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Respiratory gas exchange, nitrogenous waste excretion, and fuel usage during aerobic swimming in juvenile rainbow trout

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Abstract The types of fuel burned by juvenile rainbow trout (17 g) during a 58-h period of aerobic sustained exercise were studied by respirometry. Attempts to measure fuel usage by depletion (the *compositional approach*) in these same fish were unsuccessful due to lack of detectable changes in proximate body composition. O_2 consumption, CO_2 excretion, and nitrogenous waste excretion (ammonia-N plus urea-N) were measured in individual fish swum continuously at 55% and 80% of maximum sustainable swimming speed and in non-swimming controls. O_2 consumption and CO_2 excretion increased with swimming speed, and decreased over time. Absolute rates of N excretion were independent of swimming speed and time. *Instantaneous* aerobic fuel use, as calculated from the respiratory quotients and nitrogen quotients, was approximately 47% lipid, 30% protein, and 23% carbohydrate in non-swimmers at the start of the experiment. With increased swimming speed there was no change in absolute rates of protein oxidation, while lipid and carbohydrate oxidation both increased. Therefore, the relative protein contribution decreased with increasing speed but increased with swimming duration as the oxidation of other fuels declined over time. However, lipid oxidation predominated at all speeds and at all times. The relative contribution of carbohydrate increased with swimming speed and decreased over time. These results suggest that swimming becomes more efficient over time and help resolve uncertainties in the literature. We conclude that lipid is the main fuel of aerobic exercise, that protein catabolism is kept at minimum levels necessary for maintenance, and that carbohydrate oxidation becomes more important with increased white muscle recruitment at higher speed.

Key words Rainbow trout · Swimming · Respiratory quotient · Nitrogen quotient · Fuel · Protein · Carbohydrate · Lipid

Abbreviations AQ ammonia excretion per unit $\dot{M}O_2$ · $\dot{M}CO_2$ carbon dioxide production · $\dot{M}O_2$ oxygen consumption · $\dot{M}NH_3$ ammonia excretion · $\dot{M}N$ total nitrogen excretion · NQ nitrogen quotient ($\dot{M}N/\dot{M}O_2$) · RQ respiratory quotient · U_{crit} maximum sustainable swimming speed

Introduction

A great deal of research has been performed on the metabolic events which occur during "bursting" (exhaustive, largely anaerobic exercise) in fish. There is general agreement that carbohydrate as a fuel for glycolysis, and high-energy phosphates as a short-term energy supply, are the major metabolic fuels, evidenced by a depletion of glycogen, ATP, and creatine phosphate stores, a build-up of lactate, creatine, IMP, and ammonia, and a postexercise elevation in ammonia excretion and respiratory quotient (RQ > 1.0) [see Wood (1991) and Moyes and West (1995) for reviews]. However, in the wild, anaerobic exercise is probably an infrequent event, and fish spend most of their time swimming aerobically at submaximal velocities (i.e., below U_{Crit}). There is a general belief that protein/amino acids and lipid/fatty acids are the major aerobic fuels in teleost fish and that carbohydrate is of minimal importance (Driedzic and Hochachka 1978; Walton and Cowey 1982; van Warde 1983; Jobling 1994; Moyes and West, 1995). However, much less work has been performed on the metabolic support for

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this type of routine or sustainable exercise and the available evidence is contradictory.

Experimental investigations on aerobic fuel usage in fish fall into two broad categories (Lauff and Wood 1996): *compositional studies*, which measure changes in body fuel reserves over time or between treatments, and *instantaneous studies*, which assess fuel usage by respirometry. Some *compositional studies* on swimming salmonids have indicated that as aerobic activity level increases the relative contribution of protein as a fuel increases while that of lipid decreases (Krueger et al. 1968; Beamish et al. 1989), others have indicated exactly the opposite, an increasing reliance on lipid with increasing activity level (Brett 1973; Christiansen et al. 1989), while some have indicated no change (Kiessling et al. 1994). The picture is further clouded by *compositional studies* on salmonids during their anorexic upstream migration [review: Brett (1995)] which indicate that lipid is exploited first, followed by protein when lipid reserves are depleted. Notably, however, Brett (1995) emphasizes the many questionable assumptions and possible errors involved in the *compositional approach.*

The picture from *instantaneous studies* is equally confusing. Theoretically, the ratios of O_2 consumption ($\dot{M}O_2$), CO_2 production ($\dot{M}CO_2$), and nitrogenous waste excretion ($\dot{M}N$) can be used to calculate stoichiometrically the particular combination of lipid, protein, and carbohydrate actually being oxidized at any point in time, as long as the animal is undergoing only aerobic metabolism and is in a steady state condition [i.e., measured fluxes and production/consumption rates are equivalent; Kleiber (1987, 1992)]. In practice, this has never been done with exercising fish; however, partial measurements have been made in several studies. For example, Kutty (1968) measured $\dot{M}O_2$ and $\dot{M}CO_2$ in rainbow trout (*Oncorhynchus mykiss*) and found that the RQ($\dot{M}CO_2/\dot{M}O_2$) stayed at 0.96 over a range of different activity levels in aerobically exercising fish, suggesting that carbohydrate usage predominated. Van den Thillart (1986) measured very similar RQs (0.91 at rest, 0.96 at 80% U_{Crit}) in the same species, but concluded very differently that fuel usage changed from 20% lipid, 80% protein at rest to 10% lipid, 90% protein during submaximal exercise. This idea of greater reliance on protein during exercise has been supported by several studies on non-salmonids where $\dot{M}O_2$ and ammonia excretion ($\dot{M}NH_3$) were measured, therefore allowing calculation of the ammonia quotient (AQ = $\dot{M}NH_3/\dot{M}O_2$) as an index of protein utilization. Thus, AQ increased with increasing exercise duration in both *Tilapia mossambica* (Kutty 1972) and the catfish *Mystus armatus* (Sukumaran and Kutty 1977), although it was independent of actual swimming speed in the latter study. In contrast, however, Kutty (1978) reanalyzed the data of Brett and Zala (1975) to suggest that AQ decreased with spontaneous activity in sockeye salmon (*Oncorhynchus mykiss*). Similarly, Wiggs et al. (1989) reported that AQ decreased with increasing spontaneous activity in Atlantic salmon (*Salmo salar*).

Clearly, there is a need for a fresh approach to this question of fuel utilization in salmonids during aerobic exercise. Recently, we have developed both the theory and the necessary respirometric methods (measurements of $\dot{M}O_2$, $\dot{M}CO_2$, and $\dot{M}N$ as the sum of both ammonia and urea excretion) to apply the *instantaneous approach* in full to freshwater fish (Lauff and Wood 1996). Our objective in the present study was to apply this full *instantaneous approach* to the problem of fuel usage during aerobic, sustainable exercise in juvenile rainbow trout. To achieve recruitment of different muscle types (Webb 1971; Hudson 1973; Wilson and Eggington 1994; Moyes and West 1995), two levels of exercise corresponding to 55% (2.1 body lengths s^{-1}; $L\,s^{-1}$) and 80% of U_{crit} (3.1 $L\,s^{-1}$) were employed in addition to a non-swimming control treatment. Measurements were made over a continuous swimming period of 58 h to evaluate possible changes in fuel usage with exercise duration. Finally, the three test groups were analyzed for differences in proximate composition at the end of the experiment to allow comparison with the traditional *compositional approach.* The two methods when used simultaneously in our previous study had given vastly different answers to the question of fuels used during starvation (Lauff and Wood 1996).

Methods and materials

Animals

Rainbow trout (*Oncorhynchus mykiss* Walbaum; initially 8–10 g) were obtained from Rainbow Springs Hatchery (Thamesford, Ontario) and were kept in 15 °C dechlorinated Hamilton city tap water for several months prior to experimentation. Details of the water chemistry, feeding (1.0% of body weight daily), and husbandry can be found in Lauff and Wood (1996).

The effect of water quality on swimming performance

Decarbonated water [made from the acidifying dechlorinated tap water, bubbling with air, then readjusting the pH to 6.8 with NaOH, described in Lauff and Wood (1996)] was again used in the respirometry experiments to provide a low background total CO_2 against which expired CO_2 could be more accurately measured. This water had no effect on resting gas exchange in the previous study. However, since the present investigation involved exercise, we evaluated whether swimming performance was altered in decarbonated water. The standard test for critical swimming speed [U_{crit}; Brett (1964)] was used with a time increment of 30 min, a speed increment of 5 $cm\,s^{-1}$ and a starting speed of 10 $cm\,s^{-1}$. Experimental fish were transferred to decarbonated water 1.5 h before the start of the exercise test. All fish were tested at 15 °C in a Beamish style swimming respirometer (Farmer and Beamish 1969). Critical swimming speed was then calculated as follows:

$$U_{crit} = U_{1s} + \left(\frac{t_s}{t_i} \cdot U_i\right) \quad (1)$$

where U_{1s} is the speed ($cm\,s^{-1}$) of the last completed swimming period, t_s is the time (min) spent swimming at the final swimming speed, t_i is the time increment (min) and U_i is the speed increment ($cm\,s^{-1}$).

Respirometry and fuel depletion

Basic respirometry methods followed those of Lauff and Wood (1996) unless noted otherwise. Trout (17.5 ± 1.2 g, 113.7 ± 2.7 mm; means ± SEM, $n = 32$) were quickly blotted dry, weighed to the nearest 0.1 g and measured to the nearest millimeter (fork length). They were then transferred to individual Blažka style swimming respirometers [Blažka et al. (1960) in Beamish (1978); volume = 3.2l] that were submerged in the decarbonated water at 15 ± 1 °C to control temperature. Feeding was suspended once the fish were placed in the respirometers. During the 48-h acclimation period, freshly aerated, decarbonated water passed through each respirometer at ≈ 150 $ml\,min^{-1}$. At the end of the acclimation period controls were sacrificed by introducing neutralized tricaine methanesulfate (MS222, Syndel Laboratories; final concentration = 1 $g\,l^{-1}$). The fish were removed from the respirometers, blotted dry, weighed, freeze-clamped in liquid N_2 and stored at − 70 °C until analyzed for proximate body composition.

The remaining fish were divided into the following three groups: non-swimmers, low-speed swimmers (2.1 $L\,s^{-1}$ = 55% U_{crit}) and high-speed swimmers (3.1 $L\,s^{-1}$ = 80% U_{crit}); the swimming speeds were set based on the body length of the individual fish relative to the mean U_{crit} determined in the decarbonated water. The non-swimmers were exposed to a mild current of less than 1 $L\,s^{-1}$ to ensure good mixing of the water. This current was not sufficient to induce orientation in the fish.

In the high-speed group, to prevent a predictable burst (anaerobic) start (Wokoma and Johnston 1981), the fish were given 1 h in which to adjust to incrementally higher speeds until the target speed was reached. Even with this precaution several fish fell back to the rear wire mesh barrier. In these cases an attempt was made to induce the fish into swimming by lowering the water speed and bringing it up again when the fish had reoriented. If this was not successful, the trial was abandoned.

Three respirometry periods were run 5 h apart on each of the 3 days after acclimation (total test duration: 58 h). At each period, the respirometers were closed off for a fixed amount of time (a time which was predicted to deplete ambient PO_2 to no lower than 16 kPa, usually between 30 and 60 min), during which initial and final water samples were taken; flushing then recommenced immediately. Between same-day sampling periods the ports were open wide to allow a high flow rate (≈ 500 $ml\,min^{-1}$) of freshly aerated water to replenish the ambient PO_2. The water samples were measured for O_2, CO_2, total ammonia ($T_{amm} = NH_3 + NH_4^+$) and urea using methods described previously (Lauff and Wood 1996). From these, $\dot{M}O_2$, $\dot{M}CO_2$, and $\dot{M}N$ were calculated, thereby yielding RQ, NQ, and *instantaneous* fuel use. At the end of the 3-day experimental period all of the test fish were sacrificed in the same manner as the controls for measurement of proximate body composition.

Instantaneous fuel use calculations

The respiratory quotient ($RQ = \dot{M}CO_2/\dot{M}O_2$) and nitrogen quotient ($NQ = \dot{M}N/\dot{M}O_2$) were determined for each fish at each time. Note that $\dot{M}N$ was calculated from the sum of ammonia-N plus urea-N excretion; each molecule of urea contains two atoms of N. The protein use could then be calculated as follows [condensed from Lauff and Wood (1996)]:

$$P = \frac{NQ}{0.27} \tag{2}$$

where P is the fraction supplied by protein of the total fuels supporting $\dot{M}O_2$, and 0.27 is the theoretical maximum for NQ (i.e., when protein is the sole fuel source). Note that this maximum NQ does not change as the makeup of the fuel changes; the value of 0.27 reflects 100% protein use when ammonia and urea are the end products in any combination. However, the RQ reflecting 100% protein use ($RQ_{protein}$) is dependent on the particular mixture of nitrogenous wastes produced (Kleiber 1987), and was therefore calculated separately for each set of fish. $RQ_{protein}$ values were found to be 0.95 (non-swimmers and low-speed swimmers, based on 18% of total-N as urea) and 0.93 (high-speed swimmers, based on 34% urea). RQ_{lipid} and $RQ_{carbohydrate}$ are the well-known values of 0.71 and 1.0. Employing these fuel-specific RQs, it then follows (using high-speed swimmers for illustration):

$$RQ = P * 0.93 + C * 1.0 + L * 0.71 \tag{3}$$

where P, C, and L represent the fuel fractions supporting $\dot{M}O_2$ from protein, carbohydrate, and lipid respectively. Since:

$$L = 1.0 - P - C \tag{4}$$

then by substitution (Eqs. 2 and 4 into Eq. 3):

$$RQ = 0.81 * NQ + 0.29C + 0.71 \tag{5}$$

The equation can be solved for C, and L determined by difference. These percentage contributions to $\dot{M}O_2$ could then be converted to percentages based on carbon usage via the fuel-specific RQs. The total carbon usage was reflected in the $\dot{M}CO_2$ data of Fig. 1, which was then apportioned to absolute carbon expenditures of the three fuel types using these C-based percentages (Lauff and Wood 1996). Note that the theory is only valid for aerobic RQs, so the gas exchange of an individual test fish during a test period was not used to calculate fuel use if its RQ was greater than the aerobic maximum of 1.0.

Body composition

The bodies from the control and three experimental groups were analyzed for protein, glucose, glycogen, lactate, total lipids, inorganics (ash) and water by methods identical to those described by Lauff and Wood (1996). The sum of glucose, glycogen and lactate are reported as total carbohydrate.

Statistics

Values are reported as means ± standard error (N). A one-way ANOVA was used to determine significance in body composition and weight changes between groups of fish, while a paired t-test was used to determine if the pre- and post-experimental body weights differed. An independent t-test was used to compare critical swimming speeds in the two water types. A test for differences in slope was used to determine if the three groups represented different populations during the respirometry trials; if no difference was found, a test for different elevation was used to confirm colinearity (Zar 1974). Regressions were fitted by the method of least squares and were tested for significance using the Pearson linear correlation in the Fig. P graphics package (Biosoft, Ferguson, Mo.). For all tests, $P < 0.05$ was considered significant.

Results

The effect of water quality on U_{crit}

The critical swimming speed of fish swimming in decarbonated water (3.84 ± 0.15 $L\,s^{-1}$, mean ± SEM;

$n = 11$) was not significantly different from that of fish swimming in normal tapwater (3.98 ± 0.23 L s^{-1}, $n = 14$).

Respirometry and N-waste excretion

Both groups of swimmers showed steady decreases in gas exchange rates, whereas the non-swimmers were relatively stable over the 3-day experimental period (Fig. 1). The high-speed fish consumed O_2 initially at 12 μmol g^{-1} h^{-1} but this decreased to 9.5 μmol g^{-1} h^{-1} by the end of the experiment ($P < 0.001$). Low-speed swimmers had $\dot{M}O_2$ values about 75% of the high speed group. $\dot{M}O_2$ in non-swimmers was about 5.5 μmol g^{-1} h^{-1} throughout. CO_2 excretion rates ranked in the same order as the $\dot{M}O_2$ values. Non-swimmers had a stable excretion of 4.0 μmol g^{-1} h^{-1}; the low-speed group had excretion rates that declined from 8.0 to 5.5 μmol g^{-1} h^{-1} ($P < 0.001$), whereas the high speed group exhibited a non-significant drop from almost 10 μmol g^{-1} h^{-1} to 8.5 μmol g^{-1} h^{-1} ($P > 0.05$). Overall RQ averaged about 0.90, and there were no significant differences in RQ between the three groups or against time when all values were included (Fig. 2). When values of the RQ greater than 1.0 (i.e., bouts of anaerobicity) were removed the mean RQs dropped to about 0.85, but there were still no differences between groups or against time.

Total nitrogenous waste excretion was relatively stable in each group at approximately 0.55 μmol g^{-1} h^{-1}, with no significant changes over time ($P > 0.05$; Fig. 3).

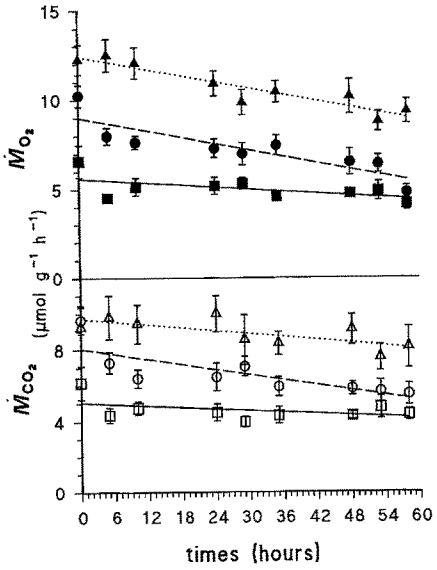

Fig. 1 Oxygen consumption and carbon dioxide excretion in non-swimming (*squares* and *solid lines*), low-speed swimming (2.1 L s^{-1}; *circles* and *dashed lines*) and high-speed swimming (3.1 L s^{-1}; *triangles* and *dotted lines*) in juvenile rainbow trout over the 3-day test. Means ± SEM

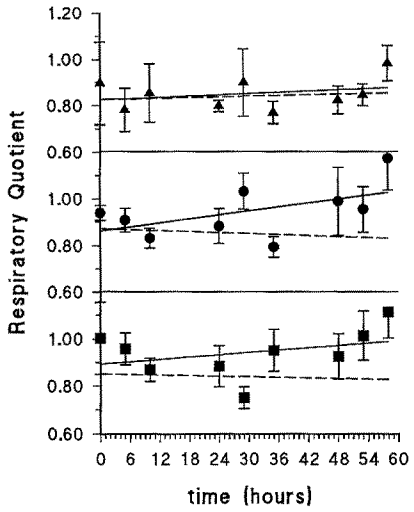

Fig. 2 The complete respiratory quotient (*symbols* and *solid regression line*) and the aerobic respiratory quotient (*dashed line*) for non-swimming (*squares*), low-speed swimming (2.1 L s^{-1}; *circles*) and high-speed swimming (3.1 L s^{-1}; *triangles*) juvenile rainbow trout over the 3-day test. Means ± SEM

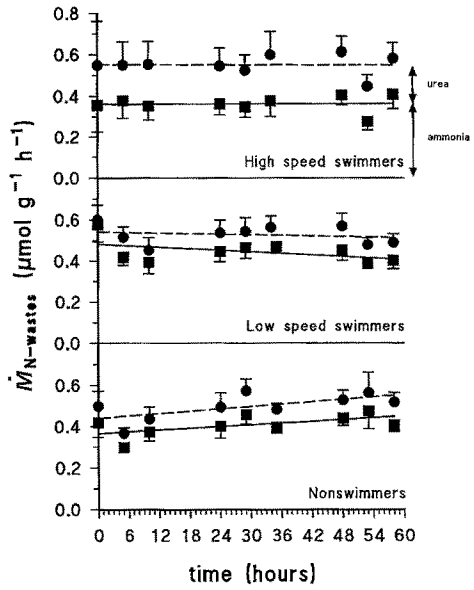

Fig. 3 Total nitrogenous waste (*t*-Nitrogen as the sum of ammonia-N and urea-N: *circles*) and ammonia-N (*squares*) excretion for the non-swimming, low-speed swimming (2.1 L s^{-1}) and high-speed swimming (3.1 L s^{-1}) juvenile rainbow trout over the 3-day test period. Means ± SEM

However, the proportion of the total nitrogen excreted as urea was 34% in the high-speed fish, whereas it was only 18% in the low-speed fish and non-swimmers, a significant difference ($P < 0.01$). These stable excretion values, in conjunction with the decreasing $\dot{M}O_2$ values (in the swimmers), gave rise to increasing NQs with time in the non-swimmers and low-speed group only ($P < 0.001$; Fig. 4). The high-speed swimmers had the lowest NQ (stable at 0.050; $P > 0.05$), while the

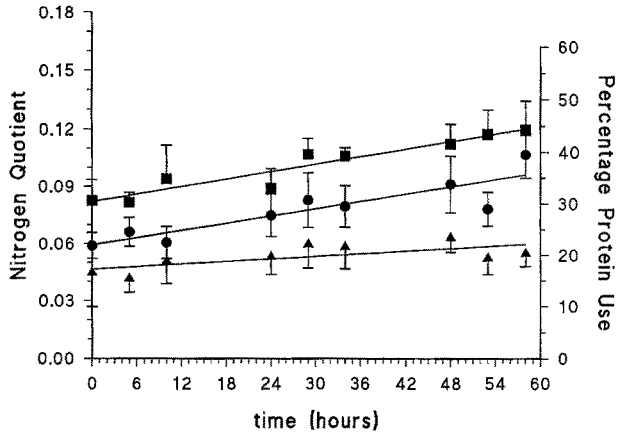

Fig. 4 Nitrogen quotient (*left* axis) and protein use (*right* axis) for non-swimming (*squares*), low-speed swimming (2.1 $L s^{-1}$; *circles*) and high-speed swimming (3.1 $L s^{-1}$; *triangles*) juvenile rainbow trout over the 3-day test. Means ± SEM

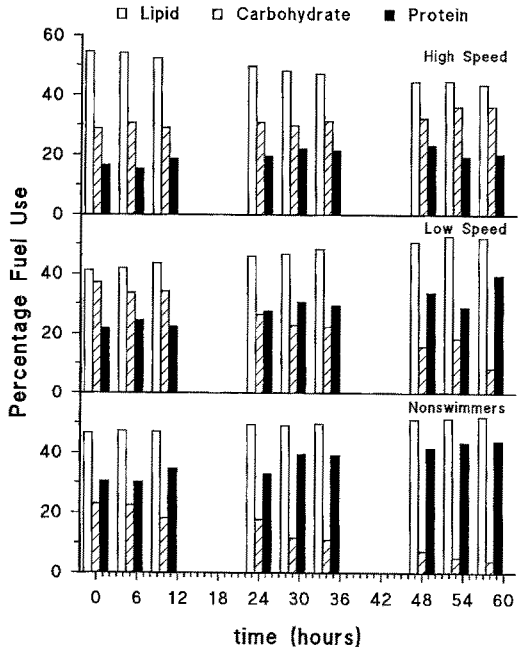

Fig. 5 Percentage use of lipid (*open bars*), carbohydrate (*hatched bars*) and protein (*solid bars*) in non-swimming, low-speed swimming (2.1 $L s^{-1}$), and high-speed swimming (3.1 $L s^{-1}$) juvenile rainbow trout over the 3-day test

non-swimmers had the highest NQ (increasing from 0.08 to 0.12). NQ is directly correlated with the contribution of protein catabolism, as shown using the right axis of Fig. 4.

Trends in *instantaneous* metabolic fuel use for all three substrates are summarized in Fig. 5 in terms of their percentage contribution to $\dot{M}O_2$. At all speeds and at all times lipid oxidation predominated, accounting for 42–54% of total $\dot{M}O_2$. Over time, both the non-swimmers and low-speed swimmers showed an increase in the contribution of protein to the total fuel

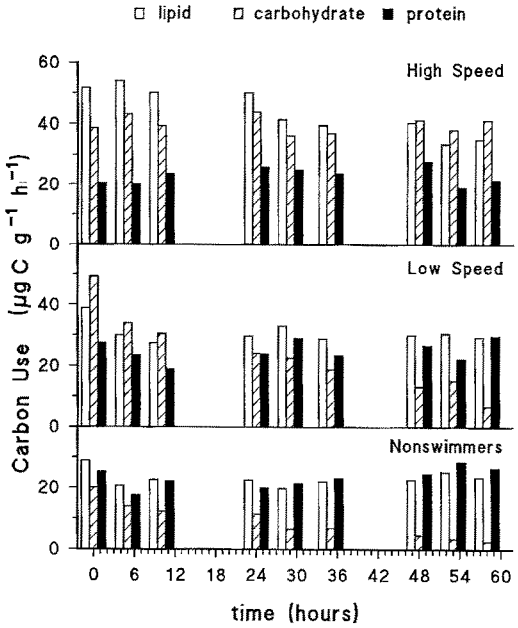

Fig. 6 The contribution of carbon from lipid (*open bars*), carbohydrate (*hatched bars*) and protein (*solid bars*) in non-swimming, low-speed swimming (2.1 $L s^{-1}$), and high-speed swimming (3.1 $L s^{-1}$) juvenile rainbow trout over the 3-day test.

mixture for $\dot{M}O_2$ (from 30 to 45% and from 20 to 36%, respectively), whereas the high-speed swimmers had a protein contribution that stayed relatively constant at 20%. The non-swimmers and low-speed swimmers showed similar trends in all fuels, whereas the high-speed group showed opposite trends in carbohydrate and lipid oxidation. The contribution of carbohydrate as a fuel for $\dot{M}O_2$ dropped in both the non-swimmers (from 23 to 5%) and the low-speed swimmers (from 38 to 8%) over the course of the experiment. There was a concurrent (albeit slight) increase in lipid use. However, in the high-speed swimmers lipid initially represented 54% of the total fuel mixture but diminished to 43%; carbohydrates increased from 29 to 38%. Especially late in the experiment, there was a general trend for the contribution of carbohydrate to increase with swimming speed.

The absolute carbon use rate is reflected in the CO_2 excretion curves of Fig. 1, and partitioned to specific fuels in Fig. 6. Unsurprisingly, the total C use rate was greatest in the high-speed swimmers and lowest in the non-swimmers. Protein C usage was not affected by either swimming speed or duration. In the non-swimmers the approximately equal contributions of lipid and protein to total C remained relatively constant at about 25 $\mu g C g^{-1} h^{-1}$ for each fuel. Similarly, the low-speed swimmers showed a constant lipid usage of 31 $\mu g C g^{-1} h^{-1}$, while protein C usage stayed at about 25 $\mu g C g^{-1} h^{-1}$ (Fig. 6). In contrast, in the high-speed group, protein C usage again stayed at this level but lipid C usage dropped from a first day average

Table 1 Body compositions (mg 100 mg^{-1}, wet weight) of the four groups of juvenile rainbow trout. Total carbohydrate includes glucose, glycogen and lactate. There are no significant differences between groups. Means ± SEM

	Lipid	Total carbohydrate	Protein	Inorganic	Water
Controls ($n = 6$)	4.7 ± 0.7	0.28 ± 0.03	14.2 ± 0.4	2.60 ± 0.09	77.2 ± 0.6
Non-swimmers ($n = 9$)	4.7 ± 0.5	0.21 ± 0.02	14.2 ± 0.2	2.66 ± 0.08	77.5 ± 0.5
Low speed ($n = 7$)	5.1 ± 0.7	0.20 ± 0.02	13.2 ± 0.7	2.68 ± 0.17	77.8 ± 1.2
High speed ($n = 12$)	5.1 ± 0.5	0.24 ± 0.02	13.9 ± 0.6	2.62 ± 0.05	77.0 ± 0.5

of 52 μg C g^{-1} h^{-1} to a third day average of 36 μg C g^{-1} h^{-1}. Carbohydrate use stayed relatively constant (39 μg C g^{-1} h^{-1}) in the high-speed swimmers, whereas in the low-speed swimmers and the non-swimmers it dropped by approximately 75% over the 3 days. Both carbohydrate and lipid contributed more C than did protein in the high-speed swimmers; carbohydrates always contributed less C than did the other fuels in the non-swimmers. No such comparative generalization could be made for the low speed swimmers.

Proximate body analysis

There were no significant differences in any parameter of body composition between any groups of test fish or the controls (Table 1). Water made up about 77.4% of the mass of the fish, with carbohydrate, lipid, protein, and inorganics (ash) making up on average 0.2%, 4.9%, 13.9%. and 2.7%, respectively. Body weight decreased in all groups, including the controls ($P < 0.05$). The controls were sacrificed at the end of the 48-h acclimation period, prior to the 58-h swimming period. The weight loss in the controls was 2.9 ± 1.0% (over the acclimation period only), whereas for the experimentals (over the 48 h of acclimation plus the 58 h of test), the weight losses were 3.0 ± 0.9% in the non-swimmers, 4.5 ± 0.9% in the low-speed swimmers, and 7.0 ± 1.0% in the high-speed swimmers. Thus, weight loss tended to increase with swimming speed but only the high speed swimmers were significantly different from the controls ($P < 0.05$).

Given this fact and the unchanged proximate composition amongst groups, estimation of fuel usage by depletion was statistically justified only in the high-speed swimmers. Calculations were performed using the standard calculation procedures of the *compositional approach* [see Tables 2 and 3 of Lauff and Wood (1996) for basic principles and oxycalorific conversion factors], and applying the control-corrected weight loss to the data of Table 1. The results indicate that per gram of wet body weight at the start of exercise, the high-speed swimmers lost 0.5 mg of carbohydrate, 8.7 mg of protein, and actually gained 1.9 mg of lipid. Protein would therefore account for essentially all fuel usage. Predicted mean $\dot{M}O_2$ from this net change in fuels over the swimming period would have been only 5.3 μmol g^{-1} h^{-1}, about half the observed value (Fig. 1), whereas predicted mean $\dot{M}N$ would have been 1.8 μmol g^{-1} h^{-1}, approximately three-fold the observed value (Fig. 2). These data illustrate the limitations of the compositional approach for short-term studies of this nature.

Discussion

The present results provide a clear picture of *instantaneous* fuel use during exercise, thereby clarifying the uncertainties raised in the Introduction. They also point out the need for precision in discussing fuel use (i.e., differences between absolute and relative uses, and differences between support of $\dot{M}O_2$ and supply of C), and the superiority of the *instantaneous approach* over the *compositional approach* for short-term laboratory studies.

General observations

The decline in $\dot{M}O_2$ and $\dot{M}CO_2$ with increasing duration of aerobic exercise (Fig. 1) has been commonly observed (Brett 1995). This increase in the efficiency of locomotion probably represents a progressive "settling down" by the fish, so that with increased experience in the swim tunnel less time and energy is expended on extraneous activity. It is also possible that progressive food deprivation played a role but this was likely of minor importance. The present experiments were not started until 48 h after the last meal, and were completed within a further 58 h. Our previous starvation study with juvenile rainbow trout demonstrated that resting metabolic rates were stable during this period (Lauff and Wood 1996).

In non-swimming control fish at the start of the experiment, the division of *instantaneous* fuel use (in terms of support of $\dot{M}O_2$) was approximately 47% lipid, 30% protein, and 23% carbohydrate. These figures may be compared with 62% lipid, 14% protein, 24% carbohydrate in the smaller trout of Lauff and Wood (1996) under comparable conditions. While there is some difference in the lipid/protein ratio, the two studies are in agreement that lipid is the major fuel, that carbohydrate use is appreciable, and that the

contribution of protein is rather less than commonly assumed, e.g., "over 40%" (van Waarde 1983). Indeed, as pointed out by Lauff and Wood (1996), calculations based on AQ in two previous salmonid studies (Brett and Zala 1975; Wiggs et al. 1989) yield similarly low values (19–36%) for the protein contribution.

The influence of exercise on instantaneous fuel use

The present data clearly show that over the 58 h of exercise the absolute rate of protein usage, as reflected in $\dot{M}N$ (Fig. 3) and in absolute C usage from protein (Fig. 6), remained constant and was essentially independent of swimming speed. This constant rate of protein utilization likely represented the so-called *endogenous* fraction of N-metabolism, necessitated by the processes of normal body maintenance (Wood 1993). There was no evidence that protein oxidation increased with either swimming speed or swimming duration. This does not, however, mean that protein turnover stayed constant; Houlihan and Laurent (1987) demonstrated that rates of both protein synthesis and protein degradation increased at moderate exercise levels in rainbow trout. In the two swimming treatments, absolute C usage from protein was smaller than that from either lipid or carbohydrate, at least during the early hours of swimming (Fig. 6). The "extra" fuel to power exercise at both levels came from elevations in both lipid and carbohydrate oxidation, with the former playing the larger role.

In hindsight, these results seem entirely reasonable. Protein, unlike lipid and carbohydrate, cannot be stored in mobilizeable depots, and it makes little sense to burn the motor (muscle protein) which is powering the exercise. Additionally, protein only supplies 60% of the calories that an equal mass of lipid can (Kleiber 1987). Why do these results disagree with so much of the previous literature? We would argue that the reasons are partly theoretical, and partly methodological. See, for example, the detailed explicit criticism by Brett (1973) of the assumptions used by Krueger et al. (1968) to conclude that coho salmon show increasing reliance on protein during sustained swimming. The respirometric approach used by van den Thillart (1986) to conclude that protein oxidation contributed 80% at rest and 90% during exercise in rainbow trout did not measure N excretion, did not admit the possibility of carbohydrate oxidation, and assumed that lipid and protein oxidation were the only two possibilities. The respirometric studies of Kutty (1968) admitted the possibility of carbohydrate oxidation (and indeed concluded that it was the major fuel of aerobic exercise), but again did not measure N excretion. The actual raw RQ data of both studies were in fact very close to those of the present investigation (Fig. 2), so it is the interpretation, rather than the data themselves, which differs.

Relative fuel usage (i.e., on a percentage basis; Fig. 5) yields a somewhat different picture. In accord with the absolute picture, the relative protein contribution decreased as swimming speed increased (Figs. 4, 5). This finding concurs with *compositional studies* indicating that relative reliance on lipid increases, and on protein decreases during exercise in salmonids (Brett 1973; Christiansen et al. 1989). It also concurs with previous studies which indicated that AQ decreased as spontaneous activity level increased in salmonids (Kutty 1978; Wiggs et al. 1979). However, as swimming duration increased in the present study, the relative contribution of protein oxidation increased, at least in the low-speed swimmers (and non-swimmers; Figs. 4, 5). This effect reflected the drop in $\dot{M}O_2$ that occurred over time as swimming became more efficient (Fig. 1). As absolute protein oxidation stayed constant, the oxidation of lipid and especially carbohydrate (Fig. 6) dropped, resulting in an increased NQ, and therefore an increased relative utilization of protein (Figs. 4, 5). This suggests that in addition to the basic swimming activity powered by lipid and carbohydrate, the extraneous activity which was gradually lost as swimming experience increased had also been powered by lipid and carbohydrate.

The literature is not entirely congruent as to the swimming speed at which white (mosaic) muscle becomes active during aerobic exercise in trout (Webb 1971; Hudson 1973; Wilson and Egginton 1994; Moyes and West 1995). Nevertheless, the two swimming speeds were chosen based on evidence that low-speed swimming at 55% U_{crit} would be powered mainly by red muscle, whereas high-speed swimming at 80% U_{crit} would involve additional recruitment of white (mosaic) muscle. Clearly, lipid was the major fuel at both speeds, but the contribution of carbohydrate tended to increase with increasing swimming speed, especially late in the exercise period (Figs. 5, 6). This suggests that the white muscle uses carbohydrate as an important aerobic fuel. This conclusion seems reasonable inasmuch as white muscle represents the largest depot of glycogen in the body (as well as glucose and lactate) and is well known to exploit this store as a fuel during anaerobic exercise (Wood 1991; Moyes and West 1995). It may well use glycogen as an important aerobic fuel. Interestingly, a similar phenomenon is seen in mammals, whereby carbohydrate oxidation becomes increasingly important relative to lipid oxidation as exercise intensity increases (Brook and Mercier 1994).

The other interesting difference associated with swimming speed was the greater percentage of N excretion occurring in the form of urea (34% *versus* 18% in the non-swimmers and low-speed swimmers; Fig. 3). One possible explanation is an increased uricolytic production of urea from a greater turnover of adenylates associated with white muscle recruitment (Wood 1993). If this is the explanation, N excretion from

protein metabolism may actually be decreasing slightly during high-speed swimming.

In addition to differences between relative and absolute fuel usages, a point which is often overlooked is the difference between the support of $\dot{M}O_2$ and supply of C. This difference is simply due to the very different oxycalorific equivalents of the various fuels. Comparison of Figs. 5 and 6 at any one point and at any one swimming speed (i.e., at constant $\dot{M}O_2$) illustrates the rather different, yet simultaneously correct, conclusions yielded by the two approaches. For example, in low-speed swimmers in the final three periods, lipid is a much more important fuel than protein in terms of support of $\dot{M}O_2$ (Fig. 5). However, in terms of C "burned", the two fuels make an equal contribution (Fig. 6). While this sort of difference is obvious in a respirometric study, it is often neglected in depletion studies using the *compositional* approach.

The instantaneous approach versus the compositional approach

Recently, it has been pointed out that since the *compositional approach* depends on measurements of mean *differences* in proximate composition and weight between different groups of fish, its accuracy is compromised by real biological variability between individuals and the limits of analytical precision (Brett 1995; Lauff and Wood 1996). These limitations are amply illustrated by the compositional data (Table 1) and weight change data of the present study. In the only group where there was a statistically justified basis for applying the *compositional approach*, the high-speed swimmers, the results were completely unrealistic. Much longer periods of exercise and/or much larger sample sizes would be needed to overcome these problems.

However, it should also be realized that even when these problems are overcome, the *compositional approach* and the *instantaneous approach* will not necessarily yield the same answers, as they measure different processes. The latter measures the fuels actually being oxidized at a particular time, the former measures the fuels depleted over time. As noted earlier (Lauff and Wood 1996), the two may disagree because of interconversion of fuels prior to oxidation, and because of excretion of fuels without oxidation. For example, if protein excretion is significant in rainbow trout, as reported by Olsen and Fromm (1971), then protein utilization by depletion will appear larger than by respirometry. A similar complication may be protein losses in mucus, a component which may well become greater as fish swim faster. For all these reasons, the *instantaneous approach* based on respirometry appears preferable for short-term physiological studies.

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