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EFFECTS OF ENVIRONMENTAL TEMPERATURE ON THE METABOLIC AND ACID–BASE RESPONSES OF RAINBOW TROUT TO EXHAUSTIVE EXERCISE

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Summary

In vivo experiments were conducted to determine how the physiological response to exhaustive exercise in rainbow trout (*Oncorhynchus mykiss*) is affected by environmental temperature. The white muscle acid–base status (e.g. pH, P_{CO_2}, HCO_3^- and ΔH_m^+) and metabolite (e.g. lactate, phosphocreatine, ATP and glycogen) content, and the acid–base status and lactate concentrations in the blood, were measured at rest and during recovery from burst exercise in rainbow trout acclimated to either 5 or 18 °C.

Trout acclimated to the warmer temperature had higher resting levels of white muscle phosphocreatine (PCr) and also utilized a greater proportion of their muscle ATP and glycogen stores during burst activity compared with trout acclimated to the colder temperature. Recovery of muscle PCr and glycogen levels was independent of acclimation temperature, but muscle ATP levels recovered faster at 18 °C. Exhaustive exercise resulted in a similar lactacidosis in the muscle of trout acclimated to either temperature. In contrast, temperature had a marked influence on the lactacidosis in the blood. Blood lactate and metabolic proton concentrations following exercise were about twofold greater in fish acclimated to 18 °C than in fish acclimated to 5 °C. Despite the more severe acidosis and the greater lactate accumulation in the plasma of fish acclimated to warmer temperatures, the time required for recovery of these variables was similar to that at 5 °C. Taken together, these results suggest that acclimation temperature does not significantly affect anaerobic capacity in rainbow trout, but may account for much of the documented variability in the dynamics of the lactacidosis in blood following exhaustive exercise in fish.

Introduction

The physiological and biochemical mechanisms associated with burst-type exercise in fish have been well studied and are generally understood (Milligan and Wood, 1986; Dobson and Hochachka, 1987; Wood, 1991). It is also evident, however, that there is considerable variability in the physiological responses to exhaustive exercise within individual species of fish (Dalla Via *et al.* 1989; Goolish, 1991; Ferguson *et al.* 1993). Several factors may contribute to this intraspecific variability, but their relative importance is often difficult to ascertain in comparisons between different studies.

Key words: temperature, metabolites, muscle, blood, pH, acid–base, exhaustive exercise, trout, *Oncorhynchus mykiss*.

It is known that a number of physiological and biochemical processes are modified when fish are acclimated to different temperatures (Hochachka and Somero, 1984; Bowler and Fuller, 1987; Prosser, 1991). At the cellular level, adaptations that occur in response to changes in water temperature involve modifications in enzyme concentrations (Blier and Guderley, 1988), membrane fluidity (Hazel, 1988) and substrate availability for energy production (Walesby and Johnston, 1980). Temperature acclimation may also lead to changes in cardiac output (Farrell, 1984), perfusion rates to the tissues (Barron *et al*. 1987), muscle fibre recruitment (Rome *et al.* 1984) and muscle contractile properties (Johnson and Johnston, 1991). Taken together, these results suggest that temperature may have a significant influence on the physiological response to exhaustive exercise *in vivo*. To date, however, most studies have focused on the physiological effects of temperature acclimation *in vitro*. Moreover, the limited number of *in vivo* studies have produced somewhat conflicting results. According to Brett (1964) and Beamish (1978), burst swimming speeds in fish are virtually independent of acclimation temperature. More recently, however, studies by Wieser *et al.* (1986) and Dalla Via *et al.* (1989) suggest that anaerobic energy production in fish may be influenced by acclimation temperature. Thus, further studies are clearly warranted regarding the effects of acclimation temperature on the physiological responses of fish to exhaustive exercise *in vivo*.

In view of the previously documented physiological and biochemical changes occurring in fish in response to temperature acclimation, we hypothesize that environmental temperature will have a significant influence on (i) the storage, utilization and/or recovery of the fuels (e.g. PCr, ATP and glycogen) required for burst exercise and (ii) the production and removal of lactate and metabolic protons following burst exercise in fish. This study, therefore, examined the effect of acclimation temperature on the acid–base (e.g. pH, P_{CO_2}, $[HCO_3^-]$ and ΔH_m^+) and metabolite (e.g. [lactate], [PCr], [ATP] and [glycogen]) status of the white muscle and the acid–base status and lactate concentrations in blood at rest and during recovery from exhaustive exercise in trout.

Materials and methods

Experimental animals and acclimation protocol

Adult rainbow trout [*Oncorhynchus mykiss* (Walbaum)] ranging in length from 36 to 41 cm (total length) were obtained from Linwood Acres Trout Farm, Ontario, and held indoors in large flow-through tanks continuously supplied with aerated, dechlorinated Kingston water. Following a period of about 2 weeks, fish were transferred to acclimation tanks where they were slowly acclimated (in increments of about 0.5–1 $^\circ C\,day^{-1}$) to either 5 or 18 $^\circ$C. Once the designated water temperature had been reached (i.e. 5 or 18 $^\circ$C), the fish remained at their respective temperature for at least 8 weeks. Deviations from the set temperature were never more than ±0.5 $^\circ$C. During the acclimation period, fish were fed a commercial trout food every second day to satiation. Five days prior to experimentation, feeding was suspended to reduce possible dietary influences on acid–base and metabolite status.

Muscle experiments

Individual fish were removed by net from their acclimation tank, transferred to a smaller circular tank filled with water maintained at the acclimation temperature, and immediately exercised to exhaustion (except for control fish) by manual chasing for 5 min. It should be noted that the lengths of the fish were not significantly different between the two temperature groups (two-way analysis of variance, ANOVA, $P>0.1$) or between sample periods (two-way ANOVA, $P>0.05$). Exercise for 5 min was adequate to exhaust the fish: they no longer responded to chasing after this. It should also be noted that we did not attempt to quantify the work done during exercise and that this may have been influenced by temperature. We chose instead to exercise the fish to a behavioural state of exhaustion at each temperature and have examined the magnitude of the physiological disturbance and the characteristics of recovery. However, the time required to reach exhaustion was not significantly different at either temperature (approximately 4 min). Samples of white muscle were taken immediately following exercise (0 min of recovery, $N=6$) or after 1 h ($N=6$), 4 h ($N=6$) or 8 h ($N=6$) of recovery in blackened Perspex boxes. Fish sampled immediately following exercise (i.e. time 0) were placed directly into the anaesthetic prior to removal of muscle. For all the other sample times, the flow to the Perspex box was stopped and a buffered solution of MS-222 (pH 7) was added. After 2–3 min, during which the fish remained quiescent, the trout were fully anaesthetized. We chose to anaesthetize the fish prior to muscle sampling because this method has been shown to reduce any metabolic and acid–base changes associated with the handling of a conscious animal (see Tang and Boutilier, 1991, for critique of technique). This is particularly important for highly labile metabolites such as phosphogens and adenylates (see Dobson and Hochachka, 1987). Following anaesthetization, a sample of white muscle was removed from the epaxial musculature, behind the operculum and well above the lateral band of red muscle. Samples were immediately freeze-clamped in pre-cooled aluminium tongs and stored in liquid nitrogen. The time between removing the fish from the box and freeze-clamping the tissue was less than 10 s. Control (i.e. resting) values were obtained in a manner similar to that described above, except that individual fish were isolated in separate Perspex boxes for at least 24 h prior to sampling and were not exercised.

Frozen muscle tissues were analyzed for pH, total CO_2, lactate, glycogen, ATP and phosphocreatine (PCr) concentrations, non-bicarbonate buffer capacity (β) and water content.

Analytical techniques and calculations

Acid–base status. The acid–base status of white muscle was determined using the technique of Pörtner *et al*. (1990). About 75–100 mg of muscle was ground, from behind a Plexiglas shield, under liquid nitrogen using a pre-cooled mortar and pestle. The ground muscle was transferred to a tube containing 200 μl of ice-cold metabolic inhibitor (150 mmol l^{-1} potassium fluoride; 6 mmol l^{-1} nitrilotriacetic acid, KF-NTA; see Pörtner *et al.* 1990, for details). Following this, another 200 μl of ice-chilled metabolic inhibitor was added to the tube, and the mixture was briefly stirred and vortexed. The tube was

weighed and quickly centrifuged (for a maximum of 8 s at 15 000 revs min^{-1}). Samples of the supernatant were then immediately taken for measurements of pH and total CO_2 content (C_{CO_2}). The pH of 80 μl of the supernatant was determined using a PHM 73 pH meter and associated micro-pH unit (Radiometer, Copenhagen, Denmark) thermostatted to the respective acclimation temperature (5 or 18 °C). The C_{CO_2} of 200 μl of the supernatant was measured with a Corning model 965 CO_2 analyzer (CIBA Corning Canada Inc.). C_{CO_2} and pH measurements were carried out in duplicate and the mean was calculated. The C_{CO_2} of the tissue water was calculated using the equations provided by Pörtner *et al*. (1990). Measured values of muscle pH and C_{CO_2} were used to calculate P_{CO_2} and [HCO_3^-] using a rearrangement of the Henderson–Hasselbach equation. The values for α_{CO_2} were determined according to the formulae provided by Heisler (1984). The values for pK were obtained from an experimental analysis of the non-bicarbonate buffering capacity of tissue homogenates under metabolic control (Pörtner, 1990). The concentration of metabolic protons over any given period (i.e. time 1 to time 2) was calculated according to the following equation:

$$\Delta H^+ = ([HCO_3^-]_1 - [HCO_3^-]_2) - \beta(pH_1 - pH_2),$$

where β is the non-bicarbonate buffer value (see below).

Tissue extraction and metabolite levels. Samples of trout muscle were ground to a fine powder as described above. Approximately 1–2 g of powder was transferred to a tared 15 ml vial to which 4 vols of ice-cold 8 % perchloric acid (PCA) solution containing 1 mmol l^{-1} of EDTA was added. This mixture was vortexed briefly to form a slurry. An extraction period (5 min) followed, during which the slurry was inverted and slowly rotated at 5 °C. The slurry was then divided into 1.5 ml Eppendorf tubes and centrifuged for 2–3 min at 5 °C. A known volume of supernatant was neutralized with 2 mol l^{-1} KOH containing 0.4 mol l^{-1} KCl and 0.4 mol l^{-1} imidazole, and immediately centrifuged (for 45 s in a clinical centrifuge at high speed). The neutralized supernatant was divided into labelled Eppendorf tubes, flash frozen and stored in liquid nitrogen. Concentrations of all the metabolites, except for glycogen, were determined enzymatically on neutralized PCA extracts according to the methods of Lowry and Passonneau (1972). Tissue glycogen concentrations were determined using the method of Hassid and Abraham (1957). All metabolite assays were performed in duplicate, and the two measurements never differed by more than 10 %. Appropriate metabolite standards (Sigma) were used with each assay.

Water content. Approximately 300–1000 mg of muscle tissue was transferred to a dried and tared 1.5 ml Eppendorf tube. Following determination of wet mass, the samples were placed into an oven at 80 °C and dried for several days until a constant mass was obtained. The percentage water content of the tissue was then calculated using the following formula:

$$H_2O\,(\%) = 100 - [(100 \times \text{dry mass})/\text{wet mass}].$$

Non-bicarbonate buffering capacity (β). Non-bicarbonate buffering capacity was determined by the methods of Heisler and Piiper (1971) and Pörtner (1990). Briefly, 1–2 g of ground muscle tissue was placed into a pre-weighed 15 ml tube containing four times the tissue volume of the KF-NTA metabolic inhibitor as above. This inhibitor has been

shown to reduce the rate of homogenate metabolism and therefore to minimize accumulation of inorganic phosphate (for details, see Pörtner, 1990; Pörtner *et al*. 1990). This slurry was briefly vortexed and placed into precooled tonometer vessels. The homogenates were then equilibrated for 30–40 min to various levels of P_{CO_2} (1–9 % CO_2, in air) using a Wöstoff gas-mixing pump (Bochum, Germany) at their respective acclimation temperature (5 or 18 °C). Samples were taken at each P_{CO_2} level and immediately centrifuged (for 4 min at 15 000 revs min^{-1}) in Eppendorf tubes. The supernatant was then analyzed for pH and C_{CO_2} as described above. Bicarbonate levels were determined from the C_{CO_2} values and were used to calculate the non-bicarbonate buffer capacity (see Heisler and Piiper, 1971; Pörtner, 1990).

Blood experiments

Prior to these experiments, all fish (N=6, for each temperature) were fitted with dorsal aortic cannulae using the method of Smith and Bell (1964). Following this procedure, fish were placed in darkened Perspex boxes and allowed to recover for 24–48 h prior to experimentation.

Experimental protocol

Prior to exercise, control blood samples (800 μl) were removed from the fish using gas-tight Hamilton syringes. Duplicate haematocrit measurements were made on about 150 μl of blood. The remaining blood was centrifuged (for 4 min at 15 000 revs min^{-1}) and the pH and P_{CO_2} of plasma were determined from 80 and 100 μl samples, respectively. Samples of plasma were also added to known volumes of chilled 8 % perchloric acid (PCA) for the subsequent determination of plasma lactate concentrations.

After the control samples had been taken, fish were exhaustively exercised by manual chasing as outlined for the muscle experiments (at either 5 or 18 °C). They were then returned to the Perspex box and another 800 μl sample of blood (time 0 sample) was removed, and analyses similar to those described for the control sample were performed. Samples were also taken 1, 4 and 8 h following exhaustive exercise. Throughout the experiment, blood samples were replaced with a similar volume of saline. As a control, an identical sampling protocol was performed on an additional six fish (at each temperature) that had not been exhaustively exercised.

Another series of experiments was conducted to determine the influence of temperature on the non-bicarbonate buffer value of true plasma. Samples of blood (about 8 ml) were removed from resting cannulated trout, adjusted to various haematocrits and equilibrated in an intermittently rotating glass tonometer for 30 min at various gas tensions (0.2, 1 and 3 % CO_2 in air). Following the equilibration period, samples were taken anaerobically, rapidly centrifuged (for 4 min at 15 000 revs min^{-1}) in a capped 1.5 ml Eppendorf tube, and the true plasma was analysed for pH and C_{CO_2}. Non-bicarbonate buffer values were determined by examining the slope of the relationship between bicarbonate concentration and pH, as outlined for the muscle experiments. The non-bicarbonate buffer values were then plotted against haematocrit to determine the relationship between the two variables at each acclimation temperature.

Analytical techniques and calculations

The pH of the true plasma was determined using a PHM 73 pH meter and associated micro-pH unit (Radiometer, Copenhagen, Denmark) thermostatted to the respective acclimation temperature (5 or 18 °C). C_{CO_2} content of the true plasma was measured with a Corning model 965 CO_2 analyzer (CIBA Corning Canada Inc.). Arterial plasma CO_2 tension (Pa_{CO_2}) and bicarbonate concentration ($[HCO_3^-]$) were calculated using a rearrangement of the Henderson–Hasselbach equation. Constants for plasma pK′ and CO_2 solubility to be used in these calculations were obtained from Boutilier *et al.* (1984). The concentration of metabolic protons added to the plasma was calculated in a manner similar to that for the muscle experiment. The concentration of lactate in the plasma was measured on thawed samples using the assay outlined in the muscle experiments. The amount of lactate added to the plasma over any given time (t) was calculated according to the equation:

$$\Delta[\text{lactate}] = [\text{lactate}]_t - [\text{lactate}]_R ,$$

where $[\text{lactate}]_R$ is the lactate concentration at rest. The proton deficit was then obtained by subtracting the metabolic protons from Δ[lactate] (i.e. $\Delta[\text{lactate}] - \Delta H^+_m$).

Statistics

All values are presented as means ± 1 S.E.M. For the muscle experiments, one-way analyses of variance (ANOVAs) were used to assess the significance of observed differences in both the 5 and 18 °C experiments. If the ANOVA indicated significance ($P \leqslant 0.05$), a Sheffe's multiple comparison test was then used to determine significant differences ($P \leqslant 0.05$) between resting values and post-exercise values at each temperature. We also used two-way ANOVAs to compare directly the overall effect of acclimation temperature for any variable measured in the muscle experiment. This allowed us to assess whether any interactive term existed between temperature and time period. Unpaired *t*-tests were used to compare the 5 °C fish with the 18 °C fish at any given sample time. For the blood experiments, a repeated-measures analysis of variance was used. If the ANOVA indicated significance, a Dunnett's multiple-comparisons test was used to determine significant differences between resting values and post-exercise values. Unpaired *t*-tests were also used to compare values for the 5 °C fish with those for the 18 °C at each sample time. Finally, an analysis of covariance (ANCOVA) was used to compare the relationship between the non-bicarbonate buffer value and haematocrit at the two acclimation temperatures. In all cases, $P \leqslant 0.05$ was the accepted level of significance.

Results

Muscle

Phosphocreatine (PCr), ATP and carbohydrate status

The resting ATP and glycogen content of the white muscle were unaffected by acclimation temperature (Fig. 1A,B). Acclimation to a warmer temperature did result in significantly higher resting levels of PCr (Fig. 1C). PCr levels following exercise,

however, were unaffected by temperature. Levels of ATP declined significantly following exhaustive exercise in both groups of fish, but fish acclimated to 18 ˚C utilized a greater amount of ATP (about 75 %) than cold-acclimated fish (<55 %) (Fig. 1A). Recovery of ATP was also faster at warmer temperatures; ATP levels were not significantly different from pre-exercise after 1 h at 18 ˚C and at 4 h at 5 ˚C (Fig. 1A).

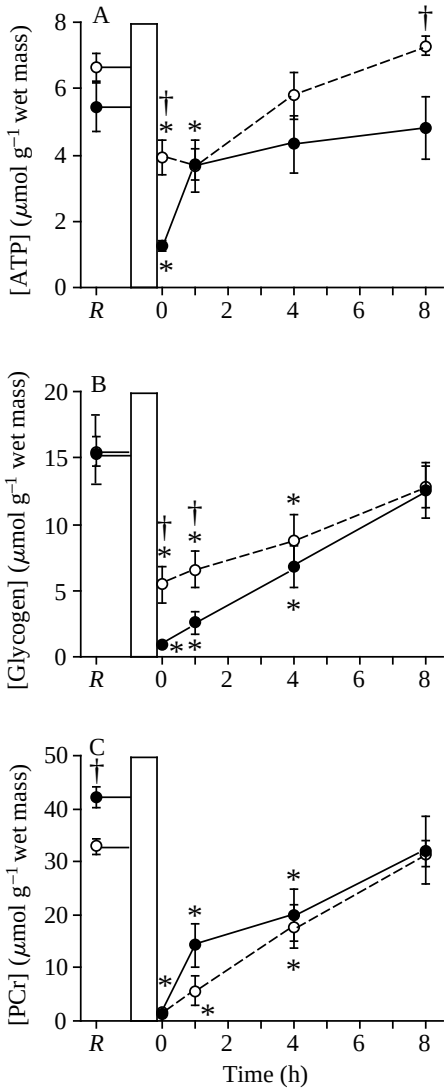

Fig. 1. Changes in white muscle ATP (A) glycogen (B) and phosphocreatine, PCr (C), concentrations prior to, and following, exhaustive exercise in rainbow trout acclimated to either 5 ˚C (open circles) or 18 ˚C (filled circles). Values given are means ± 1 S.E.M. *R* indicates rest, the open column indicates the 5 min period of exercise, and 0 indicates the sample immediately after exercise. *N*=6 for each time period. * indicates a significant ($P \leq 0.05$) difference from the corresponding rest value. † indicates significant (unpaired *t*-test, $P<0.05$) differences between the two temperatures at any given sampling period.

Levels of glycogen also declined significantly following exercise at both temperatures, but fish acclimated to warmer temperatures utilized a greater proportion of this metabolite. Recovery of glycogen required up to 8 h for both groups of fish (Fig. 1B).

Lactate levels and acid–base status

At rest, white muscle lactate concentrations were similar at the two acclimation temperatures (Fig. 2A). At exhaustion, lactate levels increased significantly, but the magnitude of the increase in muscle lactate concentration was not influenced by temperature (Fig. 2A). Lactate was cleared relatively quickly from the muscle at both temperatures and, by 8 h, resting levels were measured. There was also an increase in the concentration of metabolic protons associated with the post-exercise increase in lactate concentration within the muscle (Fig. 2B). The time course of recovery of metabolic protons was very similar to that for lactate (Fig. 2B). The non-bicarbonate buffer value of muscle was also similar at the two temperatures ($-47.1\Delta[HCO_3^-]/\Delta pH$ and $-49.2\Delta[HCO_3^-]/\Delta pH$, for 5 and 18 ˚C, respectively) and there was no significant influence of acclimation temperature on muscle water content (two-way ANOVA; temperature $P>0.05$).

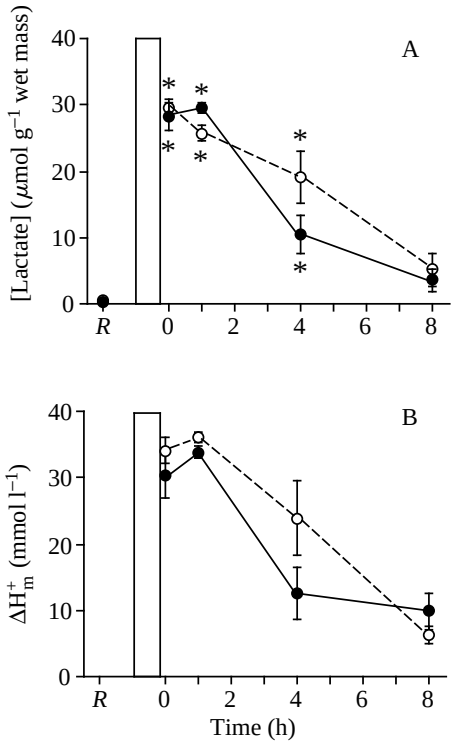

Fig. 2. Changes in white muscle [lactate] (A) and metabolic proton concentrations (ΔH^+_m) (B) prior to and following exhaustive exercise in rainbow trout acclimated to either 5 ˚C (open circles) or 18 ˚C (filled circles). Other details as in Fig. 1. Note: the data in Fig. 2B are presented as a difference between time periods, whereas the data in Fig. 2A are absolute values.

There were significant differences in the resting white muscle pH between fish acclimated at 5 ˚C and 18 ˚C (Fig. 3A). Exhaustive exercise resulted in a pronounced, but similar, acidosis in the white muscle of fish acclimated to either temperature (Fig. 3A). Muscle pH did not show any signs of recovery until between 1 and 4 h and required up to 8 h for full recovery in both groups (Fig. 3A). The acidosis was of metabolic (Figs 2B, 3B) and respiratory (Fig. 3C) origin. Although the resting P_{CO_2} and $[HCO_3^-]$ were very similar at the two acclimation temperatures, the pattern of changes in muscle $[HCO_3^-]$ following exhaustive exercise differed between the two temperatures. Muscle HCO_3^- concentrations fell immediately following exhaustive exercise in fish acclimated to 18 ˚C,

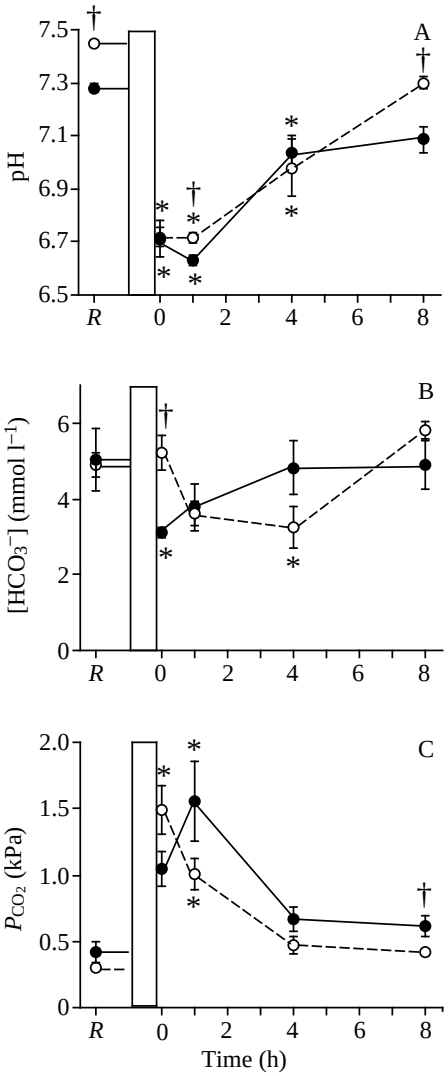

Fig. 3. Changes in white muscle pH (A) $[HCO_3^-]$ (B) and P_{CO_2} (C) prior to, and during recovery from, exhaustive exercise in rainbow trout acclimated to either 5 ˚C (open circles) or 18 ˚C (filled circles). Other details as in the legend of Fig. 1.

but recovered fully within 1 h (Fig. 3B). Fish acclimated to 5 °C, however, required 8 h for the full recovery of muscle [HCO_3^-] (Fig. 3B).

Blood

Repetitive sampling did not affect the status of acid–base variables or lactate concentration in the blood of control (non-exercised) fish (Table 1). Temperature acclimation, however, had a significant effect on the blood acid–base status in control fish. Control fish acclimated to 5 °C had significantly higher plasma pH values and HCO_3^- concentrations than fish acclimated to 18 °C at most sample times (Table 1). In contrast, plasma P_{CO_2} and lactate concentrations were almost unaffected by acclimation temperature in control fish (Table 1).

Following exhaustive exercise, plasma lactate concentrations were markedly influenced by temperature, being about twofold greater in the 18 °C fish than in their 5 °C counterparts (Fig. 4A). Although the plasma lactate concentrations were not equivalent between groups following exercise, [lactate] peaked 1 h after exercise in both groups of fish (Fig. 4A). Temperature also influenced the recovery of plasma lactate concentrations; levels returned to pre-exercise values by 8 h at 18 °C. In contrast, however, lactate levels did not return to resting levels during the experimental period in

Table 1. *The influence of acclimation temperature on plasma pH,* P_{CO_2}*, [*HCO_3^-*] and [lactate] in control (non-exercised) rainbow trout*

Temperature (°C)	Rest	Exercise			
		0 h	1 h	4 h	8 h
pH					
5	8.10±0.03	8.15±0.03	8.08±0.03	8.10±0.02	8.04±0.05
18	7.96±0.02	7.98±0.03	7.96±0.03	7.99±0.03	7.97±0.02
	$P<0.05$	$P<0.05$	$P<0.05$	$P<0.05$	NS
P_{CO_2} (kPa)					
5	0.26±0.02	0.23±0.02	0.27±0.02	0.26±0.02	0.28±0.02
18	0.24±0.01	0.23±0.01	0.24±0.01	0.22±0.01	0.24±0.01
	NS	NS	NS	$P<0.05$	NS
[HCO_3^-] (mmol l^{-1})					
5	9.78±0.46	9.32±0.58	9.51±0.55	9.62±0.57	9.15±0.79
18	6.98±0.34	7.03±0.29	6.98±0.31	6.89±0.37	7.06±0.36
	$P<0.05$	$P<0.05$	$P<0.05$	$P<0.05$	$P<0.05$
Lactate (mmol l^{-1})					
5	0.40±0.14	0.52±0.18	0.58±0.18	0.52±0.16	0.84±0.20
18	0.40±0.19	0.41±0.21	0.46±0.23	0.95±0.59	1.27±0.83
	NS	NS	NS	NS	NS

Values are means ± 1 S.E.M., $N=6$, for each group.

$P<0.05$ represents a significant difference at the same sampling time between fish acclimated to 5 and 18 °C.

NS indicates a non-significant difference at the same sampling time between fish acclimated to 5 and 18 °C.

fish acclimated to 5 ˚C (Fig. 4A). Similarly, the change in metabolic proton concentration peaked in plasma 1 h following exercise for both groups of fish (Fig. 4B). Although the post-exercise changes in the concentration of metabolic protons were much higher in the 18 ˚C fish, they had returned to levels which were no longer different from resting values by 4 h (Fig. 4B). In contrast, approximately 8 h was required for the metabolic proton load to return to resting values at 5 ˚C (Fig. 4B). Temperature also had a large effect on the proton deficit in plasma during recovery (Fig. 4C). Immediately following the

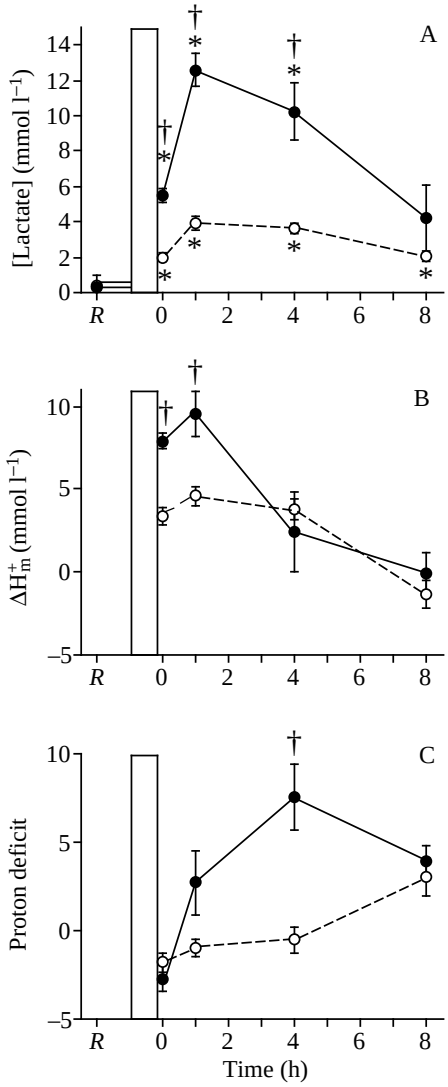

Fig. 4. Changes in plasma lactate (A) metabolic proton load (B) and proton deficit (C) prior to, and during recovery from, exhaustive exercise in rainbow trout acclimated to either 5 ˚C (open circles) or 18 ˚C (filled circles). Other details as in Fig. 1. Note: the data in Fig. 4B are presented as a difference between time periods, whereas the data in Fig. 4A are absolute values.

exercise period, plasma ΔH^{+}_{m} exceeds plasma Δ[lactate], and the proton deficit was therefore negative in both groups of fish. In fish acclimated to 18 °C, this relationship was reversed after 1 h, and the proton deficit then remained positive throughout the recovery period. In contrast, fish acclimated to 5 °C had a proton deficit that remained negative throughout the entire recovery period, with the exception of the 8 h sample. It should be noted that the non-bicarbonate buffering capacity of plasma was unaffected by acclimation temperature (5 °C, β=−0.39Hct−0.82, r=0.97; 18 °C, β=−0.45Hct+0.53, r=0.96; ANCOVA: slope and y-intercept P>0.05).

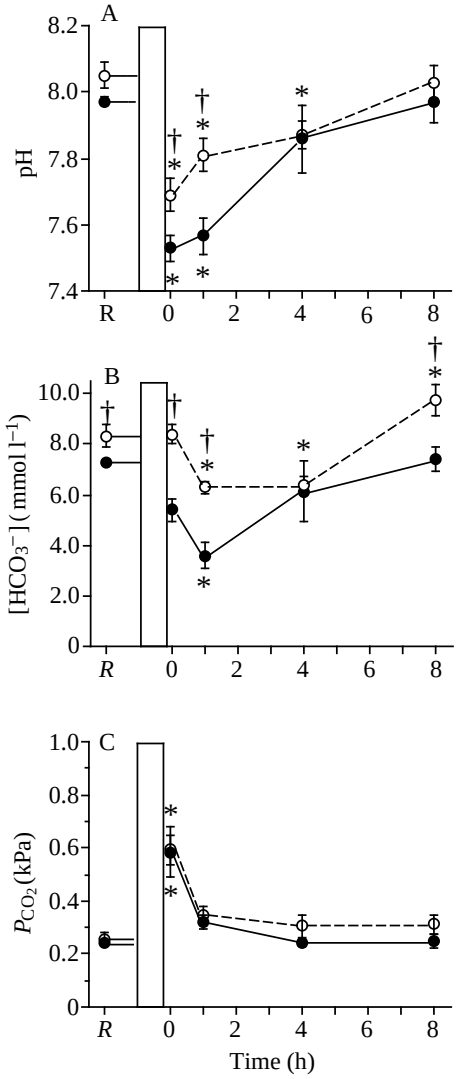

Fig. 5. Changes in plasma pH (A) [HCO_3^-] (B) and P_{CO_2} (C) prior to, and during recovery from, exhaustive exercise in rainbow trout acclimated to either 5 °C (open circles) or 18 °C (filled circles). Other details as in Fig. 1.

Exhaustive exercise resulted in a large and immediate drop in plasma pH in both groups of fish. The plasma acidosis was more severe, however, in the 18 °C group (a decrease of 0.443 pH units) of fish than in the 5 °C fish (a decrease of 0.357 pH units) (Fig. 5A). Temperature also influenced the recovery pattern for plasma pH (Fig. 5A). Fish acclimated to warmer temperatures required less time (4 h) for full recovery than fish acclimated to colder temperatures (8 h). The acidosis in plasma had both metabolic (Fig. 5B) and respiratory (Fig. 5C) components. Temperature acclimation did not influence the recovery of P_{CO_2} in plasma following exercise (Fig. 5C). In contrast, temperature acclimation had a marked impact on plasma HCO_3^- concentrations during recovery from exhaustive exercise. Fish acclimated to 18 °C experienced an immediate depression of plasma [HCO_3^-] following exercise, and recovery occurred within 4 h. In fish acclimated to 5 °C, however, the low level of plasma [bicarbonate] was maintained until 4 h post-exercise (Fig. 5B), and more than 8 h was required for full recovery.

Discussion

In general, we found that the anaerobic capacity of trout white muscle was not markedly influenced by environmental temperature. However, the concentration gradients for both lactate and metabolic protons from the muscle to the blood and their rate of clearance from the blood were markedly affected by acclimation temperature. This study therefore demonstrates that environmental temperature may have an important influence on the physiological strategy for recovery from exhaustive exercise in fish.

ATP, glycogen and phosphocreatine

At rest, both the ATP and glycogen content of white muscle were similar in trout acclimated to 5 and 18 °C. As in other species, however, acclimation to warmer temperatures did result in an increase in PCr stores (Fig. 1C; Walesby and Johnston, 1980; Dehn, 1992). The reasons for the increase in [PCr] at higher temperatures are not fully understood. Nonetheless, since PCr is generally the first energy source to be utilized during burst swimming (Dobson and Hochachka, 1987), these results suggest that fish acclimated to warmer water may have a greater capacity for immediate burst activity.

Trout acclimated to warmer temperatures also used more ATP and glycogen during exhaustive exercise and required less time for [ATP] to recover to resting levels than trout acclimated to colder temperatures (Fig. 1A,B). The faster recovery of [ATP] at higher temperatures is probably related to the overall increase in aerobic metabolic rate (e.g. Fry, 1971) and, therefore, an increased rate of oxidative phosphorylation, at elevated temperatures. It has been proposed that glycogen resynthesis does not begin until muscle pH has been restored to a level compatible with glyconeogenesis (Milligan and Wood, 1986; Walsh and Milligan, 1989). Our data support this hypothesis, as pH does not begin to recover until after 1 h post-exercise at both temperatures (Fig. 3A), and this generally corresponds to the period during which [glycogen] begins to recover (Fig. 1B).

White muscle [lactate] and acid–base balance

In white muscle, both lactate accumulation and recovery were generally independent of temperature (Fig. 2A). This result is somewhat surprising, given that there were

significant differences in glycogen depletion following exhaustive exercise. It is noteworthy, however, that the magnitudes of the post-exercise increases in metabolic proton concentration (Fig. 2B) were also similar at the two experimental temperatures and coincide quite well with the magnitudes of the observed increases in muscle lactate concentration. Thus, it appears to be the magnitude of the changes in muscle [glycogen] at different temperatures that is somewhat difficult to reconcile. The reason that glycogen depletion and lactate production deviate from the expected 1:2 ratio (i.e. Wardle, 1978; Hochachka and Somero, 1984) at cold temperatures is presently unclear and probably warrants further investigation.

The observation that temperature did not affect the accumulation of lactate in the white muscle in the present study is in contrast to previous results on juvenile roach (Wieser *et al.* 1986; Dalla Via *et al.* 1989). Wieser *et al.* (1986) suggested that reduced production of lactate at colder temperatures in roach may be due to a greater inhibition of lactate dehydrogenase (LDH) by pyruvate compared with that at higher temperatures. In contrast, the absence of a temperature effect on lactate production in our study might be expected, given the presence of temperature-specific isoenzymes of lactate dehydrogenase (LDH) in rainbow trout muscle (Somero, 1978). As for lactate accumulation, temperature did not affect metabolic proton accumulation in muscle of rainbow trout following exhaustive exercise (Fig. 2B). The lack of any temperature-dependent effects on the white muscle non-bicarbonate buffering capacity in trout also appears to be in contrast to the previous results (Nelson and Magnuson, 1987) for yellow perch (*Perca flavescens*). Differences between the present study and that of Nelson and Magnuson (1987) may again be attributable to species differences. It is noteworthy, however, that the methods for determining muscle buffering capacity differed between these two studies. The buffering value of muscle tissue in our study was determined by P_{CO_2} equilibration of metabolically inhibited homogenates, whereas Nelson and Magnuson (1987) titrated muscle homogenates with strong acids and bases in a bicarbonate-free, closed system. Pörtner (1990) provides a summary of the attributes and methodological cautions of each technique and suggests that metabolic inhibition of the homogenate may be critical in the determination of the buffer value of muscle.

Temperature also affected the changes in muscle [HCO_3^-] following exercise in rainbow trout (Fig. 3B). The magnitudes of the decrease in muscle [HCO_3^-] were similar at the two temperatures, but the recovery of [HCO_3^-] was faster at warmer temperatures (Fig. 3B). Regulation of the bicarbonate ion is generally a very slow process in fish (Heisler, 1982) and is usually accomplished by the transmembrane transfer of ions. The present results suggest that regulation of muscle [HCO_3^-] may be even slower at cooler temperatures, possibly because of an effect of temperature on ion transport processes.

Plasma [lactate] and acid–base balance

In contrast to the situation in muscle, the dynamics of lactate levels and metabolic protons in the blood of exercised rainbow trout were markedly affected by acclimation temperature (Fig. 4A). Following exercise, the concentrations of both lactate and metabolic protons were much higher in the plasma of 18 °C fish. A comparison of the peak blood lactate concentrations previously documented in exhaustively exercised trout

(Table 2) suggests that environmental temperature may also account for some of the variability that has been found between studies. It should be recognized that there are a number of important differences between these studies with regard to sampling times, exercise protocols and whether plasma or whole-blood lactate concentrations were measured. However, it is noteworthy that the lowest documented blood lactate concentrations have occurred at temperatures between 5 and 8 °C, whereas the highest documented concentrations have occurred at 15–18 °C (Table 2). Taken together, the available data therefore strongly support our original hypothesis that environmental temperature will influence the dynamics of lactate levels in the blood of rainbow trout following exhaustive exercise.

The temperature-related differences in lactate and proton accumulation observed in the blood during recovery were not the result of differences in the amount of lactic acid produced in the white muscle (Fig. 2), but were the result of temperature-dependent changes in the muscle-to-blood gradients for lactate and metabolic protons (Figs 2 and 4). These results indicate that environmental temperature may have an important influence on (i) the rate of diffusion of metabolic end-products out of the muscle and/or (ii) the blood perfusion to the muscle. Support for these assumptions also arises from several previous *in vitro* and *in vivo* studies. For example, it has been shown that acclimation to different temperatures causes changes in the fluidity of cellular membranes (Hazel, 1988) and may also affect the diffusion of small molecules within the muscle tissue (Tyler and Sidell, 1984; Sidell and Hazel, 1987). Notably, Sidell and Hazel (1987) showed that the diffusivity of non-metabolizable analogues of lactic acid in a cytosolic preparation of perch white muscle decreased with reductions in acclimation temperature. Differences in

Table 2. *Maximum lactate concentrations following exhaustive exercise in rainbow trout at various temperatures*

Duration of exercise (min)	Peak [lactate] (mmol l^{-1})	Time of peak [lactate] (min)*	Water temperature (°C)	Reference
5	12.6 [a]	60	18	Present study
5	14.0[a]	120	15	Holeton *et al.* (1983)
10	9.6[a]	60	15	Ferguson and Tufts (1992)
6	17.6[b]	120	15	Milligan and Wood (1986)
6	12.5[b]	120	14.5	Wood *et al.* (1983)
10	10.0[b]	70	10	Tang *et al.* (1989)
10	9.5[b]	120	8	Pagnotta and Milligan (1991)
5	4.0[a]	60	5	Present study

[a]Represents plasma values.

[b]Represents whole-blood values.

Fish were exercised to exhaustion by manual chasing in all except Holeton *et al.* (1983), where exercise was induced by mild electric shock.

*Time of peak [lactate] refers to the sample period at which the lactate was measured at its highest level. Note, however, that the sampling protocol (i.e. time course) varied between studies.

environmental temperature are also associated with changes in cardiac output in fish (Farrell, 1984). Recently, Barron *et al.* (1987) showed that an increase in acclimation temperature resulted in an increase in the perfusion of blood to white muscle in trout. In the present study, the greater concentrations of lactate and metabolic protons in the blood of warmer fish therefore probably result from temperature-related increases in both the rate of diffusion of lactate and the perfusion of the white muscle tissue following exercise.

Although the concentrations of lactate and metabolic protons were much higher in the blood of warmer fish, the recovery time for these variables was similar to that in the colder fish (Fig. 4A,B). Thus, warmer temperatures also appear to increase the rate of removal of metabolic protons and/or lactate from the blood during recovery. It has been proposed that the cells of various tissues, such as red muscle, heart, kidney and blood, may be important in the removal of post-exercise lactate in fish (Bilinski and Jonas, 1972; Milligan and Wood, 1986; Wood *et al*. 1990; Milligan and Farrell, 1991; Milligan and Girard, 1993). At the warmer temperatures, one can speculate that the increased metabolic rate of these lactate-consuming tissues may also contribute to the faster removal of this metabolic end-product from the plasma.

The metabolic proton deficit (Δ[lactate]$-\Delta H^{+}_{m}$) within the plasma was also much greater in trout acclimated to warmer water (Fig. 4C). These results suggest that metabolic protons may be removed from the blood at a faster rate at warmer temperatures. It is well established that the gills are important in the removal of metabolic protons from the blood to the environmental water (Holeton *et al.* 1983; Wood, 1988; McDonald *et al.* 1989). Thus, the transfer of acid–base equivalents across the gills may also be influenced by temperature. In this regard, it is noteworthy that Leino and McCormick (1993) recently showed that juvenile largemouth bass (*Micropterus salmoides*), acclimated to overwintering temperatures (i.e. 4 °C), had greatly thickened gill epithelia compared with fish at 20 °C. These authors suggested that a thickened respiratory epithelium could present a greater barrier to ion loss. Further experiments will be required, however, to determine whether environmental temperature also affects the branchial ion transport processes involved in acid–base regulation.

In conclusion, our results largely support our initial hypothesis and indicate that environmental temperature has a significant impact on several aspects of the physiological and biochemical responses of rainbow trout to exhaustive exercise. The present results also demonstrate that the effect of acclimation temperature on the muscle-to-blood gradients for lactate and metabolic protons following exercise may account for some of the previously documented differences in these variables between studies. Finally, significant differences in the metabolic proton deficit in plasma following exercise suggest that the relative importance of the gill in acid–base regulation may also be a function of environmental temperature. Further experiments are currently under way to elucidate the physiological basis of these temperature-related differences in the responses of rainbow trout to exhaustive exercise.

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