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Influence of seasonal temperature on the repeat swimming performance of rainbow trout *Oncorhynchus mykiss*

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Summary

While the temperature dependence of exercise performance in fishes is reasonably well documented, information on the temperature dependence of metabolic recovery and reperformance is scant. This study examined the recovery of swimming performance after exhaustive exercise in rainbow trout *Oncorhynchus mykiss* at seasonal temperatures ranging from 5 to 17°C and explored the relationship between performance and preceding metabolic state. The primary objective of the study was to test the hypothesis that increased temperature increases the capability of rainbow trout to repeat a critical swimming speed (U_{crit}), as assessed by two consecutive critical swimming speed tests separated by a 40 min rest interval. An additional expectation was that certain plasma ionic, metabolic and humoral parameters would be correlated with how well fish reperformed and so plasma levels of lactate, potassium, ammonia, osmolality, sodium and cortisol, as well as hematocrit, were monitored before, during and after the swim challenges *via* an indwelling cannula in the dorsal aorta. As expected, performance in the first U_{crit} test (U_{crit1}) was positively related to temperature. However, the relationship between U_{crit1} and reperformance (U_{crit2}) was not dependent on acclimation temperature in a simple manner. Contrary to our expectations, U_{crit2} was less than U_{crit1} for warm-acclimated fish (14.9±1.0°C), whereas U_{crit2} equaled U_{crit1} for cold-acclimated fish (8.4±0.9°C). Cold-acclimated fish also exhibited a lower U_{crit1} and less metabolic disruption compared with warm-acclimated fish. Thus, while warm acclimation conferred a faster U_{crit1}, a similar swimming speed could not be attained on subsequent swim after a 40 min recovery period. This finding does not support the hypothesis that the ability of rainbow trout to reperform on U_{crit} test is improved with temperature. Both plasma lactate and plasma potassium levels were strongly correlated with U_{crit1} performance. Therefore, the higher U_{crit1} of warm-acclimated fish may have been due in part to a greater anaerobic swimming effort compared with cold-acclimated fish. In fact, a significant correlation existed between the plasma lactate concentration prior to the start of the second test and the subsequent U_{crit2} performance, such that U_{crit2} decreased when a threshold plasma lactate level of around 12.2 mmol l^{-1} was surpassed for the initial swim. No other measured plasma variable showed a significant relationship with the U_{crit2} performance. We conclude that warm-acclimated fish, by apparently swimming harder and possibly more anaerobically compared with cold-acclimated fish, were unable to recovery sufficiently well during the fixed recovery period to repeat this initial level of performance, and this poorer repeat performance was correlated with elevations in plasma lactate levels.

Key words: fish, rainbow trout, *Oncorhynchus mykiss*, critical swimming speed, temperature acclimation, repeat swimming, plasma, lactate threshold, ammonium.

Introduction

An extensive literature exists on the recovery of metabolites and ions following exhaustive exercise in fish (see reviews by Driedzic and Hochachka, 1978; Milligan, 1996; Kieffer, 2000). Considerably fewer studies have measured how quickly or how well swimming performance recovers following exhaustive exercise (e.g. Stevens and Black, 1966; Randall et al., 1987; Brauner et al., 1994; Jain et al., 1998; Farrell et al., 1998, 2001, 2003). Given that metabolic recovery in skeletal muscle (muscle lactate, ATP and glycogen, but not PCr) occurs more rapidly at warm than cold temperatures in exhausted rainbow trout *Oncorhynchus mykiss* and Atlantic salmon *Salmo salar* (Kieffer et al., 1994; Wilkie et al., 1997; Kieffer, 2000), the expectation is that swimming performance is restored faster at a higher temperature. This expectation would be consistent with the known increase in both maximum oxygen uptake and maximum cardiac output with temperature (e.g. Butler et al., 1992; Farrell, 1997; Taylor et al., 1997) because an improved oxygen delivery system could support a more rapid recovery of the metabolic debt incurred with exhaustive exercise. However, when Atlantic salmon were angled rather than chased to exhaustion, muscle glycogen, intracellular pH and lactate were restored more rapidly under cold conditions than warm conditions (Wilkie et al.,

1996). Therefore, given this uncertainty and the absence of any study that has directly measured how acclimation temperature affects the recovery of swimming performance, the primary objective of the present study was to test the hypothesis that the ability of rainbow trout to repeat a critical swimming speed (U_{crit}) test is improved with temperature.

A second objective of the present study was to search for correlations between the ability to reperform after an exhaustive U_{crit} swim and the alteration in plasma levels of ions, metabolites and hormones during exercise. In particular, possible linkages were sought between the recovery of swimming performance and the post-exhaustion levels of plasma potassium, lactate and total ammonia concentrations (T_{amm}), all of which have been linked with muscular exhaustion in both mammals and fish. For example, high intensity exercise in mammals produces a potassium loss from the muscle (Sjøgaard et al., 1985; Vøllestad et al., 1994; Hallén, 1996), which could decrease the muscle membrane excitability and compromise tension development (reviewed by Sjøgaard, 1991). Plasma potassium levels increase in rainbow trout just prior to U_{crit} and, moreover, exercise training increased U_{crit} while blunting and delaying the increases in plasma potassium and lactate just prior to exhaustion (Holk and Lykkeboe, 1998). Plasma lactate concentration has long been considered a useful indicator of aerobic limitations and anaerobic capabilities in exercise studies. Indeed, rainbow trout refused to perform repetitive bouts of burst exercise when plasma lactate concentration exceeded 13 mmol l^{-1} (Stevens and Black, 1966) and a poorer repeat U_{crit} was found for sockeye salmon *Oncorhynchus nerka* when plasma lactate concentration was >10 mmol l^{-1} (Farrell et al., 1998). In mammals, elevated plasma T_{amm} has been implicated in exercise fatigue (reviewed by Mutch and Banister, 1983) due to inhibitory influences on anaerobic metabolism (Zaleski and Bryla, 1977; Su and Storey, 1994), aerobic metabolism (McKhann and Tower, 1961; Avillo et al., 1981) and neuromuscular coordination (Binstock and Lecar, 1969; O'Neill and O'Donovan, 1979). Plasma T_{amm} also increases in rainbow trout during exercise (Turner et al., 1983; Wang et al., 1994; but see Beaumont et al., 1995a,b). Furthermore, when routine T_{amm} was elevated in brown trout *Salmo trutta*, as a result of exposure to acidic, copper-containing water, the subsequent U_{crit} performance was inversely related to pre-exercise plasma T_{amm} concentration (Beaumont et al., 1995a). Thus, because plasma levels of potassium, lactate and T_{amm} are good indicators of exhaustion in fish, we anticipated that they are potentially strong indicators of repeat swimming capability in rainbow trout. If this is the case, the expectation is that individual variation in these plasma variables prior to a second swim would be correlated with individual variation in the performance of a second U_{crit} test compared to the initial performance.

Materials and methods

Fish

Rainbow trout *Oncorhynchus mykiss* Walbaum [mass=871.49±43.34 g (mean ± standard error of the mean, S.E.M.); fork length (*FL*)=40.95±0.77 cm, *N*=15] were obtained from a local hatchery (Sun Valley Trout Farm, Mission, British Columbia, Canada). They were held outdoors in a 2000 liter round fiberglass aquarium provided with aerated and dechlorinated Vancouver municipal water, pH 6.7, hardness 5.2–6.0 mg l^{-1} $CaCO_3$, and ambient temperature 5–17°C. Experiments were performed between November 1997 and April 1998, and September–October 1998. All experimental work conformed to the guidelines set out by the Canadian Council on Animal Care, as approved by the Simon Fraser University Animal Care Committee.

Swim tunnel

Fish were swum in a modified Brett-type swim tunnel, similar to that described by Gehrke et al. (1990). The swim chamber was 21 cm diameter and 97 cm length, with a metal grid at each end. The rear grid was equipped with an electrical pulse generator (4 V) that, when contacted by the fish, provided a mild stimulation to encourage the fish to swim forward. Water speed was uniform across the swim tunnel throughout the speed range used in these experiments. The water current in the tunnel was produced by a 3-phase induction motor and a centrifugal pump attached to a tachometer whose readings (Hz) were calibrated with known water velocities, as measured with a Valeport current meter (Valeport Marine Scientific Ltd., Dartmouth, UK).

Protocol for arterial cannulation

The dorsal aorta was cannulated to permit sampling of blood prior to and during the swimming tests, and during the recovery periods. Arterial cannulation was performed under anesthesia (0.1 g l^{-1} buffered MS-222; Syndel Laboratories, Vancouver, BC, Canada), using the method of Smith and Bell (1964). Fish mass, fork length, maximum width and maximum depth were also measured at this time. Cannulated fish were either placed in the swim tunnel to recover or returned to the outdoor tank, where they recovered for up to 3 days before being placed in the swim tunnel. During subsequent transfer from the outdoor tank to the tunnel, fish were lightly and briefly anaesthetized (0.05 g l^{-1} buffered MS-222). There was no significant relationship between post-cannulation recovery time and measured swimming performance (data not presented).

Habituation to the swim tunnel and high water velocities

Fish recovered from anesthesia in the tunnel at a water speed of 10 cm s^{-1} for at least 45 min. After this time, fish performed a 20 min practice swim, as suggested in Jain et al. (1997), during which water speed was increased in 9–10 cm s^{-1} increments every 2 min to a speed of ~41 cm s^{-1}. Water speed was then returned to 10 cm s^{-1} for 2 min and again increased in the same fashion to a speed of either 55 or 59 cm s^{-1}, depending on the fish's swimming capability. The practice swim, which did not exhaust the fish, prevented the training effect often observed with naive fish on a second U_{crit} (Farlinger and Beamish, 1977; Jain et al., 1997). Fish then recovered overnight (14–16 h) at a water speed of 10 cm s^{-1}.

Swimming protocol

All experiments were started between 08:00 h and 10:00 h. Fish performed a ramp-U_{crit} test (Jain et al., 1997). The first U_{crit} test was followed by a 40 min recovery period at a water speed of 10 cm s^{-1} and then a second ramp-U_{crit} test followed by another recovery period. Each ramp-U_{crit} test involved increasing water speed to ~50% of U_{crit} over a 5 min period, after which water speed was increased in 10 cm s^{-1} increments (~15% of U_{crit}) every 20 min until exhaustion. Exhaustion was taken as the point at which the fish failed to swim away from the electrified rear grid after 20 s of contact. The ramp-U_{crit} protocol produces similar values for U_{crit} to the more standard U_{crit} testing protocol in which the longer time intervals are used from the onset of the test (Jain et al., 1997).

U_{crit} values were calculated for the first (U_{crit1}) and second (U_{crit2}) swims, as described by Brett (1964):

$$U_{crit} = u_i + (t_i/t_{ii} \times u_{ii}) , \quad (1)$$

where u_i is the highest speed at which the fish swam for the full time period (cm s^{-1}); u_{ii} is the incremental speed increase (cm s^{-1}); t_i is the time the fish swam at the final speed (min), and t_{ii} is the prescribed period of swimming per speed (20 min). As the cross-sectional area of each fish was <20% but sometimes >10% of that of the swimming chamber, the calibrated water speed was corrected for the solid blocking effect according to the calculations described by Bell and Terhune (1970):

$$\text{corrected } U_{crit} = U_{crit} \times \{1 + [0.4FL / 0.5(w+d)] \times (0.25\pi dw/A_t)^{1.5}\} , \quad (2)$$

where FL is fork length (cm), w is maximum fish width (cm), d is maximum fish depth (cm) and A_t is tunnel cross-sectional area. Water temperature did not fluctuate by more than 0.5°C from ambient temperature during the period that the fish spent in the tunnel.

Blood sampling

Blood samples (0.9 ml) were taken through the dorsal aorta cannula to measure plasma ion and metabolite levels. Normally, samples were taken immediately prior to the swimming protocol (routine samples), at exhaustion for both swim tests (U_{crit} exhaustion samples), and after a 40 min recovery for both tests (recovery samples; the recovery sample for the first U_{crit} swim also served as the sample taken immediately before the second U_{crit} swim). In 14 of the 16 fish, a blood sample was taken during aerobic swimming, i.e. after 15 min at 45 cm s^{-1} (approx. 69% U_{crit}). (These data are not reported as they simply provided intermediate values between the routine and U_{crit} values.) An equal volume of physiological saline solution was used to replace all blood samples (Gallaugher et al., 1992). Routine hematocrit was never less than 23% and remained elevated throughout the swim tests (see Fig. 2D).

Analytical techniques

Hematocrit was measured in microcapillary tubes after centrifugation at 2000 ***g*** for 3 min. The remainder of the blood was centrifuged at 10 000 ***g*** for 5 min to obtain plasma, which was stored at –80°C. Within 1 week of testing, plasma lactate and glucose concentrations were measured on 25 μl samples using a YSI 2300 lactate/glucose analyzer (Yellow Springs, OH, USA) that calibrated automatically every five samples. Plasma potassium and sodium concentrations were measured using a model 510 Turner flame photometer (Palo Alto, CA, USA). Plasma (5 μl) was diluted 1:200 with a prepared 15 mEq l^{-1} lithium diluent for analysis. The machine was calibrated prior to use and checked against a standard approximately every six samples. The measurement was repeated if there was disagreement between duplicates beyond 2% of absolute value. Osmolality was measured on duplicate 10 μl samples using a calibrated Wescor Vapour Pressure Osmometer, Model 5500 (Wescor, Logan, UT, USA). The measurement was repeated if there was disagreement between duplicates beyond 3% of absolute value. The thermocouple heads were cleaned periodically in order to maintain consistency. Plasma cortisol concentration was measured using a commercial radioammunoassay kit (ICN Biomedicals, Inc., Costa Mesa, CA, USA), with a detection limit of 1.5 ng ml^{-1}. Plasma ammonia concentration (T_{amm}) was measured spectrophotometrically on 0.1 ml plasma samples (Sigma Diagnostics kit no. 171, St Louis, MI, USA) with a calibration every seven samples.

Data analysis

All plasma metabolites and ions were measured in duplicate and averaged for individual data. Fish were subdivided into two temperature acclimation groups based on their swimming performance (see Results) and values (mean ± S.E.M.) are presented for cold- and warm-acclimated fish. One warm-acclimated female fish that was overtly gravid was not included in the statistical analysis to eliminate any confounding effect, because reproductive maturity is known to negatively affect U_{crit} performance in salmon (Williams et al., 1986). Statistical comparisons within temperature groups were made with a one-way repeated measures analysis of variance (ANOVA) followed by a *post hoc* Tukey test. With this test, the values associated with each fish were compared to other levels at other sampling times for the same fish to determine whether either swimming speed or metabolite levels changed throughout testing. Comparisons of swimming performance and metabolite levels between temperature groups were made using *t*-tests. U_{crit1} was compared to U_{crit2} using a Bland–Altman plot. Bland and Altman (1986, 1995) introduced this method of graphical analysis to assess the equivalency of two testing approaches (here U_{crit1} and U_{crit2}), while removing the bias that comes from assuming that one method represents the true value (independent variable). The Bland–Altman plot uses the mean of both methods as the independent variable and the difference between the two testing methods as the dependent variable. If the linear regression of the points is non-significant, then the two testing procedures (i.e. U_{crit1} and U_{crit2} here) can be considered to be

equivalent testing procedures. Sub-groups can be identified within a data set in a Bland–Altman plot by demonstrating different significant linear regressions from each other. In the present study, different regressions would identify sub-groups with different relationships between U_{crit1} and U_{crit2}. Relationships between U_{crit} values and plasma variables were fitted with the best-fitting regressions using the options provided in Sigma-Plot (SPSS Inc.; Chicago, IL, USA). $P<0.05$ was used to establish statistical significance.

Results

Swimming performance

As water speed increased, fish progressed from a steady swimming mode to one that included periods of burst-and-glide swimming. In conjunction with higher speeds, fish ram-ventilated their gills, except during burst-and-glide swimming when active ventilation was observed. Visually, swimming behavior did not appear to be different for the first and second U_{crit} tests.

A Bland–Altman plot revealed that U_{crit1} and U_{crit2} were equivalent testing procedures ($P=0.98$), but visual inspection of the plot revealed that overall the fish could be divided into two sub-groups each with a different and significant linear relationship (Fig. 1A). Each of the two sub-groups corresponded to different acclimation temperatures and hereafter are termed warm- and cold-acclimated fish (14.9±1.0°C and 8.4±0.9°C, respectively; see Table 1).

U_{crit1} performance was temperature dependent (Fig. 1B; $r^2=0.74$, $P<0.05$). U_{crit1} (78.9±1.0 cm s^{-1}) for warm-acclimated fish was significantly greater ($P<0.05$) than that for cold-acclimated fish (59.1±2.5 cm s^{-1}; Table 1). However, U_{crit2} did not show any temperature dependency. Unexpectedly, U_{crit2} performance (65.8±2.70 cm s^{-1}) for warm-acclimated fish was significantly lower than U_{crit1}, whereas U_{crit2} for cold-acclimated fish (58.0±4.2 cm s^{-1}) was

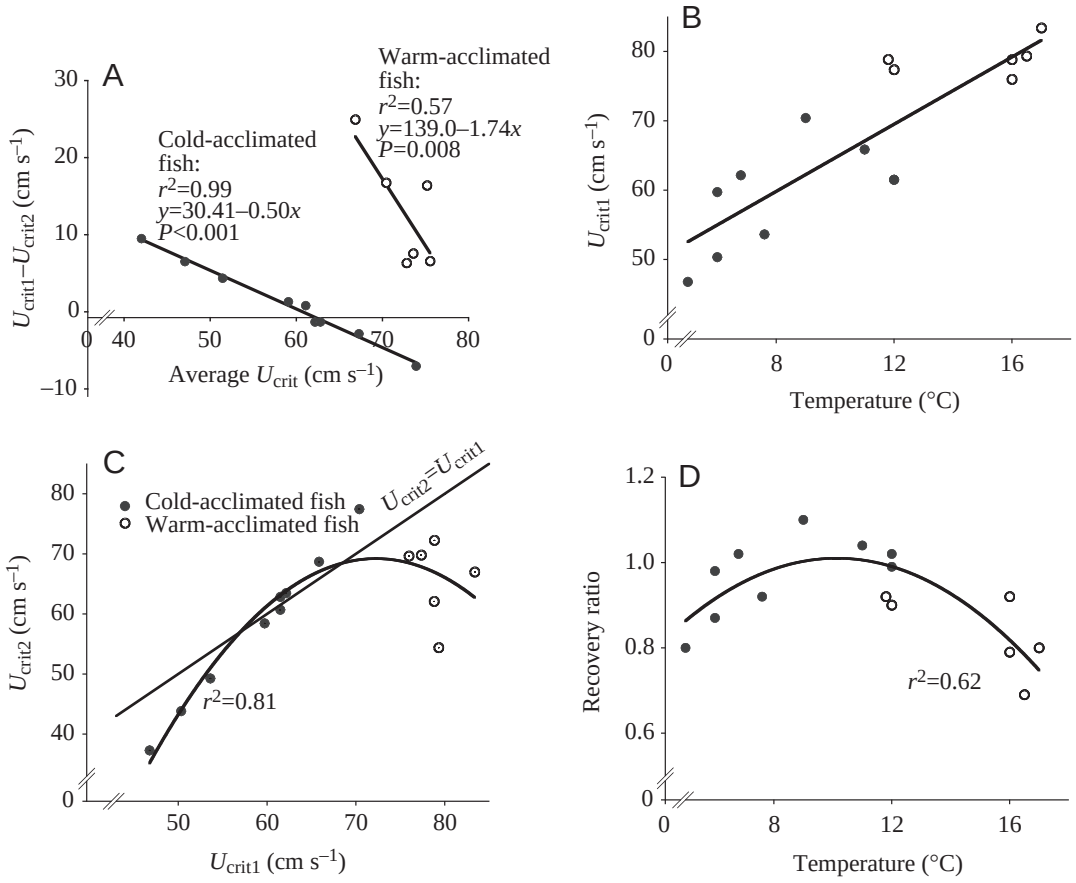

Fig. 1. (A) Bland–Altman plot comparing consecutive U_{crit} tests (U_{crit1} and U_{crit2}) performed by rainbow trout, separated by a 40 min recovery period. Regression lines indicate the existence of two sub-groups, cold-acclimated (filled symbols) and warm-acclimated (open symbols) fish, based on the visible groupings in this graph. (B) U_{crit1} *versus* ambient water temperature for rainbow trout. Fish are divided into two sub-groups, cold-acclimated (filled symbols) and warm-acclimated (open symbols) fish. Regression: $y=40.44+2.42x$, $r^2=0.74$; $P<0.001$. (C) U_{crit2} *versus* U_{crit1} for individual rainbow trout performing two U_{crit} tests separated by a 40 min recovery period. Fish are divided into cold-acclimated and warm-acclimated groups. The thin line is the line of identity where $x=y$, i.e. the predicted line if $U_{crit1}=U_{crit2}$ independent of temperature, and this was not the case. Regression (thick line): $y=-204.3+7.57x-0.05x^2$, $r^2=0.81$, $P<0.001$. (D) Recovery ratios for individual rainbow trout as a function of acclimation temperature (filled symbols, cold-acclimated group; open symbols, warm-acclimated group). The regression line for these data illustrates that warm-acclimated fish could not attain the same U_{crit} after a 40 min recovery.

Table 1. *Critical swimming speed of the cold- and warm-acclimated groups of rainbow trout for the first and second swim tests*

	Cold-acclimated fish (N=9)	Warm-acclimated fish (N=6)
U_{crit1} (cm s^{-1})	59.1±2.5	78.9±1.0[a]
U_{crit2} (cm s^{-1})	58.9±4.2	65.9±2.7[b]
U_{crit1} (*FL* s^{-1})	1.51±0.10	1.84±0.04[a]
U_{crit1} (*FL* s^{-1})	1.48±0.14	1.54±0.08[b]

Cold-acclimation temperature = 8.4±0.9°C; warm-acclimation temperature = 14.9±1.0°C.

U_{crit1}, first swim test; U_{crit2}, second swim test; *FL*, fork length.

Values are means ± S.E.M.

[a]Statistically significant difference (P<0.05) compared with cold sub-group; [b]statistically significant difference (P<0.05) between comparable U_{crit1} and U_{crit2} values.

the same as their U_{crit1} values (Table 1). As a result, the overall relationship between U_{crit1} and U_{crit2} was best described by a polynomial equation (y=–204.3+7.57x–0.05x^2; P<0.001; Fig. 1C), with cold-acclimated fish lying close to the line of identity and warm-acclimated fish lying below the line of identity. Thus, while warm acclimation conferred a faster U_{crit1}, a similar swimming speed could not be attained after a 40 min recovery period, as shown by recovery ratios that are less than unity for warm-acclimated fish (Fig. 1D).

Plasma status before, during and after U_{crit} *tests*

There were no significant differences between the cold- and warm-acclimated groups of fish in terms of routine values for plasma levels of lactate, potassium, T_{amm}, sodium, glucose, cortisol and osmolality and hematocrit. When cold-acclimated fish were exhausted at U_{crit1}, plasma levels of lactate, potassium and T_{amm}, as well as hematocrit, all increased significantly (Fig. 2A–D). Plasma cortisol (Fig. 2E) and sodium (Fig. 2F) levels were unchanged at exhaustion for U_{crit1}. After a 40 min recovery from U_{crit1}, plasma lactate increased significantly beyond the level observed at exhaustion, plasma T_{amm} decreased to the routine level, and plasma potassium and hematocrit remained elevated at the same level. As a result, plasma lactate and potassium levels, and hematocrit were all significantly elevated at the outset of the U_{crit2} test.

For cold-acclimated fish exhausted at U_{crit2}, plasma levels of lactate, potassium, sodium and T_{amm}, and hematocrit, were again significantly elevated compared with the routine values, but no more so than for U_{crit1}. In fact, compared with the recovery values for U_{crit1}, plasma lactate levels had decreased significantly (Fig. 2A) at exhaustion for U_{crit2}, while T_{amm} had increased significantly (Fig. 2C). Similar to U_{crit1}, plasma lactate increased during the 40 min recovery from U_{crit2} to a level significantly higher than that observed at exhaustion, plasma T_{amm} decreased to the routine level, and plasma potassium and hematocrit remained elevated at the same level. As a result, none of the recovery values for U_{crit2} in cold-acclimated fish were significantly different to those for U_{crit1}. Plasma levels of cortisol, glucose and osmolality remained unchanged throughout both swimming protocols (data not shown). Therefore, the second swim for cold-acclimated fish had no additive effects on any of the measured plasma variables.

When warm-acclimated fish were exhausted at U_{crit1}, plasma T_{amm} and hematocrit increased by the same amount as for cold-acclimated fish (Fig. 2C,D). In contrast, the faster U_{crit1} of the warm-acclimated fish was associated with significantly larger increases in plasma levels of lactate and potassium (Fig. 2A,B) compared with cold-acclimated fish. Furthermore, warm-acclimated fish significantly increased plasma sodium and cortisol levels at exhaustion for U_{crit1} (Fig. 2E,F), unlike cold-acclimated fish. After a 40 min recovery from U_{crit1}, the levels of plasma lactate, potassium, T_{amm}, sodium and cortisol, as well as hematocrit all remained significantly elevated in warm-acclimated fish, whereas only plasma levels of lactate, potassium and hematocrit remained elevated in cold-acclimated fish (Fig. 2). In addition, plasma lactate, potassium, sodium and cortisol remained elevated in warm-acclimated fish at levels that were significantly greater than those observed in cold-acclimated fish during recovery. In fact, the plasma lactate level was about threefold higher and plasma potassium almost twofold higher. These results suggest that the higher U_{crit1} of warm-acclimated fish may have been partly due to a greater anaerobic swimming effort compared with cold-acclimated fish, and (or) lactate and potassium were released from muscle to plasma to a greater extent.

Compared with cold-acclimated fish, warm-acclimated fish clearly began the second U_{crit} test with a greater plasma ionic and metabolic disruption and, as a result in these fish, U_{crit2} was significantly lower than U_{crit1}. In addition, while U_{crit2} for warm-acclimated and cold-acclimated fish was the same, warm-acclimated fish displayed a significant, further increase in plasma potassium levels (Fig. 2B) at exhaustion and a significant, further increase in plasma lactate levels (Fig. 2A) during the recovery from U_{crit2}. However, plasma T_{amm} did not recover to a routine level, as it did in the cold-acclimated fish (Fig. 2C). Therefore, the second U_{crit} swim of warm-acclimated fish produced significant additive effects on some of the plasma variables, unlike in cold-acclimated fish where there were none.

Correlational analysis

The initial swimming performance of individual fish was related to the appearance of lactate in the plasma. Plasma lactate concentrations measured at U_{crit1} and after a 40 min recovery were both linearly related to U_{crit1} (Fig. 3; r^2=0.73, P<0.05 and r^2=0.79, P<0.05, respectively). As might be expected from Fig. 3, plasma lactate concentrations were highly correlated with each other (2nd exhaustion with 1st exhaustion: r^2=0.95, P<0.05; 1st exhaustion with 1st recovery: r^2=0.92, P<0.05; 2nd exhaustion with 1st recovery: r^2=0.94, P<0.05; 2nd recovery with 2nd exhaustion: r^2=0.94, P<0.05).

The difference in swimming performance between U_{crit1} and U_{crit2} was significantly related to the plasma lactate concentration prior to the second U_{crit} test (Fig. 4). This relationship was described by either a polynomial (r^2=0.74), or a 2-parameter power (r^2=0.65) regression. Both types of analysis suggest that the reduction in U_{crit2} relative to U_{crit1} occurred when fish reached a plasma lactate of 12.2 mmol l^{-1} (95% confidence intervals of 7.9 and 16.5 mmol l^{-1}) 40 min after being exhausted by an initial U_{crit} swim test. Only warm-acclimated fish reached this threshold plasma lactate level.

Swimming effort in the initial swim was also related to the appearance of potassium in the blood. Plasma potassium concentration measured at U_{crit1} was linearly related to U_{crit1} (r^2=0.60, P<0.05). However, there was no significant correlation between plasma potassium levels and performance on the second swim. Plasma T_{amm} at exhaustion was not significantly related to U_{crit1}, but T_{amm} values for the 1st

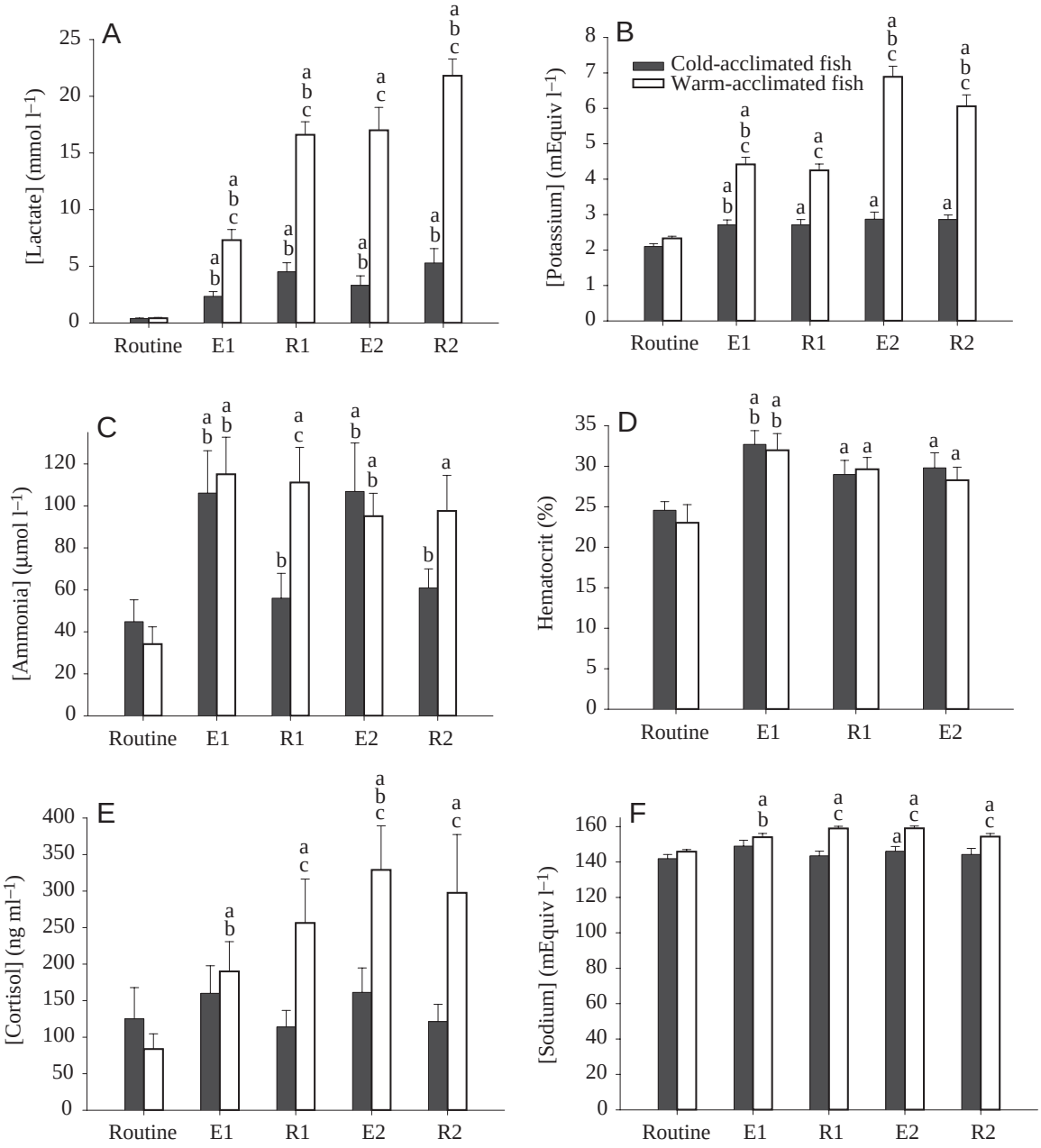

Fig. 2. Blood parameters in cold-acclimated (N=8–9) and warm-acclimated (N=5–6) fish before testing (Routine), at failure in the first U_{crit} test (E1), after a 40 min recovery (R1; this was also immediately before the start of the second U_{crit} test), at failure in a second U_{crit} test (E2), and after another 40 min recovery period (R2). [a]Level different from the routine value; [b]level different from the previous sampling time; [c]value for warm-acclimated fish different from the corresponding value for cold-acclimated fish. (A) Plasma lactate concentration. (B) Plasma potassium concentration. (C) Plasma ammonia concentration. (D) Hematocrit. (E) Plasma cortisol concentration. (F) Plasma sodium concentration.

recovery were related to U_{crit1} (Fig. 5; r^2=0.34, P<0.05). There were no other significant correlations for plasma T_{amm}.

The influence of acclimation temperature on the plasma ionic and metabolic responses to exercise is illustrated by the significant linear correlations that existed between plasma lactate, cortisol and potassium levels and temperature (Table 2). There were no significant correlations with temperature and the other parameters measured (T_{amm}, [sodium] and hematocrit).

One overtly gravid, warm-acclimated female fish was treated as an outlier, based on its slow swimming performance, and was not used for any correlation analysis. However, it is important to note that all the plasma changes observed in this fish were consistent with the slower swimming performance of the cold-acclimated fish.

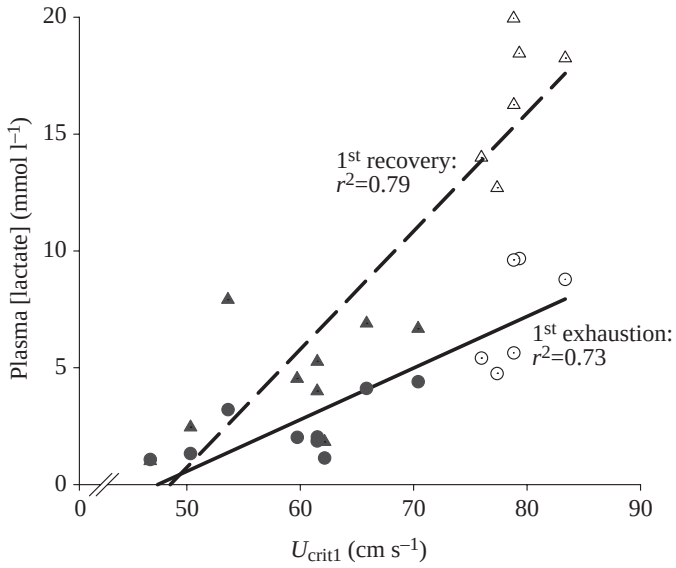

Fig. 3. Plasma lactate concentration at 1st exhaustion (circles) and 1st recovery (triangles) sampling times *versus* U_{crit1} for rainbow trout. Fish are divided into two sub-groups, cold-acclimated (filled symbols) and warm-acclimated (open symbols) fish. Regression for 1st exhaustion plasma [lactate] (solid line): y=–10.48+0.22x, r^2=0.73; P<0.001; for 1st recovery plasma [lactate] (broken line): y=–24.52+0.51x, r^2=0.79; P<0.001.

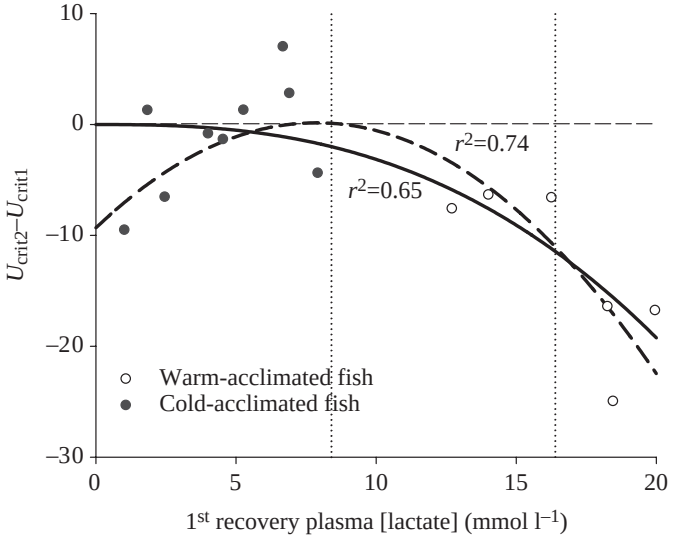

Fig. 4. U_{crit2}–U_{crit1} *versus* the plasma lactate concentration prior to the second U_{crit} test (recovery 1) for individual rainbow trout. Fish were divided into two sub-groups: cold-acclimated (filled symbols) fish and warm-acclimated (open symbols) fish. The data could be described by either a polynomial (broken line; r^2=0.74) or a 2-parameter power (solid line; r^2=0.65) relationship.

Discussion

This study tested the hypothesis that warm-acclimated rainbow trout would perform better in repeated U_{crit} swimming tests than cold-acclimated fish. The present findings, however, do not support this hypothesis because U_{crit2} was significantly lower than U_{crit1} in warm-acclimated fish than in cold-acclimated fish. At U_{crit1}, the warm-acclimated fish showed a greater metabolic disturbance in the plasma compared with cold-acclimated fish and also showed additive effects for the second U_{crit}, unlike the cold-acclimated fish. Therefore, although warm-acclimated fish swam better than cold-acclimated fish for U_{crit1}, as expected, the consequence of this faster U_{crit1} was a reduced performance on the second U_{crit} test. If anything, it appeared that warm-acclimated fish, by apparently swimming harder and possibly more anaerobically, were unable to recover sufficiently well during the fixed recovery period to repeat this initial level of performance. For cold-acclimated fish, however, the 40 min recovery period was sufficient for adequate recovery and allowed swimming performance to be repeated. Therefore, we are left with the

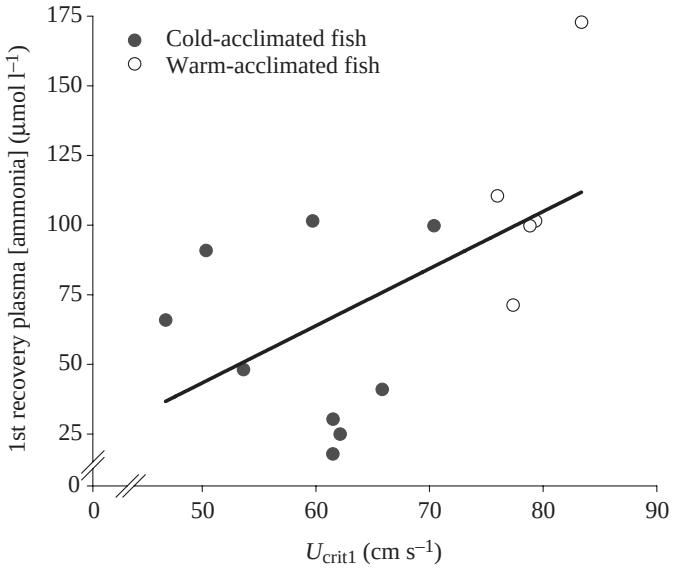

Fig. 5. Plasma ammonia concentration at first recovery *versus* U_{crit1} for rainbow trout. Fish are divided into two sub-groups, cold-acclimated (filled symbols) and warm-acclimated (open symbols) fish. Regression: y=–56.51+2.02x, r^2=0.30, P<0.05.

Table 2. *Significant linear regressions between ambient water temperature and individual plasma variables during repetitive swim tests in rainbow trout*

Plasma variable	*P*-value	r^2
[Lactate]		
1st exhaustion	<0.001	0.72
1st recovery	<0.001	0.74
2nd exhaustion	<0.001	0.68
2nd recovery	<0.001	0.69
[Potassium]		
1st exhaustion	<0.001	0.65
1st recovery	<0.001	0.69
2nd exhaustion	<0.001	0.74
2nd recovery	<0.001	0.78
[Cortisol]		
1st exhaustion	<0.01	0.41
1st recovery	<0.05	0.27
2nd exhaustion	<0.05	0.33

conclusion that overall recovery, as it pertains to repeat swimming capabilities and time allowed for recovery, was superior for the cold-acclimated compared with the warm-acclimated group of rainbow trout.

Our original hypothesis, which we now reject, was based on the established temperature dependence of post-exercise metabolic and ionic recovery when salmonids are chased to exhaustion to produce similar levels of intracellular acidosis, lactate accumulation and glycogen depletion in white muscle regardless of temperature (Kieffer et al., 1994; Wilkie et al., 1997). However, when Atlantic salmon were angled to exhaustion at a warmer temperature, essentially the opposite effect of temperature on post-exercise muscle recovery was obtained; they displayed a greater depletion of muscle glycogen, a greater intracellular acidosis and a slower recovery of muscle metabolites at the warmer temperature compared with colder temperatures (Booth et al., 1995; Wilkie et al., 1996). The present findings for U_{crit} swim tests are more in line with data obtained when fish are angled rather than chased to exhaustion because the metabolic disturbances were higher and performance recovery slower at warmer temperatures. We suggest that the disparity among studies could simply reflect differences in the degree of exhaustion and the methods used to exhaust the fish, with fish becoming more exhausted because they perceive the chasing protocol as more of a threat or provocation than either angling or U_{crit} testing. Given this possibility, cold-acclimated fish could opt to stop swimming sooner than warm-acclimated fish to preserve glycogen reserves.

A U_{crit} value, like time-to-exhaustion at a prescribed water speed (e.g. Facey and Grossman, 1990; Mitton and McDonald, 1993), allows quantification of the swimming effort, something that is not easily done when fish are chased or angled to exhaustion. U_{crit} tests also encompass a spectrum of swimming speeds, with the aerobic demands of swimming up to maximum oxygen uptake being met by cardiorespiratory adjustments, while white muscle recruitment and anaerobic metabolism increasingly supports the higher muscular power output near U_{crit} (Burgetz et al., 1998), culminating in exhaustion (Brett, 1964; Beamish, 1978). The simplest explanation for the higher U_{crit1} values obtained for warm-acclimated compared with cold-acclimated fish is a greater involvement of anaerobic swimming, given the significantly larger alterations in plasma metabolites observed for warm-acclimated rainbow trout. Certainly, the warm-acclimated fish were more stressed than the cold-acclimated fish, as judged by the greater elevation in plasma cortisol levels. However, since muscle metabolites were not measured here, we cannot exclude other possibilities. The higher levels of plasma potassium, lactate and T_{amm}, as well as the additive effects of the second swim, could simply reflect a greater release of lactate and potassium into the plasma because the release of lactate and hydrogen ions from white muscle to the blood is known to be temperature dependent (see Kieffer, 2000). Nevertheless, it is unlikely that different muscle glycogen levels were a factor since these are unaffected by acclimation temperature (Kieffer, 2000).

Rome et al. (1985) showed that acutely exposing warm-acclimated carp *Cyprinus carpio* to cold water resulted in white muscle fibres being recruited at a lower swimming speed, and this ‘compression of recruitment order’ led to earlier fatigue and a reduced sustained swimming speed. However, when the carp were cold-acclimated, they recruited white muscle at a higher swimming speed than warm-acclimated fish, presumably because cold temperature acclimation had improved the mechanical performance of the red muscle. The present findings are consistent with this earlier work with carp in that the cold-acclimated rainbow trout appeared to rely less on anaerobic white muscle than warm-acclimated fish, but the two studies differ in that cold-acclimated rainbow trout had a lower U_{crit} than warm-acclimated rainbow trout whereas cold-acclimated carp swam to the same maximum speed as warm-acclimated fish (Rome et al., 1985). Rome et al. (1985) suggested three possible physiological differences in cold-acclimated fish compared with warm-acclimated fish: (1) a higher mechanical power output from aerobic muscle, (2) limitations on the neural control of locomotory muscle and (3) limitations of the respiratory and circulatory systems in supplying oxygen. The present findings suggest a fourth possibility: fish may opt to swim to different states of exhaustion depending on either the temperature or a resulting physiological condition. One benefit of limiting the level of exhaustion under cold conditions appears to be a more reasonable recovery rate, which allows for repeated performance. At warm temperatures, fish benefit from a higher initial level of performance but, by exhausting themselves to a relatively greater degree, have the disadvantage of a more prolonged recovery period. An additional disadvantage, but for unknown reasons, is that exhaustive exercise at warm, but not at cold temperatures, can result in appreciable levels of post-exercise mortality (see Kieffer, 2000).

The present conclusions are also in line with the results of McKenzie et al. (1996) working with Nile tilapia *Oreochromis*

nilotica. They found that warm-acclimated fish had a greater cost of recovery (a higher and more prolonged post-exercise oxygen consumption) after being chased to exhaustion than cold-acclimated fish. Interestingly, white muscle lactate accumulation was similar for 16°C-acclimated and 23°C-acclimated tilapia, suggesting that muscle lactate may not always be a reliable measure of post-exercise recovery. However, 23°C-acclimated tilapia excreted over twice the amount of ammonia post-exercise than 16°C-acclimated fish. Kieffer et al. (1998) similarly found that ammonia excretion at 75% U_{crit} was almost threefold higher for 15°C-acclimated than 5°C-acclimated rainbow trout, while protein utilization at 75% U_{crit} was 30% at 15°C *versus* 15% at 5°C. Likewise, in the present study, we observed a significantly higher plasma T_{amm} in warm-acclimated rainbow trout. As discussed by McKenzie et al. (1996), the elevated ammonia production could be a result of either increased protein metabolism to fuel locomotion or increased protein degradation from tissue damage. Since elevated T_{amm} is thought to have inhibitory actions on neural and muscle activity in fish (Beamount et al., 1995a), the larger elevation in plasma T_{amm} in warm-acclimated fish is perhaps critical to survival post-exhaustion. On the other hand, tissue damage might negatively affect U_{crit2}.

U_{crit} values were comparable to those reported earlier by Jain et al. (1997) for rainbow trout of the same size in the same swim tunnel [1.64–1.66 body lengths (*BL*) s^{-1}] and higher than those reported for 822–1118 g rainbow trout (0.94 *BL* s^{-1} and 0.53 *BL* s^{-1} at 11°C and 18°C, respectively; Taylor et al., 1996). Comparisons also can be made with studies on smaller rainbow trout, which are expected to attain slightly higher U_{crit} values (Brett, 1964) than the 879 g fish used here. U_{crit} values of 1.8 to 2.0 *BL* s^{-1} are reported for 530–730 g rainbow trout at 18–19°C (Gallaugher et al., 1992) and 2.13 *BL* s^{-1} for 431–483 g rainbow trout at 7–11°C (Burgetz et al., 1998). For 320–520 g brown trout, U_{crit} values were 2.2 *BL* s^{-1} at 15°C and 1.85 *BL* s^{-1} at 5°C (Butler and Day, 1993; Butler et al., 1992).

As anticipated, a 40 min recovery period allowed full recovery of swimming performance for cold-acclimated fish. Originally it was suggested that salmonids be given 4 h between U_{crit} tests (Brett, 1964) to ensure a return to routine O_2 consumption but not necessarily to routine glycogen levels. Subsequently, recovery times of 2 h (Brauner et al., 1994), 1 h (Randall et al., 1987), 45 min (Farrell et al., 1998, 2003) and 40 min (Jain et al., 1998) have all been shown to be sufficient for salmonids to repeat U_{crit} tests without any significant decline in performance. Here fish were provided with a low speed water current during recovery and this may have aided their recovery, since recent studies with rainbow trout (Milligan et al., 2000) and coho salmon *Oncorhynchus kisutch* (Farrell et al., 2001) have shown that low to moderate swimming post-exhaustion greatly aids metabolic recovery through a warm-down effect. In contrast, recovery time without a warm-down is >2 h for optimal performance on a time-to-exhaustion test (Mitton and McDonald, 1994). Wang et al. (1994) reported that muscle phosphocreatine and ATP levels were restored within 30 min of rainbow trout being chased to exhaustion, while the post-exercise decline of oxygen consumption lasted 3–3.5 h (Scarabello et al., 1991). However, routine oxygen consumption does not have to be restored before adult sockeye salmon can repeat a second U_{crit} test (Farrell et al., 1998, 2003).

There was generally good agreement between the routine plasma variables reported here and those reported in previous studies (Butler and Day, 1993; Eros and Milligan, 1996; Pagnotta et al., 1994; Thorarensen et al., 1994; Wang et al., 1994). However, the plasma lactate concentrations at U_{crit} in this study, especially those for the warm-acclimated fish (7.3 mmol l^{-1}), were at the high end of literature values for U_{crit} swimming (1.5–5.5 mmol l^{-1}) (Butler and Day, 1993; Gallaugher et al., 1992; Thorarensen et al., 1993; Holk and Lykkeboe, 1998; Farrell et al., 1998). Milligan (1996) cites a range for plasma lactate levels of 2–13 mmol l^{-1} immediately after chasing, increasing to peak values of 12–20 mmol l^{-1} at 2 h post-exercise. The values reported here for cold-acclimated fish of 4.3 mmol l^{-1} at U_{crit} and 8.9 mmol l^{-1} 40 min later are at the low end of this range, whereas those for the warm-acclimated fish (7.3 mmol l^{-1} at U_{crit} and 16.6 mmol l^{-1} 40 min later) are at the upper end of the range and approached the level reached (17.8 mmol l^{-1}) approximately 90 min after a hypoxic U_{crit} test (Farrell et al., 1998).

The second objective of the present study was to determine whether any of the measured metabolites displayed threshold levels that, if surpassed in the first swim challenge, were indicative of a metabolic condition that negatively affected subsequent swimming performance. Plasma lactate level was the only candidate: the plasma lactate level before U_{crit2} was significantly correlated to the subsequent swimming performance (U_{crit2}). The threshold plasma lactate level of approximately 12.2 mmol l^{-1} (95% CI 7.9–16.4) agrees with that of 13 mmol l^{-1} reported by Stevens and Black (1966) for burst exercise with rainbow trout and 10 mmol l^{-1} for sockeye salmon (Farrell et al., 1998). In the earlier studies, fish refused to swim if the lactate threshold was surpassed. However, no fish refused to swim outright in the present study and instead U_{crit} performance was reduced by 8–31%. Thus, because anaerobic metabolism is increasingly required to support swimming speeds greater than 70% U_{crit} (Burgetz et al., 1998), elevated levels of lactate above the lactate threshold is probably indicative of a failure to fully recruit anaerobic metabolism in white muscle (e.g. through decreased muscle pH and glycogen stores). This idea needs further study, however, because plasma lactate dynamics are complex, reflecting rates of production in the muscle, rates of release from the muscle and rates of clearance from the blood. While the present study suggests that production may be greater at warmer temperatures, release rate is dependent on temperature (Keiffer et al., 1994) and clearance rate is inversely related to temperature (Kieffer and Tufts, 1996).

Beaumont et al. (1995a,b) reported that copper-exposed brown trout in water of low pH had poor U_{crit} values and

suggested that the elevated plasma T_{amm} inhibited white muscle activity either directly or through CNS inhibitory mechanisms, because elevated plasma T_{amm} levels were correlated with the reduced U_{crit} values. In the present study, we found no significant correlations between swimming performance and plasma T_{amm}. However, our data are not necessarily at odds with the suggestion of Beaumont et al., (1995a,b) because the plasma T_{amm} levels reported in the present work were half those measured in copper-exposed brown trout and, in the earlier studies, U_{crit} was not reduced appreciably until plasma T_{amm} reached levels >200 μmol l^{-1}. A plasma T_{amm} level >600 μmol l^{-1} resulted in fish refusing to swim. In the present study, T_{amm} reached only 100 μmol l^{-1} and was restored between U_{crit} tests for cold-acclimated fish, although not for warm-acclimated fish (Fig. 2).

Several studies report a temperature optimum for U_{crit}. For sockeye salmon, 15°C was the optimum temperature for U_{crit}, metabolic scope (Brett, 1964) and cardiac performance (Brett, 1971; Davis, 1968). The preferred temperature for sockeye salmon, however, appears to be slightly cooler (10–12°C; Birtwell et al., 1994; Spohn et al., 1996). Garside and Tait (1958) suggested a preferred temperature range for rainbow trout of 11–16°C, which coincides with the optimum temperature range suggested for cardiac performance (Farrell et al., 1996; Taylor et al., 1997; Farrell, 2002). The present experiments show that the shift in responses to repeated swimming for cold- and warm-acclimated fish occurred at around 12°C. Therefore, the fish's preferred temperature may reflect sub-maximal rates for certain activities because of negative consequences in terms of rates of recovery.

In summary, we provide evidence that warm-acclimated rainbow trout have a higher U_{crit} than cold-acclimated fish, but associated with this higher U_{crit} is a greater metabolic and ionic disturbance. A consequence of this elevated disturbance is that warm-acclimated fish do not recover well enough after a 40 min rest to perform a second test at the same level as the first one, whereas cold-acclimated fish do. Elevations in plasma lactate (but not plasma potassium, T_{amm} and cortisol) were significantly correlated with the poorer repeat swimming performance.

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