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SHORT COMMUNICATION

ASSESSMENT OF MAXIMUM SUSTAINABLE SWIMMING PERFORMANCE IN RAINBOW TROUT (*ONCORHYNCHUS MYKISS*)

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Levels of swimming activity in fishes have been divided into three categories on the basis of the time a given speed can be maintained before the onset of fatigue (Beamish, 1978): sustained (more than 200 min), prolonged (20 s to 200 min) and burst swimming (less than 20 s). The locomotory capacity of a given species reflects both its lifestyle and its body form, although definitions of performance may vary. It is generally accepted that only the aerobic ('red') muscle fibres should be active at truly sustainable swimming speeds, i.e. at speeds that can be maintained indefinitely without fatigue. However, the standard laboratory method of evaluating the maximum sustainable swimming speed (U_{crit}; Brett, 1964) almost certainly entails the recruitment of at least some of the rapidly fatigable fast glycolytic ('white') fibres at sub-critical speeds and undoubtedly complicates the evaluation of maximal cardiovascular performance. It would therefore be useful to have an objective and reproducible measure of truly sustainable performance that, by definition, relies solely on aerobic muscle activity. Electromyography (EMG) has been used to examine the pattern of white muscle recruitment following thermal acclimation in striped bass, *Morine saxatilis* (Sisson and Sidell, 1987). We wished to incorporate this method into a study of the acclimatory responses to chronic changes in environmental temperature of the cardiovascular and locomotory systems in rainbow trout (Wilson and Egginton, 1992). The present communication presents results on the cardiovascular performance and blood chemistry, at rest and during maximal aerobic exercise, of rainbow trout acclimated to 11 °C, as a validation of the methodology currently in use with fish acclimated to seasonal temperature extremes (Taylor *et al.* 1992). Different acclimation temperatures are known to produce compensatory changes in the relative proportions of red and white muscle mass (Sidell and Moerland, 1989). The aim of these continuing investigations is to compare the anatomical, cardiovascular and locomotory limitations to aerobic exercise over the full temperature range of a eurythermal fish species.

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Rainbow trout [*Oncorhynchus mykiss* (Walbaum); 707–950 g] were obtained from Leadmill trout farm, Hathersage, Derbyshire, and acclimated to the seasonally appropriate temperature of 11 °C for at least 4 weeks prior to experimentation. Under tricaine methane sulphonate anaesthesia (MS222; $0.1\,g\,l^{-1}$ initial dose, $0.06\,g\,l^{-1}$ maintenance dose), trout were fitted with dorsal aortic and caudal artery catheters. Bipolar hook electrodes were then implanted in lateral red and deep white muscle tissues on contralateral sides of the body for EMG recording (Sisson and Sidell, 1987). Once implanted, catheters and trailing electrode wires were sutured to the skin at the posterior base of the dorsal fin. Trout were then partially revived and transferred to a Blazka-type water channel (swim chamber 65 cm long and 15 cm diameter, total volume 200 l) and allowed to recover for 48–72 h at a water velocity of $5\,cm\,s^{-1}$ and with the temperature controlled at 11±0.5 °C. During experiments, catheters and electrode wires were fed through a small hole in the upper surface of the swim channel to allow sampling, injection and recording.

Following recovery, experiments were carried out over two separate days. On the first day, dorsal aortic blood samples were taken at rest (water speed at $5\,cm\,s^{-1}$) and following 45 min at the maximum aerobic swimming speed to quantify the aerobic nature of this exercise protocol in terms of blood chemistry. Total fish length (snout–caudal fin) was used to scale swimming speed in terms of body lengths per second ($BL\,s^{-1}$). The threshold velocity for white muscle (WM) recruitment (U_{WMcrit}) was established by stepwise increases in water speed, using $10\,cm\,s^{-1}$ increments and allowing a 30 min stabilisation period at each new speed. As the predicted speed of white muscle recruitment was approached, the re-adjustment of water speed proceeded more gradually (approximately $1\,cm\,s^{-1}$ increments every few minutes) to enable us to record the distinct point at which anaerobic white muscle EMG activity began (Sisson and Sidell, 1987). As soon as U_{WMcrit} was established, the water speed was reduced until all traces of intermittent WM activity disappeared (on average at 94 % of U_{WMcrit}), and the fish were left undisturbed to swim for 45 min at this aerobic maximum whilst red (RM) and white muscle activity, dorsal aortic blood pressure and heart rate were monitored. Following the exercise blood sample, water speed was returned to $5\,cm\,s^{-1}$ and the fish were left overnight to recover. Forty-five minutes is sufficient time to allow stabilisation of cardiovascular variables (Randall and Daxboeck, 1982) and blood gas and acid–base status (Thomas *et al.* 1987).

During sampling, 1 ml of blood was drawn *via* the dorsal aortic catheter into a chilled gas-tight syringe (Hamilton 1001LT) and immediately replaced by injecting 750 μl of saline. Arterial whole-blood pH, oxygen tension (P_{aO_2}), oxygen content (C_{aO_2}) and haematocrit (Hct), and plasma total carbon dioxide content were analyzed immediately upon collection. The blood used for P_{aO_2} measurement (250 μl) was then retrieved and re-injected into the animal. Plasma was obtained by centrifugation (10 000 ***g*** for 2 min) and stored at −20 °C for later analysis of cations and protein, or at −70 °C for determination of catecholamine levels. The techniques used for the analysis of whole-blood [Hb] and lactate and the above blood and plasma constituents (other than catecholamines), and calculations used to estimate carbon dioxide tension (P_{aCO_2}), plasma

bicarbonate ($[HCO_3^-]_p$) and mean corpuscular haemoglobin content (MCHC) are as described previously (Wilson and Taylor, 1993). The concentration of plasma noradrenaline (NA) was determined using reverse-phase, ion-paired HPLC with electrochemical detection (Ehrenstrom and Johansson, 1985).

On the second day, the same exercise protocol was repeated, except that regional blood flow and cardiac output were assessed at rest and after 45 min at 94 % U_{WMcrit} by injecting either ^{46}Sc- or ^{113}Sn-radiolabelled microspheres (NEN, DuPont) *via* the dorsal aortic catheter. The methodology used was similar to that of Neumann *et al.* (1983). For each injection, approximately 3×10^5 to 5×10^5 of the appropriately labelled microspheres (15 μm diameter; suspended in 0.8 ml of 10 % dextran) were mixed continuously right up to the point of injection with the aid of a stainless-steel ball within a 1 ml syringe. The microspheres were then injected whilst simultaneously withdrawing blood *via* the caudal artery catheter with a syringe pump (Braun Perfusor VI; 0.654 ml min^{-1}) to allow estimation of the cardiac output and absolute blood flows by the indicator dilution technique. Following microsphere injection, the dorsal aortic catheter was flushed with 0.5 ml of blood (previously acquired from the same animal) followed by 0.5 ml of saline. Following the 45 min exercise period, fish were infused with the second microsphere dose and killed 10 min post-infusion by injection of an overdose of MS222. Samples of deep white and lateral red muscle (from both sides of the body in the subdorsal region), heart, intestine and skin were dissected out and weighed. Microsphere activities in tissues and withdrawn blood samples were then determined with a multi-channel gamma counter (Packard Auto-Gamma 5650) and absolute blood flows were calculated by scaling specific activity to that in the reference blood sample after correction for background and crossover. Effective mixing of microspheres in the arterial blood was indicated by the equality of counts obtained in contralateral samples of red muscle. Consequently, data from left- and right-side samples have been pooled for red and white muscle.

Data have been expressed as mean ± S.E.M. (*N*=number of animals). Changes in measured variables between the resting and exercised states were compared using a Student's paired two tailed *t*-test at the 5 % level of significance.

White muscle recruitment was initiated at a mean swimming speed of 55.2±3.8 cm s^{-1} (1.35 BL s^{-1}) in rainbow trout of mean mass 812.4±41.5 g and total length of 40.8±1.1 cm (*N*=6). The subsequent speed adopted for 45 min of maximal aerobic swimming was 51.7±3.6 cm s^{-1} (1.27 BL s^{-1}). These values are low compared both with the U_{crit} values normally obtained for this size of unoperated rainbow trout (approximately 2 BL s^{-1}; Kiceniuk and Jones, 1977) and with U_{WMcrit} values for either smaller trout (18 cm) or striped bass (16–18 cm) with only EMG wires attached (Johnston and Moon, 1980; Sisson and Sidell, 1987). However, they are within the range of U_{crit} values for similarly operated trout (0.5–1.5 BL s^{-1}; Kiceniuk and Jones, 1977), where the effects of surgery and the combined drag of catheters and electrodes are significant.

It should be pointed out that trout white muscle is a mosaic of both anaerobic (fast glycolytic) and aerobic (fast oxidative) muscle fibres. However, the latter occupy only 5–10 % of cross-sectional area, depending on position along the body. In practice, activity in these aerobic fibres is detected as low-amplitude background 'noise' from the white

muscle EMG electrodes. When the truly anaerobic fast glycolytic fibres are used, the amplitude of the EMG signal is many times larger and easily distinguishable from the former. When U_{WMcrit} speeds are first encountered, these large-amplitude EMG signals do not occur with every tailbeat, signifying the start of intermittent anaerobic burst swimming activity. We believe that using a water speed where the occurrence of any large-amplitude white muscle EMG traces is avoided (94 % of U_{WMcrit}) forms a suitable definition of the maximum speed for sustainable swimming without recruitment of any of the anaerobic (fast glycolytic) white muscle. In many ways, this may be a more precise definition of the maximum purely aerobic swimming speed than using the standard $\dot{V}_{O_2max}$ or U_{crit} determinations, since both the latter methods may involve the recruitment of some rapidly fatigable fast glycolytic fibres and the production of excess lactic acid. Indeed, our U_{WMcrit} values seem reasonable in the light of results from continuous monitoring of lake-dwelling brown trout using ultrasonic tags, which gives the upper limit of free-range swimming to be around half our value (0.6 BL s^{-1}; Schulz and Berg, 1992). It would appear that, under natural conditions, trout operate well within their aerobic scope.

The only changes in blood chemistry after swimming at the highest speed where WM remained quiescent (94 % of U_{WMcrit}) were small, parallel increases in blood oxygen content, Hct and haemoglobin concentration (Table 1). Since the MCHC and plasma [protein] (indicators of red cell and plasma volume, respectively) remained unchanged, this suggests that a small splenic release of red cells occurred as part of a response to improve the delivery of oxygen to the working red muscle. As circulating NA levels were unaffected by imposed activity (Table 1), this presumably indicates a neurally mediated response. The lack of changes in blood acid–base status and lactate concentration confirm

Table 1. *Haematological and blood chemistry changes in rainbow trout in response to 45 min of maximal aerobic exercise*

	Rest	Exercise
pHa	7.828±0.021	7.820±0.043
Pa_{O_2} (kPa)	14.36±0.92	14.42±1.32
Pa_{CO_2} (kPa)	0.35±0.03	0.39±0.03
$[HCO_3^-]_p$ (mmol l^{-1})	8.07±0.81	8.80±1.08
Ca_{O_2} (mmol l^{-1})	2.13±0.37	2.43±0.38*
Hct (%)	13.8±0.37	16.0±1.8*
[Hb] (g dl^{-1})	4.29±0.83	4.54±0.76*
MCHC (g dl^{-1})	25.2±2.5	26.7±2.3
$[Na^+]_p$ (mmol l^{-1})	148.5±2.2	150.3±1.8
$[K^+]_p$ (mmol l^{-1})	2.54±0.09	2.86±0.18
$[Ca^{2+}]_p$ (mmol l^{-1})	2.25±0.10	2.18±0.09
[Lactate] (mmol l^{-1})	1.17±0.29	1.46±0.62
$[Protein]_p$ (g dl^{-1})	2.66±0.27	2.23±0.38
$[Noradrenaline]_p$ (nmol l^{-1})	6.89±1.28	8.67±2.48

p subscripts indicate plasma values; all other variables are derived from whole-blood measurements.

Data are expressed as mean ± S.E.M. (N=6), and asterisks indicate values significantly different from the resting value (P<0.05).

that oxygen delivery was sufficient to meet the demands of the tissue and, hence, that the experimental protocol generates truly sustainable, aerobic exercise conditions. Indeed, none of the changes usually associated with anaerobic burst exercise in trout (e.g. lactacidosis, elevated catecholamine concentrations, extreme haemo- and plasma concentration, red cell swelling, highly elevated plasma [K^+]) was apparent in our exercised trout. It is worth noting that we found no changes in blood gas tensions during exercise (similar to Randall and Daxboeck, 1982), unlike the reciprocal changes in $P_{a_{O_2}}$ and $P_{a_{CO_2}}$ reported by Thomas *et al.* (1987) for aerobically swimming trout fitted with an extracorporeal circuit. This could reflect strain differences in the degree of ventilatory and circulatory compensation in the face of increased demand for respiratory gas exchange.

Table 2 shows cardiovascular variables and tissue blood flows for resting and aerobically exercised trout in four of the six animals used for blood chemistry analysis. On the basis of past reports of cardiac output ($\dot{V}$b) measured by a variety of techniques in resting rainbow trout held between 6 and 18 °C, Barron *et al.* (1987) found a tight linear relationship between $\dot{V}$b and temperature. The mean value for resting $\dot{V}$b in our fish (29.8 ml min^{-1} $kg^{-1)}$ conforms very well to that predicted by Barron *et al.* (1987) for trout at 11 °C (30.6 ml min^{-1} kg^{-1}). Resting perfusion rates (ml min^{-1} g^{-1}) are similar to previously reported values for RM but slightly lower than past reports for WM (Neumann *et al.* 1983; Barron *et al.* 1987). However, this is the first time that cardiac output and tissue blood flow distribution have been measured simultaneously during maximal aerobic swimming in trout. The intensity of the aerobic exercise protocol is clearly reflected in a 480 % increase in cardiac output and a 140 % elevation of mean arterial blood pressure ($\bar{P}$a), leading to a 27-fold increase in blood flow to the working red muscle. The simultaneous 44-fold increase in the vascular conductance of red muscle indicates a substantial decrease in peripheral resistance of either neural or metabolic origin. It is interesting to compare this with RM perfusion rates in largescale suckers *Catostomus macrocheirus*, which increased even more dramatically (by 60-fold) during

Table 2. *Cardiovascular performance and regional blood flow in rainbow trout in response to 45 min of maximal aerobic exercise*

	Rest	Exercise	Percentage of resting conductance
Heart rate (beats min^{-1})	41.8±2.1	73.0±1.9*	
$\bar{P}$a (kPa)	3.15±0.23	4.37±0.28*	
$\dot{V}$b (ml min^{-1} kg^{-1})	29.8±3.0	143.0±38.5*	
$\dot{Q}_{RM}$ (ml min^{-1} g^{-1})	0.076±0.018	2.075±0.297*	4450
$\dot{Q}_{WM}$ (ml min^{-1} g^{-1})	0.009±0.001	0.013±0.004	101
$\dot{Q}_{skin}$ (ml min^{-1} g^{-1})	0.017±0.005	0.005±0.015	33
$\dot{Q}_{intestine}$ (ml min^{-1} g^{-1})	0.232±0.106	0.207±0.085	94
$\dot{Q}_{heart}$ (ml min^{-1} g^{-1})	0.0013±0.00051	0.0139±0.0076*	1061

Data are expressed as mean ± S.E.M. (N=4), and asterisks indicate values significantly different from the resting value (P<0.05).

$\dot{V}$, cardiac output; $\bar{P}$a, mean arerial blood pressure; $\dot{Q}$, tissue blood flows; RM, red muscle; WM, white muscle.

exercise (Kolok *et al.* 1993), although absolute RM perfusion rates were much smaller in these weaker swimmers. For a more direct comparison, the RM hyperaemia we observed in trout was far greater than the changes seen in trout following either burst exercise (Neumann *et al.* 1983) or exercise at 80 % U_{crit} (Randall and Daxboeck, 1982) and highlights the significance of a protocol which can objectively select swimming speeds where red muscle is maximally active and white muscle remains inactive.

Significant changes in absolute blood flows to the skin, gut and white muscle, typical of exercise in other vertebrates, were not apparent, and only in the skin was a significant reduction in vascular conductance observed. It would appear that an 'active' redistribution of blood from the quiescent white muscle to the working red muscle is not required during sustained aerobic exercise. Instead, the increased oxidative demand of RM is largely met by an increased cardiac output and systemic perfusion pressure, together with a large vasodilatation within the red muscle itself ('steal' effect). A 10-fold increase in blood flow to the heart reflects the increased demand on the cardiovascular system *per se*, although anatomical considerations mean that absolute values are a little unreliable. It is clear that in trout acclimated to 11 °C, increases in cardiovascular performance are able to maintain the supply of oxygen and fuels to the working RM without any of the disturbances to blood chemistry normally associated with non-sustainable exercise and with no redistribution of blood flow from the inactive white muscle. This protocol is currently being used to identify the same aerobic end-point in trout throughout the year, in order to determine the importance of cardiovascular adjustments during aerobic exercise over a range of environmental temperatures.

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