Metabolic recovery of most fish from a bout of exhaustive exercise, akin to what fish experience when subjected to ‘angling stress’ (e.g. Schwalme and Mackay, 1985; Booth et al., 1995), is quite a lengthy process. Most studies report that full metabolic recovery, as indicated by replenishment of muscle glycogen stores and clearance of muscle lactate load, requires anywhere from 8 to 24 h (Black, 1957, 1958; Black et al., 1959, 1960, 1962, 1966; Turner et al., 1983; Milligan and Wood, 1986; Dobson and Hochachka, 1987; van Dijk and Wood, 1988; Tang and Boutilier, 1991; Ferguson et al., 1993; Wang et al., 1994). This time frame is considerably slower than that seen in amphibians, reptiles and mammals (Gleeson, 1991). Some of the differences between fish and other vertebrates are due to differences in body temperature. Most fish studies are carried out at 10–20 °C compared with body temperatures of 30–37 °C for reptiles and mammals. However, temperature alone does not fully account for the slow metabolic recovery typically observed in fish (Kieffer et al., 1994).

There is mounting evidence that the elevation of plasma cortisol levels associated with exhaustive exercise and recovery period delays the restoration of metabolite and acid–base status to pre-exercise levels (Pagnotta et al., 1994; Eros and Milligan, 1996). The post-exercise elevation of cortisol concentration is prevented by pharmacological intervention, then fish recover fully from exhaustion within 2–3 h. This raises the question as to the potential benefit of the elevation in plasma cortisol concentration to fish if it prolongs their recovery from exhaustive exercise. Perhaps the cortisol elevation associated with exhaustive exercise and recovery is not relevant to fish in more natural conditions. Almost all the studies cited are laboratory-based and have used the experimental model of exercising fish to exhaustion and then placing them in individual chambers to recover. Under these circumstances, the fish are unable to swim freely.

One of us (G.B.H.) has observed that, when trout are caught in the wild and subsequently released, they seek refuge in current and continue swimming, rather than seeking refuge in still water. Swimming fish have lower plasma cortisol levels than fish in still water (Boesgaard et al., 1993; Postlethwaite and McDonald, 1995), implying that swimming presents less of a stress than still water. These observations, coupled with the fact that elevated cortisol levels prolong the recovery from exhaustive exercise, led to the hypothesis that sustained swimming at low velocity following a bout of exhaustive exercise may lead to lower plasma cortisol levels and hasten the recovery process. To test this hypothesis, we exercised fish to exhaustion and then allowed them to recover either in current or in still water, using a swimming velocity that could be fuelled exclusively by aerobic metabolism (0.9 BL s⁻¹, where BL is fork body length; Bone et al., 1978).

**Summary**

Sustained swimming at 0.9 BL s⁻¹, where BL is fork body length, following a bout of exhaustive exercise enhanced recovery of metabolite and acid–base status in rainbow trout compared with fish held in still water. The most striking effect of an active recovery was a total absence of the elevation cortisol concentration typically associated with exhaustive exercise. In fish swimming at 0.9 BL s⁻¹, plasma cortisol levels averaged 20–25 ng ml⁻¹ throughout the 6 h recovery period. In contrast, plasma cortisol increased to a peak level of 128.4±11.2 ng ml⁻¹ (mean ± s.e.m., N=6) in fish recovering in still water. Muscle glycogen was completely resynthesized and lactate cleared within 2 h of exercise in swimming fish compared with more than 6 h required in the fish held in still water. Similarly, blood lactate level and acid–base status were restored more quickly in the swimming fish. These observations suggest that the prolonged recovery usually associated with exhaustive exercise in rainbow trout is due to elevations in plasma cortisol concentration and that the stimulus for cortisol release is not exercise per se, but rather post-exercise inactivity.

Key words: exercise, cortisol, catecholamine, glycogen, lactate, muscle, swimming, fatigue, rainbow trout, Oncorhynchus mykiss.

**Introduction**

Metabolic recovery of most fish from a bout of exhaustive exercise, akin to what fish experience when subjected to ‘angling stress’ (e.g. Schwalme and Mackay, 1985; Booth et al., 1995), is quite a lengthy process. Most studies report that full metabolic recovery, as indicated by replenishment of muscle glycogen stores and clearance of muscle lactate load, requires anywhere from 8 to 24 h (Black, 1957, 1958; Black et al., 1959, 1960, 1962, 1966; Turner et al., 1983; Milligan and Wood, 1986; Dobson and Hochachka, 1987; van Dijk and Wood, 1988; Tang and Boutilier, 1991; Ferguson et al., 1993; Wang et al., 1994). This time frame is considerably slower than that seen in amphibians, reptiles and mammals (Gleeson, 1991). Some of the differences between fish and other vertebrates are due to differences in body temperature. Most fish studies are carried out at 10–20 °C compared with body temperatures of 30–37 °C for reptiles and mammals. However, temperature alone does not fully account for the slow metabolic recovery typically observed in fish (Kieffer et al., 1994).

There is mounting evidence that the elevation of plasma cortisol levels associated with the exhaustive exercise and recovery period delays the restoration of metabolite and acid–base status to pre-exercise levels (Pagnotta et al., 1994; Eros and Milligan, 1996). If the post-exercise elevation of cortisol concentration is prevented by pharmacological intervention, then fish recover fully from exhaustion within 2–3 h. This raises the question as to the potential benefit of the elevation in plasma cortisol concentration to fish if it prolongs their recovery from exhaustive exercise. Perhaps the cortisol elevation associated with exhaustive exercise and recovery is not relevant to fish in more natural conditions. Almost all the studies cited are laboratory-based and have used the experimental model of exercising fish to exhaustion and then placing them in individual chambers to recover. Under these circumstances, the fish are unable to swim freely.

One of us (G.B.H.) has observed that, when trout are caught in the wild and subsequently released, they seek refuge in current and continue swimming, rather than seeking refuge in still water. Swimming fish have lower plasma cortisol levels than fish in still water (Boesgaard et al., 1993; Postlethwaite and McDonald, 1995), implying that swimming presents less of a stress than still water. These observations, coupled with the fact that elevated cortisol levels prolong the recovery from exhaustive exercise, led to the hypothesis that sustained swimming at low velocity following a bout of exhaustive exercise may lead to lower plasma cortisol levels and hasten the recovery process. To test this hypothesis, we exercised fish to exhaustion and then allowed them to recover either in current or in still water, using a swimming velocity that could be fuelled exclusively by aerobic metabolism (0.9 BL s⁻¹, where BL is fork body length; Bone et al., 1978).
Materials and methods

Experimental animals

Rainbow trout *Oncorhynchus mykiss* (Walbaum) (207±15 g; 20.1±0.4 cm) were obtained from Rainbow Springs Trout Hatchery and held indoors in 4001 plastic tanks continuously aerated and supplied with dechlorinated London (Ontario) tap water at 12±1 °C. Fish were fed a daily maintenance ration of commercial trout pellets. Fish were held for at least 2 weeks prior to experimentation.

Experimental protocol

Fish were surgically fitted with a dorsal aortic cannula (Soivio et al., 1972) while under MS-222 anaesthesia (1:10000 dilution, adjusted to pH 7.0 with NaHCO₃). Fish were allowed to recover for at least 48 h in black acrylic fish boxes continuously supplied with aerated, dechlorinated tap water at the experimental temperature. The fish were not fed during this period. The catheters were checked daily and filled with heparinized (50 i.u. ml⁻¹ sodium heparin; Sigma) saline.

Individual fish were terminally sampled while at rest, immediately after exercise to exhaustion (time 0) or at 1, 2, 4 or 6 h of recovery in either still water or while swimming at 0.9 BL s⁻¹. To exhaust fish, they were transferred to a circular swim tank and manually chased around the tank for 5 min, after which those fish not sampled at time 0 were randomly assigned to one of two groups: swimming recovery or still-water recovery. For swimming recovery, fish were placed in an open swim flume (Vogel, 1981) at a current velocity of 0.9 BL s⁻¹ for the duration of the recovery period. For non-swimming recovery, fish were allowed to recover in a chamber with dimensions identical to the swim section of the swim tunnel. Both chambers were continuously supplied with aerated, dechlorinated tap water at the experimental temperature. The middle portion of each chamber was covered in black plastic, which allowed the fish a place of refuge and minimized the disturbance associated with blood sampling and fish capture.

At 1, 2, 4 or 6 h after exercise, 500 µl of blood was withdrawn from the catheter into a gas-tight Hamilton syringe and placed on ice until analyzed. The fish was then carefully netted, to avoid disturbing other fish, and transferred to a 201 bucket containing 2.0 g l⁻¹ buffered MS-222. Once ventilatory movements ceased (usually within 60 s), the fish was removed from the bucket and a sample of white epaxial muscle taken. Muscle was freeze-clamped between aluminium plates pre-cooled with liquid N₂ and stored at −80 °C prior to analysis.

Blood pH was measured on 40 µl of whole blood, and 50 µl of blood was used for measurement of total CO₂ concentration and 10 µl for determination of haemoglobin concentration. An additional 50 µl of whole blood was added to 200 µl of ice-cold 8 % HClO₃, mixed, then centrifuged at 10000 g for 5 min. The supernatant was withdrawn and stored at 4 °C until analyzed for lactate. The remaining blood was centrifuged at 10000 g for 5 min, and total CO₂ was measured on 50 µl of plasma. The remaining plasma was stored at −80 °C for analysis of cortisol and catecholamines.

Analytical techniques and calculations

Arterial blood pH (pHa) was measured on 40 µl samples injected into a Radiometer pH microelectrode maintained at 12 °C and linked to a Radiometer PHM 73 blood gas monitor. Total CO₂ was measured on 50 µl of whole blood or plasma using a Corning 965 CO₂ analyzer (Medfield, MA, USA). Haemoglobin was measured on 10 µl blood samples by the cyanmethaemoglobin method using Sigma reagents (Sigma Chemical, St Louis, MO, USA). Whole-blood lactate was measured enzymatically on 100 µl of the deproteinized HClO₃ extract using Sigma lactate reagents and procedures. Cortisol was measured on 25 µl duplicate samples of plasma using a commercially available radioimmunoassay kit (ICN Biomedicals, Costa Mesa, CA, USA). The lower limit of detection of this assay was 1.5 ng ml⁻¹, and the intra- and inter-assay coefficients of variation were 7 and 8 %, respectively. Plasma adrenaline and noradrenaline levels were measured on alumina-extracted samples using high-performance liquid chromatography (HPLC; Beckman System Gold) with electrochemical detection (ESA Coulochem II) according to the method of Woodward (1982). 3,4-Dihydroxybenzylamine hydrobromide (DHBA) was used as an internal standard in all samples.

For measurement of muscle lactate levels, muscle was ground to a fine powder in a liquid-N₂-cooled mortar. Approximately 100 mg of powdered tissue was added to 1.0 ml of ice-cold 8 % HClO₃ and homogenized on ice with three 10 s bursts of a Tissue Tearor (Biospec Products, Bartlesville, OK, USA). Homogenates were centrifuged for 5 min at 10000 g, and the supernatant was withdrawn and stored at 4 °C for up to 1 week until analyzed for lactate concentration, as described above for blood. For measurement of muscle glycogen, approximately 100 mg of frozen muscle was placed directly into 1.0 ml of 30 % KOH and digested in a boiling water bath. Glycogen was isolated as described by Hassid and Abraham (1957) and measured as free glucose following digestion with amyloglucosidase (Bergmeyer, 1965).

Plasma P<sub>CO₂</sub> was calculated from measured plasma total CO₂ using the Henderson–Hasselbach equation as described by Milligan and Wood (1986) and constants reported for rainbow trout plasma by Boutillier et al. (1985). The metabolic proton load to the whole blood, ΔH<sub>pm</sub>, was calculated as described by Wood et al. (1977). The non-bicarbonate buffer capacity of blood was estimated from whole-blood haemoglobin concentration using the relationship determined by Wood et al. (1982).

Results

When fish were placed in current (0.9 BL s⁻¹) after a bout of exhaustive exercise, they were able to swim and maintain their position in the water column. A sheet of black plastic was placed over the centre portion of the swim chamber to provide cover for the fish, and at no time during the 6 h recovery period did any individual fish drift downstream. Periodic observations indicated that these fish did not simply rest on the bottom, but
were actually swimming to maintain station. Fish placed in a similarly sized chamber but in still water (i.e. no detectable current) also maintained their position under the black plastic for the duration of the recovery period, although with minimal swimming movements. These fish used mainly pectoral fin movements to maintain position.

Swimming after exhaustive exercise had a significant impact on recovery of metabolic and acid–base status (Figs 1–4). The most striking observation is that plasma cortisol level did not increase at all in fish swimming post-exercise. In contrast, cortisol concentration increased approximately fivefold in fish in still water (Fig. 1A). There was also a lower blood lactate load in swimming fish (Fig. 1B). Blood lactate peaked at approximately 13 mmol l\(^{-1}\) at approximately 2 h post-exercise in fish in still water, whereas peak lactate levels were only approximately 5 mmol l\(^{-1}\) and seen at 1 h post-exercise in fish held in a water current. Blood lactate was cleared within 4 h in the fish held in a water current compared with more than 6 h in fish in still water. The response of plasma adrenaline and noradrenaline concentrations was the same in both groups of fish (Fig. 2).

Exhaustive exercise led to a typical blood acid–base disturbance, with pH dropping from approximately 7.85 to a minimum of 7.38 (Fig. 3A) as a result of a combination of elevated \(P_{\text{CO}_2}\) (Fig. 3B) and metabolic acid load (\(\Delta H^+_{\text{m}}\);
Fig. 3C). Recovery of blood pHa (Fig. 3A) to pre-exercise levels was complete within 2 h in fish swimming at 0.9 \( BL/s \), despite a persistent elevation of \( PCO_2 \) (Fig. 3B). The swimming fish developed a relative metabolic alkalosis (Fig. 3C), which compensated for the persistent respiratory acidosis and contributed to the increase in pHa at 6 h. In contrast, still-water fish restored \( PCO_2 \) more quickly (Fig. 3B) but, because the metabolic acid load persisted (Fig. 3C), blood pHa did not return to pre-exercise levels until 6 h (Fig. 3A).

Paralleling the rapid recovery of blood lactate and acid–base status was a rapid restoration of muscle glycogen (Fig. 4A) and clearance of muscle lactate (Fig. 4B) in fish swimming at 0.9 \( BL/s \) compared with still-water fish. Muscle glycogen and lactate levels had both returned to pre-exercise values within 2 h in swimming fish compared with more than 6 h required in the still-water fish.

**Discussion**

The approach developed over the years to describe the physiological consequences of exhaustive exercise in teleost fish...
generally involves exercising the fish, placing them in still water to recover (for a review, see Milligan, 1996) and monitoring various parameters. The consensus emerging from these studies is that recovery from exhaustion, as measured by the time required for restoration of muscle metabolic and acid–base status, is quite lengthy with some studies reporting recovery times in excess of 12 h (e.g. Black et al., 1962; Turner et al., 1983).

The data presented in the present paper clearly contradict this view: recovery from a bout of exhaustive exercise can be complete within 2 h if the fish are placed in a current of low velocity. The most striking effect of placing fish in a water current after exhaustion was the absence of an elevation in cortisol concentration. These data suggest that the stress usually attributed to this type of exhaustive exercise (chasing fish around a tank) is not due to the exercise per se, but rather to the exposure to still water post-exercise. This view is supported by the kinetics of the cortisol elevation seen in fish after a bout of exhaustive exercise. Typically, the elevation in plasma cortisol level is delayed, with increases usually not seen until 30–60 min after the cessation of swimming (Fig. 1A; Milligan and Wood, 1987; Pagnotta et al., 1994; Eros and Milligan, 1996). Elevation of plasma cortisol levels in response to stress is fairly rapid; for example, in coho salmon (Oncorhynchus kisutch), plasma cortisol was significantly elevated within 8 min of the onset of confinement stress (Sumpter et al., 1986). Rarely is cortisol level elevated in the first blood sample obtained immediately after exhaustive exercise (approximately 6–8 min from the start of the swim; Pagnotta et al., 1994). The response of plasma catecholamines was identical in both groups of fish, suggesting that catecholamine release is in direct response to exercise.

The more rapid recovery of blood acid–base status and muscle glycogen and lactate levels in the swimming fish is probably related to the absence of cortisol elevation. There is now mounting evidence that elevation of plasma cortisol concentration during recovery from exhaustive exercise actually prolongs the recovery period. Pagnotta et al. (1994) and Eros and Milligan (1996) have shown that, if the rise in plasma cortisol level is pharmacologically inhibited, then recovery of metabolic and acid–base status is hastened; recovery is complete within 2–4 h compared with more than 8 h in control fish. The emerging model is that low cortisol levels promote lactate-based in situ glycogen synthesis. As a consequence, blood lactate levels are lower because of a reduced transfer from muscle to blood as the lactate is rapidly channelled to glycogen synthesis. Alternatively, the lower blood lactate levels in swimming fish may reflect enhanced removal of lactate from the blood or a decrease in the transfer of lactate from muscle to blood. The rapid onset of metabolic alkalosis suggests that lactate uptake in the swimming fish was enhanced relative to the fish in still water, since lactate is thought to cross cell membranes via a lactate/H+ symporter (Gladden, 1996). In humans, active recovery from a bout of anaerobic exercise also results in lower blood lactate levels almost exclusively as a result of increased oxidation (Choi et al., 1994) to fuel the increased aerobic demands of the active recovery. Undoubtedly, swimming at 0.9 BL s⁻¹ imposes an increased aerobic energy demand on the fish because of the increased cardiac output and ventilation rate associated with swimming (Jones and Randall, 1978) and the reliance upon red muscle for propulsion at low swimming speeds (Wilson and Egginton, 1994). This, taken together with the demonstrated abilities to oxidize lactate (Bilinski and Jonas, 1972; Milligan and Farrell, 1991), makes cardiac, opercular and axial red muscles obvious candidates for increased lactate utilization.

In the fish swimming at 0.9BL s⁻¹, net muscle glycogen synthesis was evident within 1 h and was coincident with reduced muscle and blood lactate levels. This may explain, in part, the observations of Farrell et al. (1998) that, in critical swimming speed (Ucrit) tests after exhaustion, the majority of adult sockeye salmon tested were able to attain the same swimming performance when faced with repeated challenges. The challenges were interspersed with a 45 min recovery period during which the fish swam at 0.4BL s⁻¹. Sustained swimming in this case probably facilitated the recovery process. Interestingly, in humans, active aerobic recovery from exhaustive exercise delays glycogen resynthesis (Choi et al., 1994).

The results of the present study provide compelling evidence that the prolonged recovery period associated with exhaustive exercise is due to elevations in plasma cortisol concentration. Furthermore, the stimulus for cortisol release is not the exercise per se, but rather post-exercise inactivity. Thus, the traditional view of post-exercise recovery metabolism in fish may have to be modified to allow for the idea that post-exercise inactivity leads to artificially long recovery times (i.e. at least 8 h; e.g. Wang et al., 1994) through the actions of a cortisol-mediated stress response. It is also possible that the recovery profile of fish swimming during recovery from exhaustive exercise is more relevant to the natural environment. In the wild, prolonged recovery times would leave fish vulnerable to predation because their capacity for subsequent ‘burst’ activity would be limited. Any behaviour prolonging metabolic recovery would surely be selected against.

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References


