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THE INTERACTIVE EFFECTS OF FEEDING AND EXERCISE ON OXYGEN CONSUMPTION, SWIMMING PERFORMANCE AND PROTEIN USAGE IN JUVENILE RAINBOW TROUT (*ONCORHYNCHUS MYKISS*)

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Summary

The impacts of feeding on the rate of O_2 consumption ($\dot{M}_{O_2}$), aerobic swimming performance, nitrogenous waste excretion (ammonia-N and urea-N) and protein utilization as an aerobic fuel were investigated in juvenile rainbow trout. Feeding trout to satiation (in groups of 120) resulted in rapid growth and elevated routine $\dot{M}_{O_2}$ by 68 % relative to fasted fish and by 30 % relative to trout fed a maintenance ration of 1 % of body mass daily. This in-tank $\dot{M}_{O_2}$ of satiation-fed trout was approximately 70 % of the $\dot{M}_{O_2max}$ observed at the critical swimming speed (U_{Crit}) when trials were performed on individual trout in swimming respirometers. Feeding increased $\dot{M}_{O_2}$ at all swimming speeds; the absolute elevation (specific dynamic action or SDA effect) was dependent on ration but independent of swimming velocity. There was no difference in $\dot{M}_{O_2max}$ at U_{Crit} amongst different ration treatments, but U_{Crit} was significantly reduced by 15 % in satiation-fed fish relative to fasted fish. These results suggest that the irreducible SDA load reduces swimming performance and that $\dot{M}_{O_2max}$ is limited by the capacity to take up O_2 at the gills and/or to deliver O_2 through the circulatory system rather than by the capacity to consume O_2 at the tissues. Ammonia-N and urea-N excretion increased with protein intake, resulting in a 6.5-fold elevation in absolute protein use and a fourfold elevation in percentage use of protein as an aerobic fuel for routine metabolism in satiation-fed trout (50–70 %) relative to fasted fish (15 %). Urea-N excretion increased greatly with swimming speed in all treatments, but remained a minor component of overall nitrogen excretion. However, even in satiation-fed fish, ammonia-N excretion remained constant as swimming speed increased, and protein did not become more important as a fuel source during exercise. These results suggest that the reliance on protein as a fuel is greatly dependent on feeding quantity (protein intake) and that protein is not a primary fuel for exercise as suggested by some previous studies.

Key words: rainbow trout, *Oncorhynchus mykiss*, routine and swimming oxygen consumption, feeding, critical swimming speed, nitrogen quotient, fuel, protein.

Introduction

The ingestion of food is followed by an increase in metabolic rate in most animals (Kleiber, 1961; Garrow, 1974), a phenomenon known as specific dynamic action (SDA). In teleost fish, SDA is thought to represent all the metabolic expenditures associated with the nutritive process including the energy required for ingestion, digestion, absorption, metabolic transformation of nutrients and growth (Jobling, 1981; Beamish and Trippel, 1990; Brown and Cameron, 1991; Lyndon *et al.* 1992).

The SDA effect of feeding can be very large. LeGrow and Beamish (1986) reported that O_2 consumption of juvenile trout (*Oncorhynchus mykiss*) after a meal routinely increased to 60–80 % of the maximum rate of oxygen consumption ($\dot{M}_{O_2max}$) reported by Rao (1968). $\dot{M}_{O_2max}$ is the rate exhibited when fish are swimming at their critical swimming speed (U_{Crit}). Soofiani and Hawkins (1982) and Soofiani and Priede (1985), working on juvenile cod (*Gadus morhua*), found that repeated feeding to satiation elevated the rate of O_2 consumption to a level actually higher than for cod swimming at U_{Crit}.

In nature, fish must eat and swim simultaneously, but there is little information on how the energetic costs of the two processes might interact. Virtually all swimming studies, including those cited above, have been performed on fasted fish, which are obviated from the need to partition O_2 between the demands of nutrition and the demands of locomotion. Only a few investigations (Muir and Niimi, 1972; Beamish, 1974; Furnell, 1987; Blaikie and Kerr, 1996) have examined O_2 consumption in fish which were both fed and swum aerobically, and in none of these were fish exercised up to U_{Crit}.

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Circulatory constraints might well become important when feeding and exercise are combined. Blood flow to red muscle increases substantially, but visceral blood flow either falls or remains unchanged when fasted fish are swum aerobically (Randall and Daxboeck, 1982; Axelsson and Fritsche, 1991; Thorarensen *et al.* 1993; Kolok *et al.* 1993; Wilson and Egginton, 1994). Conversely, visceral blood flow increases when resting fish are fed (Axelsson *et al.* 1989; Axelsson and Fritsche, 1991). Unfortunately, it is not yet known what happens to red muscle and visceral blood flow when fed fish are swum. Alternatively or additionally, ventilatory or diffusive constraints at the gills (Wood and Perry, 1985; Perry and McDonald, 1993; Gallaugher *et al.* 1995) may limit the absolute rate of O_2 supply, thereby compromising SDA or exercise metabolism or both.

Three scenarios are possible. The first is that SDA and exercise metabolism proceed independently. If this is the case, fed fish should have the same (or higher) U_{Crit} as starved fish and a higher $\dot{M}_{O_2max}$ corresponding to the SDA effect. The second is that swimming metabolism is sacrificed so as to sustain the SDA effect of feeding. In this case, the fish should have a lower U_{Crit} but the same $\dot{M}_{O_2max}$ as fed fish. The third is that nutritive metabolism is sacrificed so as to sustain swimming performance, resulting in a disappearance of the SDA effect at high swimming speed, i.e. unchanged $\dot{M}_{O_2max}$ and unchanged U_{Crit} in fed *versus* starved fish. The present study employs different ration levels to examine the general effects of feeding on O_2 consumption in juvenile rainbow trout under routine conditions and then during exercise conditions designed to discriminate between these three scenarios.

This design facilitated a second objective, assessment of the quantitative importance of protein as an aerobic fuel. It is commonly believed (e.g. Brett and Groves, 1979; van Waarde, 1983; van den Thillart, 1986; Jobling, 1994; Weber and Haman, 1996) that protein is a major aerobic fuel in fish, particularly during exercise, but experimental evidence is scarce and contradictory (reviewed by Lauff and Wood, 1996*b*). Recently Lauff and Wood (1996*a*,*b*) employed a respirometric approach to show that the absolute rate of protein oxidation was low at rest and did not increase during aerobic swimming in fasted rainbow trout. However, the situation may well be different in fed fish. In the present study, we employ the respirometric method to examine the contribution of protein as a fuel in relation to both exercise and ration level.

Materials and methods

Approximately 1100 juvenile rainbow trout [*Oncorhynchus mykiss* (Walbaum)] were obtained from Rainbow Springs Hatchery (Thamesford, Ontario) and kept at 15±1 °C in dechlorinated, fully aerated Hamilton city tapwater for 3 weeks prior to experimentation. The water was of the following ionic composition (in mmol l^{-1}); Ca^{2+}, 1.0; Mg^{2+}, 0.2; Na^{+}, 0.6; Cl^{-}, 0.7; K^{+}. 0.05; titratable alkalinity to pH 4.0, 1.9 mmol l^{-1}; total hardness, 140 p.p.m. as $CaCO_3$; pH 8.0. The fish were fed commercial trout food (Zeigler's fish food; Table 1) and received 1 % of their body mass per day (0.5 % once in the morning and 0.5 % in the afternoon) until the start of the experiments. This was slightly above a maintenance ration, permitting a slow rate of growth.

Table 1. *Composition of Zeigler's fish food*

Constituent	Content (%)*
Crude protein (minimum)	50
Crude fat (minimum)	15
Crude fibre (maximum)	2
Water	12
Sodium	0.5
Calcium	2.3
Phosphorus	1.8

*Partial analysis only.

Routine metabolic rate and protein utilization

One week prior to experimentation, 720 fish (mass 4–8 g) were randomly divided among six identical flow-through tanks (volume 211 litres, 120 fish per tank) while still maintained on the 1 % body mass per day diet. Water flow into the tanks was 2.5 litres min^{-1}, and P_{O_2} was maintained at >90 % air saturation. Photoperiod was 10 h:14 h L:D.

On day 0 of the experiment, duplicate tanks were assigned to one of three treatment groups on the basis of feeding quantity: group 1, fasting; group 2, 1 % body mass per day; and group 3, satiation feeding. Group 2 were fed 0.5 % body mass at 08:00 h and 0.5 % at 16:00 h. Group 3 were hand-fed to satiation at the same times. The procedure for satiation feeding was as follows: a small amount of food was spread over the water surface every minute until there was food left on the surface at the end of the minute. If at the end of the next minute the food was eaten, then the fish were fed again, but if there was food remaining after the subsequent minute the fish were considered satiated. The amount of food eaten was recorded gravimetrically. The ration for the 1 % body mass per day groups was calculated as 1 % of the bulk mass of each tank. Food consumption was then calculated as g $fish^{-1}$ day^{-1} by dividing the amount of food consumed each day by the number of fish in the tank. Faeces and organic debris were siphoned out of the tanks daily.

The fish from each tank were bulk-weighed weekly by removing all of the fish from one tank at a time and placing them in a sieve within a container of water. The container was weighed, the fish were removed with the sieve and placed back in their tank, and the sieve was replaced in the container. The entire apparatus was reweighed, with the mass of the fish equal to the difference in mass between the two measurements.

The rates of oxygen consumption and nitrogen excretion were measured on days –4 ($\dot{M}_{O_2}$ only), –1, 1, 2, 4, 6, 9, 13 and 17 of the experiment in one tank of each duplicate set. On these days, starting at 07:00 h, the surface of the tank was sealed with a tight-fitting, transparent lid of heavy plastic, and the flow of fresh water to the tanks was stopped as well as aeration. The tank water was then recirculated by means of a pump (Little Giant Company; 10 litres min^{-1}) which drew water from the

bottom of the tank and returned it back into the upper region of the tank. At the end of the hour, the regular 08:00 h feeding was performed. Over the next 9 h period, the tanks were sealed off from the air for 1 h, every other hour, and during these periods in-tank P_{O_2} levels were monitored continuously with a Cameron E101 oxygen electrode connected to a Cameron OM-200 oxygen meter. In the hour between measurements, air saturation was re-established by vigorous aeration and the calibration of each electrode was checked. Water P_{O_2} levels in the tanks never dropped below 70 % of the air saturation values. Water samples were taken at the beginning and end of the 9 h period and frozen at –20 °C for later analysis of nitrogen waste products (ammonia and urea).

Blank trials were carried out to determine the contribution of bacterial processes (from faeces, food, etc.) to measured O_2 consumption and nitrogen waste excretion rates by feeding a comparable group of trout to satiation for several days and then removing them from the tank. The measured rates in the absence of the fish amounted to approximately 5 % of the measured rate of O_2 consumption 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.

Ammonia concentrations were determined by the method of Verdouw *et al.* (1978). To determine the small differences in urea concentrations of the water samples, resolution was increased 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).

The following formula was used to calculate the absolute O_2 consumption rate from P_{O_2} levels:

$$\dot{M}_{O_2} = (\Delta P_{O_2} \times \alpha_{O_2} \times v)/(m \times t), \quad (1)$$

where ΔP_{O_2} (mmHg) is the measured change in P_{O_2} values between the beginning and end of each 1 h test period, v is the volume (litres) of water in each tank (211 litres), m (g) is the total mass of fish in the tank, t is time (h) and α_{O_2} (μmol l^{-1} $mmHg^{-1}$) is the solubility constant for O_2 in water (Boutilier *et al.* 1984). An analogous equation was used to calculate the excretion rates of the two nitrogenous wastes, using a 9 h time period and substituting total ammonia-N or total urea-N (μmol l^{-1}) for $\Delta P_{O_2} \times \alpha_{O_2}$.

The total N-excretion rate was then calculated as:

$$\dot{M}_{Ntotal} = 2\dot{M}_{Urea} + \dot{M}_{Amm}. \quad (2)$$

The urea excretion rate was multiplied by 2 to account for the two nitrogen atoms per urea molecule.

The instantaneous relative use of protein as an aerobic metabolic fuel was calculated as outlined by Lauff and Wood (1996*a*). The nitrogen quotient (NQ) was first calculated as:

$$NQ = \dot{M}_{Ntotal}/\dot{M}_{O_2}. \quad (3)$$

The protein component of fuel usage was then determined as:

$$\% \text{ Protein} = NQ/0.27, \quad (4)$$

where 0.27 is the theoretical maximum for NQ in a teleost fish (i.e. when protein is the fuel which is being metabolized, as derived by van den Thillart and Kesbeke, 1978).

The fish fed to satiation increased in mass by over 70 % during the course of the 21 day experiment, while the fasted fish lost 17 % of their body mass (see Fig. 1). Smaller fish are known to have a higher metabolic rate on a per gram basis than larger fish, so compensation for the size differences was made by mass correction, to that for a 1 kg fish, of the rates of oxygen consumption and nitrogen waste excretion (ammonia and urea) data to the exponent 0.824, as determined for rainbow trout by Cho (1992).

Swimming metabolism

All swimming tests were performed using small-volume (3.23 litres), variable-speed Blazka-type respirometers similar to those described by Beamish *et al.* (1989). The respirometers could be run as open (flow-through) or closed systems and were maintained at 15±1 °C by submergence in a temperature-controlled wet table. The respirometers were thoroughly cleaned prior to each trial, and blank rates of O_2 consumption and N-production were negligible.

Prior to the start of the swimming metabolism tests, a preliminary experiment was performed to evaluate the time course of $\dot{M}_{O_2}$ change after the ingestion of a meal. In-tank fish (N=12) were fed a 1 % body mass meal, then immediately placed in individual Blazka respirometers at a flow rate of 20 cm s^{-1} (approximately 2 BL s^{-1}, where BL is body length). After a 1 h settling period, $\dot{M}_{O_2}$ was measured sequentially over the following 19 h to determine the period over which the metabolic rate was elevated but stable. This period was found to be 1–6 h after the initial in-tank feeding; $\dot{M}_{O_2}$ declined progressively thereafter. Therefore, all swimming metabolism tests were performed during this 5 h window of elevated, stable metabolic rate.

Juvenile rainbow trout (mass 10–20 g, length 9–12 cm) were placed in three identical tanks as above (100 fish per tank), and each tank received a different ration in the same fashion as for the routine metabolism experiment: group 1, fasting; group 2, 1 % body mass per day; and group 3, satiation. The fish were kept on their respective diets for at least 4 days prior to swimming tests to allow for the oxygen consumption and nitrogen excretion rates to differentiate and stabilize (see Figs 2, 3; there were no significant changes in any of the groups after day 3).

At the start of a swimming experiment, the fish in the tanks were fed their morning ration (aside from the fasting treatments). Individual fish were then immediately removed and placed into the Blazka respirometers. The fish were allowed to adjust to the respirometers for 1 h before the exercise tests began. All swimming tests were performed between 1 and 6 h after feeding, the period of elevated and stable metabolic rate as determined in the preliminary experiment above.

The critical swimming speed tests (U_{Crit}) (Brett, 1964) were performed by increasing the water velocity by increments of

10 cm s^{-1} every 40 min until the fish became exhausted. Beamish (1978) and Hammer (1995) state that the conclusions of such tests are independent of the interval chosen as long as it is in the range 20–60 min. We chose 40 min as the minimum practical time for oxygen consumption measurements. During each period, the respirometer was closed off for the first 30 min for these measurements. Water samples were drawn at 10 min intervals and injected into a thermostatted Cameron E101 oxygen electrode connected to a Cameron OM-200 O_2 meter. $\dot{M}_{O_2}$ values were calculated as before using equation 1 and mass-corrected to the exponent 0.824 (Cho, 1992). The respirometer was flushed with fully air-saturated water at 15±1 °C for the last 10 min of each swimming speed period to prevent the oxygen levels from dropping below 70 % of air saturation. 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 had occurred.

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

$$U_{Crit} = V_f + [(T/t)dV], \quad (5)$$

where U_{Crit} is in cm s^{-1}, V_f is the velocity prior to the velocity at which exhaustion occurred (the last velocity which was swum for the entire 40 min period), dV is the velocity increment (10 cm s^{-1}), t is the time swum at each velocity (40 min) and T is the time swum at the final velocity before exhaustion.

After exhaustion, the fish were anaesthetized with MS222, blotted dry and weighed to the nearest 0.01 g, and fork length measured to the nearest millimetre. In order to deal with variations in length of up to 30 % amongst fish in this series, swimming velocities in cm s^{-1} were converted to body lengths per second (BL s^{-1}). Regression analyses of $\log\dot{M}_{O_2}$ *versus* linear swimming velocity (see Fig. 1 of Wilson *et al.* 1994) were employed to interpolate $\dot{M}_{O_2}$ values to common speeds of 0, 1.0, 2.0, 3.0, 4.0 BL s^{-1}, etc., for the purposes of averaging, corresponding approximately to the velocity increments employed. All regression relationships were significant (P<0.05). 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).

Protein utilization during exercise

A separate series of experiments was performed to investigate the contribution of protein to aerobic fuel use at different swimming speeds. The same three ration groups as in the swimming metabolism tests were employed, and the experimental protocols were similar. Oxygen consumption, nitrogenous waste excretion, NQ and protein utilization were measured during each swimming interval of the U_{Crit} swimming tests. However, in this series, it was necessary to extend the periods for each swimming speed to 90 min and to avoid flushing until the end of the period to allow for a measurable accumulation of ammonia and urea in the respirometers. In order to achieve this without the oxygen levels dropping below 70 % of saturation, the respirometer was gently bubbled with air after the end of $\dot{M}_{O_2}$ determination (i.e. at 30–90 min of each period). Another consequence was that the tests routinely ran for 1–2 h longer than the previously established 1–6 h post-feeding window of stable metabolic rate, and 'ideal' $\log\dot{M}_{O_2}$ *versus* linear swimming speed relationships were not obtained. It was therefore not possible to interpolate accurately to common speeds in terms of BL s^{-1}, and data were averaged instead at the common absolute velocity (cm s^{-1}) intervals used.

Statistical analyses

Data are expressed as means ±1 S.E.M. (number of fish). Regression analyses of oxygen consumption rates (on a logarithmic scale) against swimming speed were performed for each individual fish in the swimming metabolism tests to permit interpolation to common swimming velocities in BL s^{-1} and to facilitate extrapolations to U_{Crit} to determine the maximum metabolic rates ($\dot{M}_{O_2max}$) and to zero velocity to predict 'minimum' metabolic rates. Wilson *et al.* (1994) provide a typical example in Fig. 1 of their study. One-way analysis of variance (ANOVA) was performed on feeding and mass data, on O_2 consumption and nitrogen waste excretion rates at the different speeds and on the U_{Crit} values for the three feeding regimes. If the result of the ANOVA was significant, then a Fisher's test for multiple comparisons was applied to test for significant differences among treatments. The fiducial limit of significance was 5 %.

Results

Routine metabolic rate

Fish fed to satiation consumed, on average, approximately 3 % of their total body mass per day or approximately three times as much as fish on the limited 1 % ration, a significant difference (Fig. 1A). Fish masses from the three feeding treatments all changed over the 21 day period. Fasted fish lost approximately 1 g per fish or 17 % of their mass by day 21, while fish on the 1 % diet gained approximately 1 g or 17 %. Fish fed to satiation increased in mass by 5.6 g over the 21 days, an increase of 73 % (Fig. 1B). These patterns were consistent between duplicate tanks, and the differences amongst groups were all significant by day 21. In retrospect, it must be noted that the fish destined to become the satiation-fed group were significantly larger at day 0 than the fish in the other two groups, by approximately 20 %. Despite this fact, the subsequent differentiation in growth between the groups is far greater than their initial variation; by day 21, the satiation-fed fish were approximately 161 % larger than the starved group and 74 % larger than the 1 % ration group.

Prior to day 0, $\dot{M}_{O_2}$ values were very similar amongst the three groups. Once the respective feeding regimes were initiated, $\dot{M}_{O_2}$ values differentiated rapidly (Fig. 2). By day 2, the differences were fully established and remained relatively stable over the next 19 days. Fish fed to satiation consumed,

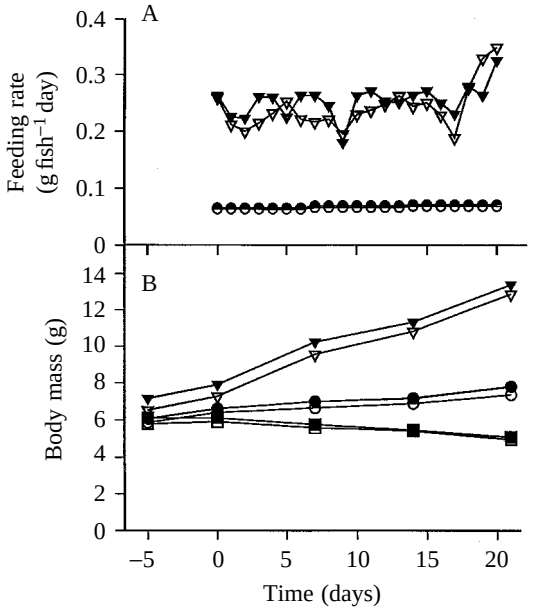

Fig. 1. (A) Feeding rate and (B) mean individual juvenile rainbow trout mass for replicated tanks of fish fed to satiation (triangles), fed 1 % body mass per day (half circles/circles) and fasted (squares) over the 21 day experiment. Prior to day 0, all fish were maintained on a 1 % body mass per day ration.

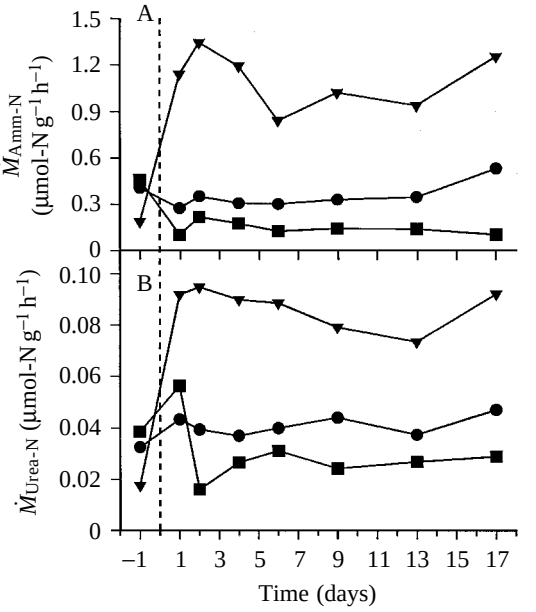

Fig. 3. Absolute nitrogenous waste excretion for (A) ammonia ($\dot{M}_{Amm\text{-}N}$) and (B) urea ($\dot{M}_{Urea\text{-}N}$) of juvenile rainbow trout fed to satiation (triangles), fed 1 % body mass per day (circles) and fasted (squares). Rates have been mass-corrected to the exponent 0.824 (Cho, 1992).

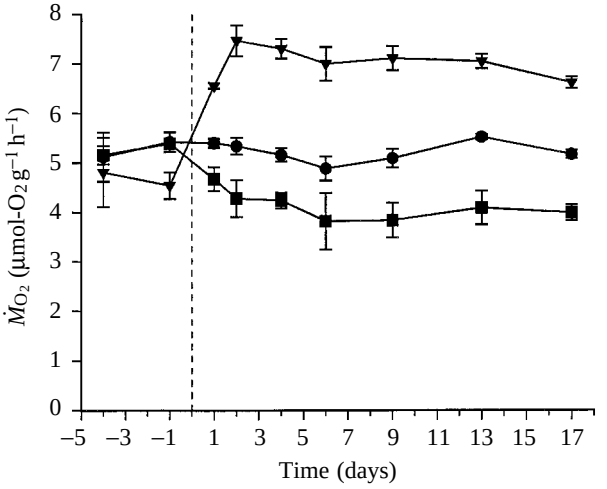

Fig. 2. Rate of oxygen consumption ($\dot{M}_{O_2}$) of juvenile rainbow trout fed to satiation (triangles), fed 1 % body mass per day (circles) and fasted (squares). Rates have been mass-corrected to the exponent 0.824 (Cho, 1992). Values are means ± S.E.M. (N=5).

on average, 30 % more oxygen than fish fed 1 % body mass per day and 68 % more than fasted fish. The absolute $\dot{M}_{O_2}$ values in satiation-fed fish were approximately 70 % of the $\dot{M}_{O_2max}$ subsequently measured when these fish were swum at U_{Crit} (see Fig. 6B).

Only single daily values (over 9 h) of ammonia and urea excretion were obtained for each treatment, but these rates differentiated more rapidly than $\dot{M}_{O_2}$ once the fish were placed on the different rations (Fig. 3). By day 1 of the experiment, the ammonia excretion levels had maximally separated to their respective levels over the experiment. Fish fed to satiation excreted over three times more ammonia than the fish fed 1 % body mass per day and over six times more ammonia than fasted fish. The pattern of urea excretion (Fig. 3B) was similar to that for ammonia (Fig. 3A), although the absolute rate of urea-N excretion was only approximately 10 % of ammonia-N excretion rates in each treatment.

Conversion of these in-tank $\dot{M}_{O_2}$ and $\dot{M}_N$ data through the NQ (equation 3) to percentage protein use (equation 4) revealed that protein supplied 50–70 % of the aerobic fuel in the satiation-fed group, approximately 25 % in the fish on 1 % ration, and only approximately 15 % in fasted fish (Fig. 4).

Swimming metabolism

In the swimming respirometers where activity levels were controlled, absolute $\dot{M}_{O_2}$ values at moderate swimming speeds ($\leqslant$2.0 BL s^{-1}; Fig. 5) were actually lower than in the holding tanks (cf. Fig. 2) where spontaneous activity and social interactions could occur. However, the effect of ration was clearly maintained. $\dot{M}_{O_2}$ was significantly elevated by approximately 50 % (or 1.4 μmol g^{-1} h^{-1}) in both the satiation-fed fish and the 1 % ration fish relative to the fasted group at the lowest speed (1.0 BL s^{-1}). $\dot{M}_{O_2}$ increased exponentially with swimming speed in all three ration groups, and the same absolute elevation in $\dot{M}_{O_2}$ persisted in the satiation-fed fish relative to the fasted group at all comparable swimming speeds up to the respective U_{Crit} values. $\dot{M}_{O_2}$ values in the 1 % ration group tended to be intermediate throughout. Thus, at any given swimming speed, $\dot{M}_{O_2}$ was lowest for fasted fish, intermediate

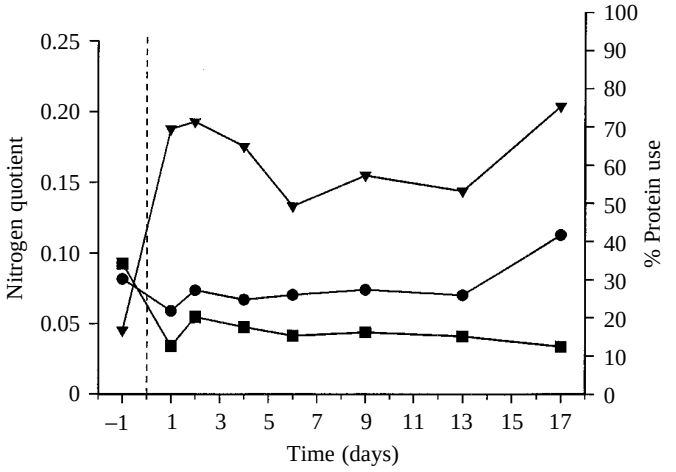

Fig. 4. Nitrogen quotients (left-hand axis) and percentage protein use as an aerobic fuel for routine metabolism (right-hand axis) of juvenile rainbow trout fed to satiation (triangles), fed 1 % body mass per day (circles) and fasted (squares).

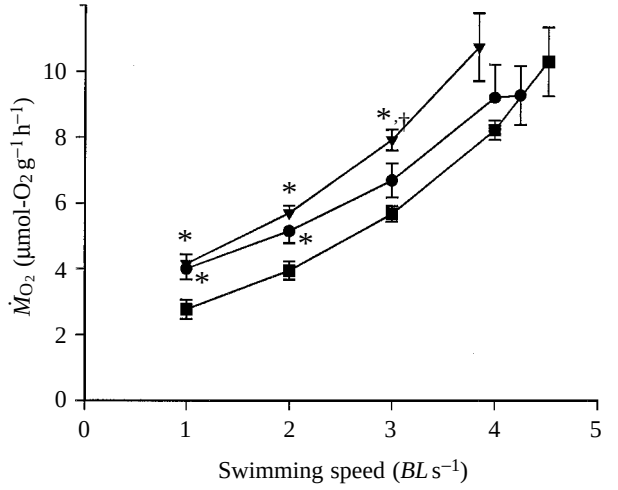

Fig. 5. Rates of oxygen consumption *versus* swimming speed for juvenile rainbow trout fed to satiation (triangles), fed 1 % body mass per day (circles) and fasted (squares). The fastest swimming speed for each group is the mean critical swimming speed (U_{Crit}). $\dot{M}_{O_2}$ rates have been mass-corrected to the exponent 0.824 (Cho, 1992). Values are expressed as means ± S.E.M. (N=6–7 fish). The asterisk (*) denotes a significant difference from the fasted oxygen consumption rate, while the dagger (†) denotes a significant difference from the rate for fish fed 1 % body mass per day (one-way ANOVA followed by Fisher's test, P<0.05). *BL*, body length.

for fish fed 1 % body mass per day and highest for fish fed to satiation.

Fasted fish had the highest U_{Crit} (4.52±0.18 BL s^{-1}), whereas U_{Crit} for the fish fed to satiation was 15 % lower (3.85±0.16 BL s^{-1}), a significant difference (Fig. 6A). U_{Crit} values for the 1 % ration fish were intermediate (4.25±0.21 BL s^{-1}, 6 % slower than the fasted fish) but not significantly different from values for either of the other two treatments. Despite these differences in U_{Crit}, there were no

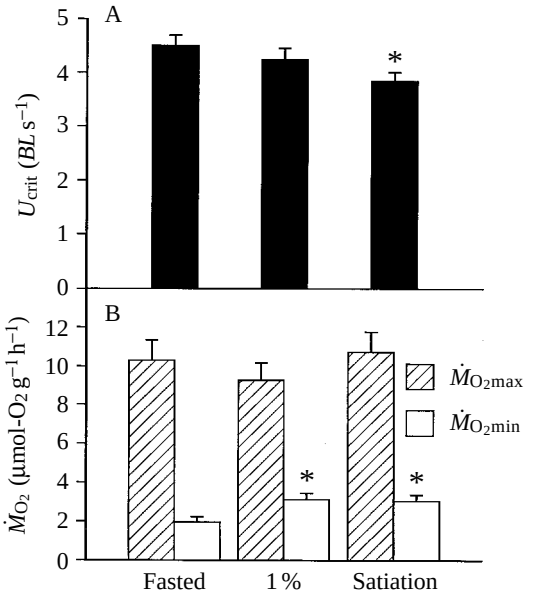

Fig. 6. (A) Critical swimming speed (U_{Crit}) and (B) minimum (at 0 BL s^{-1}) and maximum (at U_{Crit}) oxygen consumption rates [mass-corrected to the exponent 0.824 (Cho, 1992)] of fish fed to satiation, fed 1 % body mass per day and fasted. Values are expressed as means + S.E.M. (N=7 fish). The asterisk (*) denotes a significant difference from the fasted speed/rate (one-way ANOVA followed by Fisher's test, P<0.05). *BL*, body length.

differences in $\dot{M}_{O_2max}$ values (all approximately 10 μmol g^{-1} h^{-1}) at U_{Crit} amongst the three groups. However, 'minimum' $\dot{M}_{O_2}$ values, estimated by interpolation of log$\dot{M}_{O_2}$ *versus* swimming speed regressions to 0 BL s^{-1}, were significantly elevated by 55–59 % above the level in the fasted fish in both the satiation-fed trout and the 1 % ration trout (Fig. 6B).

Protein utilization during exercise

In these trials with longer swimming periods, the same basic relationship between feeding quantity, $\dot{M}_{O_2}$ and swimming performance existed (Fig. 7A). Note, however, that the exponential relationship between $\dot{M}_{O_2}$ and swimming speed, as seen with shorter periods in the previous series (cf. Fig. 5), was no longer seen in the two fed groups, presumably because the total test time extended beyond the 1–6 h post-feeding 'window of stability'. Nevertheless, U_{Crit} values were again significantly lower in the satiation-fed trout at 2.83±0.12 BL s^{-1} than in the fish fed 1 % body mass per day and the fasted fish whose U_{Crit} values were 3.59±0.07 and 3.88±0.21 BL s^{-1} respectively. There was also a clear elevation in $\dot{M}_{O_2}$ in the satiation-fed fish, at least at the lower swimming speeds (Fig. 7A).

At all swimming velocities, ammonia-N excretion rates were greatly elevated in the satiation-fed trout and lowest in the fasted fish (Fig. 7B), in accordance with the pattern seen earlier in the in-tank measurements (cf. Fig. 3A). However, ammonia excretion did not increase with swimming speed in any of the groups (Fig. 7B). Urea-N excretion was also much

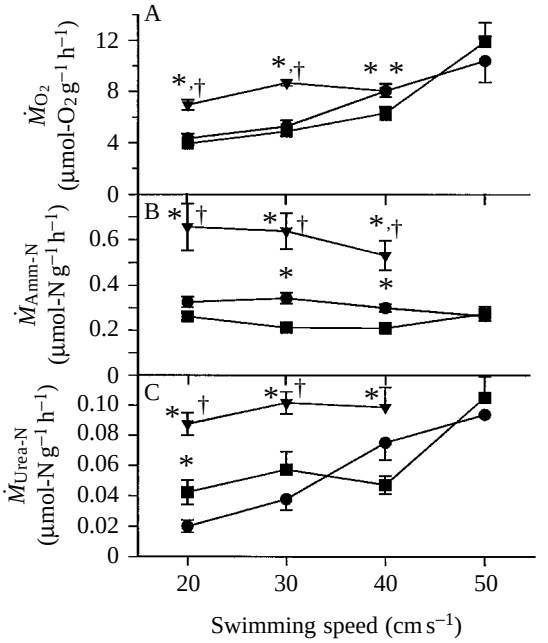

Fig. 7. (A) Rates of oxygen consumption, (B) ammonia excretion and (C) urea excretion *versus* swimming speed for juvenile rainbow trout fed to satiation (triangles), fed 1 % body mass per day (circles) and fasted (squares). Rates have been mass-corrected to the exponent 0.824 (Cho, 1992). Values are expressed as means ± S.E.M. For fish fed to satiation, N=9, 9, 6 and 0 fish at 20, 30, 40 and 50 cm s^{-1} respectively. For fish fed 1 % body mass per day, N=12, 12, 10 and 1 fish at 20, 30, 40 and 50 cm s^{-1}, and for fasted fish, N=9, 9, 8 and 5 fish at 20, 30, 40 and 50 cm s^{-1} respectively. The asterisk (*) denotes a significant difference from the fasted rate, while the dagger (†) denotes a significant difference from the rate for fish fed 1 % body mass per day (one-way ANOVA followed by Fisher's test, P<0.05).

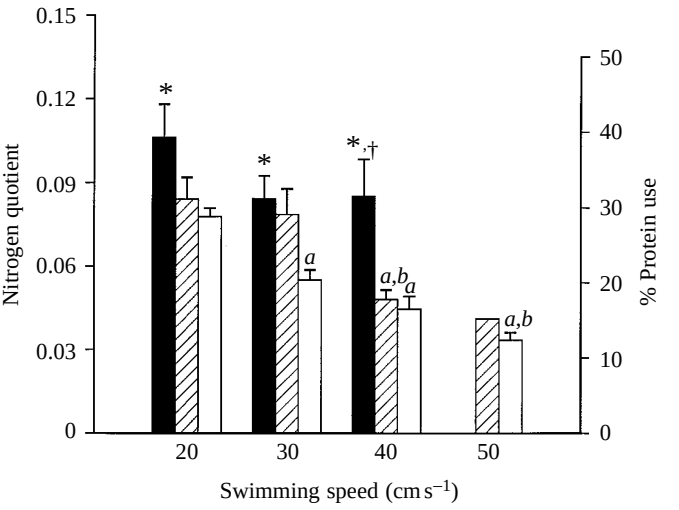

Fig. 8. Nitrogen quotients (left-hand axis) and percentage protein use (right-hand axis) *versus* swimming speed for juvenile rainbow trout fed to satiation (filled columns), fed 1 % body mass per day (hatched columns) and fasted (open columns). Values are expressed as means + S.E.M. (see Fig. 7 for values of N). The asterisk (*) denotes a significant difference from the fasted value, while the dagger (†) denotes a significant difference from the value for fish fed 1 % body mass per day. A significant difference from the value at 20 cm s^{-1} within a group is denoted by *a*, while a significant difference from the value at 30 cm s^{-1} within a group is denoted by *b* (one-way ANOVA followed by Fisher's test, P<0.05).

higher in the satiation-fed fish relative to the other two groups at all swimming speeds (Fig. 7C), as in the in-tank determinations (cf. Fig. 3B). Urea-N excretion did, however, increase with swimming speed in all three groups, an effect that was marked (2.5- to fivefold increases) and highly significant in both the fasted and the 1 % ration treatments (Fig. 7C).

Differences in NQ and percentage protein use between treatments in these swimming fish (Fig. 8) were not as marked as in the in-tank experiments (cf. Fig. 4), but again the contribution of protein was affected by dietary availability. Protein utilization was greatest in satiation-fed trout and lowest in the fasted fish at all swimming speeds (Fig. 8). The relative contribution of protein as a fuel for swimming decreased with increasing swimming speed in all three groups (Fig. 8). These reductions (approximately twofold) were greater in the fasted and 1 % ration fish; however, because the trout fed to satiation fatigued at a lower absolute velocity, the range of swimming speeds examined was reduced in this treatment. More importantly, there was no evidence that protein use as an aerobic fuel increased with swimming speed on either a relative or an absolute basis, regardless of the availability of protein in the diet.

Discussion

Routine metabolic rate

Routine in-tank metabolic rates separated very rapidly, such that by day 2 of the feeding regime, the new levels were fully established (Fig. 2). Fish fed to satiation had an $\dot{M}_{O_2}$ 68 % higher than that of the fasted fish, a difference of approximately 3 μmol g^{-1} h^{-1}. The satiation-fed metabolic rate corresponded to approximately 70 % of the $\dot{M}_{O_2max}$ measured at U_{Crit} during swimming trials (Fig. 6B). This elevation in $\dot{M}_{O_2}$ obviously reflected an important SDA component due to all the costs associated with the nutritive process, including the cost of growth (see Introduction). However, the difference in $\dot{M}_{O_2}$ between fed and fasted fish, measured in-tank, undoubtedly reflects other factors in addition to the SDA effect. Fasting reduces 'spontaneous' activity, whereas intensive feeding greatly stimulates it, an effect often referred to as excitability (Beamish, 1964, 1974; Brett and Zala, 1975; Jobling, 1994). Indeed, Brett and Zala (1975), performing similar in-tank measurements on juvenile sockeye salmon (*Oncorhynchus nerka*), found that $\dot{M}_{O_2}$ had already reached its peak immediately *prior* to the onset of the daily feeding period, an anticipatory effect. This same effect was often seen in the present study (data not shown). The cost of such spontaneous activity (turning, acceleration) per unit distance travelled may be considerably higher than the cost of steady-state swimming (Smit, 1965; Krohn and Boisclair, 1994). Furthermore, the costs of social factors (aggression, overcoming turbulence

created by neighbouring fish) may also be influenced by ration in a complex fashion (e.g. Christiansen and Jobling, 1990).

A more reliable estimate of the true SDA effect was undoubtedly obtained in the swimming respirometers, where activity was controlled and social interaction factors were removed. Here, absolute $\dot{M}_{O_2}$ levels were much lower (Fig. 5). Satiation feeding raised the measured $\dot{M}_{O_2}$ at $1\,BL\,s^{-1}$ by 50 % (Fig. 5) and the estimated minimum $\dot{M}_{O_2}$ at zero velocity by approximately 59 % (Fig. 6B), or approximately $1.4\,\mu mol\,g^{-1}\,h^{-1}$. These effects were quantitatively very similar to those reported by LeGrow and Beamish (1986), who carried out their feeding experiments with juvenile rainbow trout at $2\,BL\,s^{-1}$, at an identical water temperature.

Swimming metabolism

Other studies have looked at the effect of feeding on the oxygen consumption of swimming fish, but the present investigation appears to be the first to swim the fish to their maximum capacity and examine $\dot{M}_{O_2}$. The results demonstrate that the absolute stimulation of $\dot{M}_{O_2}$ by the SDA effect of feeding is maintained virtually constant as swimming speed increases (Fig. 5). However, fed fish are forced to stop aerobic swimming at a lower speed because of this SDA load. Fed fish therefore reach the same $\dot{M}_{O_2max}$ at a lower U_{Crit} than fasted fish (Fig. 6). Clearly, the data confirm the second of the three possible scenarios raised in the Introduction: there is an absolute limit to $\dot{M}_{O_2max}$, and swimming metabolism is sacrificed due to the SDA effect of feeding. The sites where O_2 is utilized to support swimming metabolism (skeletal muscle, predominantly red muscle) are undoubtedly different from the sites where O_2 is utilized to support the SDA effect (liver, intestine). This implies that the capacity of the fish's system to take up and/or deliver O_2, rather than the overall capacity of the tissues to consume O_2, ultimately limits $\dot{M}_{O_2max}$.

The results also suggest that there is an irreducible cost of feeding which decreases the amount of O_2 available to the muscles and reduces the swimming performance of the fish once the limiting $\dot{M}_{O_2max}$ is approached. Once nutrients from the food have reached the SDA tissues, the metabolic processes cannot be turned off. Feeding results in increased visceral blood flow in resting fish (Axelsson *et al.* 1989; Axelsson and Fritsche, 1991). The present results suggest that fed fish cannot redistribute their blood flow preferentially to red muscle (and away from the viscera) when exercised to the same extent as fasted fish (Randall and Daxboeck, 1982; Axelsson and Fritsche, 1991; Thorarensen *et al.* 1993; Kolok *et al.* 1993; Wilson and Egginton, 1994). Vasodilation by local metabolic factors in the SDA tissues and/or a different autonomic response may be involved. There is a clear need for cardiovascular studies on fish which are both fed and swum.

Few previous investigations have considered feeding *versus* swimming interactions on $\dot{M}_{O_2}$, and all were performed at submaximal velocities with wild rather than hatchery-raised fish. Nevertheless, the present results are in excellent agreement with one of these studies: Beamish (1974) reported that the absolute elevation of $\dot{M}_{O_2}$ caused by a 4 % ration was identical in smallmouth bass (*Micropterus salmoides*) swimming at 1.4, 1.9 and $2.5\,BL\,s^{-1}$. In contrast, Muir and Niimi (1972) suggested that the absolute SDA effect in the aholehole (*Kuhlia sandvicensis*) might actually increase with swimming speed because of an improved ability (greater circulatory efficiency) of swimming fish to take up O_2. Blaikie and Kerr (1996) also showed this in the Atlantic cod (*Gadus morhua*): an increase in SDA with an increase in swimming speed. However, these studies did not look systematically at ration effects at different swimming speeds. There also appears to be a direct disagreement with Furnell (1987), in the sablefish (*Anoplopoma fimbria*), who concluded that the SDA effect was gradually suppressed and eventually disappeared as swimming speed increased. This may be a real species difference; alternatively, it may be an artefact because only three individual sablefish were tested at a limited range of submaximal velocities.

Protein utilization

Nitrogenous waste excretion rates (Figs 3, 4) separated even more rapidly and to a greater relative extent than the oxygen consumption rates (Fig. 2) once fish were placed on their respective rations. The low ammonia-N and urea-N excretion rates of the fasted fish represent the so-called *endogenous* or maintenance fraction (van Waarde, 1983; Wood, 1993). The dramatic differences between the fed and fasted rates are due to the *exogenous* fraction, or the portion not retained from the absorbed food. These large differences undoubtedly resulted from the very high protein content (50 %; Table 1) of the commercial diet used. The most important factor influencing the overall rate of nitrogen excretion in salmonids is the rate of dietary protein intake (Beamish and Thomas, 1984). While all studies agree that ammonia-N excretion increases as a function of food intake, there is some disagreement as to whether the much smaller urea-N excretion responds in a similar manner (reviewed by Wood, 1993). The present results showing a strong dependence of urea-N excretion on ration (Fig. 3B) agree with several previous studies on rainbow trout (Kaushik, 1980; Beamish and Thomas, 1984), but not with the work of Brett and Zala (1975) on sockeye salmon.

The low values of in-tank NQ (approximately 0.04) in the fasting treatment (Fig. 4) indicated that only approximately 15 % of aerobic metabolism was fuelled by protein, in agreement with the recent study of Lauff and Wood (1996*a*) on juvenile rainbow trout fasted in respirometers. Relative protein use was 1.7-fold higher in fish fed a 1 % daily ration and approximately fourfold higher in fish fed to satiation (Fig. 4). Thus, feeding quantity during in-tank routine activity has a dramatic effect on protein usage (Fig. 4). Taking into account the fact that fasted fish were using less fuel overall (i.e. lower routine $\dot{M}_{O_2}$; Fig. 2), then the absolute protein use of the satiated fish was over 6.5 times that of the fasted fish. Protein use is higher in fed fish because absorbed amino acids from the food first go towards tissue protein synthesis, and excess amino acids can then be deaminated and subsequently oxidized

in the citric acid cycle for the immediate production of energy (van Waarde, 1983; Brown and Cameron, 1991; Lyndon *et al.* 1992).

With swimming fish, increased feeding elevated protein use at any given speed although, within each of the feeding groups, relative protein use decreased as swimming speed increased (Fig. 8). Although urea-N excretion was only a minor component of total-N excretion, it increased markedly with exercise, especially at the two lower ration levels (Fig. 7C). This pattern was also seen by Lauff and Wood (1996*b*) in fasted trout tested under similar conditions. The cause of this phenomenon is unknown; it may possibly reflect the increasing recruitment of white muscle fibres at faster swimming speeds (Wilson and Egginton, 1994) and the accompanying adenylate turnover, a byproduct of which is urea produced by uricolysis (Wood, 1993). This would result in an actual overestimation of protein use at higher swimming speeds.

Overall, the present data reinforce the conclusion that protein does not become more important as a fuel during exercise, even when abundantly available in the diet. This conclusion opposes a belief that is fairly widespread in the literature (e.g. Brett and Groves, 1979; van Waarde, 1983; van den Thillart, 1986; Jobling, 1994; Weber and Haman, 1996). The reasons for this disagreement have been discussed in detail by Lauff and Wood (1996*b*). In brief, the techniques used in the past have not directly measured the rates at which fuels are being burned, in contrast to the present respirometric approach. When extended to include $\dot{M}_{CO_2}$ measurements, the respirometric approach demonstrated that lipid became increasingly important as the major fuel of exercise as swimming speed increased in unfed trout, that carbohydrate was of secondary importance and that protein made the smallest contribution overall (Lauff and Wood, 1996*b*).

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