceniuk and Jones, 1977). Therefore, the possibility of a convection limitation (i.e., limited by oxygen delivery to muscle, which may be a function of either arterial or venous oxygen content) is entirely plausible. However, the differences in endurance between high and low performers exist regardless of  $PO_2$ , suggesting that another factor is also at play, and may reflect a greater

oxygen utilization capacity in muscle of high performers. In other words, high performers may have a higher oxygen extraction efficiency at the muscle.

In fish with low performance, reducing oxygen content of the water from 155 to 100 mmHg had little effect on endurance. This lack of performance decrement is likely because these fish rely primarily on substrate level phosphorylation during a sprint (i.e., PCr breakdown and glycolysis), as has previously been suggested (Chapter 5). However, the situation for high performers is different, with a clear impairment of performance upon reduction of  $P_{O_2}$ . Therefore, I suggest that high performers are more able to exploit oxidative phosphorylation during a sprint, and show greater decrements in performance when oxygen supply is not maintained to working muscles. Thus, in response to the lower  $O_2$  supplied to tissues, their performance approaches that of a low performer.

#### *Comparison of routine and post-exercise $MO_2$*

The stability of routine  $O_2$  consumption over the 2 hour measurement period suggests that values obtained in this study were truly representative of a routine metabolic rate. Furthermore, both routine and active  $O_2$  consumption equate well with previously obtained values, even though the methods used to obtain them were often different. For example, routine  $MO_2$  was obtained through in-tank measurements (Hollis et al., 1999), extrapolation of the relation between  $MO_2$  and swimming speed (Brett and Glass, 1973; Puckett and Dill, 1989; Alsop and Wood, 1997) or by stationary respirometry (McDonald et al., 1991; Scarabello et al., 1991; Gonzalez and McDonald, 1992; this study).

Routine  $O_2$  consumption was higher in fish with good sprint performance ability, however, post-exercise  $MO_2$  did not follow the same pattern (i.e., no difference in active  $MO_2$  between high and low performers). The significance of differences in routine oxygen consumption is not clear, and whether these differences are indicative of different

maximum oxygen consumptions, is unknown, and furthermore, would be difficult to measure in small fish.

### *Conclusions*

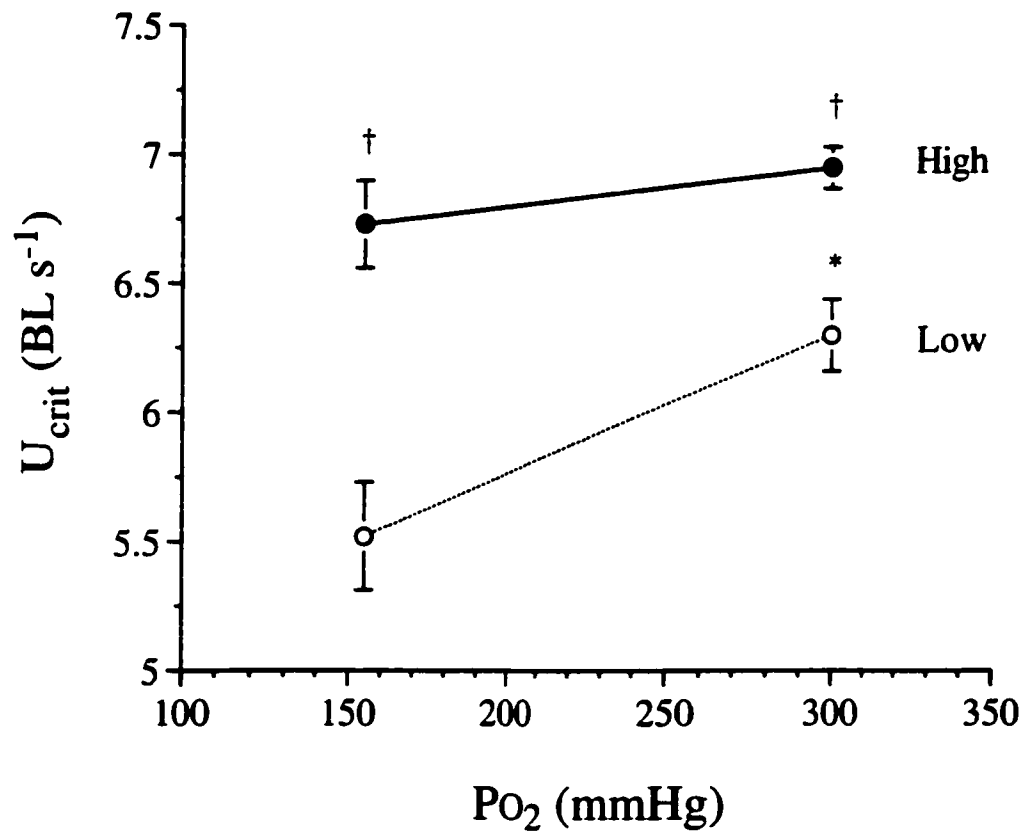
Hyperoxia was used to determine the factors limiting swim performance. I show that during a critical velocity test, low performers were more convectively limited, while high performers were limited by oxygen diffusion into the muscle, a function of the capacity of working muscles to utilize oxygen. However, during sprint exercise, endurance appears limited by the ability to adequately transport oxygen to the muscle, suggesting that convection limitations may be more important following a rapid acceleration. At this point, fish are not likely to be swimming at steady-state, and blood flow to muscle may be compromised. Hypoxic conditions led to endurance being reduced only in high performers, which was likely a function of limited oxygen delivery to muscle. Neither blood hemoglobin nor  $MO_2$  can be used to predict sprint performance. Furthermore, many different responses between high and low performers were apparent in this study, which indicates the importance of examining individual variability in the physiological response to exercise.

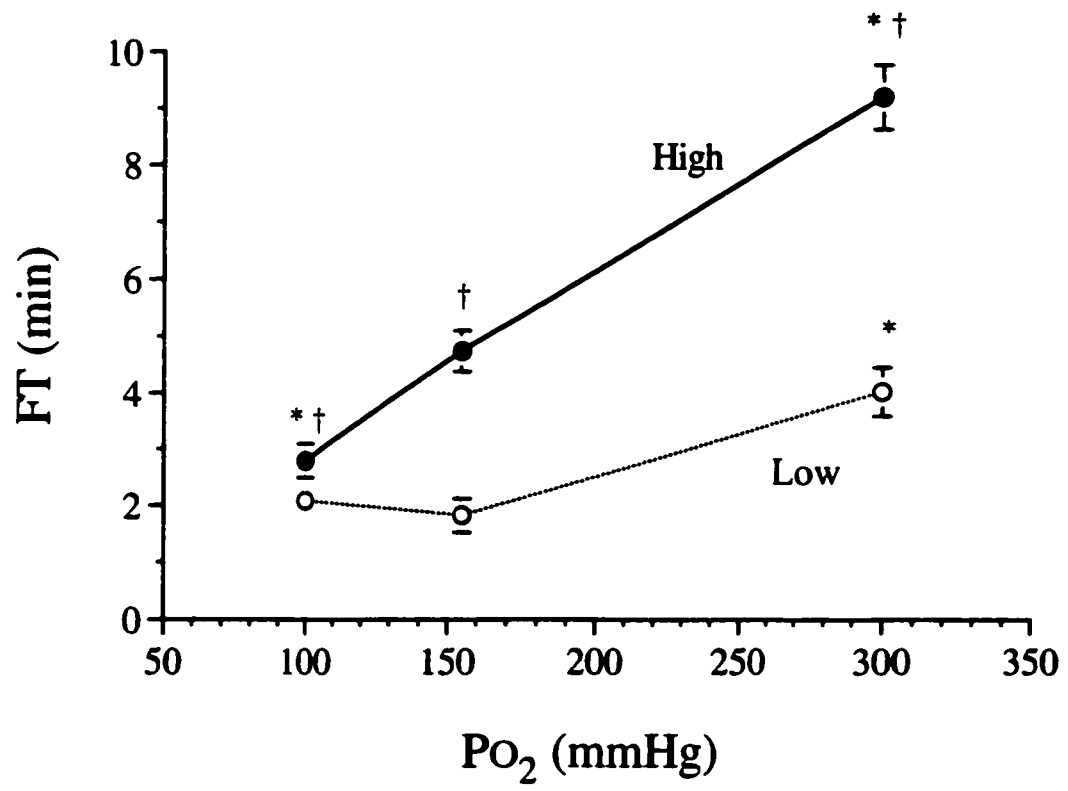
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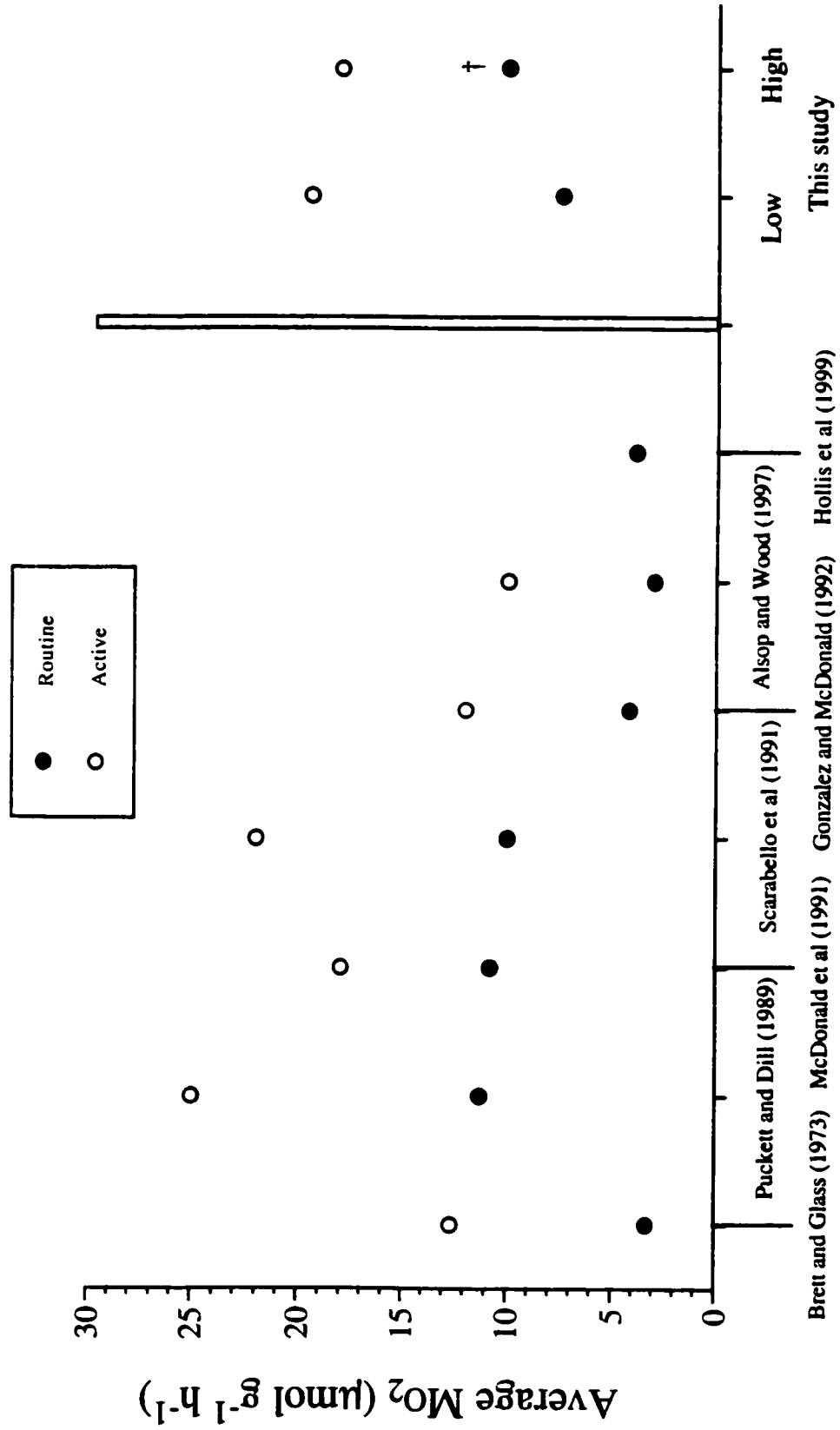
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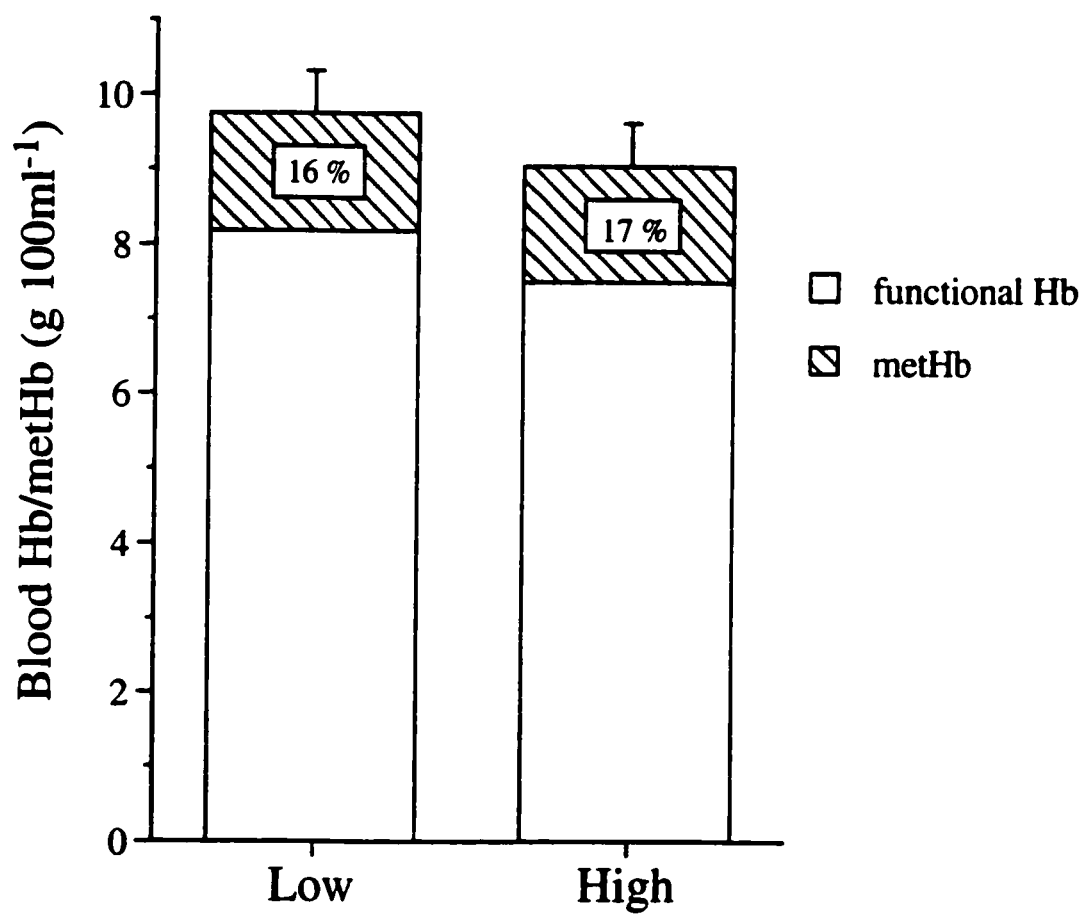
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## **CHAPTER 7**

### **GENERAL DISCUSSION**

The major themes of this study are comparisons: endurance in a  $U_{crit}$  test vs. endurance in a sprint test and the effects of acceleration rate on the latter (Chapter 2), trained vs. untrained fish (Chapters 2, 3 and 5), fatigue vs. exhaustion (Chapters 3 and 5), creatine supplemented vs. non-supplemented fish (Chapter 4), high vs. low sprint performers (Chapter 5 and 6), and diffusion vs. convection limitations with respect to oxygen supply to working muscles (Chapter 6). These comparisons have led to the following results: both slowly accelerated and periodically sprinted fish have increased endurance; the ability to train is greatly influenced by stress; reduction of ATP appears to evoke fatigue in both  $U_{crit}$  and sprint tests; glycogen levels do not determine endurance; creatine loading enhances endurance without increasing muscle creatine or PCr levels; fish with high sprint performance have a higher  $U_{crit}$ ;  $U_{crit}$  performance is diffusion-limited in high performers, but is convection-limited in low performers, yet sprint performance is convection-limited in both groups; and neither sprint nor  $U_{crit}$  performance is influenced by normal variability in blood oxygen carrying capacity.

#### *Factors limiting sprint performance*

One of the main conclusions of this study (Chapter 2) is that the slow cardiovascular response is the *key* factor limiting sprint performance. For reasons unknown, it may take several minutes or longer to increase muscle blood flow to a steady-state value when a rapid increase in velocity is imposed. Consistent with this conclusion is

the finding that a slowly accelerated fish performs better once it reaches sprint velocity compared to a rapidly accelerated fish. The implication is that if a fish is accelerated sufficiently slowly to its critical velocity ( $U_{crit}$ ), then blood flow to the muscle will have reached a steady-state value by the end of the acceleration period where oxygen supply and demand will be perfectly matched, and endurance will be indefinite (i.e., > 200 min). This is one of the reasons I have concluded that sprint performance may be partially limited by convection, or transfer of oxygen via the blood (Chapter 6). The two components that determine convective conductance are capacitance and flow. Since blood oxygen carrying capacity (i.e., level of functional blood hemoglobin) ranges considerably but does not affect performance, then blood flow must be a key variable limiting convection.

If this conclusion is right then it follows that the main effect of sprint training is to shorten the time it takes for muscle blood flow to increase to its steady-state value. What prevents a rapid increase from occurring is unknown, however I have suggested that it may be a mechanism to prevent gill damage (Chapter 2). Therefore, sprint-trained fish are probably in fact, learning that it is not detrimental to rapidly increase blood flow. An alternate explanation is that sprint trained fish are learning to swim more efficiently (i.e., they are decreasing the cost of transport). While it is possible that both mechanisms contribute to the dramatic rise in endurance the key finding that experience produced similar behavioural and metabolic changes regardless of whether performance improved, makes the latter explanation less likely.

Assuming that the training effect is a result of learning, it is clear from my results that it will only occur under certain conditions. Firstly, the effect is highly dependent on the type rather than the intensity of exercise. Only repeated rapid accelerations led to improved endurance. Fatigue was not necessary to produce training. Secondly, chronic stress impeded learning. An interesting finding in support of this theory was that fish selected for low sprint performance exhibited both high circulating cortisol levels and an

inability to train (Chapter 5). This references earlier findings (quoted in Chapter 2) that stress may directly act to suppress learning.

### *Metabolic basis of fatigue*

Fatigue, in both a  $U_{crit}$  test and a sprint test appears to be elicited by the same factors. Fatigue occurs prior to maximal anaerobic energy expenditure, and in the  $U_{crit}$  test, the amount of AEE is much smaller in relation to a sprint. In both tests, the only metabolic change within the muscle that coincides with fatigue is a drop in ATP, and thus I suggest that ATP is the metabolic trigger for fatigue (Chapter 3). PCr, although partially depleted at fatigue, is actually recruited prior to fatigue, and therefore is not a key determinant of endurance. Furthermore, muscle total creatine levels (i.e., free creatine plus PCr) do not change in response to either training or creatine supplementation, even though performance increases in both cases. However, creatine loading does produce greater endurance (Chapter 4). The improved endurance coincides with elevated muscle glycogen, but could also be due to some other mechanism, such as greater conservation of ATP stores during exercise. Another potential mechanism that is consistent with the rest of these observations is that creatine loading may be leading to better performance through increasing muscle blood flow.

In any case, if as I suggest, sprint performance is limited by oxygen delivery to the muscle, then it should not be limited by the amount of available fuel within white muscle. This explains how white muscle glycogen and anaerobic capacity can correlate with sprint performance, but not determine performance (Chapters 3 and 5). Although this study has advanced our understanding of some of the factors limiting sprint performance, there are two questions that remain unanswered. Why is the cardiovascular response so slow in fish? and why isn't blood hemoglobin more important in determining performance?

The results of this study also raise two key issues pertinent to the swimming strategies of stream-dwelling fish. The first is whether fish in nature ever swim to fatigue. Fish will either not fatigue, or as my data suggests, fish will fatigue with a very small anaerobic energy expenditure, mainly due to ATP depletion. This relatively small anaerobic debt will permit rapid recovery and repeat performance ability. The second issue is whether fish accelerating voluntarily show a similar slow cardiovascular tune-up response. If the tune-up is more rapid, performance will be greater under voluntary circumstances, as compared to when a velocity is imposed. Alternatively, if the cardiovascular response is the same with both voluntary and imposed accelerations, this suggests that under most circumstances, fish may avoid swimming at velocities near  $U_{crit}$ .