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INITIAL RECRUITMENT OF ANAEROBIC METABOLISM DURING SUB-MAXIMAL SWIMMING IN RAINBOW TROUT (*ONCORHYNCHUS MYKISS*)

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Summary

Lactate and phosphocreatine concentrations were monitored in the white muscle of rainbow trout *Oncorhynchus mykiss* following swimming at 70, 80 and 100 % of critical swimming speed (U_{crit}) using ^{31}P-nuclear magnetic resonance (NMR) spectroscopy. Lactate was detected following swimming at all speeds, but its concentration was greatest following swimming at 80 and 100 %U_{crit}. Phosphocreatine stores were reduced following swimming at 70 and 80 %U_{crit}, but were further depleted following swimming at 100 %U_{crit}. Following swimming at 70 and 80 %U_{crit}, lactate concentrations were higher in the rostral, relative to the caudal, musculature, whereas phosphocreatine stores were least depleted in the mid, relative to the rostral and caudal, musculature. There were no differences among muscle locations in concentrations of lactate and phosphocreatine following swimming to 100 %U_{crit}. Our results indicate that anaerobic metabolism is required to support swimming at speeds equal to and greater than 70 %U_{crit}. Estimates of the relative cost of anaerobic metabolism during sub-maximal swimming are presented for swimming at 70 %, 80 % and 100 %U_{crit}, and the implications of these results to energy budgets are discussed.

Key words: anaerobic metabolism, critical swimming speed, sub-maximal swimming, exercise, energy budget, rainbow trout, *Oncorhynchus mykiss.*

Introduction

The physiological effects of exhaustive swimming in the white muscle of salmonids have been studied extensively, including the dynamics and time course of recovery (e.g. Milligan and Wood, 1986; Moyes *et al.* 1992; Schulte *et al.* 1992). Although during sub-maximal swimming there is some indication that white muscle fibres are recruited (Hudson, 1973; Bone *et al.* 1978; Brill and Dizon, 1979; Rome *et al.* 1985), the anaerobic contribution to the overall energy budget is unknown. It has been suggested that anaerobic metabolism is initiated during swimming at 80 % of critical swimming speed (U_{crit}) in rainbow trout *Oncorhynchus mykiss* (Webb, 1971). The resulting energy contribution from anaerobic metabolism that may occur at and above 80 %U_{crit} has been assumed to be negligible on the basis of hydrodynamic estimates of efficiencies of aerobic and anaerobic swimming (Webb, 1971); however, no estimates of the costs of anaerobic metabolism have been made. Initial recruitment of white muscle fibres appears to be somewhat species-specific (Hudson, 1973; Johnston *et al.* 1977; Bone *et al.* 1978; Brill and Dizon, 1979; Johnston and Moon, 1980; Jayne and Lauder, 1995). The first objective of the present study was to determine the degree of anaerobic metabolism that occurs at 70 % and 80 %U_{crit} by estimating the concentration of lactate and phosphocreatine in muscle tissue following swimming.

Although increased lactate concentrations within the white muscle of fish following exhaustive exercise have been well documented, as has the rate of lactate depletion during recovery, it is not known whether lactate production is equal along the length of the fish's body. There have been some indications that white muscle fibre recruitment may vary along the musculature of the body (Jayne and Lauder, 1994, 1995). These inferences have been obtained using electromyography, and no corroborating biochemical studies have investigated unequal muscle fibre recruitment within fish. The second objective of the present study was to determine whether differential muscle recruitment occurred along the length of the body. Muscle recruitment was monitored by investigating the accumulation of anaerobic end-products in different regions of white muscle.

Anaerobic metabolism during sub-maximal swimming may, depending on its extent, have ramifications on the energy budgets of migrating salmonids. Most energy budgets for wild fish do not consider costs associated with anaerobic metabolism; therefore, if sub-maximal swimming results in the recruitment

of anaerobic fibres, energy budgets will be underestimated. This underestimate could be especially large when considering anadromous spawning migrations, which are often energetically expensive for stocks that must travel hundreds of kilometres to reach the spawning grounds (Hinch *et al.* 1996). Owing to the cessation of feeding during spawning migrations of anadromous salmonids, the recruitment of anaerobic muscle fibres below burst or exhaustive swimming speeds will have a large effect on the total energy that is available to reach the spawning grounds. If impediments such as a narrowing of the river or the creation of rapids occur within the migration path, excess energy may be required to support swimming through these sections. This additional energy expenditure may affect the success of the migration or spawning. As yet, the anaerobic component of swimming during the spawning migration has not been investigated adequately. The third objective of the present study was to assess the relative role of anaerobic metabolism during sub-maximal swimming by comparing the energy used during sub-maximal swimming bouts with aerobic energy estimates at corresponding swimming speeds.

Materials and methods

Animals

Series I rainbow trout *Oncorhynchus mykiss* (Walbaum) (mass 431.8±81.63 g; mean ± S.E.M., N=8) and sockeye salmon *Oncorhynchus nerka* (575±51.2 g, N=6) of both sexes were purchased from West Creek Trout Farm, Abbotsford, British Columbia, Canada. Series II rainbow trout (total length 35.6±1.38 cm, mass 482.7±54.5 g, N=29) were obtained from Spring Hill Trout Farm, Abbotsford, British Columbia, Canada. Fish were held for a minimum of 2 weeks in outdoor 2000 l round acrylic tanks supplied with aerated, flow-through dechlorinated Vancouver tapwater, at the Department of Zoology, University of British Columbia. During holding, fish were fed a maintenance ration of a commercially available trout chow; fish were not fed for at least 2 days prior to removal for use in experiments. The holding temperature changed seasonally from 7 to 10.5 °C.

Adult sockeye salmon (N=14) from the early Stuart stock were captured upon completion of the Hell's Gate Fishway, Fraser River, British Columbia, Canada, in July 1994. The fishways are located 180 km up-river from the ocean and are encountered by this stock during the first week of their 3 week, 1200 km migration. Sockeye were caught and handled by A. Kiessling, (Department of Agriculture and Department of Food Science, Swedish University of Agricultural Sciences, Uppsala, Sweden). Average water temperature at Hell's Gate during July 1994 was 17.5 °C. Rostral and caudal epaxial muscle was excised and placed immediately in liquid nitrogen. Samples were analysed for lactate content using standard biochemical assays.

Swimming protocol

Series I

Fish were transferred into a Brett-type flume (Gehrke *et al.* 1989) a minimum of 12 h prior to the initiation of the swimming protocol. Fresh water was flushed through the flume and the fish were left to acclimate for 12 h, with the water velocity at approximately 1 total body length s^{-1} ($BL\,s^{-1}$).

Fish were exercised to exhaustion by increasing the water velocity to the maximum swimming speed that the fish could maintain. When the fish could no longer sustain this speed, i.e. was unable to move off the downstream electrified (5 V) grid, the water velocity was decreased by approximately 30 %. If the fish could maintain this swimming speed for approximately 5 min, the water velocity was increased again. This pattern of increasing and decreasing the water velocity was maintained until the fish either lost control of balance or could not move off the back grid or swim when the water velocity had been reduced to $1\,BL\,s^{-1}$. Alternatively, fish were swum to U_{crit}, which was determined by incrementally increasing the water velocity. The initial step was approximately $0.5\,BL\,s^{-1}$, and all subsequent increases were approximately $0.25\,BL\,s^{-1}$. Each velocity was maintained for 30 min. Swimming was terminated when the fish could not move off the downstream electrified grid. U_{crit} was calculated as the product of the completed proportion of the fatigue increment and the velocity increment ($cm\,s^{-1}$) plus the water velocity of the last completed increment (Brett, 1964). The cross-sectional area of fish used in the flume was less than 10 % of the cross-sectional area of the flume, indicating that correction for solid blocking was unnecessary (Williams and Brett, 1987). The mean U_{crit} for the sample population was calculated using the U_{crit} values converted to $BL\,s^{-1}$.

Following the prescribed swimming regime, the fish were transferred from the flume to an acrylic box for ^{31}P-NMR analysis (see below).

Series II

Rainbow trout were removed by net, lightly anaesthetized with buffered tricane methanesulphonate (Syndel), and mass and total length (BL) were measured. The anaesthetised fish was then transferred either to a black acrylic box with a flow-through water supply or directly into a Brett-style flume (Gehrke *et al.* 1990). The fish quickly regained its balance and, when a low water velocity was imposed, began to swim. After 10–20 min at a low velocity (approximately $0.06\,BL\,s^{-1}$), the water velocity was increased to approximately $1\,BL\,s^{-1}$, and the fish was left to recover and to become accustomed to the flume for 10–12 h.

Following this initial period, the swimming protocol was initiated, and the fish was forced to swim to 100 %U_{crit}, 80 %U_{crit} or 70 %U_{crit}. U_{crit} was determined as described above for series I. The mean body mass, total length, swimming speed and %U_{crit} of the rainbow trout in each group are summarized in Table 1. Each fish was used for only one swimming trial, with a minimum of seven fish per group. Following the prescribed swimming regime, the fish were transferred from the flume to an acrylic box for ^{31}P-NMR analysis, as in series I. Struggling was minimized by the use of a plastic bag filled with water at ambient temperature, into which the fish swam

Table 1. *Swimming speed attained at each level of activity and the size (mass and length) of rainbow trout (*Oncorhynchus mykiss*) in each group*

		Swimming speed			
$\%U_{crit}$	*N*	(*BL* s^{-1})	(cm s^{-1})	Mass (g)	Total length (cm)
100	7	2.13±0.05	76.66±1.78	492.56±17.44	35.79±0.44
79.54±0.16	8	1.69±0.00	59.37±0.96	467.33±18.98	35.04±0.54
69.46±0.32	7	1.48±0.01	53.14±0.84	478.47±24.03	35.89±0.57
0	7			457.49±18.37	35.36±0.53

U_{crit}, critical swimming speed; *BL*, total body length.
Values are means ± S.E.M.

and was gently lifted out of the flume and into the acrylic box. Fish assigned to the resting, non-exercised group, were placed into the acrylic box for ^{31}P-NMR analysis, as described below.

NMR spectroscopy and analysis (series I)

Exercised fish were transferred immediately to a clear acrylic box for NMR analysis, lightly restrained with a perforated acrylic board and an inflated balloon, to minimize movement, and the box was closed. Continuous exchange of fresh, dechlorinated water occurred throughout the experiments. The fish was transferred into the NMR magnet and placed on top of a 4 cm, copper loop-gap resonator ^{31}P-NMR coil (Fig. 1). The elapsed time between transferring the fish from the respirometer to the NMR magnet ranged from 2 to 5 min. The NMR analyser consists of a 1.89 T horizontal superconducting magnet (Oxford Instruments, Oxford, UK) connected to a Nicolet 1280 spectrometer. The ^{31}P-NMR coil was used with a phosphoric acid standard solution for shimming, at a frequency of 32.5 MHz (tuned for ^{31}P). Each spectrum was obtained in 60 s, under the following conditions: 32 individual scans, nominal 90 pulses (42 μs), spectral window of 500 Hz, 1024 data points and a 1 s delay between pulses. Each fish was analyzed at 5–9 regions along the length of the body (ranging from approximately 0.15 to 0.75 *BL*; each region was 4 cm or 0.11 *BL*). Following NMR analysis, the fish was removed from the acrylic box and killed by a blow to the head. Fish did not struggle following removal from the NMR spectrometer, although some movement was associated with the method of killing. Muscle samples were excised from regions along the fish's body that corresponded to the regions analyzed using NMR spectroscopy. The muscle samples were freeze-clamped immediately in liquid nitrogen and stored at –80 °C until analysis. The elapsed time between the cessation of swimming and the final tissue sample was approximately 15 min (range 10–20 min).

Resting conditions were created by placing anaesthetized fish into a clear acrylic box equipped with a flow-through water supply, 10–12 h prior to ^{31}P-nuclear magnetic resonance (NMR) analysis (see below). This allowed for recovery from

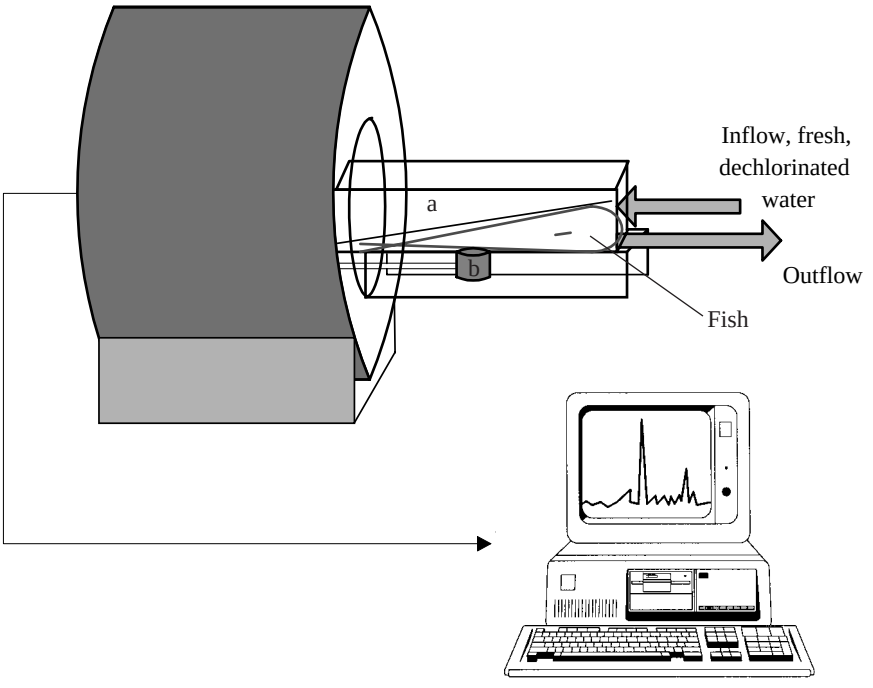

Fig. 1. Schematic diagram of fish placement within the bore of the magnet (not to scale). Fish were placed within an acrylic box (a) and restrained lightly using a plate with perfusion holes and a balloon (not shown). Continuous exchange of dechlorinated water occurred, with the inflow tube placed either in the fish's mouth or above the fish. The box was placed on the sled containing the probe coil (b), and the box and sled were placed within the bore of the magnet. Readings were taken using NMR spectroscopy, and the data were acquired and saved on computer.

handling and anaesthesia. The box was placed in the vertical position to ensure that the fish was upright. Fresh, dechlorinated water flowed into the box, which was covered with black plastic to minimize exposure to light. The room was darkened for the majority of the recovery period, after which the box was gently rotated by 90 ° and placed into the bore of the magnet.

NMR spectrum analysis

The raw data obtained from the NMR spectrometer were baseline-corrected using a Gaussian multiplication factor of 20, zero-fielded to 4096 points, Fourier-transformed and phase-shifted prior to deconvolution of the phosphocreatine (PCr) and inorganic phosphate (P_i) peaks. The PCr peak was used as the zero reference (0 p.p.m.) for the calculation of the inorganic phosphate chemical shift (δ-P_i). δ-P_i was calculated as the distance between the PCr peak and the P_i peak (see Fig. 2). PCr data was determined as the area under the PCr curve. Raw data were analyzed three times to increase the precision of the determinations. If the coefficient of variation (C.V.) for each sample was greater than 10 %, the sample was reanalyzed.

Tissue analysis

Tissue samples were ground to a coarse powder under liquid nitrogen in a prechilled mortar and pestle, and approximately 700–1000 mg of tissue was removed from each sample for analysis. The samples analyzed contained primarily white muscle; some red muscle may have been included, although this would constitute a very small proportion of the sample. Samples were subsequently transferred into preweighed tubes containing 4.0 ml of ice-cold 7 % perchloric acid, which were then reweighed. Samples were homogenized on ice three times for 15 s each using a Brinkman homogenizer. The homogenate was centrifuged at 10 000 ***g*** for 10 min at 4 °C. The supernatant was transferred to a clean, prechilled tube and neutralized with 3 mol l^{-1} K_2CO_3, 0.1 mol l^{-1} triethanolamine to a pH of 7.5. The neutralized samples were centrifuged at 10 000 ***g*** for 10 min at 4 °C, and the supernatant samples were placed into Eppendorf tubes and frozen in liquid nitrogen. The extracted tissue samples were stored at −80 °C until analysis.

Extracts were assayed for levels of lactate and PCr using enzyme assays modified from Bermeyer *et al.* (1987) for use with microplates. All assays were run in triplicate, and concentrations of lactate and PCr were validated by the use of appropriate standards. The mean and C.V. were calculated for each sample; if the C.V. was greater than 14 %, the sample was reanalyzed. Most sample analyses had a C.V. of less than 10 %.

NMR spectroscopy and analysis (series II)

Measurements were obtained from rostral (approximately 0.17–0.28 *BL*), mid (approximately 0.44–0.54 *BL*) and caudal (approximately 0.64–0.74 *BL*) regions of the white musculature. Each spectrum was obtained in 60 s. All parameters for the NMR spectroscopy were identical to the parameters used in series I. Following the measurements, the fish was removed from the acrylic box and placed in a black box to recover.

Measurements for resting fish were also obtained from three muscle regions similar to those measured for exercised trout. In some cases, additional spectra at the three regions were obtained to ensure resolution of the P_i peak. Additional spectra were obtained over 3.36 min under the following conditions: 128 individual scans, nominal pulses 90 (42 μs), spectral window of 500 Hz, 1024 data points and a 1 s delay between pulses. The additional scans allowed for easier differentiation of the P_i peak from background noise.

Oxygen consumption data analysis

Oxygen consumption data corresponding to swimming at different proportions of U_{crit} were obtained from the literature (Webb, 1971; Weatherley *et al.* 1982; Rogers and Weatherley, 1983; Bushnell *et al.* 1984; Brauner *et al.* 1994; Hughes and Biró, 1993). The data used to construct a relationship between the rate of oxygen consumption and U_{crit} were selected on the basis of the similarity in size of the rainbow trout and the temperature and test duration in each of the studies.

Statistical analyses

Series I

Systat (version 5, Systat Inc.) and Sigma Stat (versions 1.01 and 2.0, Jandel Scientific) were used for statistical analyses. The mean values from the triplicate samples analyzed for tissue lactate and PCr concentrations, the area under the NMR spectrum peak for PCr (NMR-PCr) and the chemical shift of the P_i peak were used in the analysis. Because of the preselection for precise estimates, there was no significant difference between the relationships when the mean, minimum or maximum estimate of tissue lactate or PCr concentrations were used. Simple linear regression was used to determine the relationship between biochemically measured PCr concentration and the NMR–PCr area. Polynomial regression was used to determine the relationship between tissue lactate concentration and δ-P_i. Multiple linear regression was used to test the effect of mass, species and temperature on the ratio of lactate concentration to δ-P_i and of PCr concentration on the area under the PCr peak.

Series II

The chemical shift of P_i and the area under the PCr peak were analysed three times, and each estimate was converted to either lactate concentration (μmol g^{-1} tissue) (from δ-P_i) or PCr concentration (μmol g^{-1} tissue) (from PCr area) using the regression equations obtained in series I. The mean, standard deviation and coefficient of variation (C.V.) were calculated for the converted values. If the C.V. was greater than 10 %, the sample was reanalysed in triplicate to increase the precision of the estimate.

One-way repeated-measures analysis of variance (ANOVA) was used to test for differences between regions of the fish at each swimming speed (0 %, 70 %, 80 % and 100 %U_{crit}) to determine whether the variation in metabolite levels between regions

followed the same pattern for each fish, in each group. We determined whether there was a difference in the pattern of metabolites between regions, as well as the absolute differences between regions. One-way ANOVA was used to test for differences between swimming speed within each region. Any differences were tested using the Student–Newman–Keuls *post-hoc* multiple-comparisons test. Simple linear regression was used to test for a functional linear relationship between the rate of oxygen consumption and U_{crit}. Unless otherwise stated, the probability of making a type I error (α) was set to 0.05.

Results

Series I

Appearance of NMR spectra

Clear spectra were obtained from epaxial muscle, and the peaks corresponding to inorganic phosphate (P_i) and phosphocreatine (PCr) were easily identified. A representative spectrum obtained from the epaxial muscle of an exercised rainbow trout is presented in Fig. 2.

Intramuscular lactate concentration and chemical shift of inorganic phosphate

The relationship between white muscle lactate concentration, determined biochemically, and δ-P_i, measured by ^{31}P-NMR spectroscopy, in rainbow trout and sockeye salmon is illustrated in Fig. 3. As δ-P_i decreases, the lactate concentration increases in a curvilinear fashion:

$$[\text{lactate}] = 856.38 - 306.67\delta\text{-}P_i + 27.8(\delta\text{-}P_i)^2 \quad (1)$$

(r^2=0.834, P<0.001). The ratio of lactate concentration (μmol g^{-1} tissue) to δ-P_i was tested to investigate relationships with species, mass of the fish, temperature of the experiment, region of the fish monitored and the swimming treatment used. No species effect was found (P=0.14). Although animal mass varied from 300 to 600 g, mass also had no significant effect (P=0.94). Water temperature varied during the course of the experiments (from 7.5 to 10 °C) owing to seasonal effects. Despite this variation, temperature had no significant effect on the ratio (P=0.37). Swimming treatment and the region of fish were found to be significantly predictive of the ratio of lactate concentration to δ-P_i (P<0.001 for swimming speed, P=0.03 for region monitored). The effect of both swimming treatment and fish region is probably a reflection of the non-linear relationship between lactate concentration and δ-P_i.

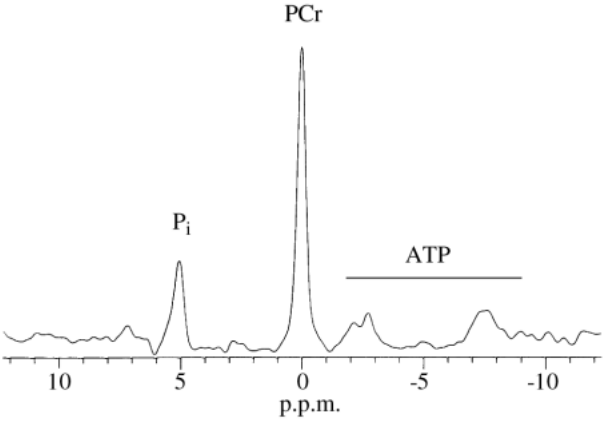

Fig. 2. Representative partial spectrum obtained using ^{31}P-NMR spectroscopy for epaxial muscle of a rainbow trout. This spectrum was obtained by scanning the white muscle of a fish exercised to 70 % of critical swimming velocity (U_{crit}). P_i, inorganic phosphate; PCr, phosphocreatine.

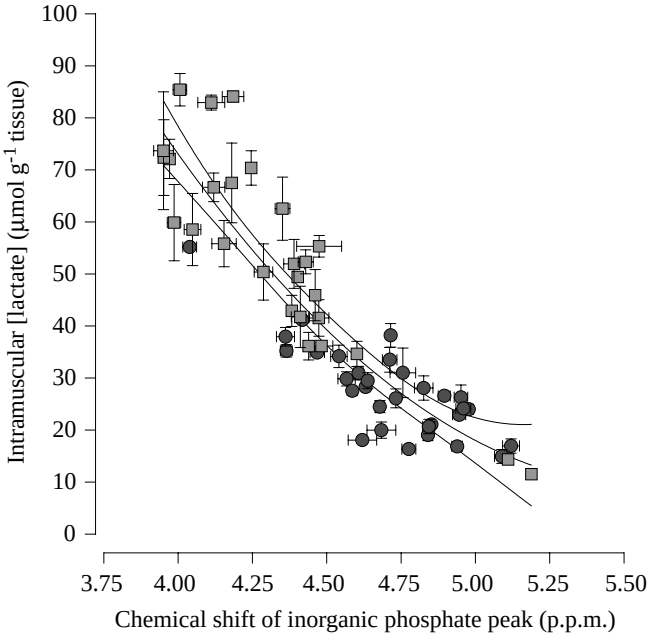

Fig. 3. Intramuscular lactate concentration measured biochemically as a function of ^{31}P-NMR-derived chemical shift inorganic phosphate peak (δ-P_i) in rainbow trout (circles, N=6) and sockeye salmon (squares, N=4). Values are means of triplicate measurements ±1 S.E.M. for both intramuscular lactate concentration and δ-P_i. Each fish was sampled several times along the length of its body (5–9 samples). The centre line represents the polynomial regression line of best fit, $[\text{lactate}]=856.4–306.7\delta\text{-}P_i+27.8(\delta\text{-}P_i)^2$ (r^2=0.834, P<0.001). The outer lines represent 95 % confidence intervals.

Intramuscular phosphocreatine measured biochemically and using ^{31}P-NMR

The linear relationship between biochemically measured PCr concentration and the area under the PCr peak in the ^{31}P-NMR-generated spectrum is shown in Fig. 4:

$$[\text{PCr}] = 0.562 + 0.021\text{NMR-PCr} \quad (2)$$

(r^2=0.80, P<0.001). Multiple linear regression was used to test whether the ratio between biochemically obtained and ^{31}P-NMR-derived PCr levels was influenced by the species, the mass of the fish, the temperature of the experiment, the experimental treatment and the region of the fish used in the NMR study. Only the region of the fish had a significant effect on the ratio (P=0.005).

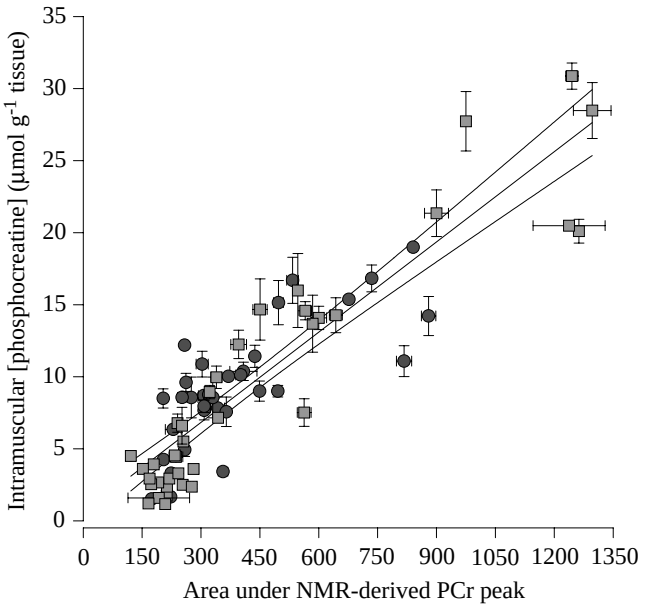

Fig. 4. ^{31}P-NMR-derived phosphocreatine (PCr) as a predictor of biochemically measured intramuscular PCr concentration in rainbow trout (circles, *N*=5) and sockeye salmon (squares, *N*=5). Values are means of triplicate measurements ± S.E.M. for both intramuscular PCr concentration and NMR-derived PCr (the area under the PCr peak; arbitrary units). The centre line represents the linear regression line of best fit, [PCr]=0.562+0.021NMR-PCr (r^2=0.80, P<0.001) and the outer lines represent 95 % confidence intervals.

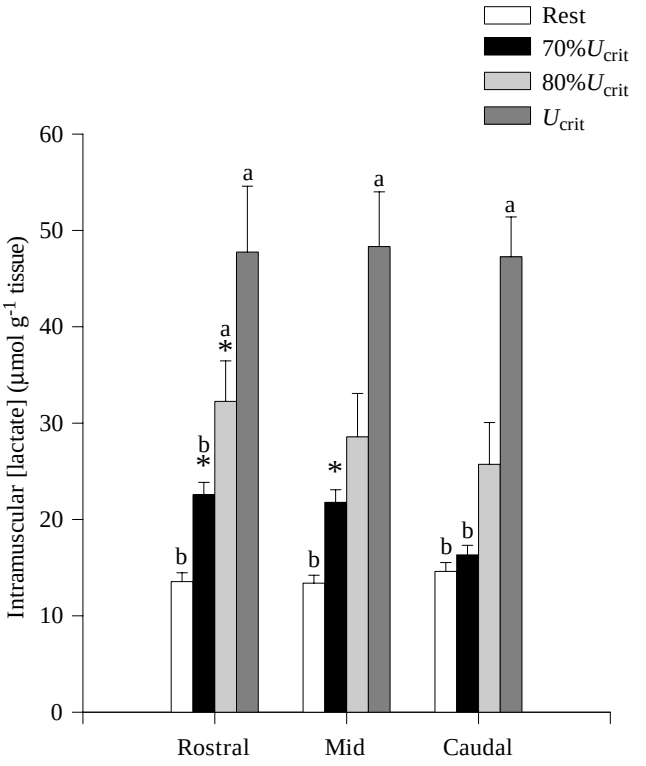

Fig. 5. Mean white muscle lactate concentration in rainbow trout white muscle following exercise and at rest, at three different regions along the body. Lactate concentration was calculated from δ-P_i values measured using ^{31}P-NMR spectroscopy and equation 1 (see text). a indicates a significant difference from the resting level, within a given region. b indicates a significant difference from the lactate concentration at critical swimming velocity (U_{crit}), within a given region. An asterisk indicates a significant difference from the caudal region, within an exercise level. Values are means + S.E.M. (*N*=7 for all regions for resting, 70 % and 100 %U_{crit}; *N*=8 for all regions for 80 %U_{crit}).

Series II

Lactate concentrations within white muscle

Lactate concentration was determined from the δ-P_i values measured using ^{31}P-NMR spectroscopy and equation 1, in the white muscle from three regions along the length of each fish. Swimming to U_{crit} resulted in significantly greater estimated lactate concentrations in all regions when compared with resting levels (Fig. 5). Generally, there was a greater concentration of lactate within the rostral musculature than in the mid or caudal regions when differences due to swimming treatment were considered. Following swimming for 30 min at approximately 70 %U_{crit}, although the estimated lactate concentration within the muscle mass was not significantly different from levels estimated for resting fish, there were significantly greater concentrations of lactate within the mid and rostral regions compared with the caudal region (Fig. 5). Swimming at 80 %U_{crit} resulted in a significantly greater concentration of lactate rostrally than caudally. No difference was observed between regions following swimming at U_{crit} or in resting fish.

Phosphocreatine concentration following exercise

The mean intramuscular PCr concentration, as estimated from equation 2 and using the area under the PCr peak from ^{31}P-NMR-generated spectra for series II fish for the three regions within the musculature, is shown in Fig. 6. The general trend across all muscle regions was a significantly greater concentration of PCr in resting muscle than in muscle from fish forced to exercise. Also, there was significantly less PCr remaining within the white muscle from rostral and mid regions following exercise to U_{crit} compared with 70 % and 80 %U_{crit}. No difference was detected between the PCr concentrations following swimming at 70 % and 80 %U_{crit} when all muscle regions were taken into account. The mean estimated intramuscular PCr concentration in resting muscle was significantly higher in the mid region than caudally (Fig. 6). Following swimming to 70 %U_{crit}, this pattern was repeated; however, there was also a significantly lower concentration of PCr within the rostral region compared with the mid region. Exercise to U_{crit} resulted in significantly lower PCr concentration compared with rest, 70 % and 80 %U_{crit} with no detectable difference between the muscle regions.

Lactate accumulation in migrating sockeye salmon

Lactate concentrations were measured biochemically in the rostral and caudal white muscle of wild-caught sockeye salmon, following migration through the Hell's Gate fishway

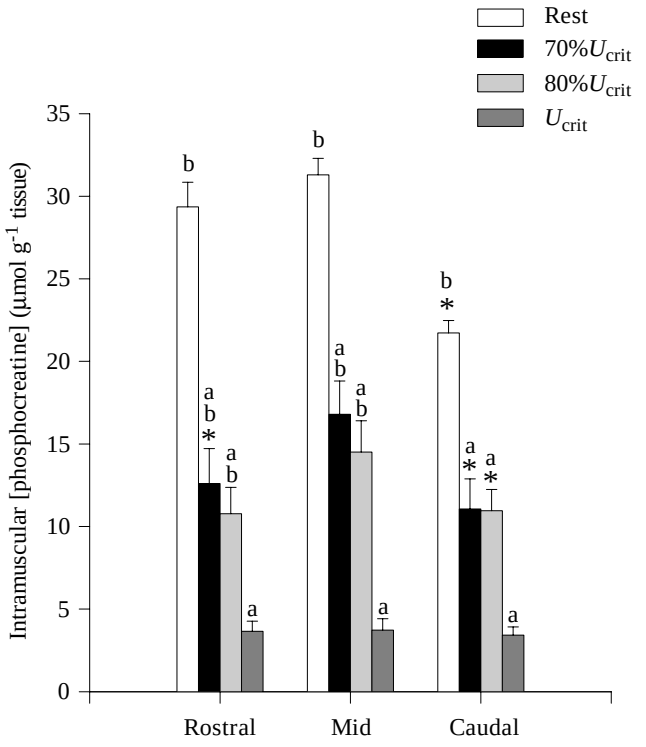

Fig. 6. Mean intramuscular phosphocreatine (PCr) concentration calculated from NMR-PCr values measured using ^{31}P-NMR spectroscopy and equation 2 (see text) in rainbow trout white muscle following exercise and at rest, at three different regions along the body. a indicates a significant difference from the resting PCr concentration, within a given region. b indicates a significant difference from the PCr concentration at critical swimming velocity (U_{crit}), within a given region. An asterisk indicates a significant difference from the mid region, within an exercise level. Values are means + S.E.M. (N=7 for all regions for 0 %, 70 % and 100 %U_{crit}; N=8 for all regions for 80 %U_{crit}).

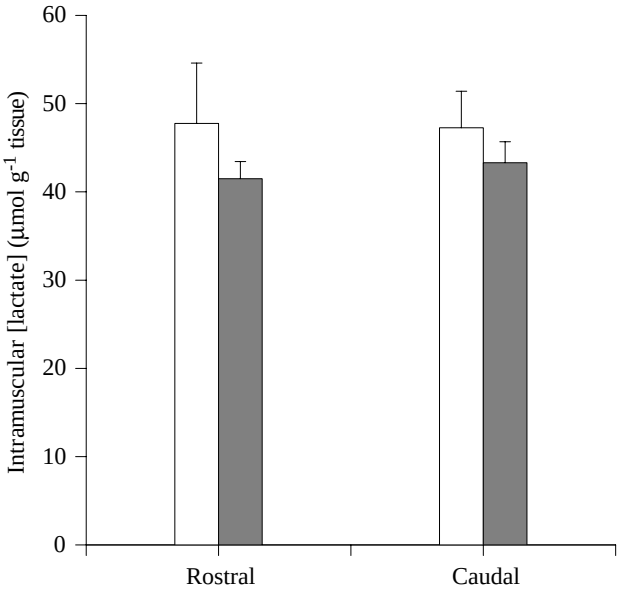

Fig. 7. Mean intramuscular lactate concentrations in the rostral and caudal musculature of rainbow trout following exercise to critical swimming velocity (U_{crit}) (open columns) and in wild-caught sockeye salmon following completion of the Hell's Gate Fishway (shaded columns). Values are mean + S.E.M. (N=4).

(Fig. 7). The levels of lactate measured were not significantly different from levels measured in rainbow trout exercised to U_{crit}; however, they were elevated compared with resting levels in farm-raised rainbow trout (see Fig. 5).

Discussion

Series I

[Lactate]/δ-P_i relationship

Biochemically measured intramuscular lactate concentration was related to the chemical shift of intramuscular P_i measured using ^{31}P-NMR spectroscopy (equation 1, Fig. 3). This predictive relationship represents a novel method of estimating lactate accumulation *in vivo* following exercise in fish. Previously, the only method available to monitor intramuscular lactate concentrations following exercise was to sample the fish terminally. Non-invasive measurement of δ-P_i *via* ^{31}P-NMR allows estimation of *in vivo* lactate accumulation following exercise that can be directly compared with values in the literature.

^{31}P-NMR has been used extensively to monitor the intracellular pH (pHi) in many systems (see Gadian, 1982; Malhotra and Shapiro, 1993) through the relationship between pHi and the chemical shift of P_i. With a change in pHi, the position of the P_i peak changes relative to the position of the PCr peak. The relationship between pHi and δ-P_i has not been extended previously to anaerobic metabolites, such as lactate. Lactate production during glycolysis is associated with a large production of protons, changing the pHi; lactate is therefore an appropriate metabolite for inference from δ-P_i following exercise. A linear relationship between pHi and lactate exists in human skeletal muscle (Sahlin, 1978). A somewhat similar relationship was derived for fish from pHi and lactate measurements from a number of studies (Milligan and Wood, 1986; Parkhouse *et al.* 1988; Tang and Boutilier, 1991; Schulte *et al.* 1992; Wang *et al.* 1994). This combined relationship can be fitted with a second-order polynomial regression (r^2=0.825). The δ-P_i data obtained in the present study was converted to pHi using the Henderson–Hasselbach equation fitted for 7 °C (pK′=6.828) and 10 °C (pK′=6.809; Kost, 1990). The pHi/[lactate] relationship from the present study were fitted with a second-order polynomial regression (r^2=0.841), which did not differ significantly from the curve calculated from the literature data only. Therefore, the data obtained from the literature and those from the present study were combined and fitted with a second-order polynomial regression (Fig. 8, r^2=0.675, P<0.001). The conversion of δ-P_i to pHi may involve an error due to the pH–temperature conversion factor taken from Kost (1990). The relationship between pH and temperature was obtained by Kost (1990) using a calibration

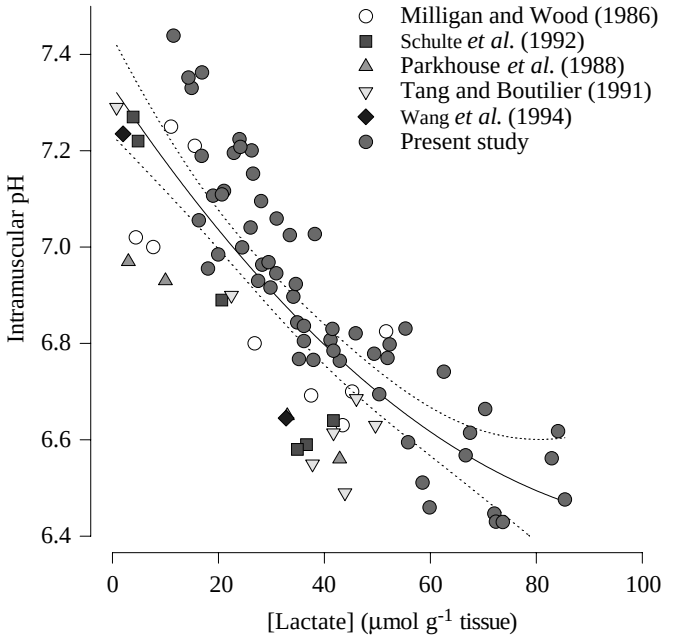

Fig. 8. Intramuscular pH of rainbow trout following exercise as a function of intramuscular lactate concentration. Intramuscular pH from the present study was derived from ^{31}P-NMR measurements of δ-P_i and the Henderson–Hasselbach equation (see text for details). The combined data set results in the second-order regression equation: $pH=7.334-0.016[lactate]+7.30\times10^{-5}[lactate]^2$ (r^2=0.675, P<0.001) (solid line). The dotted lines represent the 95 % confidence intervals for the regression line.

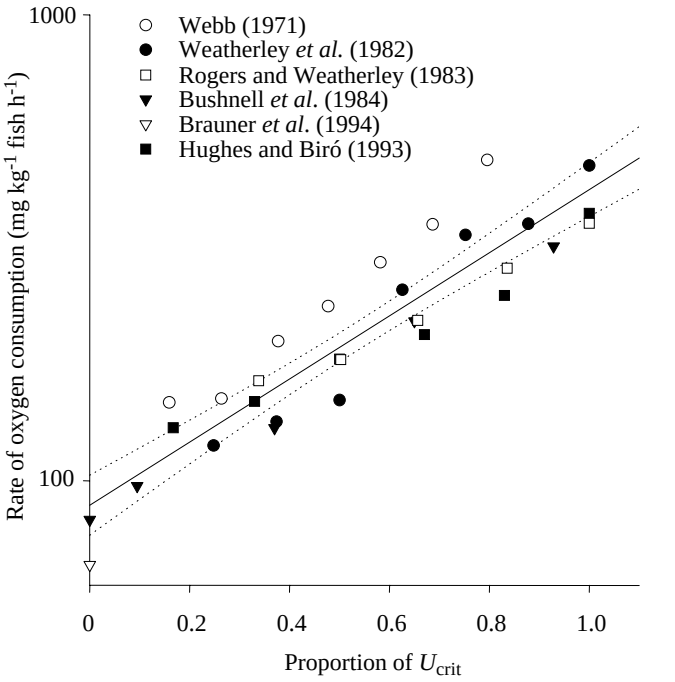

Fig. 9. The rate of oxygen consumption ($\dot{M}_{O_2}$) in rainbow trout (mass 250–500 g) during swimming at different proportions of critical swimming velocity (U_{crit}), at temperatures ranging from 10 to 15 °C, from data obtained from the literature. The solid line represents the linear regression line of best fit, $\log\dot{M}_{O_2}$=1.947+0.667(proportion of U_{crit}) (r^2=0.852, P<0.001), and the dashed lines represent the 95 % confidence intervals for the regression line.

solution designed to be of similar ionic strength to the human myocardial intracellular milieu. Carp (*Carassius carpio*) muscle pHi has been estimated by van den Thillart *et al.* (1989) using calibration curves constructed with measured pHi solutions similar to Kost's (1990) calibration solution, but containing 30 % carp muscle homogenate. The resulting curve agreed exactly with the pH calibration curve from Kost at 20 °C (van den Thillart *et al.* 1989). At present, no calibration curves are available for salmonid tissue. Therefore, some error may be associated with this conversion factor relative to the determination of intramuscular pH by more traditional biochemical methods (e.g. Pörtner *et al.* 1990).

[Phosphocreatine]/NMR-PCr relationship

The linear relationship between the area under the PCr peak in NMR spectra and the PCr concentration measured biochemically (equation 2) is important as a confirmatory measure. Not only does this confirm that NMR-PCr values estimate intramuscular PCr concentration but also that a relationship has been derived that can be used to convert the area of the PCr peak to actual concentrations. The accuracy of the biochemically measured PCr concentrations can be determined by comparing the values obtained in the present study with literature values. PCr concentrations from rainbow trout in this study are comparable with those in the literature for both resting and exhausted fish (resting, 17.49–22.69 μmol g^{-1} tissue; exhausted, 1.39–4.26 μmol g^{-1} tissue; Schulte *et al.* 1992; Parkhouse *et al.* 1988; Dobson and Hochachka, 1987). Errors associated with measured values of PCr would be an overestimation of the total concentration. This could occur if recovery of PCr has begun before the sample was obtained; however, little recovery is expected in the first 20 min following exercise (Dobson and Hochachka, 1987; Milligan and Wood, 1986).

A small discrepancy has been noted between NMR-PCr values and biochemically measured [PCr] in biopsy samples from human muscle tissue. This is assumed to be due to hydrolysis of PCr occurring between removing the sample and freezing (Dawson, 1982). However, the differences were not significant and, therefore, despite some potential loss of PCr during sampling and preparation for biochemical analysis, both methods of monitoring PCr concentrations are considered to be reliable (Dawson, 1982; van den Thillart *et al.* 1990).

Series II

White muscle phosphocreatine concentration following exercise

The changes in concentration of PCr along the length of the fish's body (Fig. 6) indicate that whole-body estimates of PCr concentration from white muscle following sub-maximal swimming and at rest may be inaccurate. For example,

overestimation of [PCr] would occur if muscle samples were taken only from the mid-lateral musculature. Phosphocreatine stores are depleted by progressively larger amounts following exercise to 70 % and 100 %$U_{crit.}$ Presumably, this reflects a progressive increase in the rate of utilization of PCr, but there may also be changes in its rate of synthesis during sub-maximal swimming.

Lactate accumulation following exercise

Many studies using electromyography have demonstrated that there is a contribution of energy from the white muscle fibres during swimming below U_{crit} (Hudson, 1973; Johnston *et al.* 1977; Bone, 1978; Bone *et al.* 1978). White muscle fibre recruitment has been shown to increase either by a change in the number of fibres recruited or in the extent of activity of the fibres recruited as swimming speed increases (Rome *et al.* 1984, 1985; Rome, 1990; Jayne and Lauder, 1994, 1995). These studies indicate that 80 %U_{crit} may be the threshold for anaerobic metabolism; however, until the present study, no biochemical measurements had been made to investigate the initiation of anaerobic metabolism. The present study demonstrates that anaerobic metabolism (measured as the accumulation of intramuscular lactate) occurs at 70 %U_{crit}, but is only significant following exercise to 80 %U_{crit}. In addition, it would appear that rostral muscle is recruited at lower swimming speeds than is caudal muscle.

Estimates of the energy associated with the anaerobic component of swimming

The accumulation of lactate within the white musculature during sub-maximal swimming has implications for energy budgets, which generally do not include estimates of anaerobic metabolism. However, our data indicate that, for migrations at speeds above 70 %U_{crit}, the anaerobic component may be important and should be incorporated. Lactate is initially converted to pyruvate, which is then used for gluconeogenesis rather than for oxidative metabolism in fish white muscle during recovery from exercise (Moyes *et al.* 1992, 1993). Intramuscular lactate levels during exercise in fish white muscle can therefore be quantitatively related to the amount of glycosyl units depleted (2 mol of lactate = 1 mol of glycosyl unit; Arthur *et al.* 1992; Moyes *et al.* 1993). During anaerobic exercise, three ATP molecules per glycogen molecule are produced during the conversion of glycogen to lactate, and five ATP molecules per glycosyl unit are required to produce glycogen from lactate during recovery (Moyes *et al.* 1990, 1993). Therefore, the quantity of ATP produced, at each level of activity in the present study, can be estimated from the lactate concentration. A caloric equivalent can then be applied to allow the anaerobic component to be added to the overall energy budget.

An oxygen equivalent for ATP can be used to compare the energy production from anaerobic glycolysis with the oxygen consumption associated with the corresponding activity level. With increasing activity, there is an increase in the rate of oxygen consumption, and relationships between the rate of oxygen consumption and swimming speed have been determined (Brett, 1964; for a review, see Beamish, 1978). The rates of oxygen consumption associated with swimming at various proportions of U_{crit} compiled from literature data for rainbow trout at 10–15 °C are presented in Fig. 9 (Webb, 1971; Weatherley *et al.* 1982; Rogers and Weatherley, 1983; Bushnell *et al.* 1984; Brauner *et al.* 1994; Hughes and Biró, 1993). A significant linear relationship between the rate of oxygen consumption ($\dot{M}_{O_2}$) and swimming speed for the combined literature data was found:

$$\log\dot{M}_{O_2} = 1.947 + 0.677(\text{proportion of } U_{crit}) \quad (3)$$

(r^2=0.852, P<0.001). Using this relationship, the anaerobic metabolism associated with a given swimming speed can be expressed as a percentage of the rate of oxygen consumption (aerobic metabolism) for that swimming speed, giving an anaerobic energy ‘tax’.

The mean lactate concentration from each region monitored, at each swimming speed, can be used to estimate the whole-body lactate production during 30 min of exercise. White muscle makes up approximately 60 % of the total body mass (Randall and Daxboeck, 1982). The distribution of white muscle mass along the length of the fish should be reflected by the shape of the fish, and crude determinations (N=3; region excised and mass of the region/total body mass determined for each region) suggest that the relative distributions of white muscle among the rostral, mid and caudal regions in rainbow trout are approximately 34, 45 and 21 %, respectively. These approximate proportions of muscle and the mean lactate concentrations calculated for series II fish in the three regions of the body were used to obtain estimates of whole-body lactate production. Two calculations were carried out, the first estimated only the cost of glycogen breakdown to lactate; the second included the cost of gluconeogenesis. The difference between the resting lactate concentration and that following exercise was used at each swimming speed for these estimates. To convert whole-body lactate production to whole-body ATP production, a factor of 1.5 mol of ATP per mole of lactate was used. This assumes that gluconeogenesis does not occur during the swimming period and does not account for the aerobically supplied ATP that is required for the resynthesis of intramuscular glycogen following exercise (2.5 mol of ATP per mol of lactate; Moyes *et al.* 1992). Although the cost of gluconeogensis is separated temporally from ATP production during anaerobic glycolysis, this cost must be accounted for in total energy budgets. To compare the quantity of ATP produced during anaerobic glycolysis with the energy demand of aerobic swimming, an oxygen equivalent of 6 mol of ATP per mol of O_2 was used (Moyes *et al.* 1993).

The calculated oxygen equivalents for swimming at 70 %, 80 % and 100 %U_{crit} are given in Table 2 and are also presented as a percentage of the estimated oxygen consumption during 30 min of swimming at the appropriate speed. Results for both sets of assumptions are presented (i.e. with and without recovery costs). It can be concluded that swimming at 70 %U_{crit} and above does represent a significant energy cost

Table 2. *Estimates of oxygen consumption equivalents during swimming*

	Oxygen equivalents ($mg\,O_2\,kg^{-1}$ fish)		Percentage of oxygen consumption	
Swimming speed	Anaerobic metabolism	Including recovery	Anaerobic metabolism	Including recovery
70%U_{crit}	32.1	85.6	24.4	65.0
80%U_{crit}	40.0	106.6	26.0	69.2
100%U_{crit}	161.7	431.3	76.9	205.0

U_{crit}, critical swimming speed.

Anaerobic metabolism is calculated as the difference between the lactate accumulated within the fish following swimming and the resting lactate concentration. Oxygen consumption is based on the oxygen consumed during 30 min of swimming at the appropriate swimming speed (see Fig. 9).

Values are calculated for the cost of glycogen breakdown to lactate (anaerobic metabolism) and also including the cost of gluconeogenesis (including recovery).

Sample calculation: Δ[lactate] = mean [lactate] after exercise minus mean [lactate] at rest. For 80%U_{crit}, Δ[lactate] = mean [lactate] after swimming at 80%U_{crit} minus mean [lactate] after swimming at 70%U_{crit} (see text for details). No recovery: Δ[lactate] ($mmol\,kg^{-1}$)×(1.5 mol ATP per mol lactate)×(1 mol O_2 per 6 mol ATP)×(32 mg O_2 per mmol O_2)×0.6 (the ratio of white muscle to whole fish mass). Including recovery: Δ[lactate] ($mmol\,kg^{-1}$)×(1.5+2.5 mol ATP per mol lactate)×(1 mol O_2 per 6 mol ATP)×(32 mg O_2 per mmol O_2)×0.6.

Percentages are calculated using the estimated oxygen consumption from equation 3 for 30 min of swimming.

because the anaerobic portion of exercise represents at least 24 % of the total oxygen consumption.

Swimming at 80 %U_{crit} for a period of 30 min was preceded by swimming at approximately 70 %U_{crit} for 30 min in these experiments. Lactate produced during swimming at 70 %U_{crit} may be either partially or entirely resynthesised to glycogen during the trial; if this is the case, then the estimate of the energy associated with anaerobic glycolysis is all that is required. This is because, if gluconeogenesis occurs during the exercise trials, the oxygen consumption measurements at 80 %U_{crit} will overestimate the aerobic demand for swimming because of the increase in oxygen consumption associated with gluconeogenesis. Therefore, if the lactate produced during swimming at 70 %U_{crit} is metabolised during the exercise trial, the lactate produced during swimming at 80 %U_{crit} results in an oxygen equivalent of 72.1 $mg\,O_2\,kg^{-1}$ of fish, or 46.8 % of the total oxygen consumption.

If any recovery metabolism occurred during swimming or prior to obtaining samples, the energy cost would have been underestimated. Recovery of lactate levels is very slow and is therefore unlikely to have influenced the results. Recovery metabolism is inhibited following exhaustive exercise by the release of cortisol (Pagnotta *et al.* 1994). At present, it is not known whether or in what quantity cortisol is released into the bloodstream during sub-maximal swimming. If there is only limited cortisol release, then it appears that the recovery time for lactate and pHi is approximately 2 h (Pagnotta *et al.* 1994). If this is the case during sub-maximal swimming, then our estimates of the energy associated with anaerobic metabolism following swimming at 70 %U_{crit} are low. The energy associated with anaerobic metabolism during swimming at 80 %U_{crit} is probably between the two estimates presented above (26.0 % and 46.8 %; that is, approximately one-third of total oxygen consumption). It is likely that some metabolism of lactate occurs during the swimming trial, but that it is incomplete. Despite the possible underestimations discussed above, the energy produced by anaerobic glycolysis expressed as oxygen equivalents is more substantial than assumed previously (Webb, 1971) and at U_{crit} may exceed that produced by aerobic metabolism.

The anaerobic component of swimming at speeds above 70 %U_{crit} results in a sufficiently large energy cost that it must be incorporated into energy budgets. As illustrated in Fig. 7, the estimates of lactate concentration following swimming at U_{crit} in rainbow trout are not significantly different from lactate concentrations measured in white muscle from wild-caught sockeye salmon. Although it is not known what swimming pattern and path through the river the wild-caught, migrating salmon took, the accumulation of lactate within white muscle at this point within the river indicates that anaerobic metabolism is required during the course of migration. Therefore, the cost of anaerobic metabolism during sub-maximal swimming is an important consideration when constructing energy budgets and in considering the effects of changing environment on migration success.

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