The Effects of Temperature and Swimming Speed on Instantaneous Fuel Use and Nitrogenous Waste Excretion of the Nile Tilapia

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ABSTRACT

The effects of acclimation temperature (30°, 20°, and 15°C) and swimming speed on the aerobic fuel use of the Nile tilapia (*Oreochromis niloticus*; 8–10 g, 8–9-cm fork length) were investigated using a respirometric approach. As acclimation temperature was decreased from 30°C to 15°C, resting oxygen consumption (Mo_2) and carbon dioxide excretion (Mco_2) decreased approximately twofold, while nitrogenous waste excretion (ammonia-N plus urea-N) decreased approximately fourfold. Instantaneous aerobic fuel usage was calculated from respiratory gas exchange. At 30°C, resting Mo_2 was fueled by 42% lipids, 27% carbohydrates, and 31% protein. At 15°C, lipid use decreased to 21%, carbohydrate use increased greatly to 63%, and protein use decreased to 16%. These patterns at 30°C and 15°C in tilapia paralleled fuel use previously reported in rainbow trout acclimated to 15°C and 5°C, respectively. Temperature also had a pronounced effect on critical swimming speed (U_{Crit}). Tilapia acclimated to 30°C had a U_{Crit} of 5.63 ± 0.06 body lengths/s (BL/s), while, at 20°C, U_{Crit} was significantly lower at 4.21 ± 0.14 BL/s. Tilapia acclimated to 15°C were unable or unwilling to swim. As tilapia swam at greater speeds, Mo_2 increased exponentially; Mo_2min and Mo_2max were 5.8 ± 0.6 and 21.2 ± 1.5 μmol O_2/g/h, respectively. Nitrogenous waste excretion increased to a lesser extent with swimming speed. At 30°C, instantaneous protein use while swimming at 15 cm/s (~1.7 BL/s) was 23%, and at U_{Crit} (5.6 BL/s), protein use dropped slightly to 17%. During a 48-h swim at 25 cm/s (2.7 BL/s, ~50% U_{Crit}), Mo_2 and urea excretion remained unchanged, while ammonia excretion more than doubled by 24 h and remained elevated 24 h later. These results revealed a shift to greater reliance on protein as an aerobic fuel during prolonged swimming.

Introduction

Members of genus *Oreochromis* exist in a wide range of water temperatures. They have been found to thrive at temperatures hotter than that of the mammalian body (Coe 1966; Narahara et al. 1996) yet can also survive temperatures as low as 11°C (Kindle and Whitmore 1986). In other species, acclimation to colder temperatures has been shown to greatly affect physiological and biochemical homeostasis. Cellular alterations such as increased activities of key oxidative enzymes (Sidell 1980; Johnston and Dunn 1987) and increased density of mitochondria and lipid droplets (which increases O_2 storage and diffusivity) have been observed with lower acclimation temperature (Egginton and Sidell 1989). Also, changes at the organ level occur, such as decreased cardiac output (reviewed by Farrell [1997]), decreased blood flow to all organs except red muscle (Taylor et al. 1993, 1996; Wilson and Egginton 1994), and increased amounts of red muscle (Sidell 1980). Whole-animal effects such as altered behavior (reviewed by Crawshaw and O'Connor [1997]) and decreased swimming performance (reviewed by Beamish [1978] and Johnston and Ball [1997]) at lower temperature have also been observed.

Considering the wide-ranging temperature tolerance of tilapia, one focus of this study was to investigate the influence of temperature on resting metabolism by examining its effect on oxygen consumption (Mo_2), carbon dioxide excretion (Mco_2), and nitrogenous waste excretion (M_{Nwaste}; ammonia-N plus urea-N). With these measurements, and the theoretical basis developed by Lauff and Wood (1996*a*) based on the original derivation of Kleiber (1987, 1992), our goal was to stoichiometrically calculate the instantaneous aerobic use of lipid, carbohydrate, and protein as metabolic fuels at different temperatures. In brief, this method employs respiratory quotients (Mco_2/Mo_2) and nitrogen quotients (M_N/Mo_2) to calculate the particular combination of fuels that are being oxidized at the time of the measurements, when the fish are undergoing aerobic metabolism in a steady state condition. Simonson and DeFronzo (1990) provide additional methodological information.

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Recently, we have shown that acclimation temperature has a marked effect on aerobic fuel use of the cold-water species rainbow trout (*Oncorhynchus mykiss*); a decrease in acclimation temperature from 15°C to 5°C resulted in an increase in carbohydrate use (from 15% to 38% of the total fuel used for aerobic metabolism) and a decrease in lipid use (from 55% to 35%), while protein use remained relatively the same (27%–30%; Kieffer et al. 1998). In this study, our objective was to examine whether or not the fuel-use patterns of warm-water and cold-water species are similar and if reliance on fuels changes in a similar manner with temperature. Optimum temperature for Nile tilapia is 25°–30°C (George 1996), while that for rainbow trout is 15°C (Elliot 1982). Experiments were conducted at three temperatures: 30°, 20°, and 15°C. Critical swimming speed (U_{Crit}) was also determined at these temperatures since changes in swimming capacity are likely to reflect intrinsic differences in energy supply and demand, as opposed to efficiency of locomotion (Taylor et al. 1997). Finally, the effects of swimming speed and swimming duration on the metabolic rate and aerobic protein use were investigated, in light of the interesting observations of Kutty (1972), where *Tilapia mossambica* excreted greater amounts of ammonia with time, while swimming for long periods.

Material and Methods

Fish Care

One hundred Nile tilapia (*Oreochromis niloticus*; 8–10 g, 8–9-cm fork length at time of testing) were obtained from Northern Tilapia (Lindsay, Ont.) and kept in a 400-L polyethylene tank at 30°C (±0.5°C) with flowing dechlorinated, fully aerated Hamilton city tap water. The water had the following ionic composition (in mM): $Ca^{++} = 1.0$, $Mg^{++} = 0.2$, $Na^{+} = 0.6$, $Cl^{-} = 0.7$, $K^{+} = 0.05$, titratable alkalinity to pH 4.0 = 1.9 mM, total hardness = 120 mg/L as $CaCO_3$, pH = 8.0. The photoperiod was 12L : 12D. Tilapia were fed to satiation three times per week using a commercial feed (fish food composition [partial analysis only]: crude protein [min], 52%; crude fat [min], 17%; crude fibre [max], 2.5%; water, 12%; Ca^{2+}, 1.4%; Na^{+}, 0.4%).

Experimental Protocol

Effect of Acclimation Temperature on Fuel Use. The 100 tilapia were divided between three 70-L tanks with water temperature set at 30°C (±0.5°C). The following week, the temperature of one tank was left unchanged, while that of the other two tanks was decreased by 1°C/d until one reached 20°C (±0.5°C) and the other 15°C (±0.5°C). Water flow into each tank was 0.5 L/min, and Po_2 was maintained at >90% air saturation. Fish were allowed to acclimate to their respective temperatures for at least 3 wk before testing.

Fish at 15°, 20°, and 30°C were not fed for 4, 3, and 2 d, respectively, before testing, to eliminate possible dietary influences on metabolite status (Brett 1964; Beamish 1978; Tang and Boisclair 1995). All experiments were performed using 3.23-L, variable-speed Blazka-type respirometers similar to those described by Beamish et al. (1989). The respirometers could be run as open systems with fresh, aerated water continuously flowing through them, or as closed systems, during which time the respirometric measurements were made. The respirometers received inflowing water from a temperature-controlled reservoir and were maintained at the required temperature by submerging them in a temperature-controlled wet table.

The day before the tests were run, the reservoir system and wet table were adjusted to the desired test temperature (i.e., 15°, 20°, or 30°C), four tilapia were removed from the acclimation tank at that same temperature, and each fish was placed in a separate respirometer. The fish were allowed to adjust to the respirometers overnight with the water velocity set at 5 cm/s, a speed that did not induce swimming but that allowed the tilapia to remain resting on the bottom of the respirometer. The respirometers received 0.4 L/min of air-saturated water during this time.

The following morning, water flow to the respirometers was shut off, duplicate 20-mL water samples were taken from the respirometers and frozen at −20°C for later analysis of nitrogenous waste products (ammonia and urea), and duplicate 4-mL water samples were placed in sealed glass vials and submerged in the wet table for later analysis of total CO_2 content. A 3-mL sample of water was withdrawn from each respirometer every 15–30 min for immediate Po_2 measurement with a thermostatted Cameron E101 oxygen electrode connected to a Cameron OM-200 oxygen meter. Oxygen concentrations were not allowed to fall below 70% of the air-saturated level. At the end of the trial, duplicate 4-mL water samples were taken again for total CO_2 analysis and submerged in the wet table, and another 20-mL sample was taken and frozen for nitrogenous waste-product analysis. The fish was then anaesthetized with MS222 (0.2 g/L), blotted dry, and weighed to the nearest 0.01 g, and fork length was measured to the nearest mm. The duration of the experiments was 1.5–4 h, depending on the water temperature of the trial. The stored water samples were then analyzed for total CO_2 as parallel "start"-"end" duplicates. Tests showed that this analytical approach yielded the most accurate estimates of CO_2 production and that there were no changes in CO_2 content during storage of the samples.

Blank tests were carried out to determine the contribution of bacterial processes to measured O_2 consumption, CO_2 excretion, and N-waste excretion. The measured rates in the absence of the fish amounted to about 5% of the measured O_2 consumption and CO_2 excretion in the presence of the fish and a negligible percentage of measured N-waste excretion; these values were considered within the error of the measurement, and corrections were not applied.

Effect of Acclimation Temperature on Swimming Performance. Critical swimming speed tests (Brett 1964) were performed at the same temperatures as the fuel-use study: 15°, 20°, and 30°C. Again, fish were transferred from the holding tanks to the respirometers the day before the tests were performed and allowed to adjust overnight. On the following day, the U_{Crit} test was started by slowly increasing the water velocity over 1 min to the first speed of the trial: 15 cm/s. Following this period, the water velocity was increased by 10 cm/s increments over 1 min every 60 min until the fish became exhausted. Fish were considered exhausted once they impinged on the rear screen and would not swim after the water velocity was temporarily lowered and then returned to the speed at which exhaustion occurred. Fully aerated water was continuously flowing through the respirometers at all times (0.4 L/min). After exhaustion, the fish were anesthetized with MS222, blotted dry, weighed, and measured.

Effect of Swimming Speed on O_2 Consumption and N-waste Excretion. The U_{Crit} test was repeated at 30°C, and, at each swimming speed (15, 25, 35, 45, and 55 cm/s), oxygen consumption and nitrogenous waste excretion were measured. After the speed was slowly increased over 1 min to the next swimming speed, fish were allowed 2 min to become accustomed to the new speed, then water flow to the respirometers was shut off for 45 min to conduct the measurements. A 20-mL water sample was taken at the beginning and end of this 45-min period and frozen at −20°C for later analysis of nitrogenous waste products. Water samples for O_2 analysis were also drawn at 15-min intervals and measured immediately as above. The respirometers were flushed with fully air-saturated water for the last 13 min of each swimming speed period to prevent the oxygen levels from dropping below 70% of air saturation.

Effect of Prolonged Swimming on O_2 Consumption and N-waste Excretion. A long-term swimming test was performed with tilapia acclimated to 30°C. Fish were placed in the respirometers the night before. The following morning, the speed was slowly brought up to 25 cm/s (2.74 body lengths/s [BL/s], approximately 50% U_{Crit}) and the respirometers were sealed off for oxygen consumption and nitrogenous waste excretion measurements between 1 and 2 h, 24 and 25 h, and 48 and 49 h. Water flowed continuously through the respirometers (0.4 L/min) during the rest of the swimming trial.

Analytical Procedures

For the experiments involving CO_2 measurements, stored water samples were analyzed immediately after the end of the experiment, using a Shimadzu GC-8A gas chromatograph equipped with a Poropak Q column. A series of $NaHCO_3$ standards were prepared in the 0–3 mM range. The output was displayed on a Shimadzu-CR3A integrator.

Water ammonia concentrations were determined by the method of Verdouw et al. (1978). To determine the small differences in urea concentrations, resolution was increased fivefold by freeze-drying 5 mL of each sample, then reconstituting the freeze-dried product to 1 mL. The urea concentration was then determined by the method of Rahmatullah and Boyd (1980).

Calculations

Respiratory Gas Exchange and N-waste Excretion. The following formula was used to calculate the absolute O_2 consumption rate from Po_2 measurements:

$$Mo_2 = \Delta Po_2(\text{torr}) \times \alpha o_2(\mu\text{mol/L/torr}) \times \text{vol(L)/mass(g)} \times \text{time(h)}, \quad (1)$$

where ΔPo_2 is the measured change in Po_2 values, vol is the volume of water in each respirometer (3.23 L), mass is the mass of each fish, and αo_2 is the solubility constant for O_2 in water (Boutilier et al. 1984). An analogous equation was used to calculate the excretion rates of CO_2 and the two nitrogenous waste products.

Log O_2 consumption versus linear swimming velocity regressions for individual fish (Wilson et al. 1994, Fig. 1) were used to estimate Mo_2 values at 0 cm/s (basal metabolic rate, Mo_2min) and U_{Crit} (Mo_2max). All regression relationships were significant ($P < 0.05$), and r values ranged from 0.863 to 0.983. N-waste excretion (which did not follow an exponential relationship) was estimated at U_{Crit} by interpolation or extrapolation, using the last two measurements for each individual fish.

Fuel Use. The instantaneous aerobic fuel use was calculated as outlined by Lauff and Wood (1996*a*). In brief, the nitrogen quotient (NQ) was first calculated as

$$NQ = M_{NWaste}/Mo_2. \quad (2)$$

The variable M_{Nwaste} is the total sum of ammonia-N and urea-N. Urea-N excretion is simply two times the urea excretion rate caused by the two nitrogen atoms per urea molecule. The contribution of protein (P) to fuel aerobic metabolism (oxygen consumption) was then determined as:

$$P = NQ/0.27, \quad (3)$$

where 0.27 is the theoretical maximum for NQ in a teleost fish when all aerobic metabolism is fueled by protein (van den Thillart and Kesbeke 1978).

Since these fish excreted both ammonia and urea-N, the respiratory quotient (RQ) of protein is dependent on the mix-

ture of these two nitrogenous waste products produced and therefore must be calculated for each set of fish with the following equation:

$$RQ_{protein} = 0.9729 - 0.001363U, \quad (4)$$

where U is the percentage of N-waste excreted as urea (Kleiber 1987). The temperature range of this study had a small effect on the contribution of urea to total M_{Nwaste}, but not enough to alter $RQ_{protein}$, which was 0.94 at all three acclimation temperatures. The values used for RQ_{lipid} and $RQ_{carbohydrate}$ were 0.71 and 1.0, respectively (Simonson and DeFronzo 1990).

The carbohydrate (C) and lipid (L) fractions could then be determined using the fuel-specific RQs:

$$RQ = P \times 0.94 + C \times 1.0 + L \times 0.71. \quad (5)$$

Since

$$L = 1.0 - P - C, \quad (6)$$

then to solve for C,

$$RQ = 0.85 \times NQ + 0.29 \times C + 0.71. \quad (7)$$

With both C and P calculated, equation (6) can then be solved for L.

The above calculations provide the percentage contributions of each individual fuel type to Mo_2. These values were then converted to percentages based on carbon (C) usage via the fuel-specific RQs. The total C usage rates (as reflected in Mco_2 data) were then calculated using the C-based percentages (see Lauff and Wood 1996*a*).

U_{Crit}. The critical swimming speed (U_{Crit}) was determined for each fish, using the equation given by Brett (1964):

$$U_{Crit} = V_f + [(T/t) \times dV], \quad (8)$$

where U_{Crit} is in cm/s, V_f is the velocity prior to the velocity at which exhaustion occurred (the last velocity that was swum for the entire 60-min period), dV is the velocity increment (10 cm/s), t is the time swum at each velocity (60 min), and T is the time swum at the final velocity before exhaustion. Swimming velocities were not corrected for the solid blocking effect because the cross-sectional area of the fish was no greater than 10% of the cross-sectional area of the swimming tube (Jones et al. 1974).

Q_{10}. Q_{10} was calculated using the formula from 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.

Statistical Analyses

Data are expressed as means ± 1 SEM (number of fish). Differences between treatments were tested for significance with a one-way ANOVA. If the result of the ANOVA was significant, a Student-Newman-Keuls test for multiple comparisons was applied to test for significant differences among treatments. The limit of significance was 5%. In addition, the relative effects of temperature, weight, Mo_2, Mco_2, M_{Amm}, and M_{Urea} on each of resting Mo_2, resting Mco_2, resting M_{Amm}, and resting M_{Urea} were modelled using a stepwise multiple linear regression analysis via the statistical program SPSS (1997). This analysis yielded the proportion of the explained variance (r^2) contributed by each factor in the individual models. This analysis was also performed using swimming speed as an additional variable and omitting Mco_2 (not measured) for the exercise results.

Results

Temperature

With a decrease in acclimation temperature from 30°C to 15°C, resting Mo_2 decreased by 55%, from 6.43 to 2.91 μmol/g/h; Mco_2 dropped 52%, from 5.55 to 2.69 μmol/g/h; and total M_{Nwaste} dropped 76%, from 0.547 to 0.130 μmol N/g/h (Fig. 1). Respiratory quotients therefore rose with decreasing temperature, from 0.86 at 30°C to 0.93 at 15°C. Acclimation temperature had a small effect on the ratio of ammonia : urea excretion. The proportion of urea to the total N-waste excretion increased, from 19% at 30°C to 26% at 15°C (Fig. 1*B*). Nitrogen quotients (NQ) decreased with decreased acclimation temperature, from 0.085 at 30°C to 0.045 at 15°C. The Q_{10}'s for ammonia excretion (2.7–2.8) were clearly higher than those for Mo_2 and Mco_2 (1.6–2.0), while those for urea excretion were intermediate (1.8–2.2; Table 1). Temperature explained 79% of the variance in resting Mo_2, while Mco_2 explained a further 4% of the variance and 52% of the variance of resting Mco_2 was explained by Mo_2. Temperature explained 70% of the variance on resting M_{Amm}, while Mco_2 added a further 4% and 33% of the variance in resting M_{Urea} was explained by M_{Amm}. No other variables were significant in the individual stepwise multiple regression models.

As acclimation temperature decreased, there were marked changes in instantaneous aerobic fuel usage. Lipid use changed from 42% to 55% to 21% at 30°, 20°, and 15°C, respectively, carbohydrate use increased from 27% to 27% to 63%, and protein use decreased from 31% to 18% to 16% (Fig. 2*A*).

Similar trends were observed when fuel use was calculated as absolute carbon usage. With a decrease in acclimation temperature from 30° to 20° to 15°C, lipid C usage dropped from 1.91 to 1.59 to 0.43 μmol C/g/h, carbohydrate C usage remained

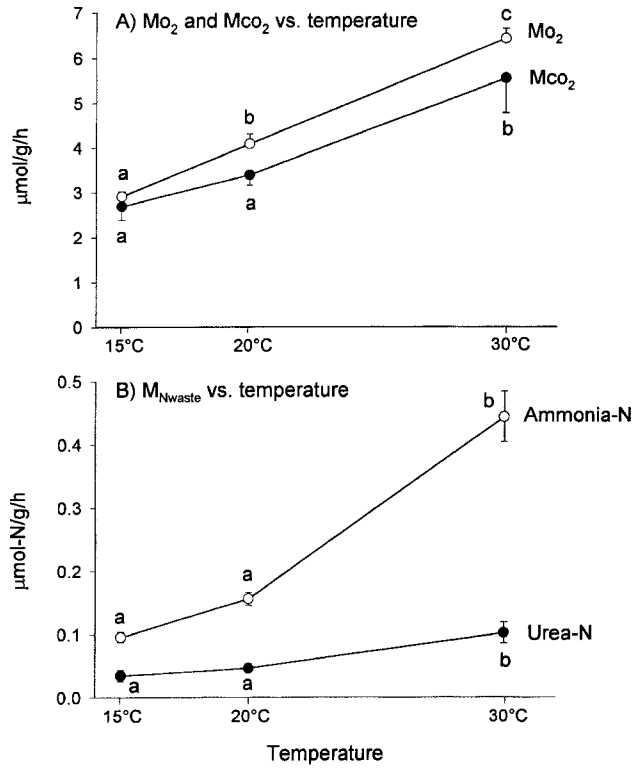

Figure 1. (*A*) Oxygen consumption (*open circles*) and carbon dioxide excretion (*filled circles*) and (*B*) nitrogenous waste excretion (ammonia [*open circles*] and urea [*filled circles*]) at three acclimation temperatures for tilapia at rest. Values are expressed as means ± SEM. At 15°, 20°, and 30°C, $n = 10$, 16, and 15, respectively. Points bearing the same letter are not significantly different ($P < 0.05$).

relatively constant at 1.73, 1.09 and 1.82 μmol C/g/h, and protein C usage dropped from 1.91 to 0.71 to 0.45 μmol C/g/h (Fig. 3).

Temperature and Swimming Performance

As temperature decreased, so did swimming performance, as seen by the reduction in U_{Crit}. At 30°C, U_{Crit} was 5.63 ± 0.06 BL/s, or 49.06 ± 1.03 cm/s, while at 20°C, U_{Crit} was significantly lower at 4.21 ± 0.14 BL/s, or 40.18 ± 1.43 cm/s. At 15°C, fish could not or would not swim for any length of time, and thus U_{Crit} could not be assessed for tilapia at this temperature.

Swimming and Respirometry

Both Mo_2 and M_{Nwaste} were assessed at different swimming speeds in tilapia at 30°C. Increasing swimming speed increased Mo_2 exponentially (Fig. 4*A*). The Mo_2max (21.18 μmol/g/h) was 3.7-fold greater than Mo_2min (5.78 μmol/g/h; Table 2). At the swimming speed above U_{Crit} (55 cm/s, a swimming velocity that only five of the eight fish were able to sustain long enough to measure Mo_2 and M_{Nwaste}), Mo_2 was 31% greater (27.66 μmol/g/h) than mean Mo_2max at U_{Crit}. As swimming speed increased, ammonia excretion rose from 0.298 μmol N/g/h at 15 cm/s, peaked at U_{Crit} at 0.592 μmol N/g/h, and dropped 18% to 0.485 at the fastest swimming speed of 55 cm/s (Fig. 4*B*). Urea excretion increased steadily with swimming speed from 0.185 μmol/g/h at 15 cm/s to 0.387 μmol/g/h at 55 cm/s (Fig. 4*B*). Of the total M_{Nwaste}, ammonia typically accounted for 55%–65% and urea made up the remaining N-excretion measured. During exercise, 74%, 53%, and 13% of the variance in Mo_2, M_{Amm}, and M_{Urea}, respectively, were explained by swimming speed. No other variables were significant in the individual stepwise multiple regression models.

Protein contributed 23% to aerobic fuel use at 15 cm/s and slowly decreased to 19% as swimming speed increased to 45 cm/s. At U_{Crit} (49.1 cm/s), protein contribution was 17% (Fig. 5). This may be compared with the resting value of 31% (Fig. 2*A*).

In the prolonged swimming trial at 25 cm/s (2.7 BL/s; approximately 50% U_{Crit}), Mo_2 and urea excretion remained constant over the 49-h period (Fig. 6). Ammonia excretion, however, increased 137%, from 0.368 μmol/g/h at 1–2 h to 0.873 μmol/g/h at 24–25 h, and remained at this elevated level at 48–49 h (Fig. 6*B*). This increased ammonia excretion in the first 24 h of swimming translated into an increase in protein use from 19.9% at 1–2 h to 32.8% at 24–25 h and 35.3% at 48–49 h (Fig. 7).

Discussion

Temperature

Decreasing acclimation temperature from 30°C to 15°C resulted in a decrease in total fuel use by approximately 55% in the Nile tilapia (indicated by Mo_2). However, the relative contributions of protein, lipid, and carbohydrate also changed. Relative protein and lipid use decreased, while relative carbohydrate use increased greatly. On an absolute basis, the decreased metabolism of both lipid-C and protein-C at lower temperatures was particularly marked.

A frequent observation of cold acclimation is an increase in plasma glucose concentration (Smit et al. 1981; Kindle and Whitmore 1986; Sun et al. 1992). Sun et al. (1992) found an increase in plasma glucose in tilapia exposed to 14°C, in comparison with those tilapia exposed to 28°C, over 86 h, though lactate was lower in 14°C-exposed tilapia. Kindle and Whitmore (1986) observed a greater than fivefold increase in plasma glucose in *Tilapia aurea* at 11.5°C, relative to 35°C. Relative carbohydrate use in this study increased 2.3-fold as temperature decreased from 30°C to 15°C. In addition, glycogen levels were observed to increase in the liver and cardiac muscle in rainbow trout acclimated to 5°C , as opposed to those trout exposed to 18°C (Dean 1969).

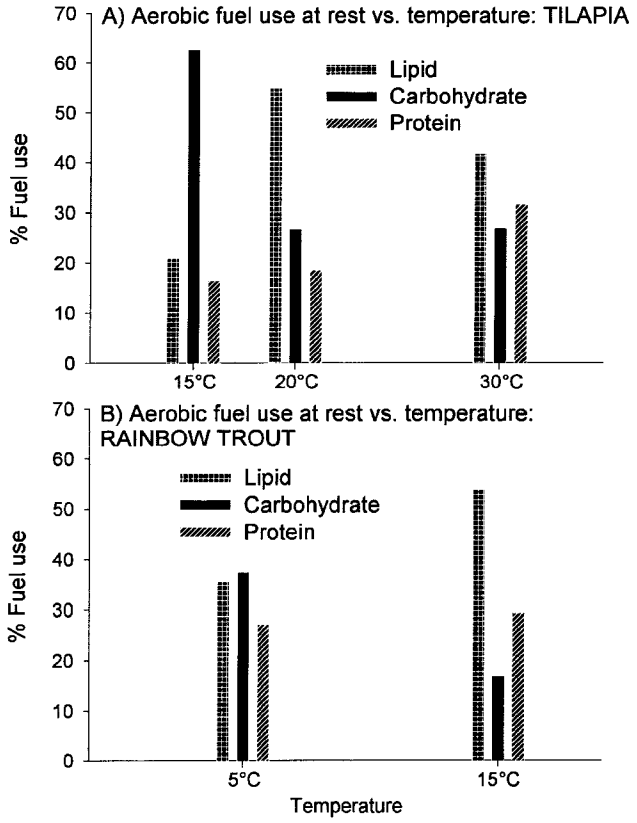

Figure 2. Percentage contribution of lipid (*grid-patterned bars*), carbohydrate (*solid bars*), and protein (*hatched bars*) to aerobic metabolism at rest (oxygen consumption) at three acclimation temperatures for (*A*) tilapia (current study) and (*B*) rainbow trout (from Kieffer et al. 1998).

Many studies have suggested that cold acclimation increases lipid oxidation. These conclusions were drawn from measurements of rate-limiting enzymes representative of carbohydrate and lipid use (glycolysis and mitochondrial β-oxidation, respectively; Crockett and Sidell 1990; Hicks et al. 1996; Patey and Driedzic 1997) or uptake of radiolabelled glucose or lipids (Dean 1969; Bailey and Driedzic 1993). The instantaneous fuel-use approach, however, is an in vivo, nonterminal technique, measuring fuels that are actually being burned at the time of the measurement, discounting interconversion of fuels and excretion of unburned fuels. Its theoretical basis has been discussed extensively by Lauff and Wood (1996*a*, 1996*b*).

When the fuel-use patterns of the cold-water species rainbow trout (Fig. 2*B*, from Kieffer et al. 1998) and the warm-water species in this study (Fig. 2*A*) are compared, an interesting pattern emerges: the fuel-use signatures are similar at the optimum temperatures of the two species, not at the same temperature. Optimum temperature for growth of Nile tilapia is 25°–30°C (George 1996) or, more specifically, 28°C (Northern Tilapia, Lindsay, Ont., personal communication), whereas optimum temperature for rainbow trout is 15°C (Elliot 1982). Resting Mo_2 for fasted tilapia at 30°C in this study is only 15% greater than for resting, fasted trout at 15°C (from Kieffer et al. 1998; trout 5.6 μmol O_2/g/h, tilapia 6.4 μmol O_2/g/h). Also, tilapia at 30°C have a basal metabolic rate only about 20% greater than similarly sized rainbow trout at 15°C (Alsop and Wood 1997). This suggests that fish at their optimum temperatures have similar basal metabolic demands, and the similarity in the fuel-use pattern of tilapia and rainbow trout at their optimum temperatures may be caused by similar basal turnover rates of the respective fuels. In addition, as the fish were acclimated to colder temperatures approaching their lower tolerance limits, the fuel-use signature changed similarly in both species: a decrease in protein and lipid use and a large increase in carbohydrate use. Decreasing temperature may pose similar metabolic restraints on fish, regardless of their absolute thermal tolerance range.

Our Mo_2 measurements are similar to those of Farmer and Beamish (1969) for *Oreochromis niloticus*, and our M_{Nwaste} measurements are similar to those of Wright (1993) on this same species at comparable temperatures. Also, the metabolic rate changes brought about by changes in acclimation temperature are similar to those recorded by McKenzie et al. (1996), working with *O. niloticus*, and Caulton (1977) working with *Tilapia rendalli* Boulenger. However, differences arise in comparison to Kutty (1972), who was working with *Tilapia mossambica* acclimated to 30°C. Kutty (1972) found that tilapia had a routine RQ of 1.03 $\pm$ 0.05 and an M_{Amm} of greater than 1 μmol/g/h. Without urea excretion measured or taken into consideration, *T. mossambica* had an ammonia quotient (AQ) of 0.23, almost 3.5 times greater than the AQ of the tilapia in this study.

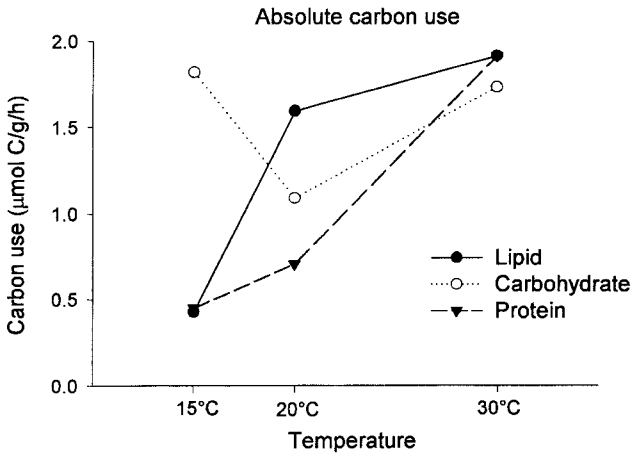

Figure 3. Absolute carbon use from lipid (*filled circles*), carbohydrate (*open circles*), and protein (*filled triangles*) for tilapia at three acclimation temperatures.

This high AQ indicates a relative protein usage of at least 85%, which seems extraordinarily high, especially in light of the RQ of 1.03 reported for *T. mossambica*. Species differences within the genus (all are now called *Oreochromis*) may be responsible, though methodological limitations in the latter study may also be involved.

Temperature and Swimming Performance

As tilapia were brought down in acclimation temperature from 30°C to 20°C, U_{Crit} decreased by 25%. At 15°C, the tilapia would not or could not swim at any speed between 15 and 55 cm/s, though they were able to burst swim (anaerobic metabolism) in the tank when we attempted to remove them for the experiments. It has been previously observed that below 16°C, growth ceases to occur, while 11°C is the lower lethal temperature for *O. niloticus* (Bardach et al. 1972). Aerobic fuel-use ability may be largely reduced at 15°C for tilapia. The fact that tilapia could still perform burst activity at relatively low tem-

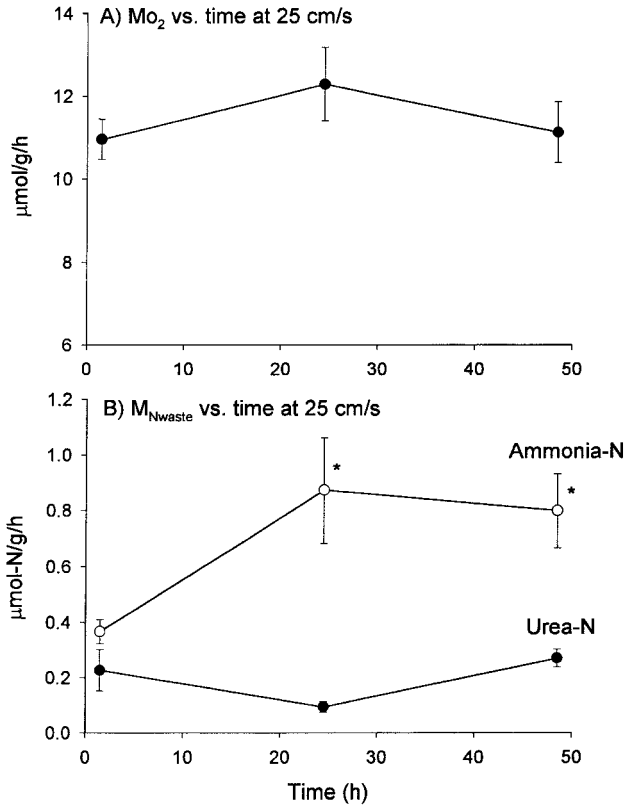

Figure 6. (*A*) Oxygen consumption and (*B*) nitrogenous waste excretion (ammonia [*open circles*] and urea [*filled circles*]) over 49 h while swimming at 25 cm/s (approximately 50% U_{Crit}). Values are expressed as means ± SEM. $n = 5$. The asterisk denotes a significant increase in ammonia excretion from the first measurement at 1–2 h ($P < 0.05$).

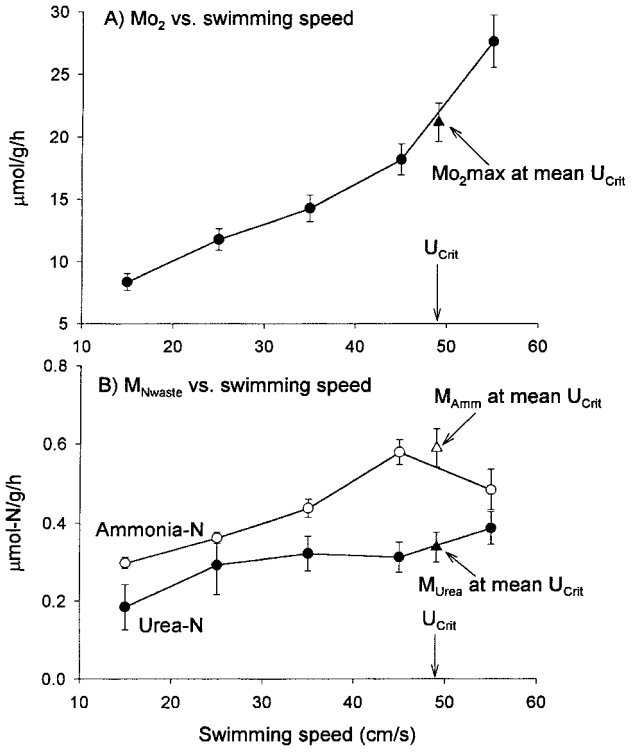

Figure 4. (*A*) Oxygen consumption and (*B*) nitrogenous waste excretion (ammonia [*open circles*] and urea [*filled circles*]) of tilapia at different swimming speeds. Swimming trials were performed at 30°C. Values are expressed as means ± SEM. At 15–45 cm/s and critical swimming speed (U_{Crit}), $n = 8$; at 55 cm/s, $n = 5$. This decrease in n reflects the fact that some fish fatigued at 55 cm/s. Triangles are mean values that have been estimated for all eight individual tilapia at their respective U_{Crit}.

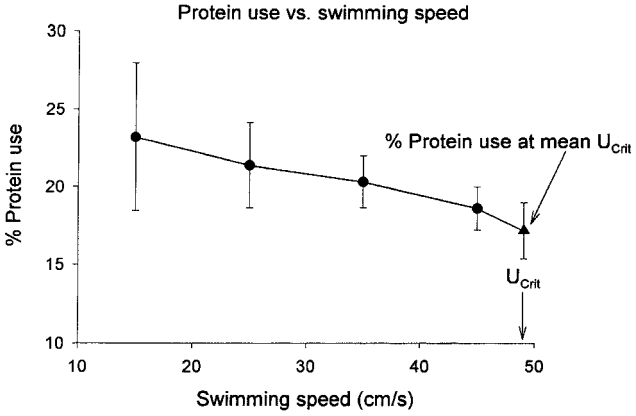

Figure 5. Percentage contribution of protein to aerobic metabolism (protein use) of tilapia at different swimming speeds. Values are expressed as means ± SEM. $n = 8$. The triangle is the mean value that was estimated for all eight individual tilapia at their respective U_{Crit}.

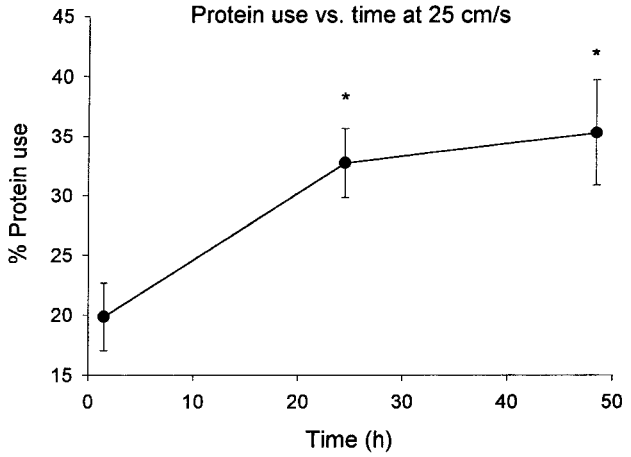

Figure 7. Percentage contribution of protein to aerobic metabolism (protein use) of tilapia over a 49-h swim at 25 cm/s (2.7 BL/s; approximately 50% U_{Crit}). Values are expressed as means ± SEM. $n = 5$. The asterisk denotes a significant increase in protein use ($P < 0.05$).

Table 1: The effect of acclimation temperature on respiratory gas exchange, nitrogen waste excretion, and critical swimming speed (U_{Crit}) for tilapia acclimated to 30°, 20°, and 15°C, as reflected in Q_{10} values

	Q_{10}		
	30°–20°C	20°–15°C	30°–15°C
Mo_2	1.6	2.0	1.7
Mco_2	1.6	1.6	1.6
M_{Amm}	2.8	2.7	2.8
M_{Urea}	2.2	1.8	2.1
U_{Crit}	1.3	...	...

Note. Tilapia would not or could not swim at 15°C, and the Q_{10} for U_{Crit} could not be determined.

peratures could indicate that anaerobic processes may be more important for this species.

The effect of decreased temperature on swimming performance has been previously documented (reviewed by Beamish [1978] and Johnston and Ball [1997]). The maximum velocity of muscle contraction decreases with decreasing temperature, which would decrease swimming performance since swimming velocity varies directly with tail-beat frequency in carp (Rome et al. 1990). A decrease in temperature has been shown to decrease power generated by the muscles, resulting in a compression of recruitment order. At colder temperatures, more aerobic muscle fibres are needed to generate locomotory power at any given swimming speed, which brings the anaerobic fibres into action earlier, and therefore exhaustion occurs at slower swimming speeds (Rome et al. 1985).

Swimming and Protein Use

As fish swam at greater velocities, oxygen consumption increased in a logarithmic fashion, as previously described by Brett (1964), working with sockeye salmon. Interestingly, tilapia at 30°C had the same Mo_2max as similarly sized rainbow trout at 15°C, as determined by Alsop and Wood (1997). However, tilapia were more efficient swimmers than trout since they had higher U_{Crit}'s (tilapia, 5.6 BL/s; trout, 4.5 BL/s), while Mo_2max values were the same in the two species.

Ammonia excretion increased slowly with increasing swimming speed. This was a slightly different pattern than that observed in trout, where ammonia excretion was constant, independent of swimming velocity (Lauff and Wood 1996*b*; Alsop and Wood 1997; Kieffer et al. 1998), as well as in catfish (Sukumaran and Kutty 1977). In terms of protein use, however, all species appear to rely less on protein as swimming speed increases. Lauff and Wood (1996*b*) and Kieffer et al. (1998) found carbohydrate use to increase with increasing swimming speed in trout. Carbohydrate use at different swimming speeds was not recorded in this study.

A large difference between species was apparent when fish swam over 49 h. Lauff and Wood (1996*b*) found a constant rate of ammonia and urea excretion for trout swimming at 55% U_{Crit} over 60 h. Tilapia swimming at 50% U_{Crit} in this experiment experienced a doubling of ammonia excretion between 1 and 24 h, indicating a switch to greater reliance on protein as an aerobic fuel. Kutty (1972) also found ammonia excretion increased greater than threefold in *T. mossambica* over the first 6 h of swimming at 2.4 BL/s. However, the ammonia excretion rates for *T. mossambica* were five to seven times greater than the tilapia in this study, so, again, large differences in metabolic processes between species within a genus can exist. Although we have observed tilapia to be excellent swimmers, they are not known to be long-distance migrators, in contrast to salmonids. It may be more important for salmonids to conserve protein during prolonged swimming.

Table 2: Gas exchange and nitrogen excretion rates for tilapia at 30°C

	Rates at 30°C
Mo_2max (μmol O_2/g/h)	21.18 ± 1.54
Mo_2min (μmol O_2/g/h)	5.78 ± .62
Resting Mo_2 (μmol O_2/g/h)	6.43 ± .21
Resting Mco_2 (μmol CO_2/g/h)	5.55 ± .77
Resting M_{Amm} (μmol N/g/h)	.444 ± .040
Resting M_{Urea} (μmol N/g/h)	.103 ± .017

Note. $n = 8$ for Mo_2max/min, and $n = 15$ for resting measurements.

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