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A RESPIROMETRIC ANALYSIS OF FUEL USE DURING AEROBIC SWIMMING AT DIFFERENT TEMPERATURES IN RAINBOW TROUT (*ONCORHYNCHUS MYKISS*)

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Summary

Instantaneous fuel usage at 5 °C or 15 °C was assessed by measurement of rates of O_2 consumption ($\dot{M}_{O_2}$), CO_2 excretion ($\dot{M}_{CO_2}$) and nitrogenous waste excretion ($\dot{M}_{nitrogen}$ =ammonia-N + urea-N) in juvenile rainbow trout (*Oncorhynchus mykiss*) at rest and during swimming at 45 % and 75 % of aerobic capacity (U_{crit}). After 2 weeks of training at approximately 1 body length s^{-1} (BL s^{-1}), critical swimming speeds (approximately 3.0 BL s^{-1}) and whole-body energy stores (total protein, lipids and carbohydrates) were identical in fish acclimated to 5 °C or 15 °C. $\dot{M}_{O_2}$ and $\dot{M}_{CO_2}$ increased with swimming speed at both temperatures and were higher at 15 °C than at 5 °C at all speeds, but the overall Q_{10} values (1.23–1.48) were low in these long-term (6 weeks) acclimated fish. The respiratory quotient ($\dot{M}_{CO_2}/\dot{M}_{O_2}$, approximately 0.85) was independent of both temperature and swimming speed. In contrast to $\dot{M}_{O_2}$ and $\dot{M}_{CO_2}$, the rate of ammonia excretion was independent of swimming speed, but more strongly influenced by temperature (Q_{10} 1.4–2.8). Urea excretion accounted for 15–20 % of $\dot{M}_{nitrogen}$, was unaffected by swimming speed and showed a tendency (P<0.07) to be positively influenced by temperature at one speed only (45 % U_{crit}). Nitrogen quotients (NQ $\dot{M}_{nitrogen}/\dot{M}_{O_2}$) were generally higher in warm-acclimated fish, remaining independent of swimming speed at 15 °C (0.08), but decreased from about 0.08 at rest to 0.04 during swimming at 5 °C. Instantaneous aerobic fuel use calculations based on standard respirometric theory showed that both acclimation temperature and swimming speed markedly influenced the relative and absolute use of carbohydrates, lipids and proteins by trout. At rest, cold-acclimated trout used similar proportions of carbohydrates and lipids and only 27 % protein. During swimming, protein use decreased to 15 % at both speeds while the relative contributions of both lipid and carbohydrate increased (to more than 40 %). On an absolute basis, carbohydrate was the most important fuel for fish swimming at 5 °C. In contrast, resting fish acclimated to 15 °C utilized 55 % lipid, 30 % protein and only 15 % carbohydrate. However, as swimming speed increased, the relative contribution of carbohydrate increased to 25 %, while the protein contribution remained unchanged at approximately 30 %, and lipid use decreased slightly (to 45 %). On an absolute basis, lipid remained the most important fuel in fish swimming at 15 °C. These results support the concept that lipids are a major fuel of aerobic exercise in fish, but demonstrate that the contribution of protein oxidation is much smaller than commonly believed, while that of carbohydrate oxidation is much larger, especially at higher swimming speeds and colder temperature.

Key words: rainbow trout, *Oncorhynchus mykiss*, aerobic swimming, respiratory quotient, nitrogen quotient, fuel, carbohydrate, protein, lipid, temperature.

Introduction

With respect to patterns of metabolic fuel use during swimming in fish, most work to date has been carried out on anaerobic burst-type exercise (for reviews, see Wood, 1991; Weber and Haman, 1996). From this large database, there is a general agreement that burst-type exercise is supported mainly by white muscle glycogen, with small contributions from white muscle ATP and phosphocreatine (Wood, 1991; Milligan, 1996). In comparison with anaerobic exercise, there is far less information and a general lack of agreement as to which metabolic fuels are used to support aerobic swimming in fish (see Lauff and Wood, 1996*a*,*b*). The earlier literature suggests that proteins and lipids (especially the former) are the major aerobic fuels in teleost fish and that carbohydrates play only a small role (Krueger *et al.* 1968; Driedzic and Hochacka, 1978; Walton and Cowey, 1982; van den Thillart, 1986; for reviews, see Jobling, 1994; Weber and Haman, 1996). However, the data of Kutty (1968) supporting an important role for carbohydrates and the analyses of Kutty (1978) and Wiggs *et*

al. (1989) suggesting a reduced role for protein oxidation during aerobic activity are notable exceptions.

These earlier studies were based either on the traditional compositional approach (which measures changes in proximate body composition between different groups of fish over time) or on a partial respirometric approach (where fuel oxidation is estimated from measured rates of consumption/production of two of the three respiratory gases, O_2, CO_2 and ammonia). However, on the basis of standard metabolic theory (Kleiber, 1992), no unequivocal conclusions can be reached from respirometry unless all three variables are measured. Recently, Lauff and Wood (1996*a*) developed the instantaneous approach, which employs simultaneous respirometric measurements of all three respiratory gases (with ammonia more accurately replaced by the sum of ammonia-N plus urea-N; Kleiber, 1987) to calculate stoichiometrically the particular combination of carbohydrate, protein and lipid that is actually being oxidized at any instant (for additional methodological considerations, see Simonson and DeFronzo, 1990). Lauff and Wood (1996*a*) concluded that the instantaneous approach was more accurate than the compositional approach (see also Brett, 1995) and, furthermore, yielded different conclusions because it measured the fuels actually being oxidized rather than the substrates being depleted over time. The latter may be affected by both excretion and interconversion of fuels.

Using this new instantaneous approach, Lauff and Wood (1996*b*, 1997) reached rather different conclusions from those of most earlier studies. In particular, for juvenile rainbow trout swimming aerobically at 15 °C, lipid was the major fuel source and carbohydrate also played a significant role, whereas protein was of lowest quantitative importance. Furthermore, as swimming speed increased, the relative importance of protein again declined and that of carbohydrate increased, although lipid still predominated overall (Lauff and Wood, 1996*b*).

Nothing is known about the relative effects of temperature on these processes; however, there are several reasons to suspect that aerobic fuel utilization might change with temperature. First, acclimation temperature alters the rate and proportion of anaerobic fuel utilization during burst-type exercise in fish (Kieffer *et al.* 1994). Second, many fundamental physiological and biochemical processes critical to aerobic performance are modified when fish are acclimated to different temperatures (Prosser, 1991; Hazel, 1993); for example, cardiac output and tissue perfusion (Farrell, 1984; Barron *et al.* 1987), muscle fibre recruitment (Rome *et al.* 1984), muscle contractile properties (Johnson and Johnston, 1991), red muscle to white muscle volume ratio (Johnston and Ball, 1997), enzyme concentrations, metabolic pathways and substrate availability (Walesby and Johnston, 1980; Sidell and Hazel, 1987; Blier and Guderley, 1988; Guderley and Gawlicka, 1992). Most importantly, Jones and Sidell (1982) and Thibault *et al.* (1997) found that cold acclimation increases the capacity for fatty acid oxidation, while early studies suggested that cold acclimation can also enhance glucose oxidation (Hochachka and Hayes, 1962).

Given this background, the objective of the present study was to use the instantaneous approach to assess how acclimation temperature (5 °C *versus* 15 °C) might affect fuel utilization in juvenile rainbow trout both at rest and during aerobic swimming at two different speeds. To compare trout swimming at the same speeds at the two different temperatures in a valid manner, it proved necessary to train the fish first. In particular, we hypothesized that cold acclimation might increase the contribution of carbohydrate and lipid metabolism in aerobically swimming trout and, thereby, decrease the contribution of protein metabolism, particularly at higher speeds. In general, our results support these hypotheses and further reinforce the conclusion that protein oxidation in aerobically swimming fish is not as great as commonly believed. The data also provide an overview of the effects of acclimation temperature and swimming speed on rates of O_2 consumption, CO_2 production and nitrogenous waste excretion in rainbow trout.

Materials and methods

Animals

Juvenile rainbow trout *Oncorhynchus mykiss* (Walbaum), approximately 8 cm long at the time of purchase, were obtained from Rainbow Springs Hatchery (Thamesford, Ontario, Canada) and kept at 11–12 °C in dechlorinated, fully aerated Hamilton city tapwater (for analysis, see Alsop and Wood, 1997) for 1 month prior to temperature acclimation. The fish were fed to satiation once daily with commercial trout food (Ziegler's; crude protein 50 %; crude fat 15 %; crude fibre 2 %; water 12 %).

Temperature acclimation

Groups of juvenile fish (see Table 1 for the physical characteristics of the fish) were transferred from the general holding area to acclimation tanks, where they were slowly acclimated (in increments or decrements of approximately 1 °C day^{-1}) to either 5 or 15 °C. Once at the designated temperature, the fish remained at that temperature for at least 6 weeks. During this period, fish were fed every second day to satiation.

Training

Preliminary experiments (see below) on swimming endurance indicated that most of the cold-acclimated fish were unable or unwilling to swim continuously for long periods and, for those that would, the maximum swimming speed was low. To overcome this problem, all fish used for proximate body composition and respirometry/fuel use experiments were trained to improve their swimming stamina (see Davison, 1997). Training consisted of placing groups of fish in a large (200 l) Beamish-style swim tunnel at a water speed of 10 cm s^{-1} (approximately 1 BL s^{-1}) for 2 weeks (see Lauff and Wood, 1997). Fresh dechlorinated water (2–3 l min^{-1}) at the respective acclimation temperature was delivered to the swim tunnel.

Table 1. *Length, mass and condition factor of the juvenile trout (acclimated to 5 or 15 °C) used during fuel use experiments*

	Length (cm)		Mass (g)		Condition factor	
Swimming speed	5 °C	15 °C	5 °C	15 °C	5 °C	15 °C
Control	11.50±0.23	11.30±0.28	15.20±1.20	18.00±1.40	0.99±0.04	1.20±0.06*
(0 cm s^{-1})	(4)	(7)	(4)	(7)	(4)	(7)
Slow swimming speed	12.20±0.30	11.60±0.19	20.60±1.70	17.50±0.80	1.10±0.03	1.10±0.02
(45 % U_{crit})	(9)	(9)	(9)	(9)	(9)	(9)
Fast swimming speed	11.40±0.25	11.10±0.15	16.80±0.90	15.10±0.70	1.15±0.03	1.10±0.03
(75 % U_{crit})	(8)	(9)	(8)	(9)	(8)	(9)

Values are means ± S.E.M. (*N*).
Condition factor=100m/l^3, where *m* is body mass and *l* is total body length.
An asterisk represents a significant difference between fish acclimated to 5 and 15 °C at a given swim speed (two-tailed, unpaired *t*-test).

Prior to all experiments, feeding was suspended for 3 days at 15 °C and for 5 days at 5 °C to reduce possible dietary influences on metabolite status and respiratory processes (Brett, 1964; Beamish, 1978; Tang and Boisclair, 1995).

Determination of critical swimming speeds

We first evaluated whether swimming performance (U_{crit}) was altered by temperature, using the standard test for critical swimming speed (Brett, 1964; Beamish, 1978). Individual fish (untrained and trained) were removed from their respective tanks and held overnight in individual Blazka respirometers (total volume 3.2 l). The U_{crit} tests were performed on individual fish by increasing the water velocity by increments of 10 cm s^{-1} every 60 min until the fish became exhausted. Water flow to the respirometers was continuous throughout the U_{crit} test. Critical swimming speed was determined for fish acclimated to either temperature using the equation given by Brett (1964):

$$U_{crit} = V_f + (T/t - \delta V), \quad (1)$$

where U_{crit} is critical swimming speed (cm s^{-1}), V_f is the speed (cm s^{-1}) of the last completed swimming period, δV is the velocity increment (10 cm s^{-1}), t is the time swum at each velocity (60 min), and T (min) is the time swum at the final velocity before exhaustion.

After the U_{crit} test, the fish were anaesthetized (neutralized MS-222 final concentration 0.25 g l^{-1}), patted dry, weighed (to the nearest 0.01 g), and the fork length (to the nearest mm) was measured. All swimming velocities (cm s^{-1}) were converted to body lengths per second (BL s^{-1}).

Respirometry and fuel use experiments

Since training influenced U_{crit} in fish acclimated to 5 °C (Fig. 1), our experiments on respirometry and aerobic fuel use were carried out on fish that had been previously trained. Five Blazka-style swimming respirometers (volume approximately 3.2 l) were used to house individual fish, as described by Lauff and Wood (1996*a*,*b*). Except for the periods of closed respirometry, air-saturated water (maintained at the acclimation temperature) flowed through each respirometer at a rate of approximately 150 ml min^{-1}. To ensure thermal equilibrium, the respirometers were submerged in the water of a wet table.

For proximate composition measurements, individual fish were quickly transferred from the training Beamish-style swim tunnel to the Blazka-style respirometers and held overnight. The next day, these fish were killed by introducing an overdose of neutralized MS-222, then removed from the respirometer, quickly blotted dry, weighed, measured and freeze-clamped in liquid nitrogen and stored at −70 °C until analyzed for proximate body composition (see below).

For the fuel use experiments, individual trained fish were randomly divided into one of three groups: control (i.e. non-swimmers), low-speed swimmers (15 cm s^{-1}, approx. 45 % U_{crit}) and high-speed swimmers (25 cm s^{-1}, approx. 75 % U_{crit}). These swimming speeds were based on the mean U_{crit} determined on a subset of trained fish acclimated to either 5 °C or 15 °C (Fig. 1). Control fish were exposed to a very low-speed water current to ensure good mixing of the water; this current did not induce swimming in the fish.

Since these experiments were designed to evaluate short-term fuel use in swimming trout, our experimental design consisted of a single period of aerobic swimming (or rest)

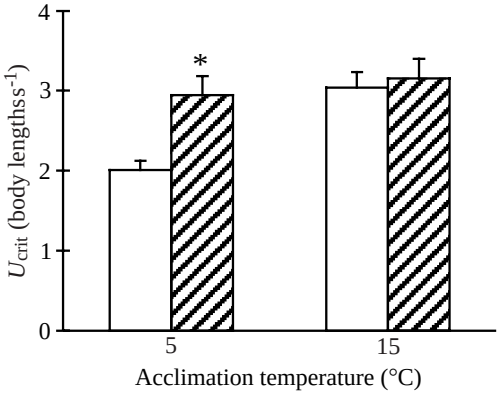

Fig. 1. Influence of training on critical swimming speed U_{crit} in fish acclimated to 5 or 15 °C. An asterisk indicates a significant difference between untrained (open columns) and trained (hatched columns) fish at a given acclimation temperature. Values are means + 1 S.E.M. (*N*=6–8, for each group).

lasting 4 h. The first 2 h was employed to allow the fish to achieve a steady-state condition at their respective swimming speed; during this period, the flow of water to the respirometers was maintained as described above. Following this period, a 40 ml sample of water was removed prior to sealing each respirometer for the final 2 h of the experiment, after which a final water sample was taken (i.e. at the end of the experiment). These water samples were quickly divided into three subsamples: (1) duplicate subsamples for CO_2 analysis were stored in glass vials which were quickly capped to prevent diffusive exchange of CO_2; (2) 15 ml samples for analysis of nitrogenous wastes were immediately frozen and stored at –20 °C; (3) oxygen (as P_{O_2}) was measured immediately on the balance of the water sample. Small water samples (5 ml) were also drawn periodically from the closed respirometer over this 2 h period for the measurement of P_{O_2}, and the experiment was terminated if the P_{O_2} dropped below 70 % saturation.

The P_{O_2} was measured on each water sample using a water-jacketed O_2 electrode (Cameron E101) coupled to a Cameron oxygen meter (OM-200) thermostatted to the appropriate acclimation temperature (i.e. 5 or 15 °C). Multiple P_{O_2} measurements on several subsamples of water indicated a high degree of accuracy (i.e. the coefficient of variation between samples was less than 2 %). Absolute oxygen consumption rates were calculated using the following formula:

$$\dot{M}_{O_2} = \frac{\Delta P_{O_2} \times \alpha_{O_2} \times V}{M \times t}, \quad (2)$$

where $\dot{M}_{O_2}$ is the molar rate of oxygen consumption (μmol O_2 g^{-1} h^{-1}), ΔP_{O_2} is the measured change in P_{O_2} values between the start and end of each oxygen consumption test period (mmHg; 1 mmHg=0.133 kPa), V is the volume of water in the respirometer (l), M is the mass of the fish (g), α_{O_2} is the solubility constant for O_2 in water (μmol l^{-1} $mmHg^{-1}$) (Boutilier *et al.* 1984) and t is time (hours). Analogous equations were used to calculate CO_2, ammonia and urea excretion rates. From these values of $\dot{M}_{O_2}$, $\dot{M}_{CO_2}$ and $\dot{M}_{nitrogen}$, the respiratory quotient (RQ), nitrogen quotient (NQ) and instantaneous fuel use (see below) were calculated.

The CO_2 content of the water samples was measured in duplicate using a Shimadzu GC-8A gas chromatograph equipped with a Poropak Q column and calibrated with a series of $NaHCO_3$ standards (0–3 mmol l^{-1} $NaHCO_3$ standards). The output was displayed on a Shimadzu-CR3A integrator. The coefficient of variation between duplicates (samples and standards) was less than 3 %.

Ammonia concentrations on the water samples were determined using the method of Verdouw *et al.* (1978). Water urea concentrations were determined using the method of Rahmatullah and Boyd (1980), with micro-modifications of the methodology outlined in Lauff and Wood (1996*a*).

Proximate body composition

Control fish (i.e. non-swum fish) were ground under liquid nitrogen. The resulting powder was freeze-dried. Total body protein, lipid, water content, glycogen, glucose and lactate (for details of the procedure, see Lauff and Wood, 1996*a*) were measured, and the sum of the latter three has been reported as total carbohydrate.

Fuel use calculations

The respiratory quotient ($RQ=\dot{M}_{CO_2}/\dot{M}_{O_2}$) and nitrogen quotient ($NQ=\dot{M}_{nitrogen}/\dot{M}_{O_2}$) were determined for each fish at each time (see Lauff and Wood, 1996*a*,*b*). $\dot{M}_{nitrogen}$ was calculated from the sum of the rates of ammonia-N plus urea-N excretion. Ammonia and urea represented the vast majority (at least 80 %) of N-waste products in these unfed fish (J. D. Kieffer and C. M. Wood, unpublished observations; for theoretical considerations, see also Lauff and Wood 1996*a*). Since the fish in our study were neither strictly ammoniotelic nor ureotelic (i.e. they excreted both ammonia and urea-N), traditional values of RQ representing protein use ($RQ_{protein}$) for 100 % ammoniotelism or 100 % ureotelism could not be used. $RQ_{protein}$ is dependent on the particular mixture of nitrogenous wastes produced and was therefore calculated for each set of fish according to the following equation:

$$RQ_{protein} = -0.001363u + 0.9729, \quad (3)$$

where u is the percentage of the N-wastes excreted as urea (Kleiber, 1987). $RQ_{protein}$ was found to be approximately 0.95 (all speeds) in fish acclimated to 15 °C and 0.94 (non-swimmers and slow-speed swimmers) and 0.93 (high-speed swimmers) in fish acclimated to 5 °C. The values employed for RQ_{lipid} and $RQ_{carbohydrate}$ were 0.71 and 1.0, respectively. Only values of $RQ \leqslant 1.0$ were used to determine instantaneous fuel use since an RQ of 1.0 represents the upper limit obtainable during aerobic metabolism (Kleiber, 1987). We found that only approximately 25 % of the data had to be discarded because the RQ values were greater than 1; this was mainly a problem for the resting fish, because of spontaneous activity.

Instantaneous fuel usage was then determined as follows:

$$P + L + C = 1.0, \quad (4)$$

where P, L and C represent the fraction of the total fuels supporting $\dot{M}_{O_2}$ from protein, lipid and carbohydrate, respectively. The protein component could be determined using:

$$P = NQ/0.27, \quad (5)$$

where P is the fraction supplied by protein and 0.27 is the theoretical maximum for NQ (Van den Thillart and Kesbeke, 1978) when all aerobic metabolism is fuelled by protein. The carbohydrate and lipid fractions could then be determined using the respective fuel-specific RQs (using low-speed swimming at 5 °C for illustration):

$$RQ = 0.94P + 1.0C + 0.71L. \quad (6)$$

Since:

$$L = 1.0 - P - C, \quad (7)$$

then, by substitution of equation 5 and 6 into equation 7:

$$RQ = 0.85NQ + 0.29C + 0.71. \quad (8)$$

RQ and NQ were determined in the experiment, so the equation can be solved for C; L can be then determined by difference. These percentage contributions to $\dot{M}_{O_2}$ could then be converted to percentages on the basis of carbon usage *via* the fuel-specific RQs (see Lauff and Wood, 1996*a*,*b*). The total carbon usage was then apportioned to the absolute carbon expenditures of the three fuel types using these carbon-based percentages and measured CO_2 excretion (see Lauff and Wood, 1996*a*).

Q_{10} calculations and statistics

Q_{10} was calculated using the formula provided by Prosser (1991):

$$Q_{10} = (k_2/k_1)^{10/(t_2-t_1)}, \quad (9)$$

where k_1 and k_2 are the rates of reaction (rate constants) at temperatures t_1 and t_2, respectively.

All values are presented as means ± S.E.M. (N). For these experiments, one-way analyses of variance (ANOVAs) were used to assess the significance of observed differences in both the 5 and 15 °C experiments. If the ANOVA indicated significance ($P \leqslant 0.05$), a Scheffe's multiple comparison test was then used to determine significant differences ($P \leqslant 0.05$) between the resting values and low-speed and high-speed swimming groups at each temperature. We also used two-way ANOVAs to compare directly the overall effect of acclimation temperature for any variable measured in these experiments. Unpaired t-tests were used to compare the 5 °C fish with the 15 °C fish at any swimming speed. Regressions were calculated by the method of least squares, using either untransformed or log-transformed data as appropriate, and the significance of Pearson's correlation coefficient (r) was assessed.

Results

The effect of water temperature and training on critical swimming speed

The critical swimming speeds (U_{crit}) of untrained trout were 2.01 BL s^{-1} and 3.04 BL s^{-1}, when acclimated to 5 °C or 15 °C, respectively (Fig. 1). Training at approximately 1 BL s^{-1} significantly improved the U_{crit} by approximately 30 % in 5 °C-acclimated fish, but did not modify the U_{crit} in 15 °C-acclimated fish (Fig. 1). U_{crit} values were not significantly different at 5 °C and 15 °C in trained fish.

Respirometry and excretion of nitrogen waste

Under control conditions (i.e. non-swimming), warm-acclimated fish had higher ($P<0.05$) oxygen consumption rates (μmol g^{-1} h^{-1}) than cold-acclimated fish. At 5 °C, $\dot{M}_{O_2}$ increased in an exponential fashion ($r=0.59$, $P<0.006$) from approximately 4 μmol g^{-1} h^{-1} under control conditions to approximately 7 μmol g^{-1} h^{-1} at the highest swimming speed. Trout acclimated to 15 °C also showed increased oxygen consumption rates with increases in swimming speed ($r=0.77$, $P<0.0001$): these values increased from 5.6 μmol g^{-1} h^{-1} under control conditions to approximately 10 μmol g^{-1} h^{-1} at the highest swimming speed (Fig. 2A). Overall, warm-acclimated fish consumed more oxygen (two-factor ANOVA, $P<0.0001$; Q_{10}=1.3–1.5, depending on the swimming speed; Table 2).

Carbon dioxide excretion rates ($\dot{M}_{CO_2}$) ranked in a similar order to the $\dot{M}_{O_2}$ values (Fig. 2B). Non-swimmers had significantly higher $\dot{M}_{CO_2}$ values at 15 °C than at 5 °C ($P<0.03$). Increases in swimming speed caused $\dot{M}_{CO_2}$ to rise from 3.5 μmol g^{-1} h^{-1} (resting values) to 5.7 μmol g^{-1} h^{-1} ($r=0.58$, $P<0.001$) at the highest swimming speed in cold-acclimated

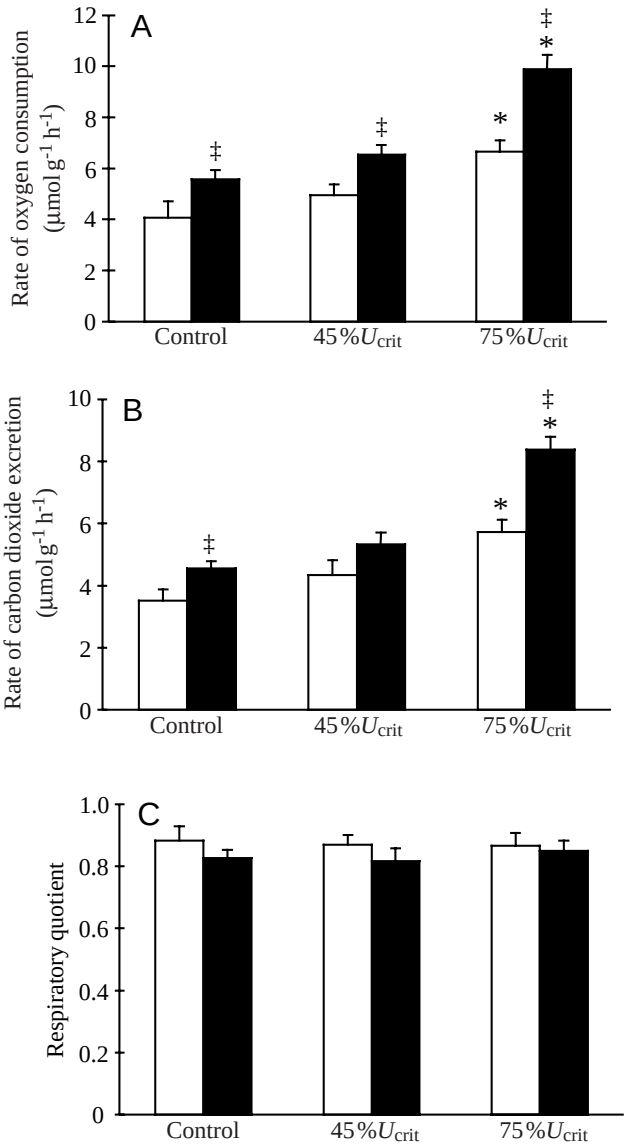

Fig. 2. Rates of oxygen consumption (A) and carbon dioxide excretion (B) and the respiratory quotient (C) of non-swimming (control), low-speed swimming (45 % U_{crit}) and high-speed swimming (75 % U_{crit}) juvenile rainbow trout acclimated to either 5 °C (open columns) or 15 °C (filled columns). Values are means + 1 S.E.M. See Table 1 for sample sizes. Asterisks indicate a significant difference from non-swimming controls at a particular acclimation temperature. Double daggers indicate a significant difference between 5 and 15 °C fish at a particular activity level.

Table 2. *The influence of acclimation temperature (i.e. Q_{10} values) on rates of excretion and consumption of various respiratory gases at various swimming speeds in juvenile rainbow trout*

	Q_{10} value		
	Non-swimming fish	45 % U_{crit}	75 % U_{crit}
Oxygen consumption	1.37	1.32	1.48
Carbon dioxide excretion	1.29	1.23	1.46
Ammonia excretion	1.4	2.88	2.84
Urea excretion	1.29	2.25	1.33

$Q_{10}=(k_2/k_1)^{10/(t_2-t_1)}$; after Prosser (1991), where k_1 and k_2 are rates of reaction (rate constants) at temperatures t_1 and t_2, respectively.

fish, and from 4.6 to 8.4 μmol g^{-1} h^{-1} (r=0.78, P<0.001) in warm-acclimated fish. Overall, warm-acclimated trout exhibited a significantly greater rate of CO_2 excretion compared with 5 °C-acclimated fish (two-factor ANOVA, P<0.0001; Fig. 2; Q_{10}≈1.2–1.5; Table 2).

Although significant relationships existed between swimming speed and both CO_2 excretion rates and O_2 consumption rates, as noted above, there was no influence of swimming speed on the respiratory quotient (RQ) in fish acclimated to either temperature (Fig. 2C). The mean RQ pooled across all experimental groups was 0.850±0.014 (N=46).

In general, urea-N excretion accounted for only 15–20 % of total nitrogenous waste excretion; $\dot{M}_{nitrogen}$ was stable (P>0.05) across all swimming speeds at cold temperatures, but higher and more variable at warmer temperatures (Fig. 3A,B). Despite a trend towards higher rates of ammonia-N and urea-N excretion at higher swimming speeds, there were no significant changes in $\dot{M}_{nitrogen}$ with increases in swimming speed (Fig. 3A,B). On average, warm-acclimated trout had higher ammonia excretion rates than cold-acclimated fish (two-factor ANOVA, P<0.001; Q_{10}=1.4–2.9, Table 2). Urea excretion rates were not influenced by acclimation temperature (Fig. 3B), except at 45 % U_{crit} where there was a tendency (P<0.07) for the rates to be temperature-dependent (Q_{10}=2.3; Table 2).

Nitrogen quotients (NQ) remained stable at approximately 0.08 across all swimming speeds in warm-acclimated trout (Fig. 4). However in cold-acclimated trout, increases in swimming speed caused the NQ to decrease significantly from 0.075 in control fish to approximately 0.04 at both low and high swimming speeds (Fig. 4). The significantly higher overall NQ of the 15 °C fish (two-factor ANOVA, P=0.025) (Fig. 4) arose from a protein use that represented approximately 30 % of the total fuel mixture based on $\dot{M}_{O_2}$. The protein use in the 5 °C-acclimated fish was similar to that of the 15 °C-acclimated fish under control conditions (approximately 30 %), but accounted for only approximately 15 % of the $\dot{M}_{O_2}$ at both swimming speeds (Fig. 4).

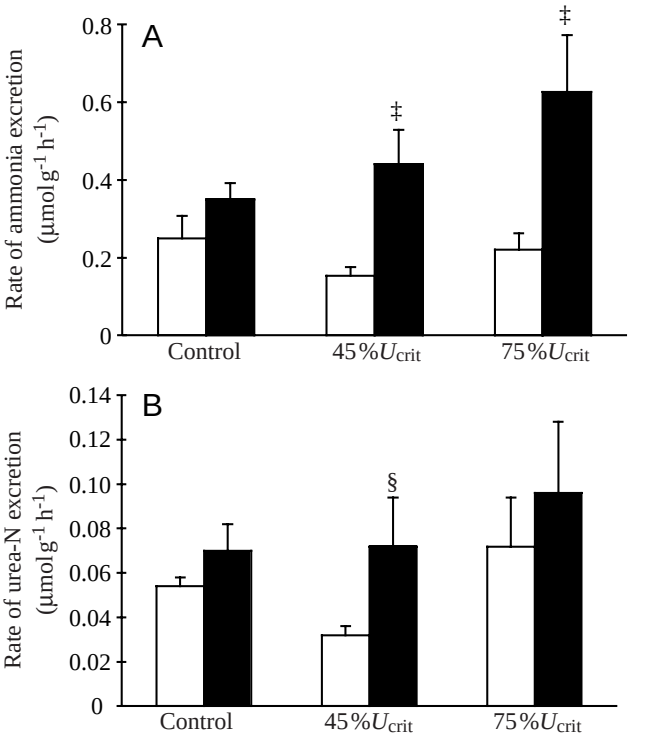

Fig. 3. Rates of ammonia excretion (A) and urea excretion (B) in non-swimming (control), low-speed swimming (45 % U_{crit}) and high-speed swimming (75 % U_{crit}) juvenile rainbow trout acclimated to either 5 °C (open columns) or 15 °C (filled columns). Values are means + 1 S.E.M. See Table 1 for sample sizes. Double daggers indicate a significant difference between 5 and 15 °C fish at a particular activity level. § indicates a near-significant difference (P=0.07).

Instantaneous fuel use

Trends in instantaneous metabolic fuel use for all three substrates are summarized in Fig. 5 in terms of their percentage contribution to $\dot{M}_{O_2}$. In warm-acclimated trout, lipid oxidation predominated, supporting 46–58 % of the total $\dot{M}_{O_2}$ at all swimming speeds (Fig. 5B). Carbohydrates made the smallest contribution (approximately 15 %) under control conditions and at low swimming speeds, but this increased to approximately 25 % at the fastest swimming speed. As noted above, the protein contribution to metabolism was stable (approximately 30 %) in warm-acclimated fish. In cold-acclimated fish, lipids were again an important fuel, but the relative importance was reduced by approximately 10 %. The contribution of lipids in 5 °C fish increased slightly from control values of 35 % to 42 % during swimming (Fig. 5A). The contribution of carbohydrates at 5 °C was much greater than at 15 °C and very comparable (approximately 42 %) to that of lipid at all swimming speeds. The contribution of protein decreased from 27 % in non-swimming fish to approximately 15 % in swimming fish (Fig. 5A). Overall, cold-acclimated fish relied on carbohydrates and lipids, whereas warm-acclimated fish relied mainly on lipids (compare Fig. 5A and Fig. 5B).

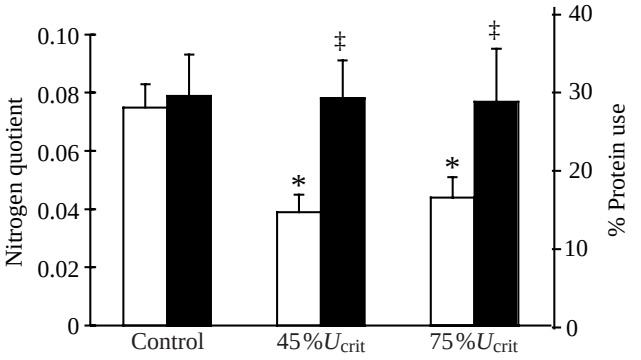

Fig. 4. Nitrogen quotient and protein use (expressed as a percentage of total fuel use, $\dot{M}_{O_2}$) in non-swimming (control), low-speed swimming (45% U_{crit}) and high-speed swimming (75% U_{crit}) juvenile rainbow trout acclimated to either 5 °C (open columns) or 15 °C (filled columns). Values are means + 1 S.E.M. See Table 1 for sample sizes. Asterisks indicate a significant difference from non-swimming controls at a particular acclimation temperature. Double daggers indicate a significant difference between 5 and 15 °C fish at a particular activity level.

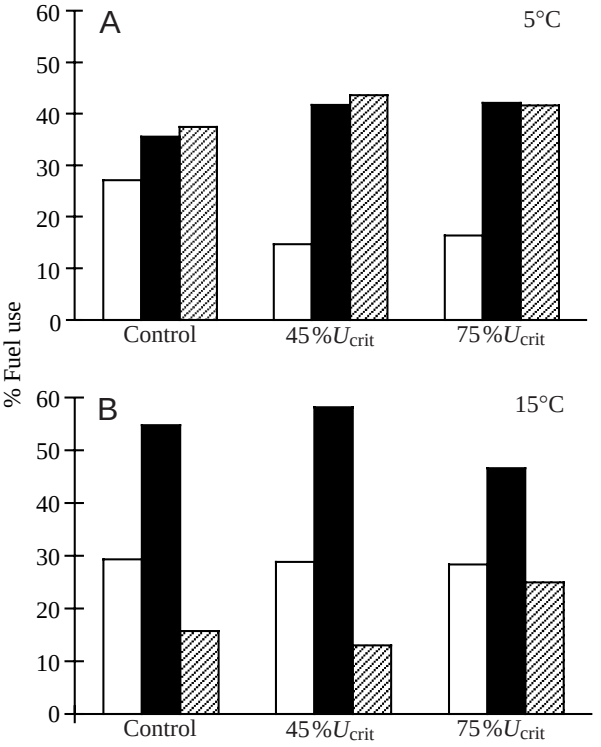

Fig. 5. Percentage use of protein (open columns), lipid (filled columns) and carbohydrates (hatched columns) in non-swimming (control), low-speed swimming (45% U_{crit}) and high-speed swimming (75% U_{crit}) juvenile rainbow trout acclimated to either 5 °C (A) or 15 °C (B). Percentages are expressed as a proportion of total $\dot{M}_{O_2}$.

At 5 °C, the rate of carbon use from proteins remained fairly constant (8–12 μg C g^{-1} h^{-1}) at different speeds, whereas at 15 °C it increased from 18 to 32 μg C g^{-1} h^{-1} with increasing speed (Fig. 6). The rates of carbon use from carbohydrates in warm and cold-acclimated fish were similar (approximately 12 μg C g^{-1} h^{-1}) under control conditions but, in general, increased with increases in swimming speed, especially at 5 °C (to 33 μg C g^{-1} h^{-1}). Overall, burning of carbohydrate carbon was slightly faster in the cold-acclimated fish (Fig. 6). Lastly, the rate of lipid carbon usage increased with increases in swimming speed in both groups of fish (i.e. from 12 to 25 μg C g^{-1} h^{-1} at 5 °C, from 26 to 38 μg C g^{-1} h^{-1} at 15 °C); typically, warm-acclimated fish used this fuel to a greater extent (1.5- to twofold higher) than their cold-acclimated counterparts (compare Fig. 6A and Fig. 6B).

Proximate body analysis

There were no significant differences in any parameter of body composition in control fish (non-swimming) between the two acclimation temperatures (Table 3). Water made up approximately 77% of the mass of the fish, with protein and lipids constituting most of the stored energy. Carbohydrates accounted for only a small fraction of the total energy metabolites stored in the fish (Table 3).

Discussion

Critical swimming speed and training effects

It is well-known that critical swimming speed (U_{crit}) is depressed at temperatures both below and above the optimal range (reviewed by Beamish, 1978; Johnston and Ball, 1997), so the lower U_{crit} value at 5 °C *versus* 15 °C in untrained trout

Table 3. *Body composition of juvenile trout acclimated to either 5 or 15 °C*

	Lipid (mg 100 mg^{-1} wet mass)	Total carbohydrate (mg 100 mg^{-1} wet mass)	Protein (mg 100 mg^{-1} wet mass)	Water (mg 100 mg^{-1} wet mass)
5 °C trout (N=9)	4.16±0.31	0.28±0.04	11.20±0.21	77.48±0.63
15 °C trout (N=10)	4.92±0.39	0.38±0.05	11.24±0.26	76.77±0.58

Total carbohydrate includes glucose, glycogen and lactate.
Values are means ± S.E.M.
Note that there were no significant differences between groups for any variable.

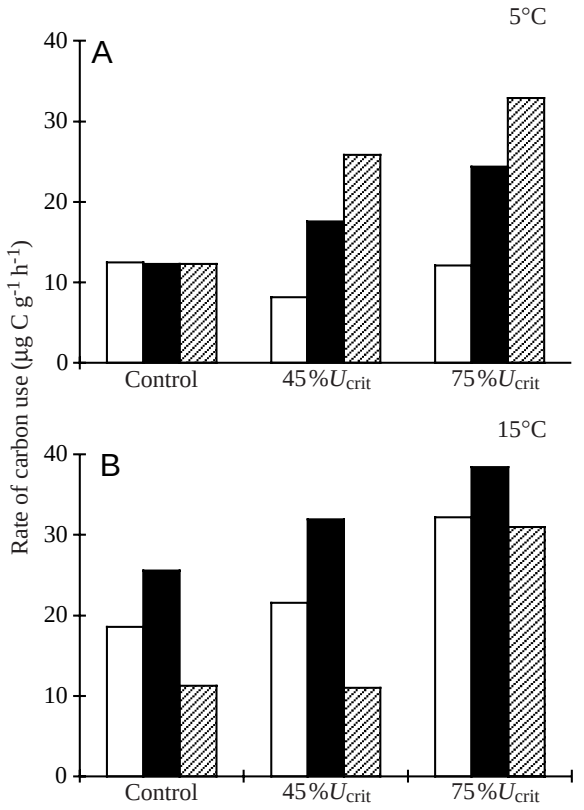

Fig. 6. The contribution of carbon from protein (open columns), lipid (filled columns) and carbohydrates (hatched columns) to total carbon use in non-swimming (control), low-speed swimming (45 % U_{crit}) and high-speed swimming (75 % U_{crit}) juvenile rainbow trout acclimated to either 5 °C (A) or 15 °C (B).

was expected (Fig. 1). However, more surprising was the fact that training enhanced U_{crit} at 5 °C but not at 15 °C. Interestingly, two studies on much larger brown trout (*Salmo trutta*), which may have been 'incidentally' trained by holding in a current at a slightly lower relative speed (approximately 0.6 BL s^{-1}), reported that U_{crit} was either slightly lower (Butler *et al.* 1992) or identical (Beaumont *et al.* 1995) at 5 °C relative to 15 °C, a pattern similar to that in the trained rainbow trout of the present investigation. The few recent studies on this topic have found either an increase or no change following training (Davison, 1997); however, we are aware of no previous data specifically on the effect of temperature on these relationships. In a recent review, Taylor *et al.* (1997) concluded that differences in energy supply, rather than mechanical efficiency, set the upper limit for swimming performance at different temperatures, but beyond this, it remains unclear which of the many aspects of energy supply set the critical limits. Since many aspects of energy supply are known to be improved by training (Davison, 1997), presumably one or more of these is critical at 5 °C, but not at 15 °C. From a practical point of view, the facts that fish acclimated to 5 °C or 15 °C had the same U_{crit} following training (Fig. 1) and that fish acclimated to both temperatures had the same amounts of 'onboard' metabolic fuels (Table 3) simplify interpretation of the present results.

The effects of swimming speed and temperature on gas exchange

The relationship between swimming speed and $\dot{M}_{O_2}$ in fish has been studied extensively (reviewed by Brett, 1995; Taylor *et al.* 1997). Our data (Fig. 2A) clearly agree with the observed pattern that $\dot{M}_{O_2}$ increases exponentially with swimming speed. However, there have been only a few studies where $\dot{M}_{CO_2}$ has been measured simultaneously with $\dot{M}_{O_2}$ in swimming fish. The present results (Fig. 2B,C) are in agreement with all previous studies on salmonids on this topic (Kutty, 1968; Van den Thillart, 1986; Lauff and Wood, 1996*b*, 1997) in showing that $\dot{M}_{CO_2}$ increases in proportion to $\dot{M}_{O_2}$ at different speeds, so that RQ changes little, if at all, and metabolism remains aerobic (i.e. RQ<1.0). Indeed, the absolute RQ values (approximately 0.85) and the absolute values of $\dot{M}_{O_2}$ and $\dot{M}_{CO_2}$ measured in the present trout at 15 °C were very similar to the values reported by Lauff and Wood (1996*b*, 1997) at similar swimming speeds in both trained and untrained trout. In contrast, Kutty (1972) observed that $\dot{M}_{CO_2}$ exceeded $\dot{M}_{O_2}$ (RQ≈1.2) in tilapia *Tilapia mossambica* during long-term swimming and concluded that this species resorts to anaerobic metabolism to partially fuel sustained exercise. This is clearly not the case in the rainbow trout.

Although temperature did influence $\dot{M}_{O_2}$ at the various swimming speeds in trout, we found only a small overall effect of temperature (i.e. Q_{10}) on these relationships (e.g. Q_{10}=1.3–1.5). This pattern is in general agreement with most previous studies where fish have been acclimated for long periods, as in the present investigation, and reflects the influence of the many compensatory processes that are homeostatic for metabolic rate (reviewed by Brett, 1995; Taylor *et al.* 1997). Similarly, acclimation temperature did not affect $\dot{M}_{CO_2}$ (Q_{10}=1.2–1.5) or the RQ (approximately 0.85).

In direct contrast to $\dot{M}_{O_2}$ and $\dot{M}_{CO_2}$, we found that the rate of nitrogenous waste excretion ($\dot{M}_{nitrogen}$) was not significantly increased by swimming speed (for both ammonia and urea) but was markedly sensitive to acclimation temperature (at least for ammonia: Q_{10}=1.4–2.9; Fig. 3A,B). The lack of a positive influence of swimming speed on $\dot{M}_{nitrogen}$ was entirely consistent with previous studies on rainbow trout (Lauff and Wood, 1996*b*; Alsop and Wood, 1997) and a freshwater catfish *Mystus armatus* (Sukumaran and Kutty, 1977). The positive influence of temperature on the rate of ammonia excretion is in agreement with the work of Kieffer and Tufts (1996) on adult rainbow trout (5 *versus* 18 °C; Q_{10}=1.4). In addition, A. J. Wiggs (unpublished data cited in Wiggs *et al.* 1989) found that the rate of ammonia excretion also increased at warmer temperatures in Atlantic salmon (*Salmo salar*). These increased ammonia excretion rates reflect a greater reliance on protein oxidation at warmer temperatures (see below).

Aerobic metabolic fuel use

The instantaneous fuel use approach employed in the present

study, which is based on respirometry, shows clearly that the relative and absolute importance of the three different fuels for aerobic metabolism (proteins, lipids and carbohydrates; Figs 5, 6) vary considerably as a function of both swimming speed and acclimation temperature. This occurs despite the fact that swimming capacity (Fig. 1) and the starting amounts of fuels 'on board' (Table 3) are both independent of temperature.

The relative contribution of protein was low (<30 %) under control conditions at both temperatures, and either did not change (at 15 °C) or decreased (at 5 °C) as swimming speed increased (Fig. 5). In terms of carbon usage, this reflected a modest increase with swimming speed at the higher temperature and essentially no change with swimming speed at the lower temperature (Fig. 6). These findings are very consistent with other recent studies on juvenile rainbow trout in our laboratory (Lauff and Wood, 1996*a*,*b*, 1997; Alsop and Wood, 1997). These data therefore oppose the traditional view, based largely on the compositional approach, that protein is a major aerobic fuel source that is exploited to an even greater extent by fish during exercise (see Introduction for references).

Furthermore, it is clear from our analysis that fish acclimated to colder temperatures utilize even less protein than warm-acclimated fish (Figs 5, 6), in accordance with our original hypothesis (see Introduction). There are no comparative data available in the literature. However, our results support a similar conclusion noted by Aldridge *et al.* (1995) for a mussel species (*Dreissena polymorpha*). One likely explanation is the well-known inhibitory effect of low acclimation temperature on protein synthesis and degradation rates in most tissues of teleost fish, including rainbow trout (McCarthy and Houlihan, 1997). Given the very high metabolic cost of protein synthesis in fish (more than 20 % of resting $\dot{M}_{O_2}$ at minimum; Smith and Houlihan, 1995) and the integral role of proteins as contractile units in locomotion, it seems reasonable that fish in general would try to conserve this important structural molecule, especially under low-temperature conditions.

Contrary to popular belief as to the unimportance of carbohydrate as an aerobic fuel in fish (see Introduction), carbohydrate made the largest relative contribution (approximately 40 %) to $\dot{M}_{O_2}$ at 5 °C (Fig. 5) and exhibited the greatest absolute increase in carbon usage during exercise (Fig. 6). At 15 °C, its contribution was much smaller but again increased on a relative and absolute basis at the highest swimming speed (Figs 5, 6). These data agree with other recent evidence, both indirect (Moyes and West, 1995) and direct (Lauff and Wood, 1996*b*, 1997), that the reliance on carbohydrates as an aerobic metabolic fuel increases with swimming speed, which in turn suggests that white muscle recruitment becomes more important with increases in swimming speed (Moyes and West, 1995). The much greater reliance on carbohydrate at 5 °C *versus* 15 °C as a fuel for both rest and exercise is in accordance with our original hypothesis (see Introduction) and seems entirely reasonable on the basis of enzymatic and muscle recruitment considerations. For example, Hochachka and Hayes (1962) have shown that cold acclimation increases the rates of glucose oxidation by the muscle of brook trout (*Salvelinus fontinalis*). In addition, Helly (1976) found that the rate of catabolism of carbohydrates *via* the pentose phosphate shunt pathway and the Krebs cycle was generally elevated in cold-acclimated relative to warm-acclimated fish. Guderley and Gawlicka (1992) found that cold-acclimated trout increased the activity of phosphofructokinase in their white muscle compared with warm-acclimated fish. In addition to the enzymatic data, electromyographic experiments indicate that, to generate equivalent muscle power at lower temperatures, fish must recruit more white muscle fibres (Rome *et al.* 1992). Furthermore, fish acclimated to low temperatures generally have a reduced volume mass of red muscle relative to white muscle (Johnston and Ball, 1997). It follows, therefore, that they would probably utilize more carbohydrates, since the white muscle represents the largest deposit of glycogen in the body. It is well known that fish are able to exploit this store as a metabolic fuel during burst-type exercise (Wood, 1991; Kieffer *et al.* 1994).

The high reliance on lipid as an energy source both at rest and during exercise at both temperatures (35–58 %; Fig. 5) and its greater absolute importance during aerobic swimming (Fig. 6) are consistent with most literature reports based on both instantaneous and compositional methods (Driedzic and Hochachka, 1978; Moyes and West, 1995; Brett, 1995; Lauff and Wood, 1996*b*, 1997). On the basis of enzyme assays and other *in vitro* tests (Dean, 1969; Jones and Sidell, 1982; Guderley and Gawlicka, 1992; Thibault *et al.* 1997; Cordiner and Eggington, 1997), we had hypothesized that lipid use should increase with cold acclimation (see Introduction). To the contrary, we found that cold-acclimated fish used a slightly smaller proportion of lipids at rest and during aerobic swimming compared with warm-acclimated fish (Figs 5, 6). Although these temperature-related differences were not enormous (averaging 10–15 %), the differences between the results of our study and those of Guderley and Gawlicka (1992) may be the result of comparing whole-animal metabolism with enzymatic profiles of specific tissues.

In conclusion, our results in general support the concept that lipids are a major fuel for aerobic exercise in fish but demonstrate that the contribution of protein oxidation is much smaller than commonly believed, while that of carbohydrate oxidation is much larger, especially at higher swimming speeds and colder temperature. Given the large effects of acclimation temperature on the patterns of metabolic fuel use in trout, it would be interesting to subject warm-acclimated and cold-acclimated trout to an acute temperature change to determine whether fuel use patterns are 'fixed' by acclimation history or labile according to moment-to-moment conditions.

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