

**AEROBIC FUEL USE IN RAINBOW TROUT, *ONCORHYNCHUS MYKISS***

THE EFFECTS OF STARVATION, EXERCISE, AND  
EXERCISE WITH PRE-TRAINING ON  
AEROBIC FUEL USE  
IN  
JUVENILE RAINBOW TROUT (*ONCORHYNCHUS MYKISS* WALBAUM)

By

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**TITLE:**       **The Effects of Starvation, Exercise, and Exercise with Pre-training on Aerobic Fuel Use in Juvenile Rainbow Trout (*Oncorhynchus mykiss* Walbaum)**

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## Abstract

Metabolic fuel use in rainbow trout (*Oncorhynchus mykiss* W.) was investigated using closed system respirometry and proximate body analysis. During short term starvation (15 days, routine activity) the utilization of protein as a substrate, as determined by respirometry, increased from 14 to 24% of total fuel supply. However, even by the end of the experiment, the contribution of protein (24%) did not approach the classically reported values for fish of between 40 and 90%. Indeed, from respirometry data, during the first quarter of the experiment lipid contributed the majority of the fuel (> 60%) while carbohydrate contributed about 20%. Thereafter, lipid and carbohydrate became essentially equivalent in importance (about 37% each). However, from proximate body analysis, a more traditional fuel mixture was found (protein, 58%; lipid, 40%; carbohydrate, 2%) suggesting the possibility that the two procedures were measuring fundamentally different parameters.

Instantaneous fuel use during sustainable swimming at different speeds was investigated by respirometry using three day test periods. While protein catabolism remained constant over time, and uniform between groups, its relative contribution tended to increase with time as total  $M_{O_2}$  declined with sustained swimming. Protein catabolism was highest in nonswimming fish (30-45%) and lowest in the high speed swimmers (20%);

lipid was the most abundant (41-55%) fuel used in all groups at all times. In the nonswimmers and lowspeed swimmers, lipid use tended to increase slightly over time whereas in the high speed swimmers, lipid use dropped from 54 to 44%. Carbohydrate use (up to 38%) was higher than predicted by earlier literature, but decreased greatly in both the nonswimmers and low speed swimmers over the three days, whereas in the high speed swimmers the contribution increased with time.

The low speed swimmers from the last set of experiments were used as controls for the final set of experiments in which another group of fish were trained for two weeks at  $1.0 \text{ L}\cdot\text{s}^{-1}$  prior to testing using an otherwise similar regime. Even though there was no difference in gas exchange, the make-up of the fuel mixture was different for the two groups. Protein use was significantly lower, while lipid use was higher in the trained fish. In addition, relative protein use in the trained fish was constant over the three day period, a feature found only in the the high speed swimmers of the previous experiment.

A critical evaluation of the respiratory quotient is given since its use by fish physiologists has been without complete conversion from that used by the mammal physiologists. In addition, the often quoted term "fuel use" is differentiated into *instantaneous fuel use* and *compositional fuel use* since the two describe fundamentally different principles, though this has not always been recognized in the literature.

**Acknowledgements**  
(The only pages that are really me!)

Well, here it is. The thesis. Fish, Frustration, and Fun. In no particular order, that almost completely describes my tenure at Mac. Add a heaping serving of enlightenment, both academic and nonacademic and you have the whole damn thing.

I've learned quite a bit here, not all of it dealing with fish callisthenics. Watching a pro baseball game is boring. I don't care if the Jays are the sole Canadian team to win the sweater or the cup or whatever it is those overgrown, overpaid crotch scratchers win, the game is boring. I've learned that. I've also learned that canoes can fly. That one is too embarrassing to detail in print, so ask me about it someday.

People care. That's important. My friends, both local and distant, helped me through some tough times and just as importantly, shared in my triumphs. My most regular electronic mail correspondents, Keith Jensen (Halifax), Carol Kroeker (Calgary), and Mungo Marsden (Victoria) provided me with advice on everything from women to freeze-driers. I think I have freeze-driers figured out now. They also kept me in touch with other distant friends and in many, many cases, gave me something to laugh about. You three can bet you made a real difference.

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And finally, the heartiest of thanks to my supervisor, Dr. Chris Wood, a brilliant scientist. I learned more than just exercise physiology under your guidance. I came to your lab with only a passing interest in fish; I leave your lab with more confidence in my research abilities, teaching abilities and drinking capacity. For all of these, I thank you (hic!).

Randolph Friedrich Lauff, B.Sc.  
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December, 1993.

...and don't ever, ever, ever forget those fish muffins.



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## Chapter 1

### General Introduction

#### Background

Exercise in fish is only one area of a vast and growing field of piscine research in Canada. Presumably, the large component of the national economy supported by commercial and recreational fishing and aquaculture, and the large portion of leisure time of individuals devoted to aquatic pursuits in this country has fueled both the financial support and interest in fish research. Other than adding to basic scientific information, knowledge garnered from some of the work in this field directly helps to feed many of the world's people, while other research helps to preserve or repair our mismanaged ecosystems.

Swimming is the only means of locomotion for the vast majority of the planet's 22 000 species, and it is often viewed from the perspective of the physiologist as exercise. Exercise can be easily induced in the laboratory to approximate a natural component of the routine activity of wild fish. As a result of this, a vast literature exists on the physiology of fish locomotion, though morphological (eg. Riddell and Leggett, 1981), biomechanical (eg. Alexander, 1970; Webb, 1971; Rome *et al.*, 1988; Daniel, Jordan and Grunbauer, 1992) and biochemical (eg. Duncan and Tarr, 1958; Henderson

and Tocher, 1987) studies exist as well.

Within the realm of physiology, researchers are piecing together the complexities of exercise from many of the classical approaches including endocrinology (Barret and McKeown, 1988; Hughes, Le Bras-Pennec and Pennec, 1988; Butler *et al.*, 1989), ionoregulation (Brauner, Shrimpton and Randall, 1992), ventilation (Jones *et al.*, 1990), respiration (Brett, 1964; Farmer and Beamish, 1969; Sukumaran and Kutty, 1977; Duthie, 1982; Hughes *et al.*, 1988), muscle function (Johnston and Goldspink, 1973b; Johnston, Ward and Goldspink, 1975; Greer Walker and Emerson, 1978), haematology (Jeziarska, Hazel and Gerking, 1982; Davie, Tetens and Wells, 1986), and others. Most of this work has been done on salmonids for several reasons: they have great economic importance (thus generate more research funding than would those species deemed "unimportant"); they are readily available from farms or hatcheries (which allows for homogenous genetic stock); since many of them are stream dwellers in the wild, they are naturally good swimmers (Scott and Crossman, 1990). Having such a large database on salmonids allows for useful comparisons between fish of different life histories or swimming styles. The current study focusses on the best-studied salmonid species, the rainbow trout, *Oncorhynchus mykiss*.

The native rainbow trout is an anadromous species of the Pacific drainage system (Scott and Crossman, 1990). It, like many other salmonids, journeys vast distances upstream in order to spawn; these feats of



endurance, while remarkable in themselves, are even more extraordinary in that in many species of Pacific salmon, though not the rainbow trout, the journey is completed without feeding (Idler and Clemens, 1959; Walton and Cowey, 1982; Henderson and Tocher, 1987). Upstream migration of the spawning adult salmon is the most costly sustained energy expenditure of all activities recorded (Brett and Groves, 1979). These observations then draw the inevitable question, "Given that there is no energy intake, what substrates are burned in order to provide the necessary energy that powers locomotion?" This question can also be expanded to include periods of inactive starvation since fish can survive for much longer periods without food than can homeotherms. Indeed, for many species, a period of fasting during the winter months is part of the natural life cycle (Henderson and Tocher, 1987).

### Metabolic Fuels in Fish

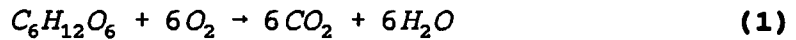
The aerobic fuel for metabolism in fish is drawn from carbohydrates, lipids and protein, but information on the preferred substrate is scarce, and what little there is seems to be inconsistent (Beamish, Howlett and Medland, 1989). Substrate utilization has been studied from an aquacultural perspective, often with the intention of enhancing the growth of the fish via specialized diets (Atherton and Aitken, 1970; Poston, 1991; Carter *et al.*, 1993), conferring resistance to an environmental stressor (Beamish and Tandler, 1990) or improving swimming performance (Beamish

*et al.*, 1989). At the other extreme of the spectrum of literature on fuel use, oxidative properties of muscle suggest that mitochondria from different fiber types are specialized for oxidation of different sets of substrates (Moyes *et al.*, 1989; Kiessling and Kiessling, 1993). For example, glutamate, proline and fatty acyl carnitines are all metabolized better in red muscle than white muscle.

Most of the presently available literature deals with the *depletion* of fuels during an experiment, or over a fish's natural migration (eg. Duncan and Tarr, 1958; Idler and Clemens, 1959; Mommsen, French and Hochachka, 1980; Virtanen and Forsman, 1987). These studies have looked at whole body (or organ) compositional changes to estimate fuel use. The results of such *depletion* studies reflect the *source* (ie. body stores) of the substrates which were either metabolized, or for instance, converted into gonadal material during migration. In general, the conclusion from such studies is that the carbon source for ATP synthesis is thought to arise mainly from lipid and protein reserves (Idler and Clemens, 1959; Henderson and Tocher, 1987).

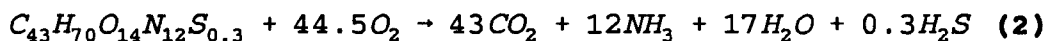
*Instantaneous* fuel use studies answer a fundamentally different question than do studies which measure the *depletion* of substrates. *Instantaneous* fuel use is that parameter measured by using ratios of gas exchange and nitrogenous waste excretion to stoichiometrically predict the fuel or combination of fuels actually being oxidized to supply energy to

support the fish's metabolism at that moment in time. For instance, in the well known equation describing the oxidation of one mole of glucose,



it can be seen that six moles of  $CO_2$  are produced for the six moles of  $O_2$  consumed in the reaction. The ratio of  $CO_2$  produced to  $O_2$  consumed during steady state is known as the respiratory quotient, and in this example (for carbohydrate) can be seen to have a value of unity. Similar equations give rise to values of 0.71 for lipid and from 0.83 to 0.97 for proteins (depending on the degree of ureotelism versus ammoniotelism; see Chapter 2 for a discussion of this issue).

Approximately 16% of protein is nitrogen which cannot be used for energy (Phillips, 1969). Since the nitrogen must be excreted, the nitrogen quotient (NQ, the ration of nitrogen excreted [ $M_{N-wastes}$ ] to oxygen consumed [ $M_{O_2}$ ]) can be used to estimate protein catabolism from the following equation (Van den Thillart and Kesbeke, 1978),



Even though this equation assumes strict ammoniotelism, the theoretical maximum NQ for protein catabolism (as a fraction of total metabolism) is not dependant on the proportion of ammonia and urea in the waste (see Chapter 2). The theoretical maximum then is,

$$\frac{12 \text{ mol } NH_3}{44.5 \text{ mol } O_2} = 0.27 \quad (3)$$

Although most work using this approach has been done on mammals (for a review, see Kleiber, 1987), a few researchers have used one or both quotients to estimate *instantaneous* fuel use in fish (Kutty, 1972, 1978; Van den Thillart and Kesbeke, 1978; Wiggs *et al.*, 1989). Protein utilization, the parameter most studied, has been estimated at 14 through 90% of total metabolism (references as above; and the review of Van Waarde, 1983). Some of the range quoted is undoubtedly due to inherent interspecific differences, though differences in ambient conditions (temperature, dissolved oxygen), absorptive state, activity levels, and experimental protocol likely make some of the values incomparable with one another.

Metabolic rates, both routine and active, are reflected in the  $M_{O_2}$  (Beamish *et al.*, 1989); in nonexhaustive swimming, the quantity of  $O_2$  consumed is proportional to the amount of work done (Beamish, 1978). A number of papers have correlated  $M_{O_2}$  with swimming speed (Wood and Randall, 1973; Kiceniuk and Jones, 1977; Puckett and Dill, 1984). Brett (1964) however, provides the most comprehensive study of  $M_{O_2}$  with sustained swimming. As with his successors, Brett found  $M_{O_2}$  rose exponentially with swimming speed.  $M_{O_2}$  rises under other conditions as well, a notable example being when fish are in the absorptive state (Brett

and Zala, 1975). The amount of work that can be done (whether in swimming, digestion, or other functions) per unit O<sub>2</sub> respired is dependant upon the type of fuel burned. The oxycalorific equivalent is the parameter which correlates the energy derived from the fuel to the amount of O<sub>2</sub> actually used. Values for carbohydrate, lipid and protein are 112, 105, and 107 kcal·mol<sup>-1</sup> O<sub>2</sub>, respectively (Kleiber, 1987).

The choice of which metabolic fuel to burn is presumably not a conscious one in fish or any organism. One factor which limits the choice though is availability (either directly from a body source or via conversion to a more preferred fuel). Several other limiting factors also exist including mobilization, transport (including plasma solubility), cellular and mitochondrial uptake, and rate of ATP synthesis (Weber, 1987). On a per-mass basis, lipid provides the most energy (9.5 kcal·g<sup>-1</sup> versus 4.0 and 5.7 kcal·g<sup>-1</sup> for carbohydrate and protein, respectively) which would seem to make it the logical form in which to store carbon reserves. However, other factors such as buoyancy control, and organ-specific fuel demands dictate that some carbon be stored as carbohydrate and proteins.

### The Sites of Fuel Utilization

In sustained swimming, fish use primarily, though not exclusively, red muscle for propulsion. Red muscle in most fish is superficial, lying in a relatively thin sheet along either flank, with its greatest thickness forming a wedge along the outer margin of the horizontal septum (Lindsey, 1978;

Sanger, 1992). In some fish (including salmonids), many of the red muscle fibers are scattered in a mosaic amongst the more numerous white (Driedzic and Hochachka, 1978a). However, based on histochemical investigations of succinic dehydrogenase, intracellular lipid and myofibrillar ATPase activity, only those red fibers of the lateral bundle are considered to be highly aerobic (Johnston *et al.*, 1975). Physiological experimentation lends support to the different roles played by the two populations of red fibers as well. While the lateral bundle red muscle is active at all swimming levels, the red fibers of the mosaic muscle are only recruited starting at 35% of the maximum sustainable swimming speed (Hudson, 1973).

White muscle is also capable of oxidative metabolism, though the concentration of the enzymes involved is much lower than in red muscle. Upon severe exertion, white muscle functions largely anaerobically, i.e., pyruvate does not enter the Krebs cycle but is instead converted to lactate (Driedzic and Hochachka, 1978a; Dobson and Hochachka, 1987). To investigate whether lactate could serve as a metabolic fuel, Weber (1991) compared the oxidation of [U-<sup>14</sup>C] lactate in resting and aerobically swimming (85% of critical swimming speed) rainbow trout. Since lactate use did not rise appreciably, Weber concluded that the ability of trout to exercise sustainably does not depend on their capacity to metabolize lactate rapidly.

## Lipid Metabolism

Total body lipids of coho salmon (*Oncorhynchus kisutch*) decreased in proportion to the distance travelled during sustained swimming (Driedzic and Hochachka, 1978) . However, lipid utilization seems to depend on swimming speed. Davison and Goldspink (1977) showed a swimming speed-dependant drop in red and white muscle lipids in *Salmo trutta*, with the higher speed fish utilizing more of their stores.

The effects of starvation on lipid catabolism have been well documented. Lipid stores are depleted steadily, with more depletion occurring in the white muscle (Cowey and Sargent, 1979; Henderson and Tocher, 1987). It is possible that the white muscle lipids are transferred to the red muscle for use. Red muscle (lateral band), in both salmonids and cyprinodonts, can oxidize free fatty acids at least 10-fold faster than white muscle. Undoubtedly, this is due to the oxidative nature of red muscle (Driedzic and Hochachka, 1978a). Some work suggests the mosaic muscle is capable of lipid utilization (Mommensen *et al.*, 1980; Johnston and Moon, 1980; Moyes *et al.*, 1989); at least some of this capacity is anaerobic (Milligan and Girard, 1993; Y. Wang, G.J. Heigenhauser and C.M. Wood, unpublished data), a role which better fits the historical perception of this organ.

When fish mobilize lipids for energy, they appear not to discriminate between the poly-unsaturates that originate solely in the diet

and the saturates and mono-unsaturates that can either originate in the diet or from the animal's biosynthetic activity. Mitochondria must therefore be capable of the oxidation of the wide range of fatty acids present in fish deposits and dietary lipids (Henderson and Tocher, 1987).

### Carbohydrate Metabolism

Both Brett and Groves (1979) and Van Waarde (1983) agreed that dietary carbohydrates are used poorly. Phillips (1969) suggested trout are physiologically incapable of utilizing high concentrations of dietary carbohydrates based on the relatively small number of insulin producing Islets of Langerhans. Ristori and Laurent (1985) found no significant changes in plasma glucose levels during sustained swimming. However, by itself, this does not suggest a lack of carbohydrate utilization since a constant turnover could be present. More significantly, the work of Van den Thillart (1986) showed that the oxidation of [<sup>14</sup>C]-glucose injected by cannula into the bloodstream of swimming trout was extremely low. Using this and respirometry, he concluded that the oxidation of carbohydrate for energy by trout was not significant. In contrast, Kutty (1978), whose work was also respirometry based, suggested carbohydrates were preferentially used by the mullet (*Rhinomugil corsula*). In several other respirometry based studies Kutty (1968, 1972) has stressed the importance of carbohydrates in fueling the sustainable swimming of fish of several species.

Davison and Goldspink (1977) found glycogen stores *increased*



dramatically in both red and white muscle of brown trout at the two lower test speeds (1.5 and 3.0 L·s<sup>-1</sup>), suggesting carbohydrates are not used as fuel over this range of speeds. However, they reported (as for the lipids) a large ( $\approx 80\%$ ) drop in glycogen at the highest speed (4.5 L·s<sup>-1</sup>).

### Protein Metabolism

Protein and/or free amino acids are known to be used as energy sources and are thought by many to provide the bulk of the energy used in sustained swimming in salmonids (Idler and Clemens, 1959; Kutty, 1972; Van Waarde, 1982; Richardson, 1983; Van den Thillart, 1986; Chamberlin *et al.*, 1991). Van den Thillart (1986) estimated 90% of energy for sustained swimming in rainbow trout came from protein. Wiggs *et al.*, (1989) clearly show ammonia quotient data for *Salmo salar* which suggests that at most only 45% of the fuel burned was protein. Idler and Clemens (1959) found sockeye salmon (*O. nerka*) used between 31 and 61% of protein reserves during migration, depending on gender and population. Mommsen *et al.*, (1980) also found evidence that proteins were used during migration. Heavy proteolysis was indicated by increasing levels of white muscle amino acids. Blood levels also rose which suggested that amino acids from white muscle were being oxidized elsewhere.

Body proteins are in a constant state of turnover (Walton and Cowey, 1982). When they are catabolized for energy they are first converted to intermediates which enter the Krebs cycle, which are then

oxidized to CO<sub>2</sub> and H<sub>2</sub>O. Regardless of the actual (and controversial) amount of protein used for energy, protein metabolism in fish is similar to other vertebrates in that gluconeogenesis is not a prerequisite for energy production, rather amino acids can be converted directly into Krebs cycle intermediates via deamination, transdeamination and nonoxidative decarboxylation (Walton and Cowey, 1977).

Protein turnover has now been measured in several studies in fish using a <sup>3</sup>[H]-phenylalanine swamping technique first developed by Garlick *et al.* (1980) for use in rats. Factors studied to date include exercise (Houlihan and Laurent, 1987), temperature (Fauconneau and Arnal, 1985), starvation (Loughna and Goldspink, 1984) and body size (Houlihan, McMillan and Laurent, 1986). In rainbow trout, Houlihan and Laurent (1987) found that gills exhibited the highest rate of protein turnover followed by ventricle, red muscle and white muscle. This order was independent of exercise, though the magnitude of the rates (except for gill) for the trained fish which swam during the test were approximately 2-fold greater than control animals. Interestingly, values for trained animals that were not swimming when tested were not significantly different from control animals. This implies then that swimming itself, not training, gives rise to high protein turnover rates.

### Objectives of the Present Study

The primary goal of the present study was to investigate instantaneous fuel use under moderate starvation, and during aerobic

swimming with and without a pretraining regime. In general, depletion studies, as noted above, have investigated compositional fuel use under a variety of conditions, including those of the present study, while respirometric techniques have not been fully exploited in any aspect dealing with instantaneous fuel use in fish. In all of the experiments of the current study,  $M_{O_2}$ ,  $M_{N-wastes}$ , and carbon dioxide excretion ( $M_{CO_2}$ ) were measured using closed system respirometry. These values were then used to calculate the respiratory and nitrogen quotients, from which instantaneous fuel use could then be calculated. Body compositions of the tested fish were compared with controls to determine depletion (or accretion) of fuels during the test period.

In the starvation component of this study (Chapter 2), the goal was to determine if the lipid and protein depletions mentioned previously would 1) be found in juvenile rainbow trout, and 2) be reflected in similar proportions in instantaneous fuel use. Over the 15 day experiment, bouts of respirometry were conducted daily in order to determine if a constant fuel mixture was burned or if a sudden or gradual change in fuel mixture occurred.

Chapters 3 and 4 on swimming fish focus on possible changes in instantaneous fuel use with intensity and duration of swimming, and pre-training. Three day test periods were used and thrice daily bouts of respirometry allowed for a finer resolution of fuel use than was possible in the starvation study. In Chapter 3, two different sustainable swimming

speeds (2.1 and 3.1 L·s<sup>-1</sup>) plus a non-swimming condition were compared. In Chapter 4, the low speed swimmers of Chapter 3 served as controls for another group of trout which had been trained for 2 weeks at 1.0 L·s<sup>-1</sup>.

An instantaneous fuel use study of this scope has not been reported previously for fish. The results demonstrate that fuel supply in juvenile rainbow trout is sensitive to starvation, to swimming speed and duration, and to exercise training. At the same time, these results challenge some current ideas about the relative importance of different fuels used by fish, and cast light on the differences between *instantaneous fuel use* and *compositional fuel use* studies. Hopefully, the present investigation provides a solid foundation for future studies which will fully integrate the two approaches.

## CHAPTER 2

### Introduction

Unlike most terrestrial animals, the majority of temperate species of fish experience a period of starvation for a substantial part of every year. They are well adapted to mobilizing their body constituents as fuel for survival (Love, 1970). It has been long assumed that the major metabolic fuel in fish is protein (Van Waarde, 1983; Van den Thillart, 1986). A high protein diet in the wild in many fish (Bever, Chenoweth, and Dunn, 1981) presumably has led to the assumption that protein would be the most abundant, and therefore quantitatively the most important fuel (Atherton and Aitken, 1970). A very wide range of protein utilization values (from 14 to 90%) has been reported (see Van Waarde, 1983, for review; Van den Thillart, 1986), which suggests either marked variation between and within species, or the methods by which the values have been determined are not comparable with one other. It is interesting to note that Brett and Zala (1975) provided data contradictory to the idea of high protein dependency, though they did not interpret their data as such. Kutty (1978) used Brett and Zala's data to show low ammonia quotients (molar excretion rate of ammonia : molar consumption rate of oxygen), and thus low protein use, during peak oxygen consumption periods.

It is generally recognized that trout do not use exogenous carbohydrates well (Brett and Groves, 1979; Watanabe, 1982; Van Waarde, 1983), though specialized diets can enhance uptake (Mazur *et al.*, 1993). Carbohydrate stores arise substantially from protein as a precursor to gluconeogenesis, again emphasizing the importance of protein (Bever *et al.*, 1981; Walton and Cowey, 1982; Van Waarde, 1983). Endogenous carbohydrate is typically thought of as the fuel reserved mainly for anaerobic metabolism in fish (Driedzic and Hochachka, 1978b).

Lipid seems to play a small but important role during routine sustained swimming (Van den Thillart, 1986; Kiessling and Kiessling, 1993), though its importance increases during high cost endeavors such as migration (Idler and Clemens, 1959; Jezierska *et al.*, 1982). From an aquacultural perspective, fish fed a high fat diet grow faster by burning the lipids, thus sparing the proteins for growth (Atherton and Aitken, 1970; Jayaram and Beamish, 1992).

The respiratory quotient (RQ,  $M_{O_2}:M_{CO_2}$ ) has been assumed by some to be a precise marker of fuel use, rather than one of several tools that should be used simultaneously to arrive at consumption. In using solely the RQ, researchers cannot be sure if the value represents a single fuel, or a combination of two or more fuels; misinterpretations of RQ do exist, as in Kutty (1972) and Brett and Groves (1979). These authors did not account for the multiple nitrogenous waste excretion products which resulted in their

misevaluations. By first calculating the protein component of the fuels used via the nitrogen quotient ( $M_{N-waste}:M_{O_2}$ ), the balance of fuels can then be determined using the RQ (Kutty, 1972; Kleiber, 1987).

During starvation, the intake of food is not sufficient to meet the metabolic demands of an organism. By necessity then, the organism must tap into its own body depots (whether they were intended as reserves or not) in order to maintain itself. The pattern of *depletion* of these reserves has been investigated in several classes of the Vertebrata, but the work done on fish has not addressed the question of *instantaneous fuel use* in any detail. In other words, the literature has addressed the compositional changes in fish during starvation, both in whole body (Idler and Clemens, 1959; Jezierska *et al.*, 1982) as well as specific tissues (Duncan and Tarr, 1958; Idler and Clemens, 1959; Robinson and Mead, 1973; Johnston and Goldspink, 1973a; Jezierska *et al.*, 1982). However, since substrates are highly interconvertible, these studies have not identified the composition of the fuel actually burned at any one time. Ideally, oxidizing lipid exclusively would seem to be in the best interest of the fish since it has the highest energy yield per unit mass (Kleiber, 1987). Over long term starvation lipid reserves do go down (citations as above), but it is interesting to note that plasma levels stay constant, even after 5 weeks of starvation (Robinson and Mead, 1973).

Since no work has followed the pattern of instantaneous fuel use

over a period greater than several hours, the current study seeks to do so. The fuel usage in rainbow trout (*Oncorhynchus mykiss*) during a fifteen day starvation was monitored via two methods. Respirometry was used to determine the gas exchange and nitrogenous waste excretion in each fish; with this technique both the respiratory quotient and nitrogen quotient were obtainable, and empirically the fuel use could then be derived. Body composition changes were also determined to see if depletion patterns mimicked the expected changes determined by respirometry. The two methods are discussed with respect to their strengths as well as to the logical interpretations to which each can give rise.



## Methods and Materials

### Animals

Rainbow trout (*Oncorhynchus mykiss* Walbaum, formerly *Salmo gairdneri* Richardsoni; 2-4g) were obtained from Rainbow Springs Hatchery (Thamesford, Ontario) and were kept in 15°C dechlorinated Hamilton city tap water, for at least two weeks prior to experimentation. The water was moderately hard, with the following composition (in mequiv l<sup>-1</sup>): Na<sup>+</sup>, 0.6; Ca<sup>++</sup>, 1.8; Cl<sup>-</sup>, 0.8; K<sup>+</sup>, 0.04; Mg<sup>++</sup>, 0.5; titration alkalinity (to pH = 4.0), 1.9; total hardness, ≈ 140 mg·l<sup>-1</sup> as CaCO<sub>3</sub>; pH 8.0. The fish were maintained on a commercial trout feed (Martin Trout Food Pellets, Tavistock, Ont; Table 1), and were fed 1.0% of their body weight daily.

### Respirometry

#### The Influence of Water Quality

Decarbonated water was used in the respirometry experiments to provide a low background total CO<sub>2</sub> against which excreted CO<sub>2</sub> could be accurately measured. M<sub>CO<sub>2</sub></sub> could not be reliably determined against the high background of total CO<sub>2</sub> (≈ 2 mM) in normal Hamilton tap water.

Decarbonated water was made up in a 750 l tank by acidifying 15°C normal tap water to ≈ pH 3 with concentrated HCl and bubbling vigorously overnight with air. The pH was brought up to 6.8 with NaOH. The final

Table 1. The nutritional composition of the feed used in this study as supplied by the manufacturer. Carbohydrate was measured as free glucose and glycogen.

component	content
crude protein	40% (min)
crude fat	12% (min)
crude fibre	3% (max)
sodium	0.35%
calcium	1.2%
phosphorus	0.85%
vitamin A	7500 i.u./kg (min)
vitamin D <sub>3</sub>	3000 i.u./kg (min)
vitamin E	100 i.u./kg (min)
ascorbic acid	800 mg/kg (min)
carbohydrate	8.4%

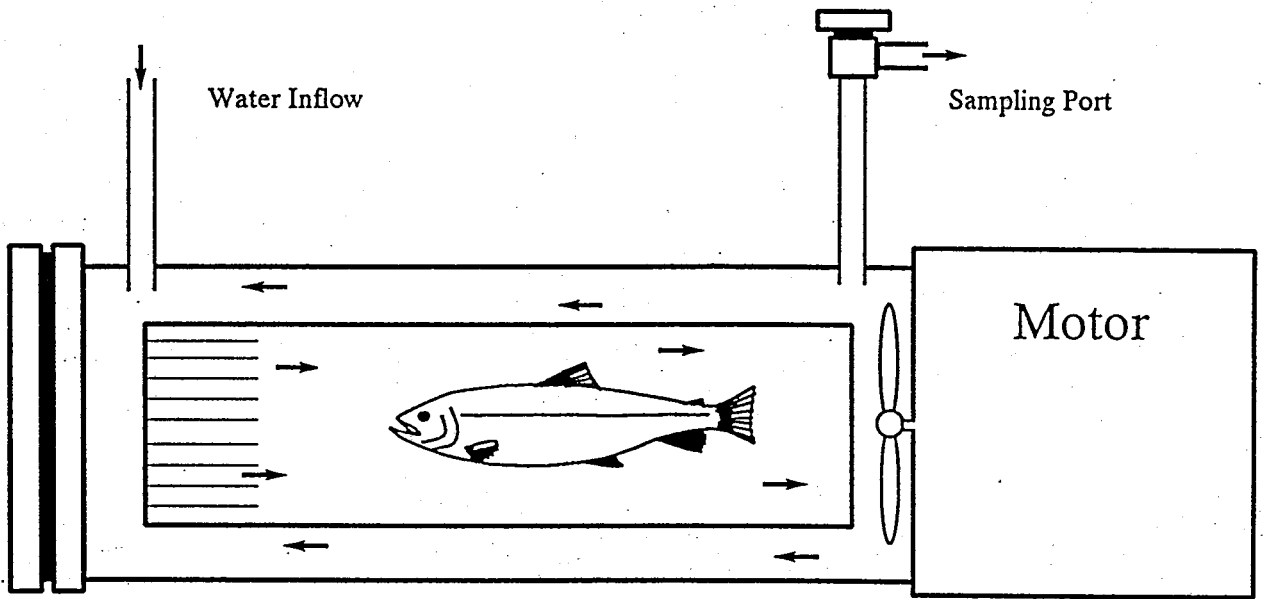
product then had a total  $\text{CO}_2$  concentration of  $20 \mu\text{M}$ ,  $3.2 \text{ mequiv l}^{-1}$  of chloride and  $1.1 \text{ mequiv l}^{-1}$  of sodium.

To test whether the transfer from normal tapwater to decarbonated water had any adverse effects on gas exchange, a simple experiment was carried out with fish in small (70 ml), opaque flow-through respirometers. The weighed fish were put into the individual respirometers and allowed to acclimate for one day. Air saturated tap water constantly flowed through at approximately  $150 \text{ ml} \cdot \text{min}^{-1}$ . The respirometry was performed under a closed system regime; a water sample was taken prior to the typical 10 minute closure, and then again afterwards. The actual duration of the bouts was calculated so as not to let the  $P_{\text{O}_2}$  of the water drop below 120 torr. The water was then analyzed for both  $\text{O}_2$  and  $T_{\text{amm}}$  (total ammonia =  $\text{NH}_4^+ + \text{NH}_3$ ; see below). Sampling periods were immediately before the changeover, as well as 10, 30 and 60 minutes, and 2, 4, and 6 hours after the changeover.

### The Influence of Starvation

Eight Blažka style swimming respirometers (Fig. 1) of known volume (3.2l) were used to house individual fish for the duration of the experiment (Blažka, Volf and Cepala, 1960, *in* Beamish, 1978). Except for the periods of respirometry, air-saturated water flowed through each respirometer at a rate of  $\approx 150 \text{ ml} \cdot \text{min}^{-1}$ . The water from the head tank of a recirculating system (total volume of the system, approx. 500l) entered the

Figure 1. A diagrammatic side view of a Blažka style respirometer used in this study. The fish, shown in the central tube is disproportionately large for purposes of illustration only. Arrows indicate water flow direction.



respirometers via one port, and exited via the sampling port, from where the water drained directly into a wet table. To ensure thermal equilibrium, the respirometers were submerged in the water of the wet table. About 300 l of the water was replaced on a daily basis.

Eight fish ( $4.5 \pm 0.1$ g, mean  $\pm$  s.e.m.) were quickly blotted dry, weighed to the nearest 0.1 g and then transferred to individual respirometers. An opaque sheet completely covered the respirometers; the sampling port and tubing from the head tank passed through holes in the sheet. The fish were not fed at any time during the tests, including the 24 hour acclimation period.

Over the subsequent 15 day test period, all fish underwent one or two closed respirometry trials simultaneously each day. Following the withdrawal of a 30 ml aliquot of water from the sampling port, both the inflow and outflow valves were shut. After three hours, the valves were opened, and an end sample was taken.  $P_{O_2}$  did not typically drop below 120 torr. Each aliquot was immediately divided into three subsamples for the analyses. The 8 ml subsample for  $CO_2$  analysis filled a precooled glass vial which was quickly capped to prevent diffusive exchange of  $CO_2$ . These samples were stored at 4°C for analysis later the same day (see below). A 15 ml subsample for analysis of nitrogenous wastes was immediately frozen and stored at -20°C. The remainder of the aliquot was used to measure oxygen (see below).

Oxygen was measured immediately with a water jacketed O<sub>2</sub> electrode (Radiometer E5046) attached to a Cameron O<sub>2</sub> meter (OM-200). The electrodes were thermostated to the temperature of the test system (15 ± 1°C).

P<sub>O<sub>2</sub></sub> was converted to oxygen concentration (C<sub>O<sub>2</sub></sub>) using tabulated solubility coefficients for freshwater (Boutilier *et al.*, 1984). M<sub>O<sub>2</sub></sub> could then be calculated via the equation,

$$M_{O_2} = \frac{\Delta C_{O_2} \cdot vol}{m \cdot t} \quad (4)$$

where M<sub>O<sub>2</sub></sub> is the molar oxygen consumption in μmol O<sub>2</sub> · g<sup>-1</sup> · h<sup>-1</sup>, ΔC<sub>O<sub>2</sub></sub> is the difference in oxygen concentration between start and end of the test period, vol is the volume of the respirometer in liters, m is the mass of the fish in grams, and t is the time in hours that the respirometer was closed.

Analogous equations were used for CO<sub>2</sub>, T<sub>amm</sub>, and urea excretion rates.

The water samples for CO<sub>2</sub> analysis were rewarmed to 15°C and measured in duplicate on a Shimadzu GC-8A gas chromatograph equipped with a Poropak Q column; the output was displayed on a Shimadzu-CR3A integrator. A series of sodium bicarbonate standards were made up in the 0-200 μM range using the test medium.

The salicylate-hypochlorite assay was used to analyze total ammonia in the water (Verdouw *et al.*, 1978). Urea was measured as in Rahmatullah and Boyd (1980) and modified by T.P. Mommsen (*pers. comm.*)

by dissolving 10mg of thiosemicarbizide and 500mg of diacetyl monoxime in 20 ml of deionized water to make the colour reagent. Due to the relatively low levels of urea production relative to the volume of the respirometer, it was necessary to freeze-concentrate water samples for urea analysis. The water samples were thawed, mixed, and duplicate 5.0 ml aliquots were refrozen in culture tubes covered with perforated parafilm. The samples were freeze-dried and rehydrated to a volume of 1.0 ml. Standards (0-10  $\mu\text{M}$ ) were treated in the same way. The assay was then run on the 1.0 ml (5x concentrated) samples and standards. Even with this 5x preconcentration step, occasional values were clearly aberrant due to sensitivity limitation. Since the fuel use calculations (see below) required the urea values, a ratio of  $T_{\text{amm}}$ :urea excretions from nonaberrant data were used to interpolate the missing values.

At the end of the experiment, the fish were sacrificed by introducing neutralized tricaine methanesulfonate (MS222, Syndel Laboratories) such that the final concentration to which the fish were exposed was  $1\text{g}\cdot\text{l}^{-1}$ . The fish were removed from the respirometers, blotted free of excess water, weighed, freeze-clamped with aluminum tongs in liquid nitrogen, and stored at  $-20^{\circ}\text{C}$  for analysis of proximate body composition (see below).

## Body Analyses

A second set of eight fish from the same stock went through the



same acclimation period as the test fish, and were then sacrificed. The mean weight of these control fish ( $4.2 \pm 0.3\text{g}$ ) was not different from the test fish ( $p > 0.05$ ). The bodies of the test fish and controls were ground in an Ika electric tissue grinder (Staufen, Germany) which was cooled with methanol/dry ice ( $\approx -77^\circ\text{C}$ ). The resulting powder was lyophilized to determine water content. One of the controls was lost after lyophilization giving an  $n = 7$  for the remainder of the tests. All assays were done on these freeze-dried samples.

Lipids were extracted using chloroform-methanol (2:1). Ten ml of the chloroform-methanol mixture were added to 100 mg of sample powder in a culture tube. The tubes were mixed and allowed to sit overnight at  $4^\circ\text{C}$  in the dark. The following day, 2.6 ml of 0.9% NaCl was added with mixing, and the tubes were again allowed to incubate overnight. All the water soluble tissue components and the methanol would then be found in the saline; only the lipid would be found in the lower chloroform phase (approximately 7ml). A 4 ml aliquot of the chloroform phase was removed with a glass/metal/teflon syringe and transferred to a preweighed culture tube, after which the chloroform was evaporated under a filtered stream of air, which left only the lipid in the tube. The tubes and remaining lipids were put into a desiccator for an hour after which they were reweighed. Thus,

i) 
$$[\text{empty tube (g)} + \text{lipid (g)}] - \text{empty tube (g)} = \text{lipid (g) in aliquot}$$

$$\text{ii) lipid (g) in aliquot} \times \text{organic phase vol (ml)} \div \text{aliquot vol (ml)} \\ = \text{sample lipid (g)}$$

Protein was determined via the Lowry method (Miller, 1959), using bovine serum albumin (Sigma) as a standard. Glucose, glycogen and lactate were assayed as in Bergemeyer (1985). Due to mechanical constraints of removing the fish from their respirometers, there was a delay of approximately one minute between anaesthetization and freeze-clamping. This delay could have resulted in decreased glycogen, and increased both glucose and lactate (Black *et al.*, 1962; Barton, Weiner, and Schreck, 1985). Therefore, the percent ( $\text{mg} \cdot 100\text{mg}^{-1}$ ) sum of glucose, glycogen and lactate is reported as total carbohydrate. Ash content was determined by heating the freeze-dried tissue to  $750^{\circ}\text{C}$  until a constant weight was obtained (approximately 4 hours).

#### Fuel Use Calculations

The respiratory quotient (RQ;  $\text{CO}_2$  expired: $\text{O}_2$  consumed, during steady state) and nitrogen quotient [NQ, ( $T_{\text{amm}}\text{-N} + \text{urea-N}$ ): $\text{O}_2$  consumed] were determined for each fish at each time. Note that  $M_{\text{urea-N}}$  was used in the calculation, not simply  $M_{\text{urea}}$ . In essence,

$$NQ = \frac{2 \cdot M_{\text{urea}} + M_{T_{\text{amm}}}}{M_{\text{O}_2}} \quad (5)$$

All values of RQ were used to calculate the mean RQ at each time, though only values of  $\text{RQ} \leq 1.00$  were used to determine instantaneous fuel use since an RQ of 1.00 represents the upper limit obtainable during aerobic

metabolism (Eckert *et al.*, 1989). In other words, fish that were anaerobic were not included in fuel use calculations. Only  $T_{\text{amm}}$  and urea were measured for the calculation of NQ since they represent the vast majority of nitrogenous end product. The error associated with neglecting other possible N-products will be addressed in the Discussion.

Since the fish were found to be neither strictly ureotelic nor strictly ammoniotelic but rather excreted 75%  $T_{\text{amm}}$ -N and 25% urea-N, traditional values of RQ representing protein use ( $RQ_{\text{protein}}$ ) for 100% ammoniotelism or 100% ureotelism could not be used. Table 2 shows the calculation employed in the present study, while the theory behind this is explained in the Discussion. Essentially, enough elemental moles as urea (column 5) and as  $T_{\text{amm}}$  (column 6) were subtracted (in the proportions found in this study) from the typical protein elemental composition (column 4) to account for all the nitrogenous wastes that would be excreted. Thus for N itself, for which there are 1.21 moles $\cdot$ 100g $^{-1}$  protein, 0.30 moles (25%) would be in excreted urea, and 0.91 moles (75%) would be in excreted  $T_{\text{amm}}$ . From the balance (column 7), the remaining oxygen is removed as water (internal oxidation, column 8). The remaining C and H (column 9) are assumed to be completely oxidized, and the required  $O_2$  for both processes are summed (column 10). The  $CO_2$  produced is divided by the total oxygen consumed to get the RQ; similarly, the total N excreted is divided by the oxygen consumed to arrive at the NQ (column 12). For the fish in this

Table 2. A calculation for obtaining the  $RQ_{\text{protein}}$  and the  $NO_{\text{protein}}$  for the fish in this experiment based on the observation that 25% of the total N wastes were in the form of urea. Columns 1-4 data from Kleiber (1987); columns 5-12 from this study.

1	2	3	4	5	6	7	8	9	10	
protein elements	At Mass $g \cdot mol^{-1}$	%age or $g \cdot 100g^{-1}$	moles $\cdot 100g \text{ protein}^{-1}$	elemental moles in urea	elemental moles in ammonia	protein minus N wastes	water loss (internal oxidation)	remaining C-H	$O_2$ required	
C	12	52	4.33	0.15	0	4.18		4.18	4.18	
H	1	7	7.00	0.61	2.73	3.66	2.57	1.09	0.27	
O	16	23	1.44	0.15	0	1.29	1.29	0		
N	14	17	1.21	0.30	0.91	0				
S	32	1	0.03	0	0	0.03	negligible			
								total:	4.45 mol	
				respiratory gases	11 moles required					12 $RQ_{\text{protein}}$ or $NO_{\text{protein}}$
				$CO_2$	4.18					0.94
				$O_2$	4.45					
				Nitrogen	1.21					0.27

study, the  $RQ_{\text{protein}}$  and  $NQ_{\text{protein}}$  were found to be 0.94 and 0.27, respectively.

Instantaneous fuel usage was then determined as follows:

$$P = \frac{NQ}{0.27} \quad (6)$$

where 0.27 is the theoretical maximum for NQ (ie. when protein is the sole fuel source; equation 3). Thus,

$$RQ = P*0.94 + C*1.0 + L*0.71 \quad (7)$$

where P, C, and L represent the fraction of the total fuels burned arising from protein, carbohydrate and lipid, respectively. Since the value of P has been determined (equation 6), only C and L need be determined. Since,

$$P + L + C = 1.0 \quad (8)$$

$$L = (1.0 - P - C) \quad (9)$$

Substituting equation 6 into equations 9 and 7,

$$L = \left(1 - \frac{NQ}{0.27} - C\right) \quad (10)$$

$$RQ = \frac{NQ}{0.27} * 0.94 + C*1.0 + L*0.71 \quad (11)$$

finally, substituting equation 10 into equation 11,

$$RQ = \frac{NQ}{0.27} * 0.94 + C*1.0 + (1.0 - \frac{NQ}{0.27} - C)*0.71 \quad (12)$$

which simplifies to

$$RQ = 0.85 \cdot NQ + 0.29 \cdot C + 0.71 \quad (13)$$

RQ and NQ were measured in the experiment, so the equation can be solved for C, and L can be determined by difference (equation 9).

While the relative contributions of the individual fuels are important, the absolute expenditure could also be calculated. The percentage contribution of each fuel, as calculated above based on *the consumption of O<sub>2</sub>* was then converted to a percentage based on *carbon usage* via the fuel-specific RQ's. The total carbon usage was reflected in the M<sub>CO<sub>2</sub></sub> data of Fig. 3, and could be apportioned then by C-based percentages to absolute C use.

The calculation of compositional fuel usage from depletion of reserves over the 15 day starvation period was based on measured changes in body composition and body weight. The test fish declined from 4.5 to 3.8g (average masses) over the experimental period. The concentration (in mg·100mg<sup>-1</sup>, wet weight) of the components of the fish bodies was converted to absolute amounts by taking the appropriate concentration of each component and correcting for the average 4.5 g body weight at the start or 3.8 g body weight at the end of starvation in the test fish. (The composition of the control fish was taken to represent that of the test fish at

the start of the experiment, since body compositions could not be done pre- and post-test on the same fish). The difference was taken to obtain a mean loss in weight. Total fuels were tallied and a percentage based on fuel weight was derived. The carbon mass of each fuel was calculated based on the percentage weight of carbon in each fuel as provided in Kleiber (1987).

As an internal check between the two methods of determining fuel use, the total O<sub>2</sub> consumed and N excreted were calculated directly by taking the area under the M<sub>O<sub>2</sub></sub> and M<sub>N</sub> graphs (Figs. 3, 5) and multiplying by the mean weight of the fish, or indirectly on the net fuel losses as calculated by the change in body composition.

## Statistics

Since there were only two groups of fish, an independent t-test was used to determine statistical significance for the body composition measurements. Regressions were fitted by the method of least squares and were tested for significance by using the Pearson linear correlation and the appropriate t-test (Fig.P graphics package, Biosoft, Ferguson, Mo.). A one way ANOVA was used to check for any variation between points in the water changeover; a  $p < 0.05$  was considered significant for all tests.

## Results

### The Effect of Water Quality

Fig. 2 shows the changeover from dechlorinated to decarbonated water had no effect ( $p > 0.05$ ) on either  $M_{O_2}$  or  $M_{NH_3}$  over the 6 hour test. Ammonia excretion remained stable at  $0.17 \mu\text{mol} \cdot \text{g}^{-1} \cdot \text{h}^{-1}$  whereas  $M_{O_2}$  remained stable at  $5.0 \mu\text{mol} \cdot \text{g}^{-1} \cdot \text{h}^{-1}$ . It was then assumed that working in decarbonated water would not give rise to any complicating effects.

### Respirometry and Waste Excretion

Both oxygen consumption ( $M_{O_2}$ ) and carbon dioxide excretion ( $M_{CO_2}$ ) decreased over the 15 day test period, though this was only significant ( $p < 0.001$ ) for oxygen (Fig. 3). In both cases, values fluctuated about the line of regression with decreased amplitudes over the second half of the run.  $M_{O_2}$  decreased from approximately  $7.5$  to  $5.3 \mu\text{mol} \cdot \text{g}^{-1} \cdot \text{h}^{-1}$  (slope =  $-7.01 \times 10^{-3} \mu\text{mol} \cdot \text{g}^{-1} \cdot \text{h}^{-2}$ ), while  $M_{CO_2}$  dropped from  $6.8$  to  $5.5 \mu\text{mol} \cdot \text{g}^{-1} \cdot \text{h}^{-1}$  (slope =  $-2.99 \times 10^{-3} \mu\text{mol} \cdot \text{g}^{-1} \cdot \text{h}^{-2}$ ). Since the slope of  $CO_2$  excretion was less than that for  $O_2$  consumption, a positively sloped (though not significantly so) RQ resulted (Fig. 4). The RQ also showed fluctuation about the line of regression, but individual means tended to remain between  $0.8$  and  $1.0$ . Since this study focused on aerobic metabolism, any value of RQ  $> 1.00$  could not be used in the fuel use calculations. Therefore, a second



Figure 2. The effect of the changeover from normal tapwater to decarbonated tapwater on oxygen consumption ( $n = 12$ ) and  $T_{amm}$  excretion ( $n = 10$ ) in juvenile rainbow trout. The changeover occurred at  $t_0$ ; there was no significant difference in either parameter. Means  $\pm$  sem.

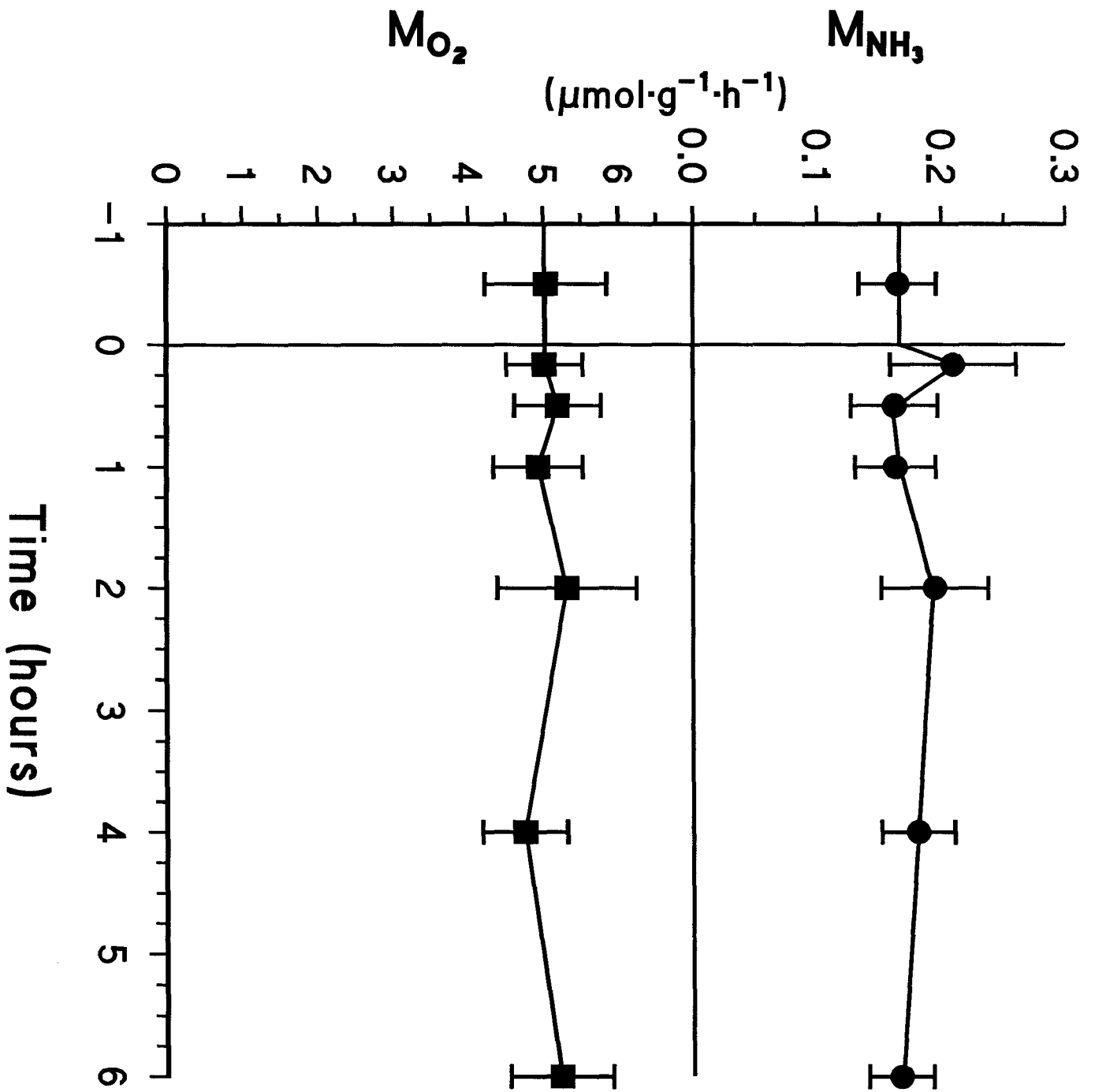


Figure 3. Oxygen consumption and carbon dioxide excretion over 15 days of starvation in juvenile rainbow trout. In both cases, the solid line represents the regression through all points. Means  $\pm$  sem, n = 8.

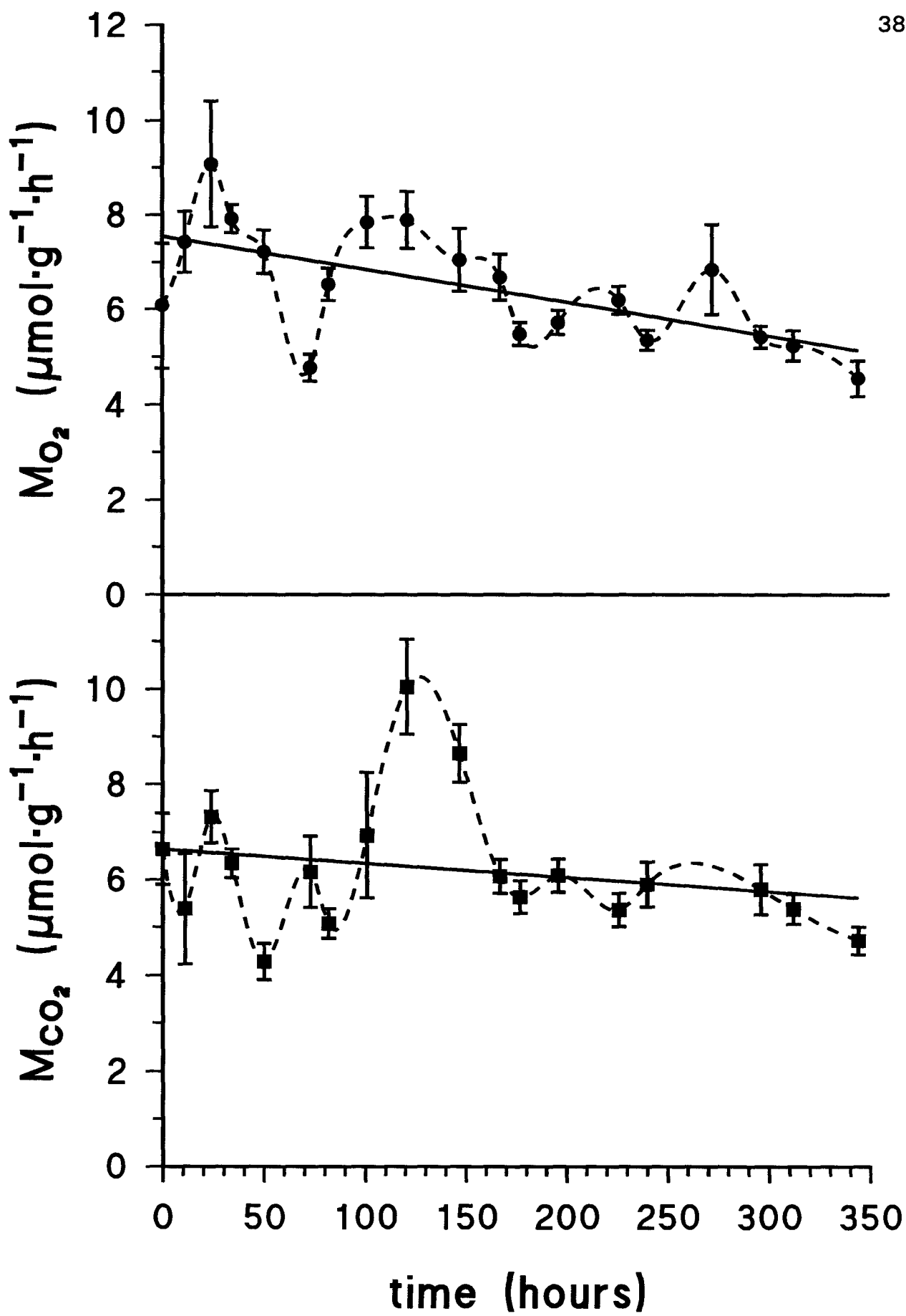
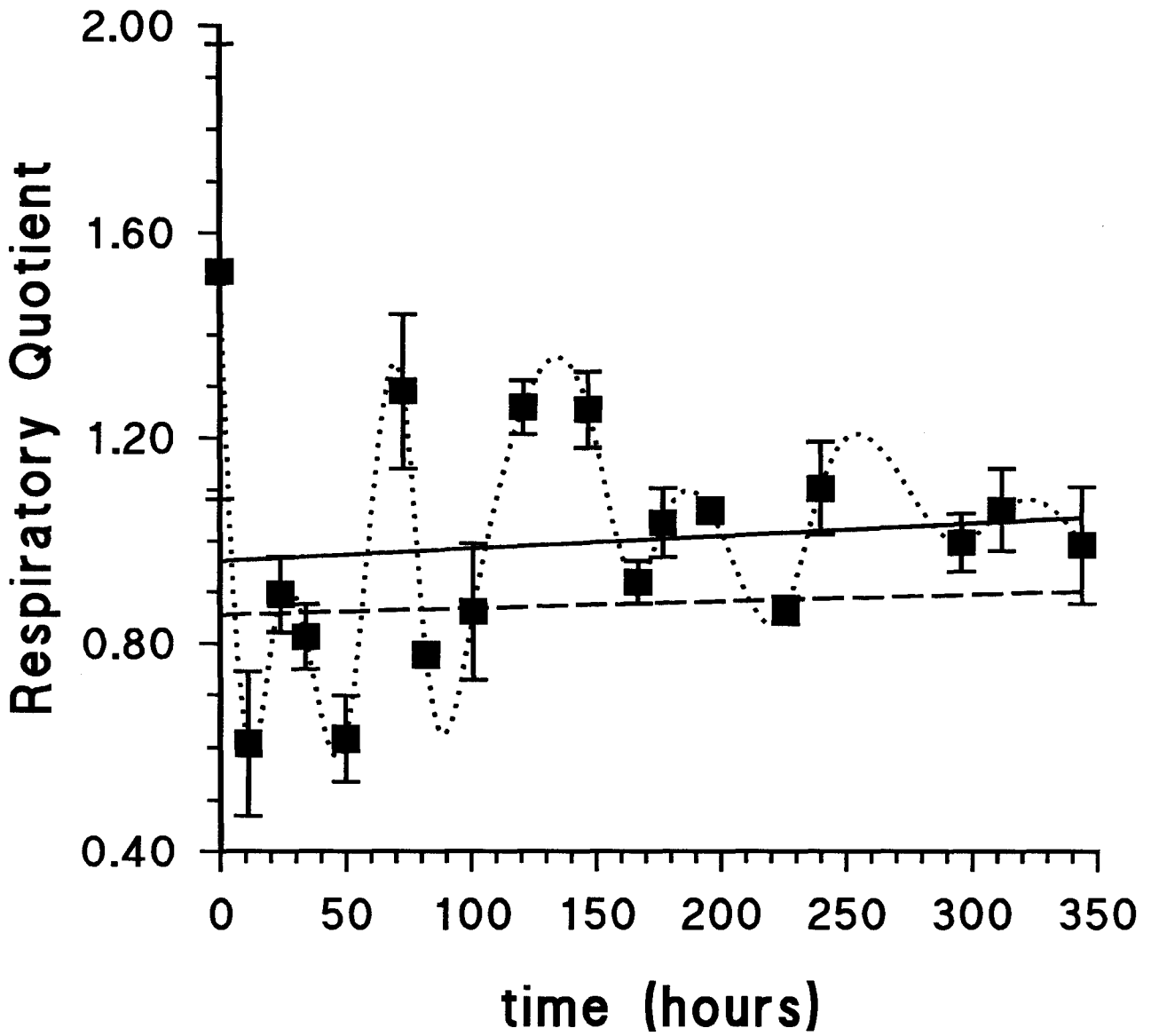


Figure 4. Respiratory quotient (solid symbols and dotted lines) and the regression of the aerobic respiratory quotients only (dashed line) over 15 days of starvation in juvenile rainbow trout. Means  $\pm$  sem, n=8.



line derived only from the values of aerobic RQ was plotted in Fig. 4 as well.

Nitrogenous waste excretion was triphasic over the duration of the experiment (Fig. 5a). The first phase (80 hours) showed a  $M_{N\text{-waste}}$  which averaged  $0.23 \mu\text{mol}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$ . During the second phase (90 hours), the fish exhibited a relatively stable 50% increase ( $0.34 \mu\text{mol}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$ ) over the first phase. In the final phase (170 hours), the excretion rate averaged  $0.25 \mu\text{mol}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$ . Total ammonia ( $\text{NH}_3 + \text{NH}_4^+$ ) represented about 75% of the total. Neither any one phase, nor the entire curve taken as a whole, showed a significant slope.

Using the decreasing and fluctuating  $M_{\text{O}_2}$  and the changing  $M_{N\text{-wastes}}$ , a linear ( $r = 0.662$ ) and increasing ( $p < 0.001$ ) relationship for the nitrogen quotient was obtained (Fig. 5b). The NQ rose from 0.037 at  $t=0\text{h}$  to 0.064 at  $t=344\text{h}$ .

#### Instantaneous Fuel Use

Given the proportional nature of the formula to determine protein use (equation 2), the shape of the curve for NQ (Fig. 5b) was mimicked in the shape of the curve for protein use (Fig. 6). At 0h, protein made up only 14% of the total fuels; at 344h, protein use had risen by almost 10%. At 73h and during the period from 147h to 177h values were somewhat higher than the line of regression. The point at 73h corresponded with a concurrent low in  $\text{O}_2$  consumption (Fig. 3a). The second peak corresponded with the second phase of nitrogen excretion (reported above). When the NQ

Figure 5. (a) Total nitrogenous waste excretion (t-Nitrogen as the sum of ammonia-N and urea-N, circles;  $T_{\text{amm}}$ , squares) and (b) nitrogen quotient (triangles with solid regression line) over 15 days of starvation in juvenile rainbow trout. Means + sem, n=8.



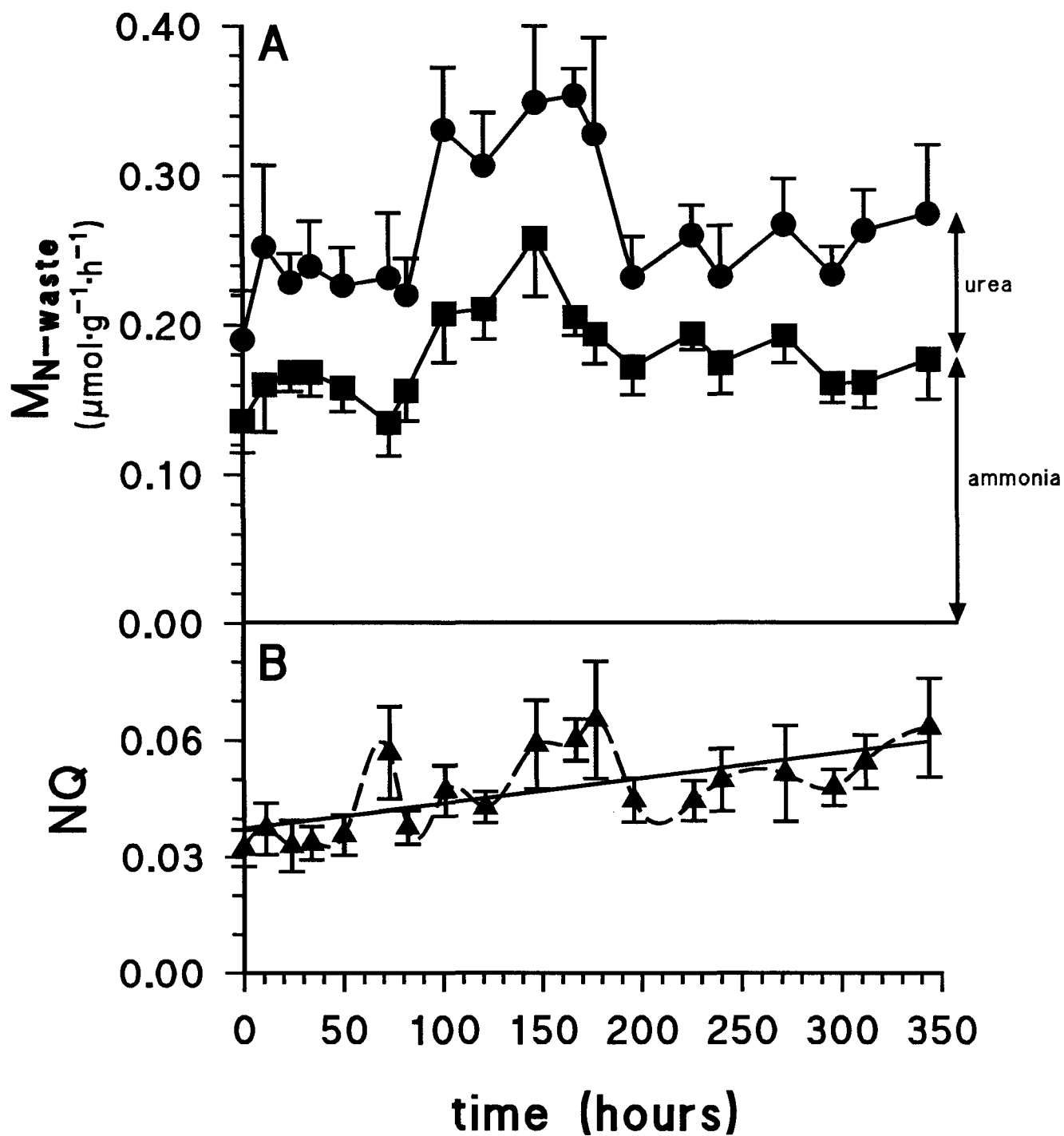
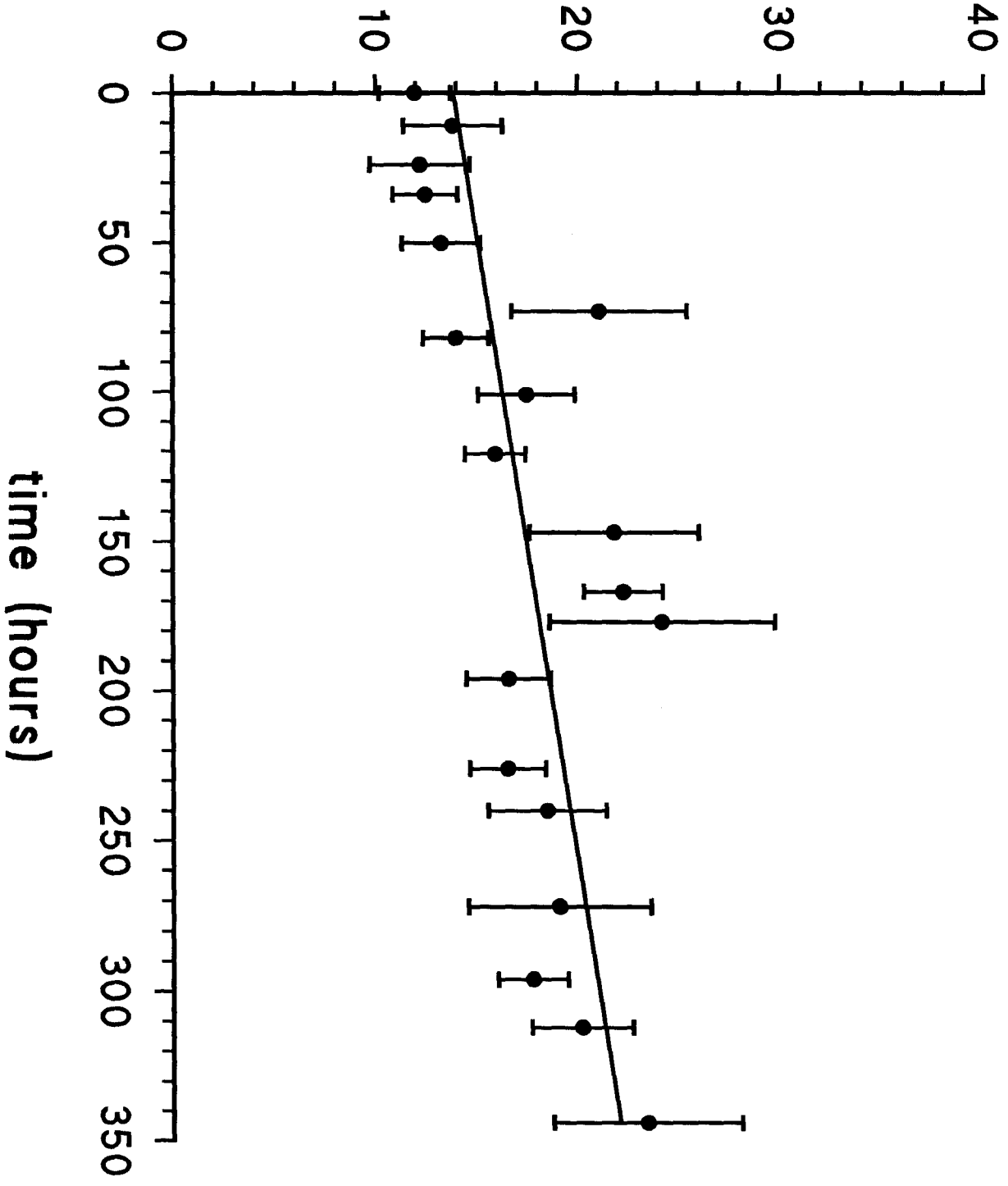


Figure 6. Percentage protein use over 15 days of starvation in juvenile rainbow trout. Means  $\pm$  sem, n=8.

# Percentage Protein Use



and the regressed values of aerobic RQ were used to calculate the generalized fuel use picture, two distinct areas of fuel use were found (Fig. 7). During the first quarter of the experiment, lipids averaged approximately 68% of all fuels burned, whereas carbohydrates represented about 20%. Over the remainder of the experiment, the two N-free fuels tended to equal out at about 37% each. Protein, of course, made up the balance of fuels over the entire time.

Carbon use from proteins was lowest over the first 82 hours where the average contribution was  $10.5 \mu\text{g C}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$  (Fig. 8). At the same time, carbon usage from lipids was at its highest at an average of  $44 \mu\text{g C}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$ . The period from 101 to 177h showed higher protein C use at  $17 \mu\text{g C}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$  after which it decreased and averaged  $14 \mu\text{g C}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$ . Thereafter, lipids only contributed between 15 and  $25 \mu\text{g C}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$ . Carbon from carbohydrate was oxidized initially at  $23 \mu\text{g C}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$ ; following a brief drop to  $10 \mu\text{g C}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$  at 82h, carbohydrates supplied carbon over the range of 19 to  $43 \mu\text{g C}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$  with an average use of  $32 \mu\text{g C}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$ .

#### Body Composition and Compositional Fuel Use

Mean weight declined from 4.5 to 3.8g over the 15 day period. Water content of the starved fish ( $76.7 \pm 0.3\%$ ) was significantly higher ( $p < 0.005$ ) than the  $75.4 \pm 0.3\%$  found in the controls, though total water content went down (Table 3). Ash also increased significantly from  $2.40 \pm 0.03 \text{ mg}\cdot 100\text{mg}^{-1}$  to  $2.68 \pm 0.05 \text{ mg}\cdot 100\text{mg}^{-1}$  ( $p < 0.005$ ) though again,

Figure 7. Percentage use of lipid (open bars), carbohydrate (hatched bars), and protein (solid bars) as calculated from respirometry and nitrogenous waste excretion data, over 15 days of starvation in juvenile rainbow trout.

### Fuel Use (%)

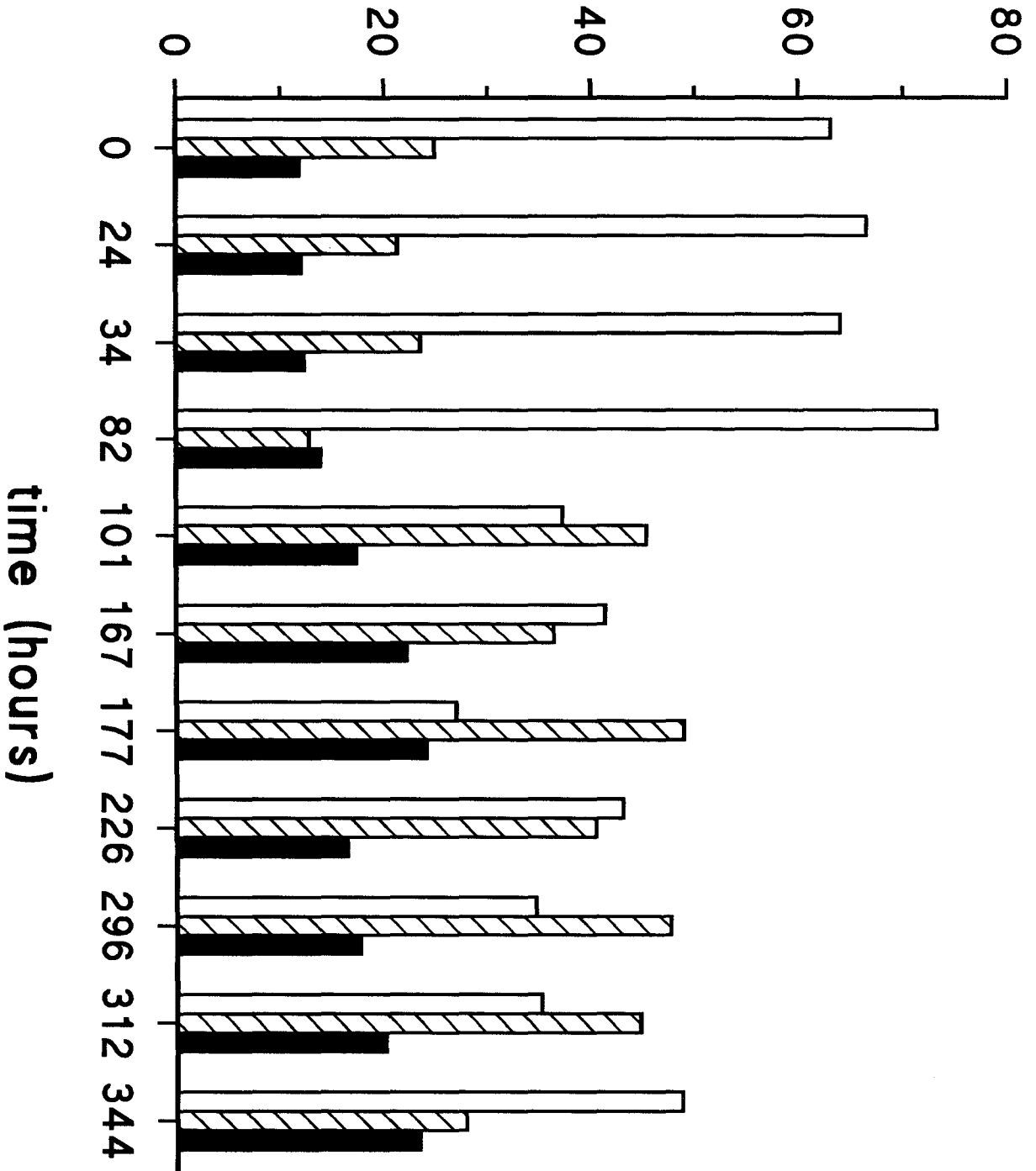


Figure 8. The contribution of carbon from lipid (open bars), carbohydrate (hatched bars), and protein (solid bars) over 15 days of starvation in juvenile rainbow trout.

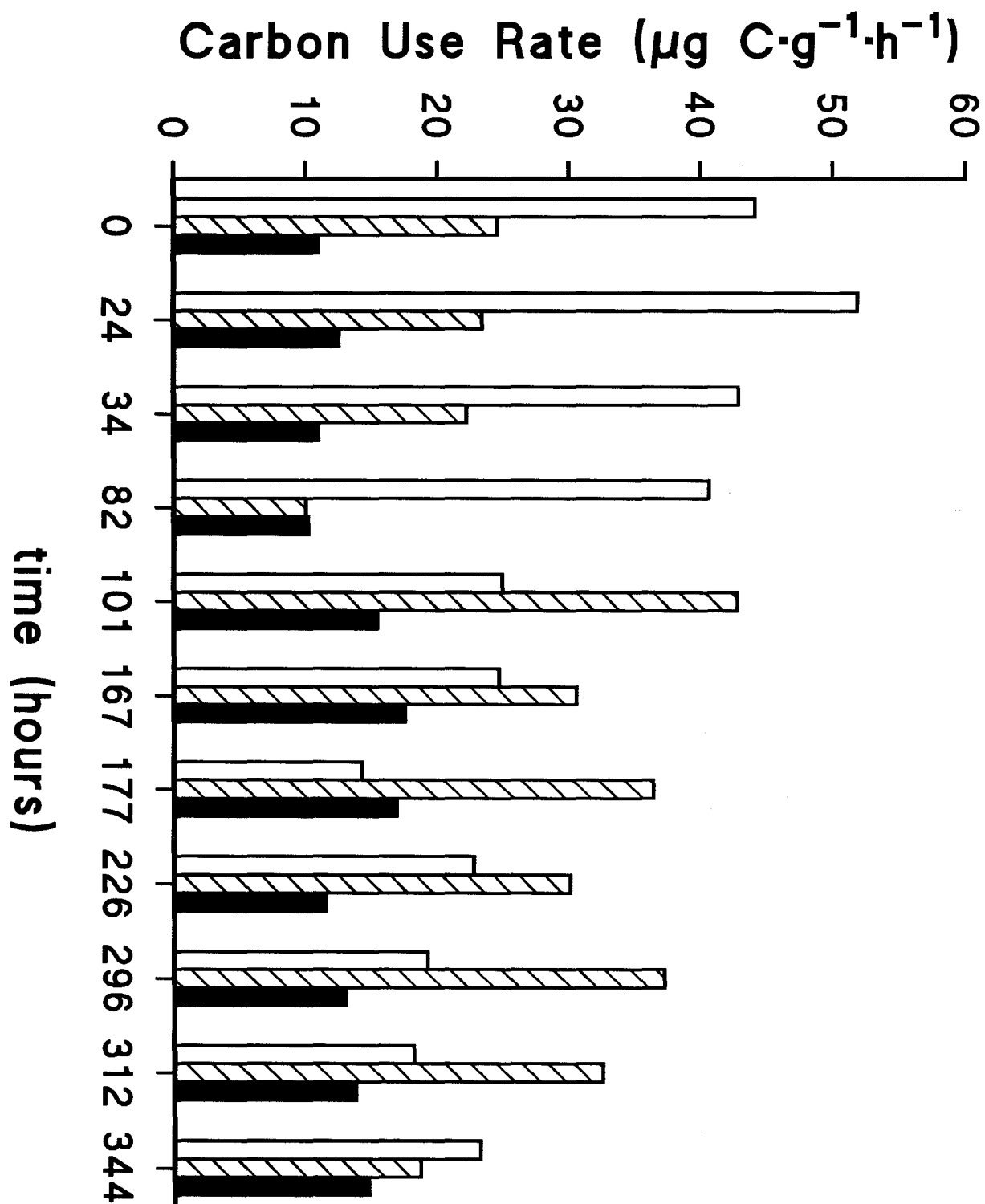




Table 3. a) Body compositions ( $\text{mg}\cdot 100\text{mg}^{-1}$ , wet weight) of pre- and post-starvation fish. Total carbohydrates includes glucose, glycogen and lactate.  $n=7$  (controls) or 8 (test fish). \*  $p < 0.025$ , \*\*  $p < 0.005$ , indicate significant difference from controls. means  $\pm$  sem.

	lipid	total carbohydrates	protein	inorganics	water
prestarvation (controls)	$7.6 \pm 0.5$	$0.25 \pm 0.03$	$14.0 \pm 0.7$	$2.40 \pm 0.03$	$75.37 \pm 0.33$
poststarvation (test fish)	$7.0 \pm 0.1$	$0.14 \pm 0.001^{**}$	$12.3 \pm 0.4^*$	$2.68 \pm 0.05^{**}$	$76.67 \pm 0.26^{**}$

b) Absolute mass of components (mg), based on average 4.5g (prestarvation) and 3.8g (poststarvation) fish.

prestarvation	341.2	11.3	628.6	107.8	3384
poststarvation	264.6	5.3	464.9	101.3	2898
Difference	76.6	6.0	163.7	6.5	486
Total fuel lost (percentage)	31.1	2.4	66.5		

c) Carbon mass in the fuels (mg).

prestarvation	259.3	4.5	326.9	total carbon
poststarvation	201.1	2.1	241.8	lost
Difference	58.2	2.4	85.1	145.7
Total C lost (%)	40.0	1.6	58.4	100.0

actual content dropped slightly. Since there was no dietary source of minerals, then presumably much of the ash decrease was due to  $\text{Ca}^{++}$  release from bone and subsequent loss to the environment. The three fuels all dropped in content, though only total carbohydrates and proteins did so significantly ( $p < 0.005$  and  $p < 0.025$ , respectively; Table 3a). The component losses of protein, lipid and carbohydrate by weight were 66.5, 31.1 and 2.4%, respectively (Table 3b), though if the fuels losses are expressed in terms of their carbon component, then the contribution to total fuel by protein, lipid and carbohydrate were 58.4, 40.0 and 1.6%, respectively (Table 3c). Finally, if a nitrogen quotient is calculated from the *predicted*  $\text{O}_2$  consumptions and N excretions (based on the measured depletion of fuels), a value of 0.125 results, in contrast to the mean of 0.042 by respirometry; this then converts to a protein use of 46.3% in contrast to the 15.4% by respirometry (Table 4).

It should be noted that the discrepancy in the percentage contributions of protein to fuel use (58.4% [Table 3c] versus 46.3% [Table 4a]) is *apparent*, rather than real, and results from the different bases used in the two calculations. The prediction arising from the NQ (compositional data, Table 4a) is based on the fate of the  $\text{O}_2$ , not on the source of the  $\text{CO}_2$  as is the case in Table 3c. The amount of  $\text{O}_2$  which would oxidize the depleted protein-C (85.1mg from Table 3c) can be calculated via:

Table 4. A comparison of predicted oxygen consumption, nitrogenous waste excretion, nitrogen quotient and protein use from body compositional changes and respirometry.

a) Body Composition

	fuels lost (mg)	caloric equivalent (kcal·g <sup>-1</sup> )	oxycalorific equivalent (kcal·mol O <sub>2</sub> <sup>-1</sup> )	O <sub>2</sub> for combustion (mmol)	N excreted as waste (mmol)	NQ	protein (%)
protein	163.7	5.7	107	8.72	1.98		
lipid	76.6	9.5	105	6.93	-		
carbohydrate	6.0	4.0	112	0.21	-		
total	246.3			15.86	1.98	0.125	46.3

b) Respirometry

O <sub>2</sub> consumption (mmol)	N excretion (μmol)	NQ	protein (%)
9.96	415.1	0.041	15.4

$$\frac{85.1 \text{ mg C}}{12 \text{ mg C} \cdot \text{mmol CO}_2^{-1}} \cdot \frac{1.00 \text{ mmol O}_2}{0.94 \text{ mmol CO}_2} = 7.5 \text{ mmol O}_2 \quad (14)$$

where the second factor in the equation is simply the inverse of the  $\text{RQ}_{\text{protein}}$ . Similar calculations give rise to 6.9 mmol  $\text{O}_2$  required for lipid oxidation and 0.2 mmol  $\text{O}_2$  required for carbohydrate for a total  $\text{O}_2$  requirement of 14.6 mmol. From the empirically derived N excretion (1.98 mmol, Table 4a), an NQ of 0.136 can be found. This gives rise to a protein use of 50.2%, a value in much closer agreement to the 46.3% of Table 4a. The remaining small discrepancy is attributable to rounding errors in the constants used.

#### A Comparison of the Instantaneous and Compositional Methods for Calculating Fuel Use

The very different conclusions arising from the instantaneous *versus* compositional methods for calculating fuel utilization (eg. 15.4% from respirometry *versus* 46.3-50.2% from the compositional approach) leads to further critical assessment. An internal check of the two methods reveals a discrepancy in the cumulative oxygen consumption over the experimental period (Table 4). Based on the changes in body composition, it would take 15.9 mmol of  $\text{O}_2$  to completely burn the substrates. The total oxygen consumption was actually 9.96 mmol, a 38% lower value. A similar internal check of the N budget also reveals an unbalanced equation. From Table 2, a fish excretes 1.21 mol N for every 100g protein ( $12.1 \mu\text{mol N} \cdot \text{mg protein}^{-1}$ ) used. From Table 3b, an average 4.5g fish in 368 hours, lost 163.7mg of protein. Therefore a total of 1.98 mmol N should have appeared in the

water, assuming all of it was excreted (Table 4a). However, from the data on N-waste excretion, (Fig. 5), a total of only 415.1  $\mu\text{mol N}$  were excreted. This leaves a balance of 1.56 mmol of unaccounted N. This amount of N would have arisen from 129 mg of protein. It is very clear that the two methods yield a fundamentally different picture of the fuel use budget. The possible reasons for these differences will be addressed in the Discussion.

## Discussion

### Critical Assessment of Theory for Fuel Use by Respirometry

In using the respiratory quotient, several assumptions must be made (Krogh, 1916 *in* Kleiber, 1987). Firstly, the only metabolizable fuels available are lipids, carbohydrates and proteins. Secondly, no synthesis takes place alongside breakdown, and finally, the rate of CO<sub>2</sub> excreted equals the rate of production. There are weaknesses in these assumptions, but in general they are considered sound.

In Figure 4, the mean RQ's were plotted along with the regressions of both the raw data and the edited data. Any individual RQ greater than 1.0 was removed from the raw data because this is the value above which anaerobic metabolism is assumed to occur. For example, in fish tolerant of hypoxic conditions (eg. *Carassius auratus*) conversion of pyruvate to ethanol involves a unique anaerobic decarboxylation (Shoubridge and Hochachka, 1980). Decarboxylations not associated with energy production (eg. the pentose phosphate pathway; Rawn, 1989) could also give values for RQ greater than 1.0. In these cases, rejection of the data would be valid and necessary.

However, values of RQ greater than one may have also reflected the anaerobic conversion of glucose to lactate without the consumption of

O<sub>2</sub> or the *direct* production of CO<sub>2</sub>. However, *indirectly*, lactic acid would have titrated bicarbonate ions in the well known HCO<sub>3</sub><sup>-</sup> dehydration reaction. CO<sub>2</sub> would then be produced, possibly resulting in a transient elevation of the RQ to a value over unity. Lactate though is well known as a metabolic fuel (Weber, 1991; Milligan and Girard, 1993), and if it were subsequently oxidized, then the HCO<sub>3</sub><sup>-</sup> pool would be re-established by the retention of CO<sub>2</sub>, and RQ would decrease. A way to test for this, which would not have been feasible with the small fish in this study, would have been to monitor the acid-base status concurrently with the bouts of respirometry. If this were the situation in the apparent bouts of anaerobicity, then RQ above 1.0 should not have been omitted, rather a time-averaged RQ taking into account both the surge above 1.0 and the compensating fall below 1.0 should have been used. As this approach was clearly not feasible in the current study, and the theory cannot deal with absolute RQ's above 1.0, I therefore chose the conservative approach of omitting all values above 1.0. If this apparent anaerobicity were occurring in the data that were deleted, an underestimate of carbohydrate use would have resulted.

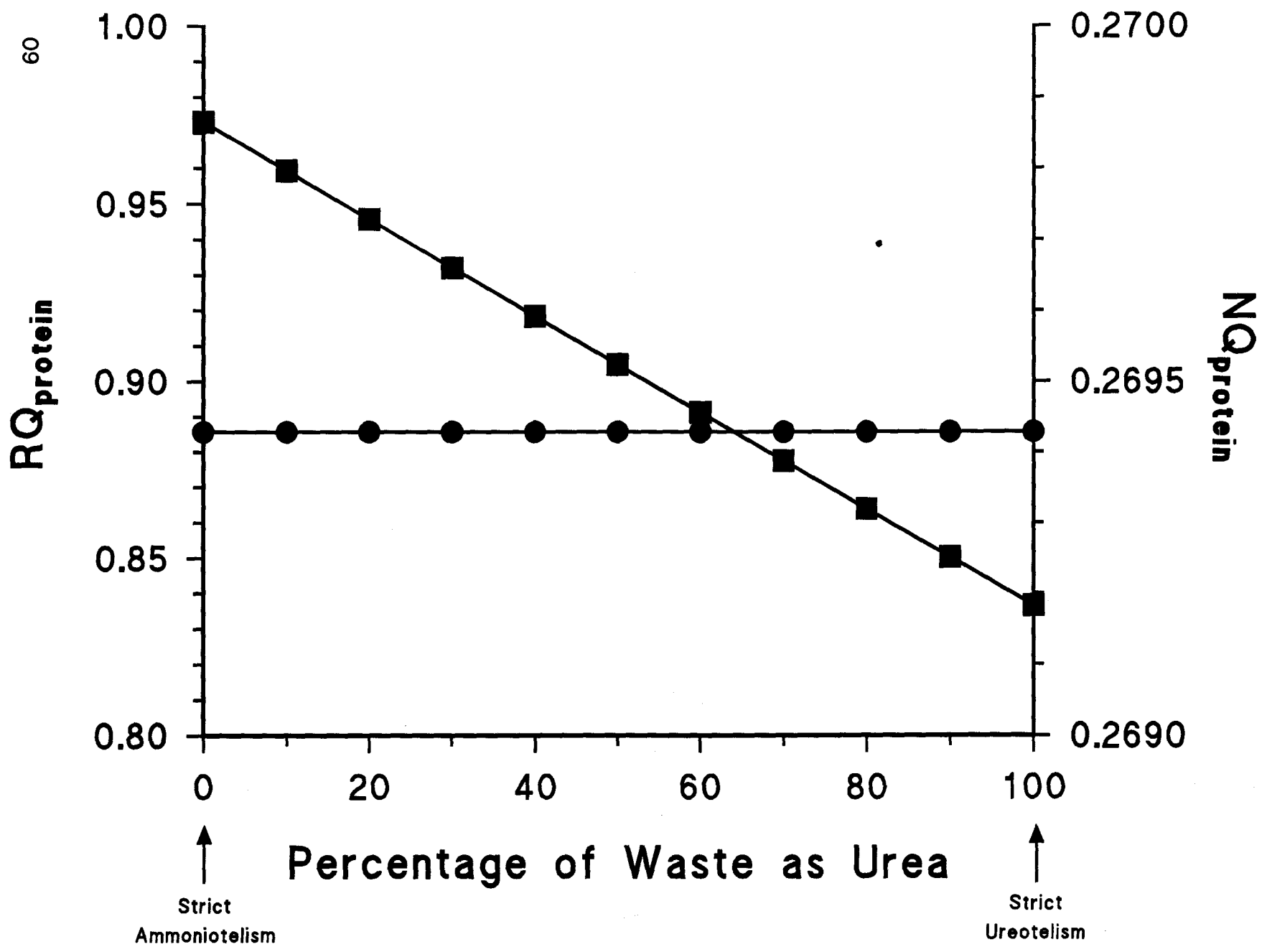
RQ's for lipid and carbohydrate are well known to be 0.71 and 1.0 respectively (eg. Blaxter, 1965; Brett and Groves, 1979). An RQ<sub>protein</sub> in strict ureoteles is an undisputed 0.83 (Kleiber, 1987). Several studies on fish metabolism have used the ureotelic RQ<sub>protein</sub> in the calculation of fuel use with the assumption that the different nitrogenous waste products would

have no effect on the value (Kutty, 1972). It was later calculated that the  $RQ_{\text{protein}}$  for ammoniotelic animals was 0.97 (Van den Thillart and Kesbeke, 1978). While this value is true if the organism is strictly ammoniotelic, very few "ammoniotelic" organisms excrete exclusively ammonia. Since the amount of carbons and hydrogens excreted with N vary with the different end products, it would be expected that the RQ would change with different proportions of the end products (Fig. 9). Trout are not strict ammoniotelic organisms as was assumed by Van den Thillart and Kesbeke (1978; equation 2, this study). In a classic paper, Smith (1929) found urea to be a secondary, though important N-excretory product in teleosts; this has been subsequently confirmed in this study and by many others (eg. Brett and Zala, 1975; Jobling, 1981; Kikuchi *et al.*, 1990; Jayaram and Beamish, 1992). These results alone indicate that whenever studies utilizing the RQ are done, the N waste products must be known in order to effectively predict fuel use (Fig. 9). In the current experiment, urea accounted for approximately 25% of nitrogenous waste excretion.

Other nitrogenous waste products do exist, though Olson and Fromm (1971) have shown that urea,  $T_{\text{amm}}$  and water-borne protein account for essentially all the N found in the water. Since those authors considered the proteins (not free amino acids) to have arisen from sloughed mucus, water proteins are not counted in the current study as contributing to the nitrogenous waste excretion *resulting from metabolism*, and hence are not



Figure 9. The relationship of  $RO_{\text{protein}}$  and  $NO_{\text{protein}}$  to the relative proportions of nitrogenous waste excretion as urea and ammonia, assuming these are the only two nitrogenous waste products.



indicative of proteinaceous energy production. Using similar techniques, Jayaram and Beamish (1992) found urea-N and  $T_{\text{amm}}\text{-N}$  to account for all (within statistical limitations) of the excreted nitrogen in *Salvelinus namaycush*. Other end products then, at least under nonextreme conditions, appear negligible.

Via the method of Kleiber (1987) an  $RQ_{\text{protein}}$  of 0.94 was found for the fish in this study (Table 2). Kleiber's typical protein composition reflects the mammalian condition, though Van Waarde (1983) considered the same composition valid for use in fish.

Several authors have used only  $T_{\text{amm}}\text{-N}$  as predictor of protein catabolism. Ming (1985) used only the  $M_{\text{NH}_3}$  to provide an index (not an absolute value) of protein use between three strains of *O. mykiss*. Had oxygen consumption been measured as well (as in Brett and Zala, 1975), an ammonia quotient would have given an absolute, albeit underestimated value for protein utilized as was done in Wiggs *et al.*, (1989) and Kutty (1978). However, as described above, the importance of urea cannot be neglected. Even though most of the urea likely arises from uricolysis (Van Waarde, 1983), the N in the purines are thought to arise originally from protein deamination (Forster and Goldstein, 1969). Walton and Cowey (1982) nicely synthesized the available literature on this topic and concluded that the N in purines is derived from glycine, aspartate and glutamate, and that uricolysis was important in linking amino acid degradation to urea synthesis.

Urea is also known to be synthesized from arginine (via arginase; Kaushik, 1980). In the extreme scenario that no waste products other than ammonia were synthesized from the protein breakdown, then the AQ would have been the more appropriate factor to use and an overestimate of protein use would have resulted. However, it appears much more probable that most of the urea, plus perhaps other unmeasured nitrogenous waste products, arise from protein catabolism, so the NQ is a more accurate index of protein usage than the AQ.

An important assumption must be made when using the NQ. The N which arises from the catabolized proteins must be excreted at the same rate at which it is deaminated. This has been shown to be true at least as far as ammonia is concerned (Van Waarde, 1983). Wood (1993) points out though that by convention, the production rate of ammonia is considered to be the *net* production and is taken to equal the excretion rate under steady state conditions.

### The Meaning of Fuel Use

In reviewing the literature, it seems apparent that there are two meanings to the term "fuel use." As referred to in the Introduction, the wide range of protein use values seems to imply that fish are either very diverse in their metabolism, or that a miscommunication between studies exists. In this study, it was sought to resolve this dilemma. Two types of fuel use shall be described, and their interrelatedness discussed.

The *instantaneous fuel use* is that quantity which is algebraically derived from the respiratory gas exchange and the nitrogenous waste excretion. It is an indicator of what substrates are actually being broken down to fuel metabolism at that point in time. Often, only the AQ is reported (from which protein catabolism has been estimated, eg. Kutty, 1972; Sukumaran and Kutty, 1977), though attempts have been made to see the complete spectrum as well (Van den Thillart, 1986).

The *compositional fuel use* is that quantity measured by changes in body components. This is the fuel use most discussed in ecophysiological studies, for instance in migration (Duncan and Tarr, 1958; Idler and Clemens, 1959). Since fish have a great ability to convert their proteins to carbohydrates and lipids (Van Waarde, 1983; Wood, 1993) neither type of fuel use alone can be used as a predictor of the other.

Van den Thillart and Kesbeke (1978) employed an  $RQ_{\text{protein}}$  of 0.97 to predict that resting rainbow trout used an instantaneous fuel mix of 80% protein and 20% lipid. They found that exogenous glucose (administered via catheter) was not used, and since Black *et al.*, (1962) found that glycogen levels in the liver and muscle remained constant during moderate exercise (1-2 L·s<sup>-1</sup> in a rotating chamber), it was assumed by Van den Thillart and Kesbeke that protein and lipid were the only fuels of significance. However, the quoted data showed great variability presumably "due to the use of fish too large for the rotating chamber" (Black *et al.*, 1962). However, if their

numbers were representative, it simply means that there was no *net* loss of glycogen, it does not rule out a constant turnover. Bever *et al.*, (1981) also interpreted the constant glycogen reserves as representative of the existence, rather than an absence of a maintenance level turnover.

In his review on ammonia production by fish, Van Waarde (1983) presents a summary of work done on ammonia quotients (Table 1 *in* Van Waarde, 1983). Three of the five references cited show "the contribution of protein catabolism to energy production is...over 40%." Presumably this was phrased in such a manner as to reflect conventional thought. An equally true statement about that table would be, "Four of the five references show the contribution of protein catabolism towards energy production to range from 14-45%." This is a more realistic phrasing which better describes the trend of instantaneous protein use in fishes.

## Starvation

Both  $M_{O_2}$  and  $M_{CO_2}$  showed fluctuation about their descending lines of regression (Fig. 3). In 1975, Brett and Zala showed that 22 day starved sockeye salmon (*Oncorhynchus nerka*) retained a diurnal cycling of  $M_{O_2}$ , but the cycle dampened as the  $M_{O_2}$  decreased with time. To show this, they performed closed system respirometry every 2 or 3 hours on 12 of the 22 test days. It was not the intention of this experiment to confirm the diurnal cycle (only 1 or 2 bouts of respirometry could be performed daily due to constraints in the methodology), though the diminished  $M_{O_2}$  over time was

confirmed (Fig. 3a). In addition, Fig. 3b shows a similar and expected pattern for  $M_{CO_2}$ .

During routine metabolism, Kutty (1972) found mean RQ's of *Tilapia mossambica* to be 1.03. Since the fish were exposed to high concentrations of oxygen, he interpreted the result (using the typical mammalian  $RQ_{protein}$  of 0.83) to mean that about 20% of the  $CO_2$  was being produced anaerobically. It was clear in the current study, that fish periodically went through bouts of anaerobiosis (Fig. 4).  $CO_2$  washout (which could give rise to an  $RQ > 1.00$ ) should not have been a problem given the small size of the animals used, and the long test period (Kleiber, 1987). If these bouts of anaerobiosis were stress induced, the source of the stress was not immediately obvious as the fish never saw the experimenter and the  $P_{O_2}$  never dropped below 120 torr. The procedure of sampling necessitated touching the respirometers, the vibrations from which may have startled the fish. Alternatively, the fish may have undergone irregular bouts of spontaneous anaerobic activity. There is no reason to assume such activity is not possible and in fact, this has been well documented by Kutty (1972).

In the current study, the overall trend in N excretion was found to consist of three phases (Fig. 5a). However the trend in nitrogen quotient, and thus protein use, remained more or less linear due to the fluctuating  $O_2$  consumption (Figs. 5b, 6, 7). This indicates that given some other influence

on metabolism which resulted in the changes to the  $M_{O_2}$ , the fish retained the trend in protein degradation instead of switching to lipid, an energetically more favourable fuel on a weight for weight basis. An increased dependence on proteins during starvation in mammals is known (Walton and Cowey, 1982) and the trend is also now apparent in fish, though only in a relative, not absolute sense (compare Figs. 6 and 7).

During starvation, breakdown of endogenous proteins and amino acids is the primary source of excreted N (Wood, 1993). Brett and Zala (1975) showed that starved *O. nerka* (mean weight 29g) show no diurnal or long term fluctuation in ammonia or urea excretion. However,  $M_{O_2}$  retained a dampened diurnal flux (see above) which resulted (by my calculations on their data) in a diurnal flux of protein use between 27% (daylight minimum) and 51% (darkness maximum). For *Tilapia mossambica* starved 36 hours, Kutty (1972) found an AQ of 0.20 (protein use of 74%) when ambient  $P_{O_2}$  was greater than 80 torr. Both of these studies are high compared with the 14-24% range found over the 15 days in the current study; species and life stage differences in the three experiments could have accounted for some of the differences.

#### Implications of the Discrepancy between Instantaneous and Compositional Estimates for the Role of Protein

Whereas respirometry (the instantaneous method) indicated that protein oxidation contributed only 14-24% of  $M_{O_2}$ , measured protein depletion (the compositional method) suggested a protein contribution of



46.3-50.2% over 15 days of starvation. Part of this disagreement could be explainable by analytical errors and the excretion of non-metabolised protein (see next section), but another likely explanation for a disagreement of this magnitude is that proteins, while supplying a basal level of carbon for direct entry into the Krebs cycle, also underwent gluconeogenesis and lipogenesis. The work of Bever *et al.*, (1981) on kelp bass (*Paralabrax clathratus*, also a carnivore) supports this in that they found a rapid rate of disappearance of injected, labelled alanine with a concomitant increase in labelled glucose. A 12-50% use of carbohydrate was predicted via respirometry in the current study, though only a low percentage of the fuel mix arose from the measured depletion of endogenous carbohydrate stores (Table 3). Therefore, gluconeogenesis with a corresponding turnover of glycogen stores may contribute a significant portion of the discrepancy. Glycogen is known as the major fuel of anaerobic metabolism (Driedzic and Hochachka, 1978a) and it now appears to be an important instantaneous fuel of aerobic metabolism as well. Given the findings of this study, it would be prudent to conduct studies on glycogen turnover and activities of gluconeogenic enzymes during starvation to confirm this hypothesis.

#### Internal Check

The test fish in this study used less oxygen than the depletion of fuels predicted (Table 4a). A similar problem was encountered by Krueger *et al.*, (1968) who found the caloric value of the material losses in rapidly

swimming fish was higher (3 fold) than that predicted by the oxygen consumption. Their study was on coho salmon (*O. kisutch*) whereas their oxygen consumption values arose from values published by Brett (1964) on *O. nerka*. Even so, they could not reconcile the 3-fold deviation to the presumably slight differences in species specific oxygen consumption.

In the current study, the indirect cumulative oxygen consumption was calculated based on the assumption that the differences measured in body composition all went directly towards powering metabolism. As explained in the previous section, it is possible that much of the protein was converted to glucose before being catabolized for energy. If the majority of the protein went to carbohydrate (as predicted by the respirometry), an overestimate of cumulative oxygen consumption (via the compositional method) would (and did) occur since the caloric equivalent of carbohydrate is 30% less than that of protein. An additional possible reason for the discrepancy is outlined below, namely the excretion of fuels (eg. protein) without oxidation. This reasoning would also explain the dilemma faced by Krueger *et al.*, (1968).

In attempting to balance the nitrogen excretion with the depletion of proteins, a 5 fold surplus was predicted from the body composition data over that actually measured via respirometry. Nitrogen from deaminations must be excreted or stored in a less harmful form. One possibility is that nitrogen could be stored in high nitrogen amino acids (eg. glutamine,

arginine, histidine) but due to osmotic considerations, this would involve special storage proteins; the protein assay would not have accounted for this high N stored in the protein. However it seems unlikely that 1.56 mmol of N (arising from the catabolism of 129 mg protein) could all have been stored in this manner. An alternate or additional possibility is that the "missing N" was excreted in an undetected form. Alternative end products for nitrogen do exist (eg. creatine, creatinine, trimethylamine oxide, amino acids; Forster and Goldstein, 1969), but again, it seems unlikely that so much nitrogen could be lost in these relatively minor forms.

A very likely source of some of the "missing" N was direct excretion as protein or amino acids. Indeed, Olson and Fromm (1971) reported that 25% of all N-waste excretion in rainbow trout (an amount equal to the urea excretion) occurred in the form of protein, presumably as a component of mucus. Bever *et al.*, (1981) followed the disappearance of radio-labelled amino acids and found that some of the amino acids were in fact incorporated into the mucus. This, together with any amino acid excretion which occurred, could have accounted for a substantial portion of the N discrepancy between the respirometry data for total N excretion and the protein depletion data. It would also help explain the discrepancy between the respirometry data for total O<sub>2</sub> consumption and the total fuel depletion, because excreted proteins and amino acids would be measured as fuel depletion, but of course would *not* have been associated with O<sub>2</sub>

consumption.

The other possible reason for discrepancies between respirometry data and fuel depletion data is experimental error, which is undoubtedly greater for the depletion measurements. Firstly, because of technological limitations, it is impossible to measure body composition on the *same* fish at the beginning and end of starvation (current methods involve sacrificing the fish), therefore, differences in composition between two groups of fish must be used. In contrast, respirometry data are collected from the same fish throughout the experiment. Secondly, the measurement of body weight changes is critical, but for fish of the size used in the present study, it was not practical to measure weight with an accuracy of more than 0.1g (two significant figures) without causing undue stress to the fish through excessive drying. This figure (0.1g) represents represents 14% of the average body weight change (0.7g) measured in the present study (Table 3). Finally, while the accuracy of the composition measurements is comparable to that of the respirometry data (similar coefficients of variation in the range of 10-30% were obtained), error is compounded because the depletion data depend on the *difference* between starting and ending composition. For example, the sum of the standard errors for protein, the fuel exhibiting the greatest depletion, was 65% of the measured difference (1.7%; Table 3). In practice, the error in compositional fuel use experiments is inversely proportional to the duration of the study and to the number of individuals

sampled. For example, in the following study (Chapter 3), no significant differences in body composition were found even though body weight dropped significantly over the five day study involving non-fed fish swimming at different aerobic intensities, yet the fish did of course excrete nitrogenous waste and use energy.

In conclusion, the compositional approach is probably best suited for very long term eco-physiological studies employing large numbers of fish to minimize variability. However, it should be recognized that a significant potential for error exists due to interconversion of fuels and/or excretion of nonmetabolized fuels. The instantaneous approach based on respirometry appears far more suitable for short term physiological studies.

## CHAPTER 3

### Introduction

Food capture, predator avoidance and reproduction are all directly related to swimming speed limits and endurance (Videler and Wardle, 1991; Weber, 1992). The physiology of swimming fish has been intensively studied from many different viewpoints. Much of the work has been done on burst performance or exhaustive exercise (McDonald, Tang, and Boutilier, 1989; Goolish, 1991; Scarabello *et al.*, 1991, 1992), a large portion of which is fuelled anaerobically, though work on routine or sustainable swimming, which occupies a vastly larger portion of a fish's time has been addressed as well (Van den Thillart, 1986; Hughes *et al.*, 1988; Beamish *et al.*, 1989; Weber, 1991; Thorarensen *et al.*, 1993).

Aerobic metabolism is used to provide energy for sustained activities (Webb, 1993). Sustained swimming speeds, by definition, are those speeds which a fish can maintain for greater than 200 minutes (Beamish, 1978). There are two noteworthy sustainable swimming speeds for each fish. The first is the maximum sustainable swimming speed, also known as the critical swimming speed, or  $U_{crit}$  (Brett, 1964); the other, the optimal swimming speed, is the speed at which energy expenditure is lowest for a given distance covered. The optimal swimming speed should then be

the average speed at which fish swim for long periods of time. For carangiform swimmers, the optimal speed was predicted by Weihs (1973) and confirmed in the field for migrating sockeye salmon (*Oncorhynchus nerka*, actually a subcarangiform swimmer) to be one body length per second (Quinn, 1987). Weihs based his prediction on energetic principles.

However, like so many other natural phenomena, it would not be surprising to find that the frequency of the tailbeat at the optimal swimming speed occurred at its natural harmonic frequency, thus saving energy. This remains to be tested in fish, though it has been confirmed in tadpoles (Oxner, Quinn and DeMont, 1993).

The ability to perform prolonged physical activity is closely linked with the aerobic capacity (Weber, 1992). The energy expended on aerobic swimming is a large part of the energy budget of individual fish (Beamish *et al.*, 1989). However, the source of this energy has remained controversial. Since there seemed to be a dichotomy in the literature as to the meaning of "fuel use", a distinction was made in the previous Chapter between two operational definitions: *instantaneous* and *compositional*. *Compositional* fuel use values result from measuring body (or organ) compositions before (via a control group) and after some test (Davison and Goldspink, 1977), or at several points (on different groups of fish) along a migration route (Idler and Clemens, 1959; Mommsen *et al.*, 1980). These experiments are also referred to as *depletion* studies. In Chapter 2, depletion analyses yielded a

figure of approximately 50% for the contribution of protein to aerobic metabolism during 15 days of starvation in juvenile *Oncorhynchus mykiss*. Alternatively, the *instantaneous* fuel use gives information on the substrates powering metabolism at a single point in time and is based on measurements of respiratory gas exchange and nitrogenous waste excretion. In Chapter 2, this approach yielded a much lower figure for the protein contribution of only 14%, increasing to 24% after 15 days of starvation. Kutty (1972) and Sukumaran and Kutty (1977) both gave ammonia quotient (AQ; ammonia excreted : oxygen consumed) data from which protein catabolism could be calculated (on *Tilapia mossambica* and *Mystus armatus*, respectively). However, in both cases, the AQ increased with both exercise intensity and duration beyond the theoretical maximum for aerobic metabolism, so the actual protein contribution was hard to interpret. Van den Thillart, (1986) estimated that protein accounted for 90% of the fuel of cannulated rainbow trout during sustained swimming (80%  $U_{crit}$ ), with the balance arising solely from lipid.

Since no study has looked at the changes in instantaneous fuel use in relation to both aerobic swimming speed and duration, the current study seeks to do so for the rainbow trout (*Oncorhynchus mykiss*). A low (2.1 body lengths·second<sup>-1</sup>; L·s<sup>-1</sup>) and a high (3.1 L·s<sup>-1</sup>) swimming speed were chosen which represented approximately 55 and 80%  $U_{crit}$  respectively. The fuel usage at both speeds were compared with nonswimming fish.



While these speeds are within the realm of aerobic exercise, they are both faster than the previously mentioned optimum swimming speed ( $1.0 \text{ L}\cdot\text{s}^{-1}$ ). Limitations of the equipment (respirometers, motors) prevented the testing of the much slower optimal swimming speed.

Fuels are of course metabolized anaerobically or under nonsteady state conditions, but these fuels would not be accurately reflected in the respiratory quotient (molar ratio of carbon dioxide excretion to oxygen consumption). This study then is confined to aerobic levels of work since respirometry is a key method in determining the substrates metabolized.

## Methods and Materials

### Animals

Rainbow trout (*Oncorhynchus mykiss* Walbaum, formerly *Salmo gairdneri* Richardson; 8-10g) were obtained from Rainbow Springs Hatchery (Thamesford, Ontario) and were kept in 15°C dechlorinated Hamilton city tap water for at least two weeks prior to experimentation. The water was moderately hard, with the following composition (in mequiv l<sup>-1</sup>): Na<sup>+</sup>, 0.6; Ca<sup>++</sup>, 1.8; Cl<sup>-</sup>, 0.8; K<sup>+</sup>, 0.04; Mg<sup>++</sup>, 0.5; titration alkalinity (to pH = 4.0), 1.9; total hardness, ≈ 140 mg·l<sup>-1</sup> as CaCO<sub>3</sub>; pH 8.0. The fish were maintained on a commercial trout feed (Martin Trout Food Pellets, Tavistock, Ont), and were fed 1.0% of their body weight daily. Approximately 20% of the fish were weighed on a weekly basis to monitor growth; the food ration was increased to meet the prescribed feeding regime. The nutritional breakdown of the diet was presented in Chapter 2, Table 1.

### The Effect of Water Quality on Swimming Performance

Decarbonated water (made from dechlorinated water) was again used in the respirometry experiments to provide a low background total CO<sub>2</sub> against which expired CO<sub>2</sub> could be more accurately measured. This water was not found to be detrimental in the static environment of the previous Chapter. However, since the current study involved swimming fish, it was

thought prudent to compare the swimming performances in both decarbonated and dechlorinated Hamilton tap water. The standard test for critical swimming speed (Brett, 1964) was used with a time increment of 30 minutes, a speed increment of  $5 \text{ cm}\cdot\text{s}^{-1}$  and a starting velocity of  $10 \text{ cm}\cdot\text{s}^{-1}$ . All fish were tested at  $15^\circ\text{C}$  in a Beamish style respirometer (Farmer and Beamish, 1969). Critical swimming speed ( $U_{\text{crit}}$ ) was then calculated as follows:

$$(15)$$

where  $U_{\text{is}}$  is the velocity (in  $\text{cm}\cdot\text{s}^{-1}$ ) of the last completed swimming period,  $t_{\text{s}}$  is the time (min) spent swimming at the final swimming speed,  $t_{\text{i}}$  is the time increment (min) and  $U_{\text{i}}$  ( $\text{cm}\cdot\text{s}^{-1}$ ) is the velocity increment.

### Respirometry and Fuel Depletion

The methods and experimental system used were similar to those in the previous Chapter. Fish (mass =  $17.5 \pm 1.2\text{g}$ ; length =  $113.7 \pm 2.7\text{mm}$ ; mean  $\pm$  s.e.m.,  $n = 32$ ) were quickly blotted dry, weighed to the nearest 0.1g and measured to the nearest millimeter (fork length). They were then transferred to individual Blažka style swimming respirometers (Blažka, Volf and Cepala, 1960 *in* Beamish, 1978) that were submerged in the decarbonated water at  $15 \pm 1^\circ\text{C}$ . In this way, thermal stability was maintained. During the 48 hour acclimation period, freshly aerated, decarbonated water constantly entered the respirometers through one port,

while mixed water left via the sampling port at  $\approx 150 \text{ ml}\cdot\text{min}^{-1}$  (Fig. 1).

The entire system in which the fish were tested was of approximately 500 l and operated under recirculating conditions. About 300 l of the water was changed on a daily basis. The fish were not fed at any time during the experiment, including the acclimation period. An opaque sheet floated on the surface of the water to prevent the fish from being affected by visual disturbances. At the end of the acclimation period, controls were sacrificed by introducing neutralized tricaine methanesulfate (MS222, Syndel Laboratories) such that the final concentration to which the fish were exposed was  $1\text{g}\cdot\text{l}^{-1}$ . The fish were removed from the respirometers, blotted dry, weighed, freeze-clamped in liquid nitrogen and stored at  $-20^\circ\text{C}$  until analyzed for proximate body composition (see below).

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

At  $t_0$ , the respirometer motors were adjusted to the appropriate speeds based on body length for individual fish in the low speed group. In the high speed group, to prevent a predictable burst (anaerobic) start (Wokoma and Johnston, 1981), the fish were given an hour 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 velocity and bringing it up again when the fish had reoriented. If this was not successful the respirometer motor was turned off and any data collected on that fish were not used in determining the fuel use. In an attempt to determine the cause of failure, initial oxygen consumptions ( $M_{O_2}$ ) and body condition factors [mass (g)·fork length<sup>-3</sup> (cm<sup>3</sup>)] were compared with those of the successful swimmers.

Three sampling periods (0.5-1.0 h) were run 5 hours apart on each of the three days after acclimation for a total test duration of 58 hours. Each sampling period consisted of taking initial water samples, closing off the respirometers for a fixed amount of time (a time which was predicted to not allow depletion of ambient  $P_{O_2}$  to lower than 120 torr, usually between 30 and 60 minutes), opening the respirometers and immediately taking new water samples. Between same-day bouts of respirometry, the sampling ports were open wide to allow a high flow rate ( $\approx 500\text{ml}\cdot\text{min}^{-1}$ ) of freshly aerated water to replenish the ambient  $P_{O_2}$ . The water samples were measured for  $O_2$ ,  $CO_2$ , total ammonia ( $T_{\text{amm}} = NH_3 + NH_4^+$ ) and urea as in the previous Chapter. Other nitrogenous waste end products do exist, though under typical conditions these represent a very small percent of the total metabolic waste (Olson and Fromm, 1971; Jayaram and Beamish,

1992). They were not measured in this study. At the end of the three day test, all of the test fish were sacrificed and treated in the same manner as the controls.

### Calculations

Respiratory quotient (RQ; CO<sub>2</sub> expired : O<sub>2</sub> consumed) was determined for each fish in each experimental period, as was the nitrogen quotient (NQ; total-N : O<sub>2</sub> used, where total-N is the sum of urea-N and ammonia-N). From these, fuel use could be determined as follows:

$$P = \frac{NQ}{0.27} \quad (16)$$

where  $P$  is the fraction of the total fuels for M<sub>O<sub>2</sub></sub> made up by protein. The maximum NQ (NQ<sub>protein</sub>) does not change as the balance of waste products change; the value of 0.27 reflects 100% protein use when T<sub>amm</sub> and urea are the end products in any combination. In contrast, a respiratory quotient reflecting 100% protein (RQ<sub>protein</sub>) must be calculated for each set of fish and is dependant on the mixture of nitrogenous waste end products (Chapter 2). RQ<sub>protein</sub> values were calculated for the three groups of fish in this study to be 0.95 (nonswimmers and low speed swimmers, based on 18% of total-N as urea) and 0.93 (high speed swimmers, based on 34% urea). RQs for lipid and carbohydrate are well known to be 0.71 and 1.0 respectively (Brett and Groves, 1979; Kleiber, 1987). It then follows for high speed fish,

$$RQ = P*0.93 + C*1.0 + L*0.71 \quad (17)$$

where  $P$ ,  $C$  and  $L$  represent the fraction of the total fuels burned for  $M_{O_2}$  arising from protein, carbohydrate and lipid, respectively. Since,

$$L = 1.0 - P - C \quad (18)$$

and substituting equations 16 and 18 in equation 17,

$$RQ = 0.81NQ + 0.29C + 0.71 \quad (18)$$

For a more detailed derivation of this equation, refer to Chapter 2.  $RQ$  and  $NQ$  were both calculated from data measured in the experiment, so only  $C$  and  $L$  need be determined. Equation 19 can be solved for  $C$ , and  $L$  can then be determined by equation 18. The fuel usages for nonswimmers and low speed swimmers were calculated similarly.

The nitrogenous waste excretion was used to determine the carbon contribution of proteins, while the relative proportions of fuels and their respiratory quotients were then used to determine the carbon contributions of the other two fuels (see Chapter 2 for the complete derivation).

### Body Analyses

The bodies were analyzed for protein, glucose, glycogen, lactate, total lipids, inorganics (ash) and water. Glucose glycogen and lactate are reported as total carbohydrate. The procedures were identical to those of the previous Chapter.

## Statistics

Values are reported as means  $\pm$  standard error. A one way ANOVA was used to determine significance in body compositional changes between groups of fish, while a paired t-test was used to determine if the pre- and post-experimental body masses differed. An independent t-test was used to compare critical swimming velocities 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, a  $p < 0.05$  was considered significant.



## Results

### The Effect of Water Quality on $U_{crit}$

The critical swimming velocity of fish swimming in decarbonated water ( $3.84 \pm 0.15 \text{ L}\cdot\text{s}^{-1}$ , mean  $\pm$  s.e.m.;  $n = 11$ ) was found to be not different than that of fish swimming in normal tapwater ( $3.98 \pm 0.23 \text{ L}\cdot\text{s}^{-1}$ ,  $n = 14$ ;  $p > 0.05$ ; Fig. 10).

### Respirometry

Both groups of swimmers showed steady decreases in gas exchange rates whereas the nonswimmers were relatively stable over the 3 day experimental period (Fig. 11). The high speed fish consumed  $\text{O}_2$  initially at  $12 \mu\text{mol}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$ , but this decreased to  $9.5 \mu\text{mol}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$  by the end of the experiment ( $p < 0.001$ ). Low speed swimmers had  $M_{\text{O}_2}$  values about 75% of the high speed group.  $M_{\text{O}_2}$  in nonswimmers was about  $5.5 \mu\text{mol}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$  throughout.  $\text{CO}_2$  excretion rates ranked in the same order as the  $M_{\text{O}_2}$  values. Nonswimmers had a stable excretion of  $4.0 \mu\text{mol}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$  ( $p > 0.05$ ); the low speed group had excretion rates that declined from  $8.0$  to  $5.5 \mu\text{mol}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$  ( $p < 0.001$ ) whereas the high speed group exhibited a nonsignificant drop from almost  $10 \mu\text{mol}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$  to  $8.5 \mu\text{mol}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$  ( $p > 0.05$ ). There was no discernable difference in RQ between the three groups, when all of the values for each fish were included (Fig. 12). When values of the RQ greater

Figure 10. A comparison of  $U_{crit}$  of juvenile rainbow trout in normal tapwater (n = 14) and decarbonated tapwater (n = 11). Means  $\pm$  sem.

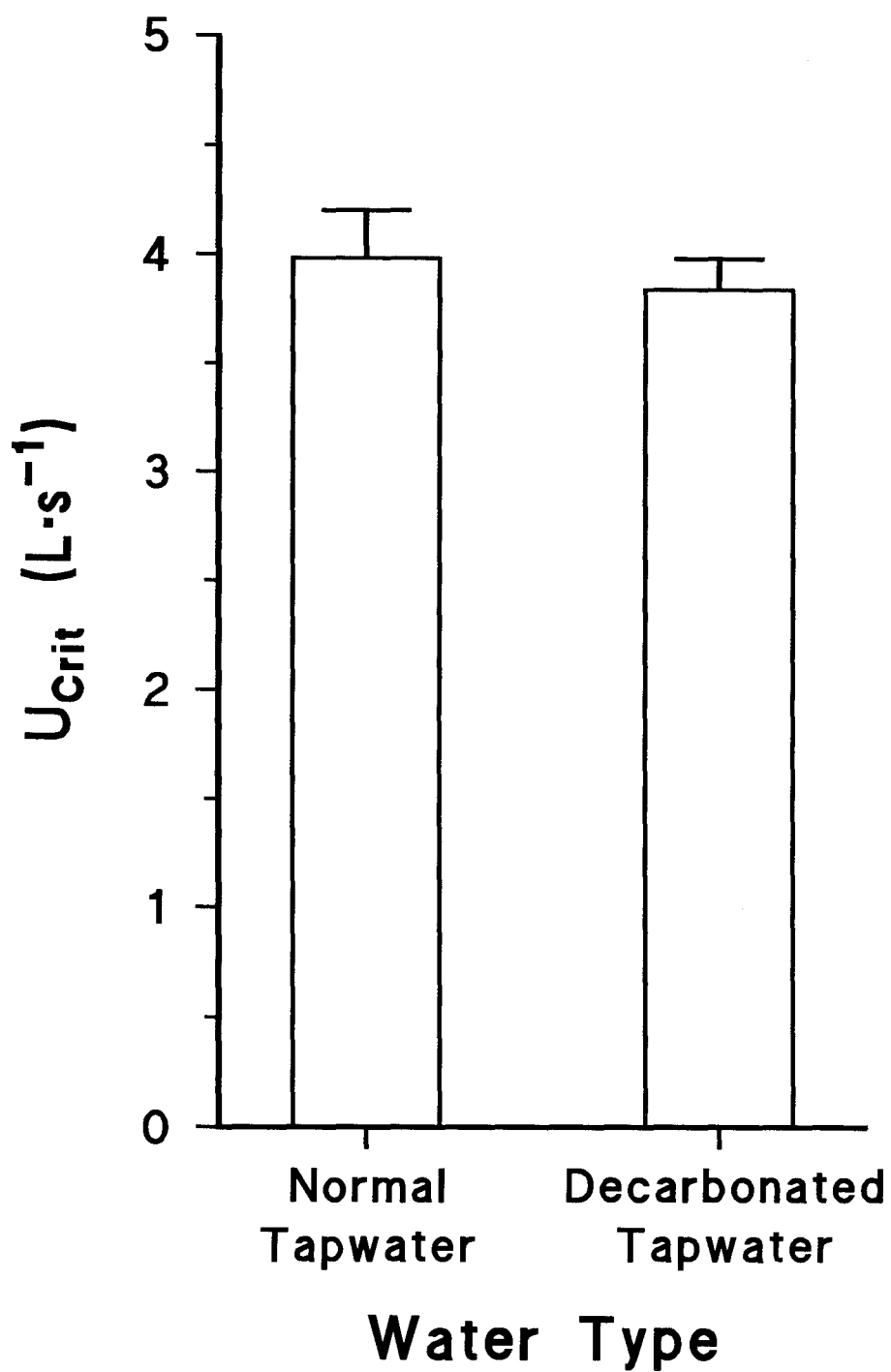


Figure 11. Oxygen consumption and carbon dioxide excretion in nonswimming (squares and solid lines, n = 11), low speed swimming (circles and dashed lines, n = 12), and high speed swimming (triangles and dotted lines, n = 7) in juvenile rainbow trout over the three day test. Means  $\pm$  sem.

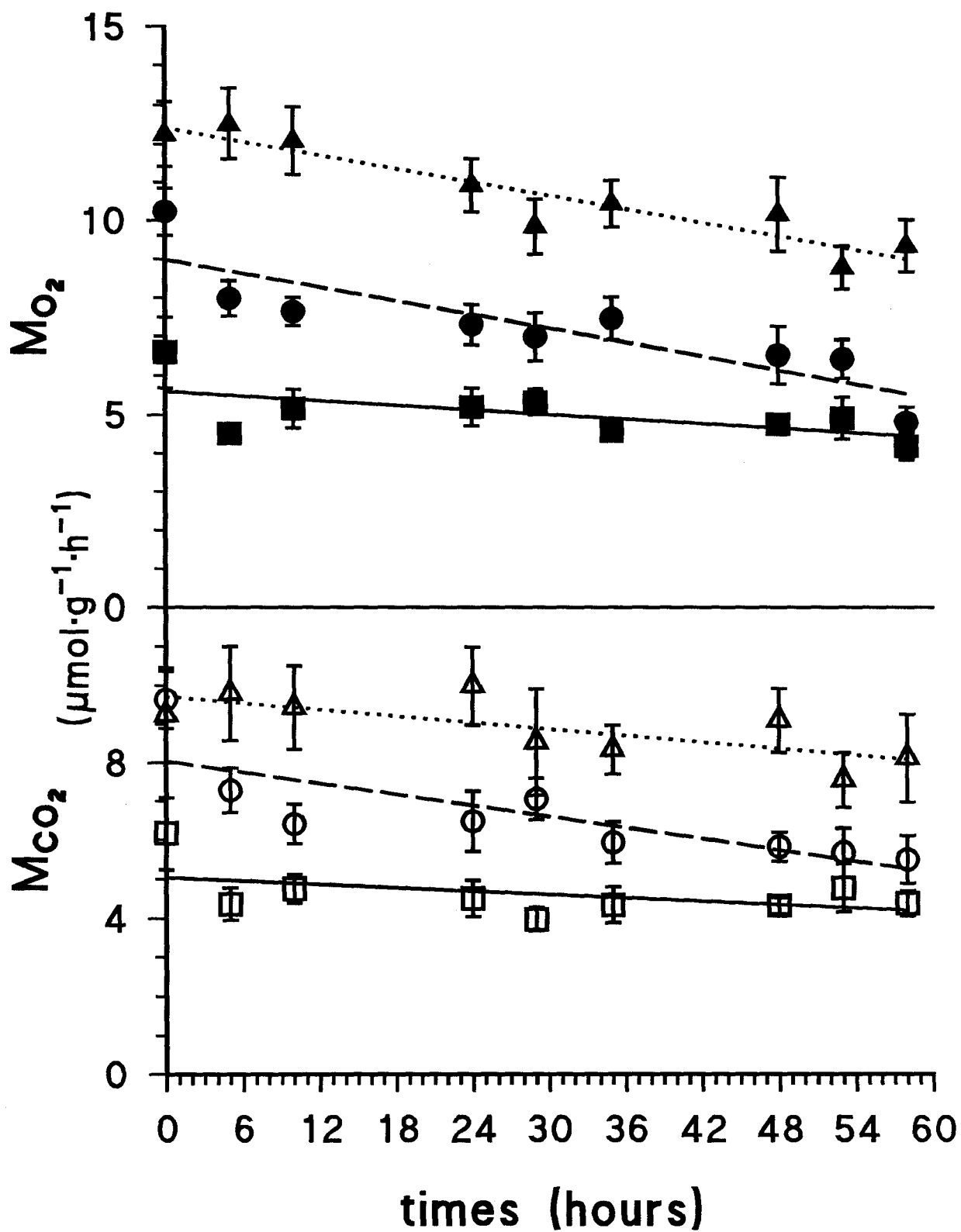
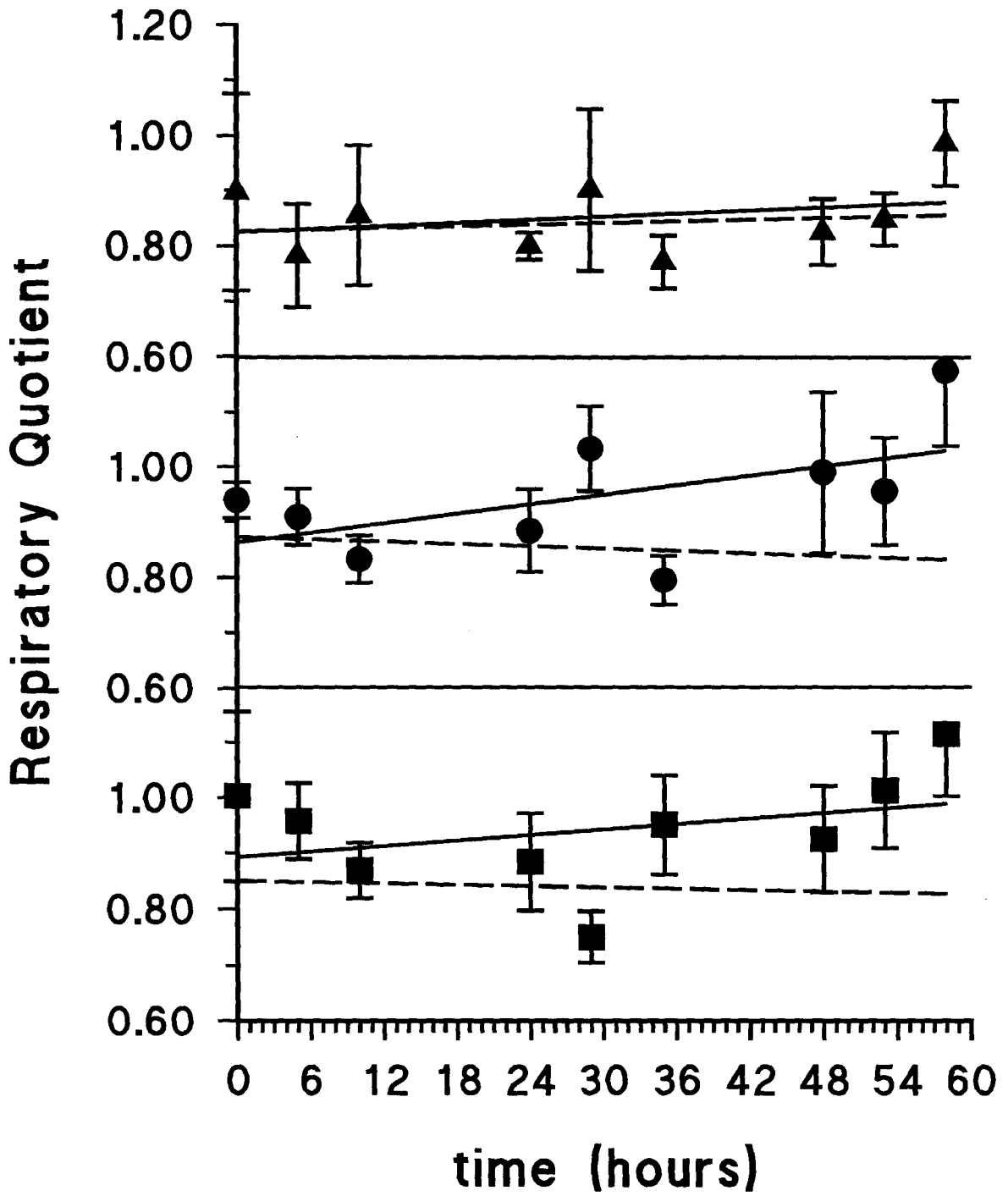


Figure 12. The complete respiratory quotient (symbols and solid regression line) and the aerobic respiratory quotient for nonswimming (squares,  $n = 11$ ), low speed swimming (circles,  $n = 10$ ), and high speed swimming (triangles,  $n = 7$ ) juvenile rainbow trout over the 3 day test. Means  $\pm$  sem.



than 1.0 (ie. bouts of anaerobicity) were removed, there was still no difference in the trend between groups (Fig. 12).

Total nitrogenous waste excretion was relatively stable in each group at approximately  $0.55 \mu\text{mol} \cdot \text{g}^{-1} \cdot \text{h}^{-1}$  ( $p > 0.05$ ; Fig. 13). 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 nonswimmers. These stable excretion values, in conjunction with the decreasing  $M_{\text{O}_2}$  values (in the swimmers), gave rise to increasing NQ's with time in the nonswimmers and low speed group only ( $p < 0.001$ ; Fig. 14). The high speed swimmers had the lowest NQ (stable at 0.050;  $p > 0.05$ ), while the nonswimmers had the highest NQ (increasing from 0.08 to 0.12). Since protein catabolism is directly correlated with the NQ (equation 16) it is also shown on Fig. 14 using the right axis. Essentially, both the nonswimmers and low speed swimmers showed an increase in the contribution of protein to the total fuel mixture for  $M_{\text{O}_2}$  (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%.

Trends in instantaneous metabolic fuel use are shown in Fig. 15. In general, the nonswimmers and low speed swimmers show similar trends in all fuels, whereas the high speed group shows opposite trends in carbohydrate and lipid oxidation. Carbohydrate use dropped in both the nonswimmers (from 23 to 5%) and the low speed swimmers (from 38 to



Figure 13. Total nitrogenous waste (t-Nitrogen as the sum of ammonia-N and urea-N; circles) and ammonia-N (squares) excretion for the nonswimming (n = 11), low speed swimming (n = 10) and high speed swimming (n = 12) juvenile rainbow trout over the three day test period. Means  $\pm$  sem.

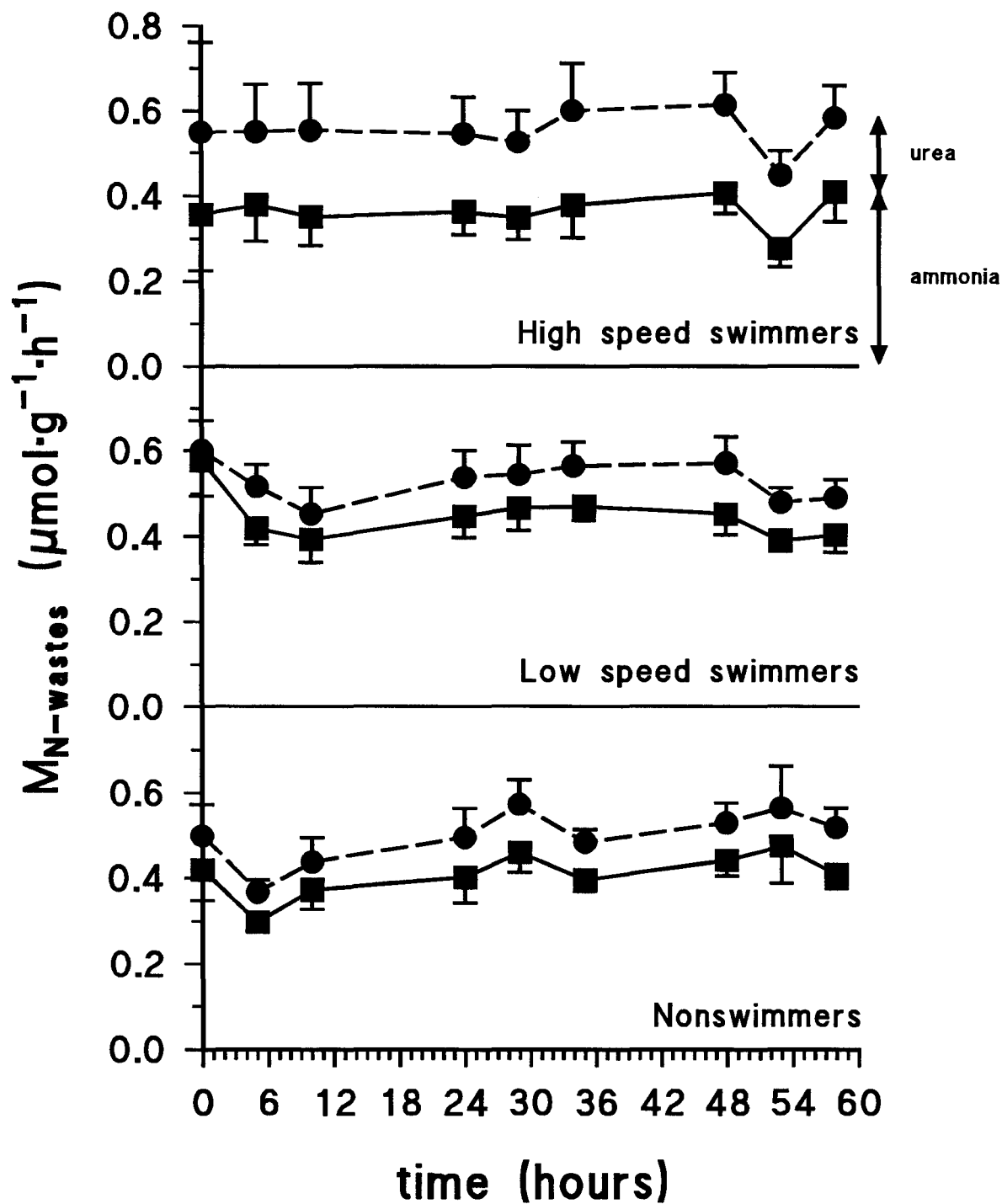


Figure 14. Nitrogen quotient (left axis) and protein use (right axis) for nonswimming (squares, n = 11), low speed swimming (circles, n = 10) and high speed swimming (triangles, n = 12) juvenile rainbow trout over the three day test. Means  $\pm$  sem.

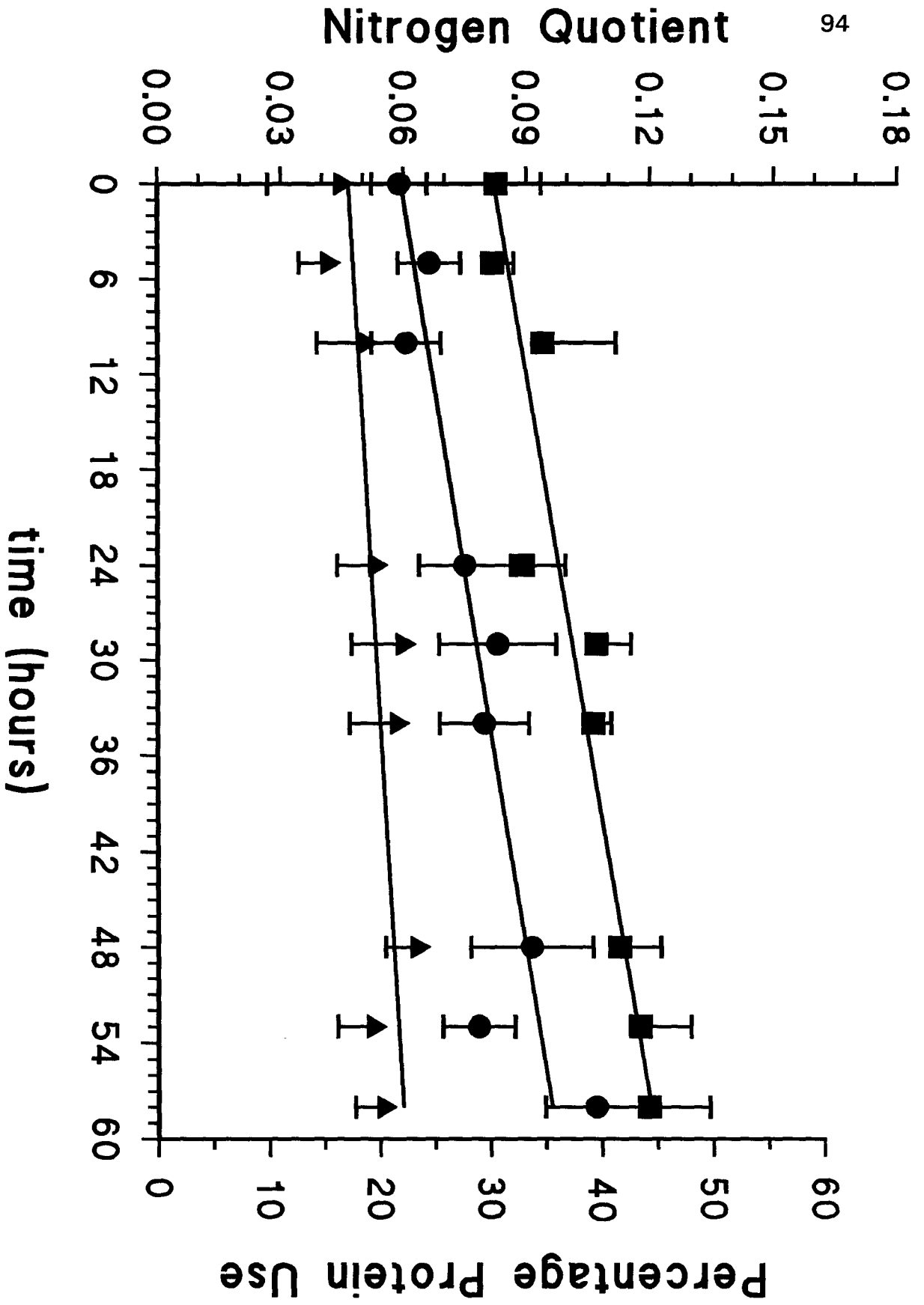
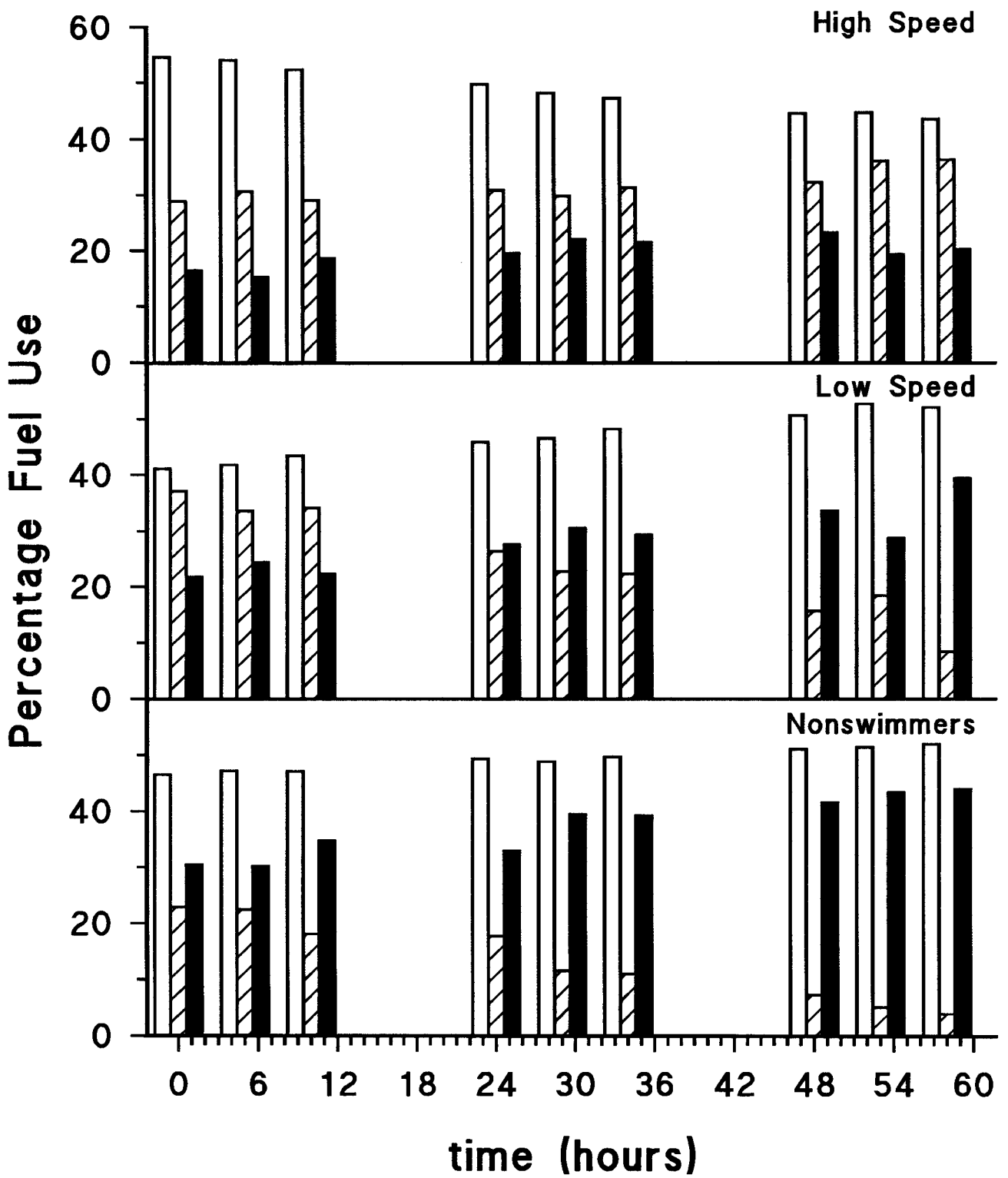


Figure 15. Percentage use of lipid (open bars), carbohydrate (hatched bars) and protein (solid bars) in nonswimming, low speed swimming, and high speed swimming juvenile rainbow trout over the three day test period.



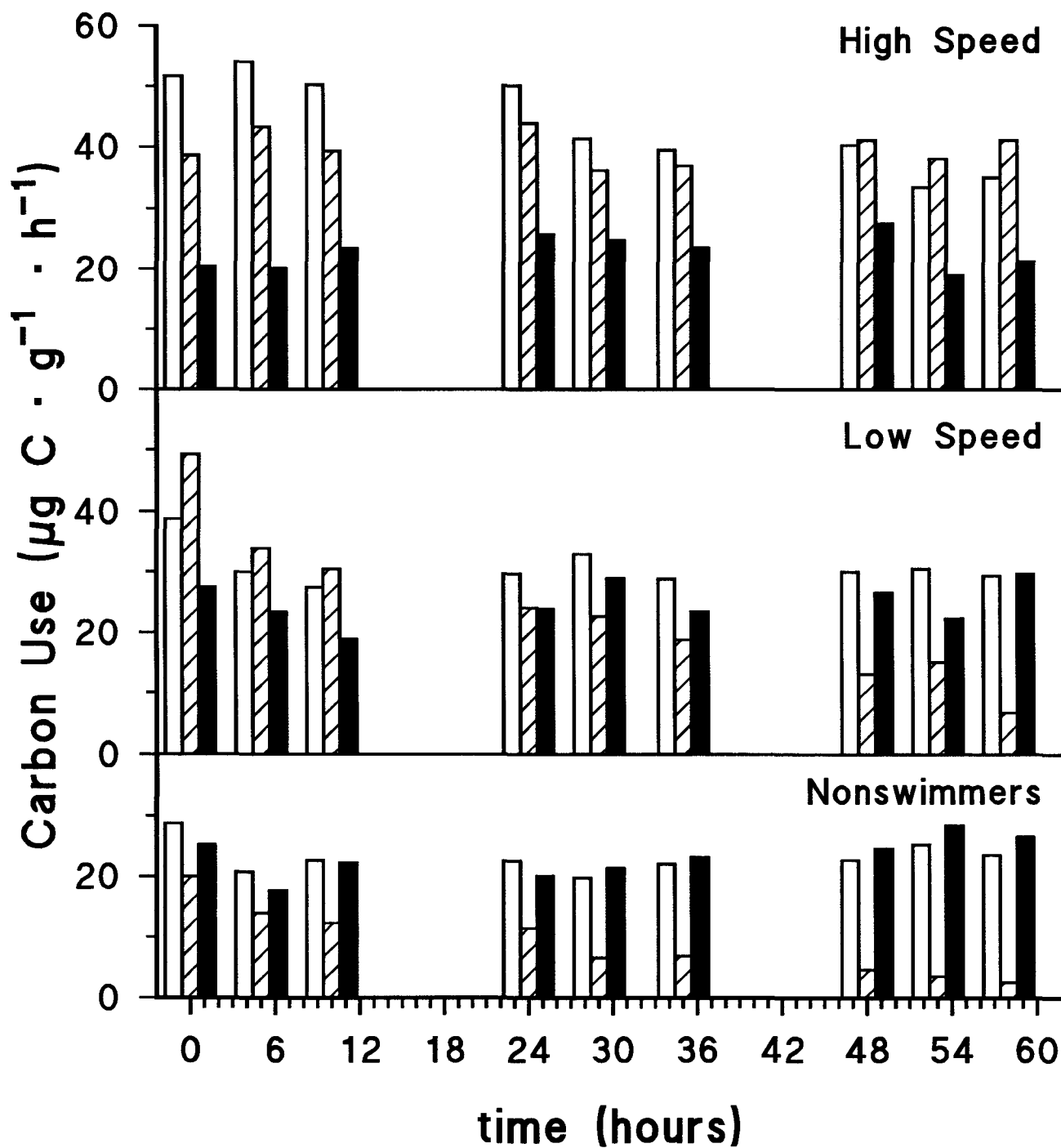
8%) over the course of the experiment. Concurrently, lipid increased in importance, but only slightly, since protein (as described above) increased in contribution as well. However, in the high speed swimmers, lipid started at 54% of the total fuel mixture, but diminished to 43%; carbohydrates increased from 29 to 38%.

The absolute carbon use rate is reflected in the CO<sub>2</sub> excretion curves of Fig. 11. Unsurprisingly, the total C use rate was greatest in the high speed swimmers, while the nonswimmers had the lowest usage of 57  $\mu\text{g C}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$ . The high speed swimmers reduced their total C use from 140  $\mu\text{g C}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$  to the third day average of 113  $\mu\text{g C}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$ ; similarly, the low speed swimmers dropped from 120  $\mu\text{g C}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$  to 53  $\mu\text{g C}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$ .

The contribution of lipid to total C remained relatively constant at 23  $\mu\text{g C}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$  in the nonswimmers; similarly, the low speed swimmers showed a constant lipid usage of 31  $\mu\text{g C}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$  (Fig. 16). In contrast, lipid dropped in the high speed group from a first day average of 52  $\mu\text{g C}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$  to a third day average of 36  $\mu\text{g C}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$ . Carbohydrate stayed relatively constant (39  $\mu\text{g C}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$ ) in the high speed swimmers, whereas in the low speed swimmers, like the nonswimmers, its contribution dropped over the three days by approximately 75%. 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 nonswimmers. No such comparative generalization could be made for the low speed swimmers.

Figure 16. The contribution of carbon from lipid (open bars), carbohydrate (hatched bars) and protein (solid bars) in nonswimming, low speed swimming, and high speed swimming juvenile rainbow trout over the three day test period.





## Proximate Body Analysis

There were no significant differences in any parameter of body composition between any groups of test fish or the controls (Table 5). Water made up 77% of the mass of the fish, with carbohydrate, lipid and protein making up on average 0.23%, 4.9% and 13.9% respectively; inorganics (ash) made up the balance of 2.64%. Body mass did decrease in all groups, including the controls ( $p < 0.05$ ). The weight loss during the acclimation period (represented by the data on control fish) represented from 55-75% of the weight loss of the other groups over the whole experiment.

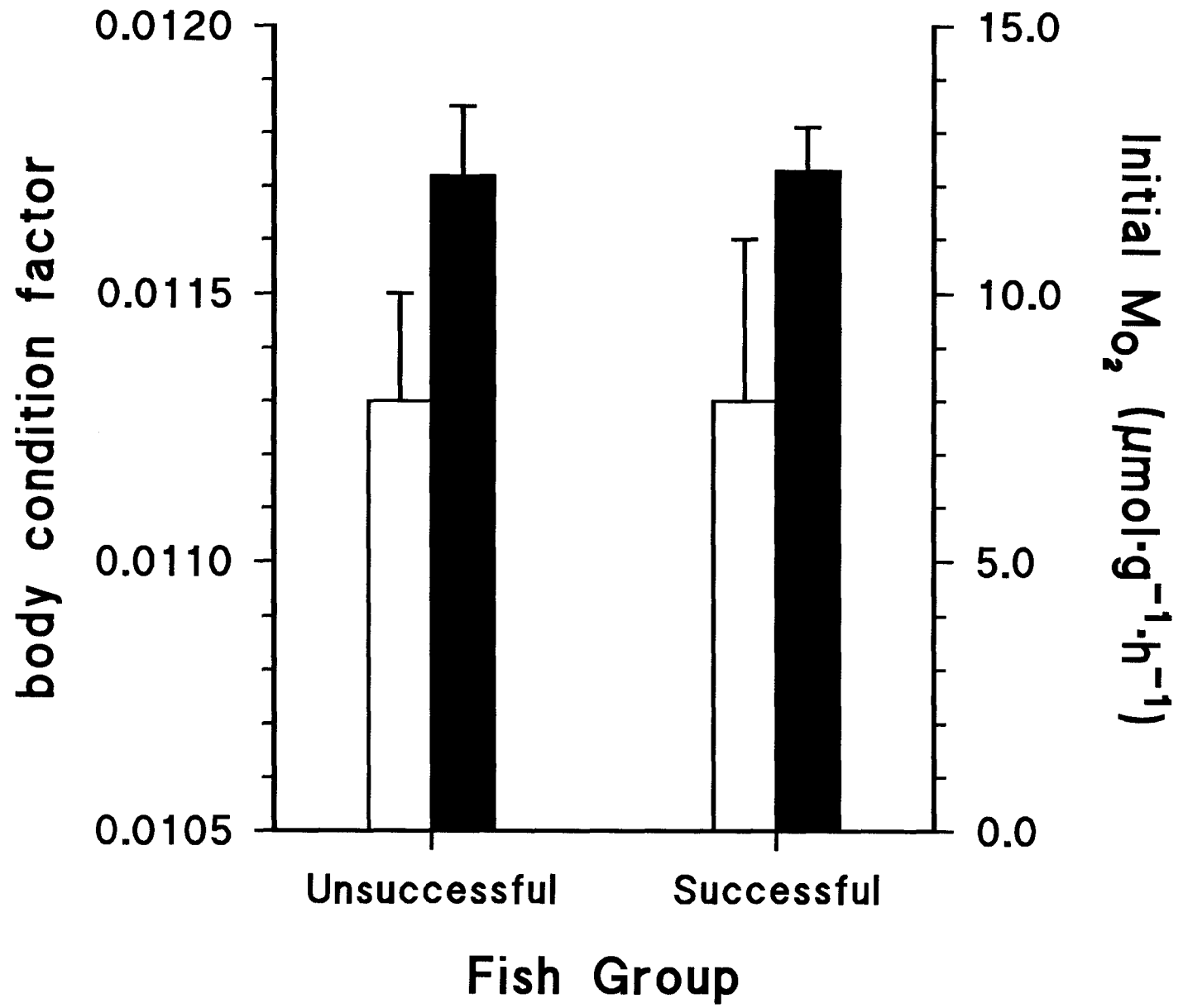
## A Comparison of Successful and Unsuccessful Swimmers

There was a 65% failure during the swimming tests in this experiment. Both the initial oxygen consumption and body condition factor were not different between the successful and unsuccessful swimmers ( $p \geq 0.05$ ; Fig. 17).

Table 5. Body compositions ( $\text{mg}\cdot 100\text{mg}^{-1}$ , 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  $\pm$  sem.

	lipid	total carbohydrate	protein	inorganic	water
controls (n = 6)	4.7 $\pm$ 0.7	0.28 $\pm$ 0.03	14.2 $\pm$ 0.4	2.60 $\pm$ 0.09	77.2 $\pm$ 0.6
nonswimmers (n = 9)	4.7 $\pm$ 0.5	0.21 $\pm$ 0.02	14.2 $\pm$ 0.2	2.66 $\pm$ 0.08	77.5 $\pm$ 0.5
low speed (n = 7)	5.1 $\pm$ 0.7	0.20 $\pm$ 0.02	13.2 $\pm$ 0.7	2.68 $\pm$ 0.17	77.8 $\pm$ 1.2
high speed (n = 12)	5.1 $\pm$ 0.5	0.24 $\pm$ 0.02	13.9 $\pm$ 0.6	2.62 $\pm$ 0.05	77.0 $\pm$ 0.5

Figure 17. A comparison of body condition factor (open bars) and initial oxygen consumption (solid bars) between the unsuccessful (n = 22) and successful high speed swimmers (n = 12). Means  $\pm$  sem.



## Discussion

### A Note on the Failure Rate of High Speed Fish

No differences between successful and unsuccessful swimmers were immediately obvious in this study. Swimming performance tests with young salmon were considered problematic by Virtanen and Forsman (1987). Krueger *et al.*, (1968) found that "shorter and lighter" salmon were less competent at a given absolute water velocity (ie. swimming speed) than their longer and heavier counterparts. This is hardly surprising given that a longer fish would see a *relatively* slower current, and a heavier fish would be stronger (assuming the extra weight was muscle). When comparing swimming velocities of fish of different size, the relative speed of body lengths per second, not the absolute speed is the more useful measure. As in the latter work, the fish in the current study that were unsuccessful in enduring the swimming regimen had similar body condition factors to successful swimmers (Fig. 8). Successful and unsuccessful swimmers were also not different with respect to initial oxygen consumption values, therefore an explanation based on burst swimming is not likely.

A plausible explanation though is that the swimming speed of individual fish was set based on an average  $U_{crit}$ . In reality, it was quite likely that speeds equivalent to 100%  $U_{crit}$  were thereby set for some of the

slower fish, rather than the intended 80%  $U_{crit}$ . Recall that the definition of sustainable swimming speeds imposes a minimum time constraint of 200 minutes and in practice is based on velocity increments over 30 minute periods, yet it sets no upper limit. If a fish managed at least 200 minutes (a time that would fall between the first and second sample periods), and then fatigued, it was swimming sustainably. It was found that the fish which were unsuccessful typically fatigued prior to this 200 minute limit (ie. within or just after the first sample period). Since the initial oxygen consumptions were not greater from the successful swimmers, it can be assumed they were swimming at speeds that were not sustainable, in other words, they were "prolonged" (Brett, 1964). Prolonged speeds can be sustained for tens of minutes via aerobic and anaerobic pathways, but eventually they end in fatigue (Webb, 1993). Ideally then, individual  $U_{crit}$ 's should have been established for each fish just prior to testing, with several days recovery allowed.

In Fig. 16, it was shown that the (successful) high speed swimmers relied heavily on lipid. Fatty acids have a low maximum power (rate of ATP synthesis) and are slow to be metabolized (Weber, 1988). It is possible then, that the unsuccessful swimmers were not able to mobilize enough lipids during the one hour speed increment phase to supply the initial energy demands.

## Fuel Use

The dynamic interplay of fuels used by the fish in this study was not predicted based on information found in the literature. Proteins, for all groups, were responsible for a uniform (or slightly increasing) contribution of C (Fig. 16) at percentages typically below that reported in other studies (Fig. 15). Lipids contributed a relatively constant (nonswimmers and low speed swimmers) or decreasing (high speed swimmers) amount of C, whereas carbohydrates supplied a higher percentage of C in all groups than has been predicted in the past. As discussed in the previous Chapter, the different techniques used in predicting fuel use are often not compatible (though often compared), as they are intended for different purposes. *Compositional* fuel use (as measured by corporeal losses in fuel) has been used successfully over long term studies (eg. Duncan and Tarr, 1958; Idler and Clemens, 1959; Mommsen *et al.*, 1980), though for short term studies such as this one, its value is questionable due to insufficient time to detect reliable changes in substrates (Table 5). Of interest though is an 8 hour study on low speed swimming in *S. salar* parr by Virtanen and Forsman (1987) in which whole body lipids were depleted by 50%. Whether this difference was due to the fact that their fish were wild caught, or more simply a species specific difference or some other factor, is unknown. Even though *instantaneous* fuel use has been investigated at least since the turn of the century in man (Rosa, 1900), relatively few have pursued this goal in fish



(exceptions being Kutty [1978] and Van den Thillart [1986]). In addition to respirometry and depletion studies, predictions based on biochemical (Moyes *et al.*, 1989) and histochemical (Hudson, 1973) procedures also exist. Instantaneous fuel use studies reveal the actual molecules oxidized for energy production, regardless of any interconversion that may have resulted between the source and the mitochondrion.

At all sustainable speeds, the red (slow) muscle of the lateral band is active, though at higher sustainable speeds, the mosaic muscle (which includes the white or fast fibers as well as interdigitating red fibers) in the rainbow trout becomes increasingly important (Hudson, 1973).<sup>1</sup> The corporeal source of metabolic fuels for the muscles has been investigated with depletion studies, and for example, has been found for proteins to be the white muscle (Mommensen *et al.*, 1980), though it is not known if an interconversion of fuels occurs at any time between the sites of mobilization and combustion. Weber (1991) considered metabolite movement between red and white muscle to be unlikely in fish on the grounds that the two tissues were spatially separate and perfusion of the white muscle was low.

The white fibers are recruited in many species below 50%  $U_{crit}$

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<sup>1</sup>It seems counter intuitive to think that fish can operate both types of fibers simultaneously. The contraction velocity of red fibers is 25% that of the white which seems to predict disharmonious contractions. However, the white muscle fibers are arranged helically, in contrast to the red fibers of the lateral band which are longitudinal. This dimorphometry allow the two sets of fibers to work in tandem at intermediate speeds (Rome *et al.*, 1988).

(Johnston and Goldspink, 1973b; Johnston and Moon, 1980; Young and Cech, 1993), and in *O. mykiss* have been recorded active at 35%  $U_{crit}$  (Hudson, 1973). In the current study, both groups of swimmers presumably were using their mosaic muscle, though obviously to different degrees.

It has generally been agreed in the previous literature that carbohydrate utilization is very low. This has typically been measured by following the disappearance of radio-labelled substrates (Walton and Cowey, 1982; Van den Thillart, 1986). Proteins are thought to play the predominant role (some estimate to 90% of all fuels), and lipid holds the intermediate position. Most of the lipid work arises from depletion studies (see below) though a good comparison of fatty acid oxidation rates exists as well (Kiessling and Kiessling, 1993). The latter study was however done at the mitochondrial level which offers very little information on *in vivo* rates, especially in the presence of competing fuels.

## Protein

Relative protein usage increased over time and decreased with increasing swimming speed in the present study, but never exceeded 50% of the total fuel mix (Figs. 14, 15). At first glance of Fig. 15, one could draw the conclusion that the high speed swimmers used less protein than did the low speed swimmers. This is only true in a relative sense; on an absolute basis there was little difference between groups (Fig. 16). Inspection of Fig. 13 shows all three groups had relatively constant

nitrogenous waste excretions which were not significantly different from each other, which implies a relatively constant protein breakdown (Fig. 16). The only way that the protein contribution could increase while the absolute amount of protein metabolized stayed the same was if the total fuel requirement of the animals went down with time (as was reflected in the CO<sub>2</sub> excretion rate, Fig. 11). It seems that in fish of this size ( $17.5 \pm 1.2\text{g}$ ) or life stage, there exists a fixed protein contribution to fueling metabolism. In contrast, the smaller fish ( $4.5 \pm 0.1\text{g}$ ) from the previous chapter had an approximately 2-fold lower excretion rate, the reason for which is unclear. Wiggs *et al.*, (1989) also found that  $M_{\text{NH}_3}$  remained constant, and AQ decreased with increasing activity. They interpreted this to mean that protein use did not increase proportionately with activity and that protein might be used at a relatively constant rate while other substrates were being oxidized to meet increased energy demands of higher activity.

Even though conventional thought assigns protein a dominant role as the carbon source for energy production in both resting and swimming fish (eg. Van den Thillart, 1986), others have assigned a quantitatively less important role to this fuel (Kutty, 1978; Chapter 2, this study), in agreement with the present results. No *specific* storage sites for fuel-destined proteins are known to exist (Driedzic and Hochachka, 1978a; Cowey and Sargent, 1979), though Mommsen, French and Hochachka (1980) found that white muscle was the primary source of amino acids during migration of *O. nerka*.

Duncan and Tarr, (1958) found that depletion of white muscle N was disproportionately greater than the whole body weight loss, suggesting that the muscles themselves acted as a fuel reservoir. Bever *et al.*, (1981), working *P. clathratus*, suggested that aerobic oxidation of amino acids probably represented only a small fraction of the protein turnover. Much of the injected radio-labelled amino acids in the fish of that study went to synthesize glucose or mucopolysaccharides. In a similar study using the catfish (*M. armatus*), Sukumaran and Kutty (1977) found that after 5 hours of swimming, high speed swimmers ( $3.0 \text{ L}\cdot\text{s}^{-1}$ ) had higher AQ's than did lower speed swimmers ( $2.0$  and  $2.5 \text{ L}\cdot\text{s}^{-1}$ ), a result opposite to that in the current study. However, the two species are neither closely related nor do they have similar niches or feeding strategies. This dichotomy is an area in comparative ecophysiology that needs more work for clarification.

## Lipid

In the present study, lipid appeared to be the quantitatively most important fuel in swimming trout. Indeed, during high speed swimming, lipid accounted initially for almost 55% percent of the total fuel (Fig. 15). While swimming near  $U_{crit}$ , the red fibers were presumably making use of their own lipid stores as well as those of the viscera (Jeziarska *et al.*, 1982) to fuel their own oxidative, lipolytic energy production; the mosaic fibers may have been able to subsist on their extracellular stores alone, though importation from the viscera or *de novo* synthesis may also have played a

role. Instead of showing a temporally decreasing commitment to lipid, the low speed swimmers and nonswimmers showed relatively constant rates of lipolysis, suggesting only a single pool was being turned over.

Robinson and Mead (1973) have shown that the red muscle has a higher lipid concentration than the white, though the total stores in the white muscle were greater than those in the red because of the much greater mass of white muscle in the trout. White muscle gave up its stores more quickly, presumably via the circulatory system to the red muscle (lateral band), which is more capable of oxidizing lipid (Moyes *et al.*, 1989). Intracellular lipid was not detectable in the white muscle (Johnston *et al.*, 1975), though  $\beta$ -hydroxybutyryl-CoA dehydrogenase, an enzyme involved in  $\beta$ -oxidation has been found to be active (Mommensen *et al.*, 1990). Recent work in this lab also shows evidence of white muscle metabolizing lipid. After exhaustive exercise, a large increase in short chain acyl-carnitines was detected in the white muscle (Y. Wang, G.J.F. Heigenhauser, and C.M. Wood, unpublished data). This of course does not imply a similar mechanism occurs during aerobic exercise, but it does mean the potential exists.

Due to its vast volume, Robinson and Mead (1973) concluded that the white muscle was the major lipid storage organ (Robinson and Mead, 1973). However, Greer Walker and Emerson (1978) found "much [fat] deposited in the mesenteries and around the alimentary canal." Jeziarska *et al.*, (1982) found that during starvation, of the four lipid pools investigated

(epaxial and hypaxial muscle, viscera and liver) visceral lipid was depleted by the greatest amount. This pool was not measured by Robinson and Mead (1973) as they assumed that the only two pools of significance in fish were the muscle and the liver, with the muscle being the only noteworthy pool in trout. In light of the fact that the work of Jezierska *et al.*, (1982) was more exhaustive, it is more likely to represent the actual condition in fish.

### Carbohydrate

The outstanding feature from Figs. 15 and 16 was the unexpectedly high contribution (up to 38%) of carbohydrate as a fuel in all groups. This was also found in the starvation study of Chapter 2, where carbohydrates represented 20% of the fuel mixture over the first four days, and 40% over the balance of the experiment (Fig. 7, Chapter 2). Trends in carbohydrate use, like those for lipid were more similar between the low speed and nonswimming fish than either of those groups were with the high speed fish. This contribution of carbohydrate was much more than literature estimates or assumptions would have predicted (Black *et al.*, 1962; Van den Thillart, 1986).

Conflicting evidence exists in the literature with respect to carbohydrate use. The general findings have been that fish absorb and metabolize dietary carbohydrates poorly (Brett and Groves, 1979). In rainbow trout, neither exogenous (as cannula-injected glucose) nor endogenous (as native glycogen) sources of carbohydrate were thought to

play a role in fueling aerobic metabolism of fish (Black *et al.*, 1962; Van den Thillart, 1986). In the kelp bass, the disappearance of radio-labelled amino acids administered via cannula was rapid (15-30 min) and the fraction that was incorporated into blood-borne glucose followed soon afterwards (Bever *et al.*, 1981). The fate of the new glucose was not determined, though it does seem contradictory to the study of Van den Thillart (1986) mentioned above. In essence, why convert amino acids to glucose, if the glucose does not get utilized? However, a common finding of many depletion studies (reviewed by Driedzic and Hochachka, 1978a) was that carbohydrate became increasingly more important as the demand on muscle increased. Indeed, Chapter 2 showed that carbohydrate was often the major fuel in the latter part of the 15 days of starvation.

Depletion studies on the coalfish (*Gadus virens* L.) clearly showed a reduction of glycogen in the red muscle and an almost parallel rise of lactate in the white over the range of sustainable swimming speeds. Depletion of glycogen in the white muscle was only detectable at sustainable speeds higher than 75%  $U_{crit}$  (Johnston and Goldspink, 1973b). The interpretation offered was that at all but the lowest swimming speeds, white muscle was active (since lactate increased) and the glycogen was being replaced from either the red muscle or liver. Again, care must be taken in the interpretation of compositional (depletion) studies. A change in substrate levels simply means there was a difference between the start of the

experiment and the end. The direct measure of substrate turnover and the interconversion between fuels has been largely ignored in compositional fuel use studies.

Lactate can be oxidized and used as a metabolic fuel (Bilinski and Jonas, 1972; Weber, 1991). Weber (1991) estimated that up to 25% of the routine metabolic rate of a fish could be fueled by lactate; the value dropped to 15% during sustainable exercise. More recently, Milligan and Girard (1993) concluded that several tissues of rainbow trout (eg. red muscle, cardiac muscle) could oxidize lactate at high rates ( $6 \mu\text{mol}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$ ), and that white muscle, even though its oxidation rate was quite low, could account for a high total amount of lactate oxidization due to its sheer bulk. However, Wokoma and Johnston (1981) found a 3-fold increase in red muscle lactate of trout after 20 minutes swimming at  $\approx 83\% U_{\text{crit}}$ , yet only a 2-fold increase in white muscle lactate. They suggested that red muscle was catabolizing white muscle derived lactate, in accord with a similar suggestion of Hulbert and Moon (1977) in the eel (*Anguilla rostrata*). More recently, Forsman and Virtanen (1989) have found similar results at  $\approx 50\% U_{\text{crit}}$  in *Salmo salar*.

### Integration of Fuel Use

During migration, adult salmonids are known to travel at their optimal cruising speeds of approximately  $1.0 \text{ L}\cdot\text{s}^{-1}$  (Quinn, 1987). Weihs (1973) showed that speeds greater than the optimum would be energetically more favourable than speeds differing by an equal amount less than the



optimum. If juveniles (from the current study) and the migrating adults (from Quinn, 1987) burn similar compositions of substrates, the typical condition in the wild was reflected more closely in our results by the low speed swimmers than by either other group. In other words, continuously swimming wild fish would tend to fuel their aerobic metabolism with a 20-30% protein commitment, a 40% lipid commitment, with the balance as carbohydrates.

If fat, by weight, is the energetically most efficient fuel, then why do fish burn any other substrate? Some organs such as the brain and gonads use glucose preferentially (Walton and Cowey, 1982). Even though protein gives rise to less than half the energy by weight than does lipid (Kleiber, 1992) the protein pool makes up 60% of the dry weight of the fish (Table 5), making the total store of potential energy much greater. Lipolysis is essentially constant in the nonswimmers and low speed swimmers (Fig. 15). In the high speed group, lipolysis over the first day is substantially greater than over the last two days, suggesting either a decreased rate in the same pool(s) of fat over the last two days, or a secondary pool, harnessed on the first day, was no longer tapped. This seems to suggest that an alternate pool of lipid may be tapped or lipogenesis increases in more stressful (higher speed) events. An increase in lipogenesis could occur by tapping a second substrate or by increasing activity on the original substrate.

White fibers are thought to use primarily glycogen as a fuel

(Johnston and Goldspink, 1973b; Driedzic and Hochachka, 1978a,b; Mommsen *et al.*, 1980). As swimming speed increases, progressively more white fibers are recruited (Hudson, 1973; Johnston and Goldspink, 1973b; Johnston and Moon, 1980); it then follows that more carbohydrate would be burned. Even though the absolute amount of C from carbohydrate was greater in the high speed swimmers, (and in contrast to the other two groups, remained high; Fig. 16) the initial contribution to the C pool by lipid was responsible for over 50% of the total fuel (Fig. 15). In addition, it would make sense not to increase the contribution of proteins, since they are directly responsible for the process of contraction (and therefore swimming) itself.

In a similar, though compositionally oriented and longer term study (on fed brown trout, *Salmo trutta*), it was found that at  $U_{crit}$ , muscle protein depletion was greater than that at lower speeds (Davison and Goldspink, 1977). At two-thirds  $U_{crit}$ , no detectable change in protein in either red or white muscle was noted after 28 days of swimming. It would be interesting to investigate the instantaneous fuel use in a companion study to the one just described.

## CHAPTER 4

### Introduction

Rainbow trout (*Oncorhynchus mykiss* W.) in the wild can almost be considered a different species from those which are farmed and used in the laboratory. They differ markedly in behaviour (Symons, 1969) and in some aspects of their physiology as well (Caldwell Woodward and Strange, 1987; Kindschi, Smith, and Koby, 1991a,b). For instance, from the moment the fry swim up from the serenity of the gravel bed, they are exposed to the stream's current (Scott and Crossman, 1990). In a sense then, they are in physical training from their first tentative tail beats. This can differ markedly from the unnatural settings of farm and laboratory holding tanks in which the fish are exposed to little or no current.

In order to better mimic the condition in the wild, several researchers have subjected current dwelling species to training periods of a variety of durations (from a few days to more than a year), and intensities (less than  $0.5 \text{ L}\cdot\text{s}^{-1}$  to  $3.0 \text{ L}\cdot\text{s}^{-1}$ ; reviewed in Davison, 1989). Many of the studies have focussed on changes in muscle parameters such as fiber size (Davison and Goldspink, 1977; Greer Walker and Emerson, 1978; Johnston and Moon, 1980; Davie *et al.*, 1986; Gamperl and Stevens, 1991), recruitment (Johnston and Moon, 1980), as well as distribution and supply

of capillaries (Davie *et al.*, 1986), though growth rates (Greer Walker and Emerson, 1978; Houlihan and Laurent, 1987), body condition factor (Davie *et al.*, 1986; Gamperl and Stevens, 1991), food conversion efficiency (Davison and Goldspink, 1977), and blood parameters (Davie *et al.*, 1986) have also been investigated. However, there have been no previous studies on how training might alter fuel utilization during swimming, which led to the present cross-sectional study.

Specifically, the previously reported metabolism and fuel use of a group of rainbow trout (the low speed swimmers of Chapter 3) swimming at  $2.1 \text{ L}\cdot\text{s}^{-1}$  were compared with those of an identically tested group that had been trained for 2 weeks at  $1.0 \text{ L}\cdot\text{s}^{-1}$ . The previous results were notable inasmuch as they tended to contradict the well established view in the literature that proteins dominate in the mix of metabolic fuels (Kutty, 1972; Van den Thillart, 1986; Davison, 1989), that lipids are important as well (Atherton and Aiken, 1970; Jezierska *et al.*, 1982; Van Waarde, 1983), and that carbohydrate is of minor importance (Black *et al.*, 1962; Van den Thillart, 1986). Therefore the goal of the present study was to test whether the surprisingly low reliance on protein (20-36%) and high reliance on carbohydrate (up to 38%) would be altered by prior training. The previous results of Chapter 3 demonstrated that relative protein usage did increase with 3 days of low speed swimming in previously untrained fish, whereas relative carbohydrate usage decreased. Such trends might be the start of a

training effect which should be well developed after 2 weeks of continuous low speed swimming. A training speed of  $1.0 \text{ L}\cdot\text{s}^{-1}$  was chosen for this test because Greer Walker and Emerson (1978) found this speed (in comparison to higher sustainable speeds and unswum controls) to be optimal for growth and muscle hypertrophy. In addition, Weihs (1973) predicted a cruising speed of  $1.0 \text{ L}\cdot\text{s}^{-1}$  to be the energetically most efficient swimming speed for salmonids while Quinn (1987) showed that migrating sockeye salmon (*O. nerka*) do travel at that speed.

Most work on fuel use has been the result of depletion (compositional) studies. This study looked at the instantaneous fuel use differences between trained and untrained fish in order to add another aspect to the wide ranging literature on training effects in fish.

## Methods and Materials

The husbandry, test regime (save for the training, detailed below), respirometry ( $O_2$  and  $CO_2$ ), water nitrogen assays (total ammonia [ $T_{amm}$ ], urea), proximate body analyses (protein, carbohydrates, lipids, water and ash), calculations for fuel use, and statistics were performed using the same methods described in the previous Chapters. The average mass of the trained fish ( $16.5 \pm 2.2g$ ; mean  $\pm$  s.e.m.;  $n = 5$ ) was not different from that of the untrained fish ( $19.8 \pm 0.8g$ ; t-test,  $n = 10$ ;  $p > 0.05$ ). All fish were weighed just prior to placing them into individual Blažka style respirometers.

The trained fish went through two weeks of swimming at  $1.0 L \cdot s^{-1}$  (body lengths per second) in a Beamish-style swim tunnel (Farmer and Beamish, 1969) before undergoing the test at  $2.1 L \cdot s^{-1}$ . The water temperature was held constant at  $15 \pm 1^\circ C$  which was the same temperature at which the untrained fish were held. The swim tunnel had a total capacity of approximately 200 l; fresh dechlorinated tapwater entered, while mixed water left continuously at  $\approx 4 l \cdot min^{-1}$ .

Both groups of fish were fed 1% of their body weight per day; no correction was made for any weight gain over the training period. It was found to be more efficient if the swim tunnel motor was turned off during

feeding. In this way, more of the food was accessible to the fish; feeding took no longer than 5 minutes each day.

## Results

### Respirometry and Instantaneous Fuel Use

In both groups, the initial oxygen consumption of fish swimming at  $2.1 \text{ L}\cdot\text{s}^{-1}$  was  $10 \mu\text{mol}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$ . Thereafter,  $M_{\text{O}_2}$  decreased and stabilized ( $p > 0.05$ ) in the trained group, but continued to decline ( $p < 0.0001$ ) in the untrained group (Fig. 18).  $M_{\text{CO}_2}$  was more variable, though it too started high. Only the untrained fish showed a significant (decreasing) slope ( $p < 0.001$ ). Essentially though, the gas exchanges were similar in both groups.

Respiratory quotient (RQ;  $M_{\text{O}_2}:M_{\text{CO}_2}$ ) was variable in both groups, though values for both tended to remain between 0.8 and 1.0 (Fig. 19). Since this study focussed on aerobic metabolism only, the data for individual fish which generated  $\text{RQ} > 1.0$  at any point were not used in calculating fuel use (since  $\text{RQ} > 1.0$  represents anaerobic metabolism); the remaining RQ values, replotted as regressions, showed almost identical and stable ( $p > 0.05$ ) curves (Fig. 19).

The nitrogenous waste excretion, except for the terminal value of the trained group, was stable ( $p > 0.05$ ) over the three day test period (Fig. 20). However, the untrained group had an average excretion rate of  $0.54 \mu\text{mol N}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$  (made up of 18% urea, 82%  $T_{\text{amm}}$ ) while the trained fish showed an excretion rate 39% lower at  $0.33 \mu\text{mol N}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$  (23% urea, 77%



Figure 18. Oxygen consumption and carbon dioxide excretion of untrained (circles,  $n = 10$ ) and trained (squares,  $n = 5$ ) juvenile rainbow trout swimming at  $2.1 \text{ L}\cdot\text{s}^{-1}$  over the three day test. Means  $\pm$  sem.

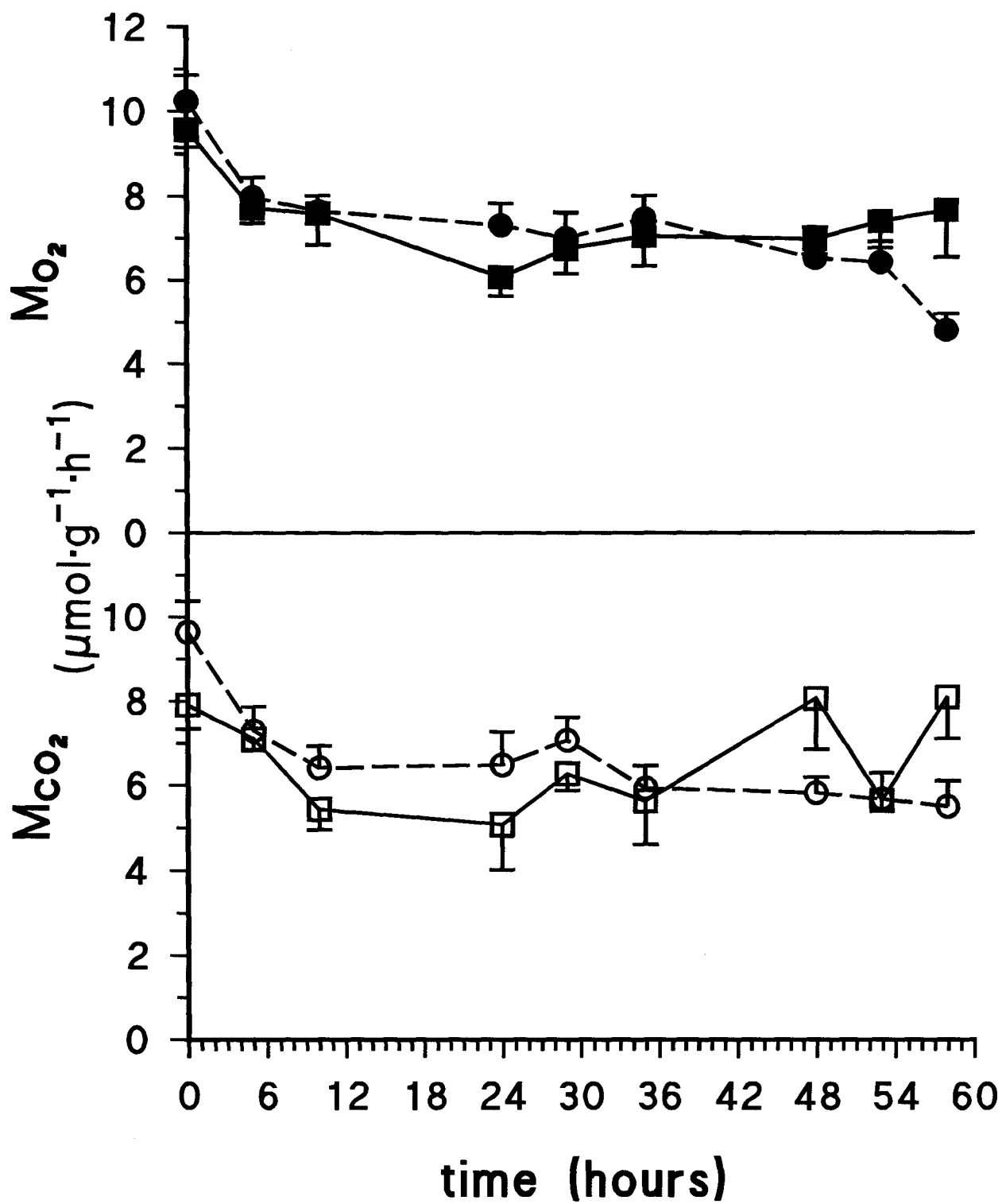


Figure 19. The complete respiratory quotient (symbols and solid regression line) and the aerobic respiratory quotient (dashed regression line) for (A) untrained ( $n = 10$ ) and (B) trained ( $n = 5$ ) juvenile rainbow trout swimming at  $2.1 \text{ L}\cdot\text{s}^{-1}$  over the three day test. Means  $\pm$  sem.

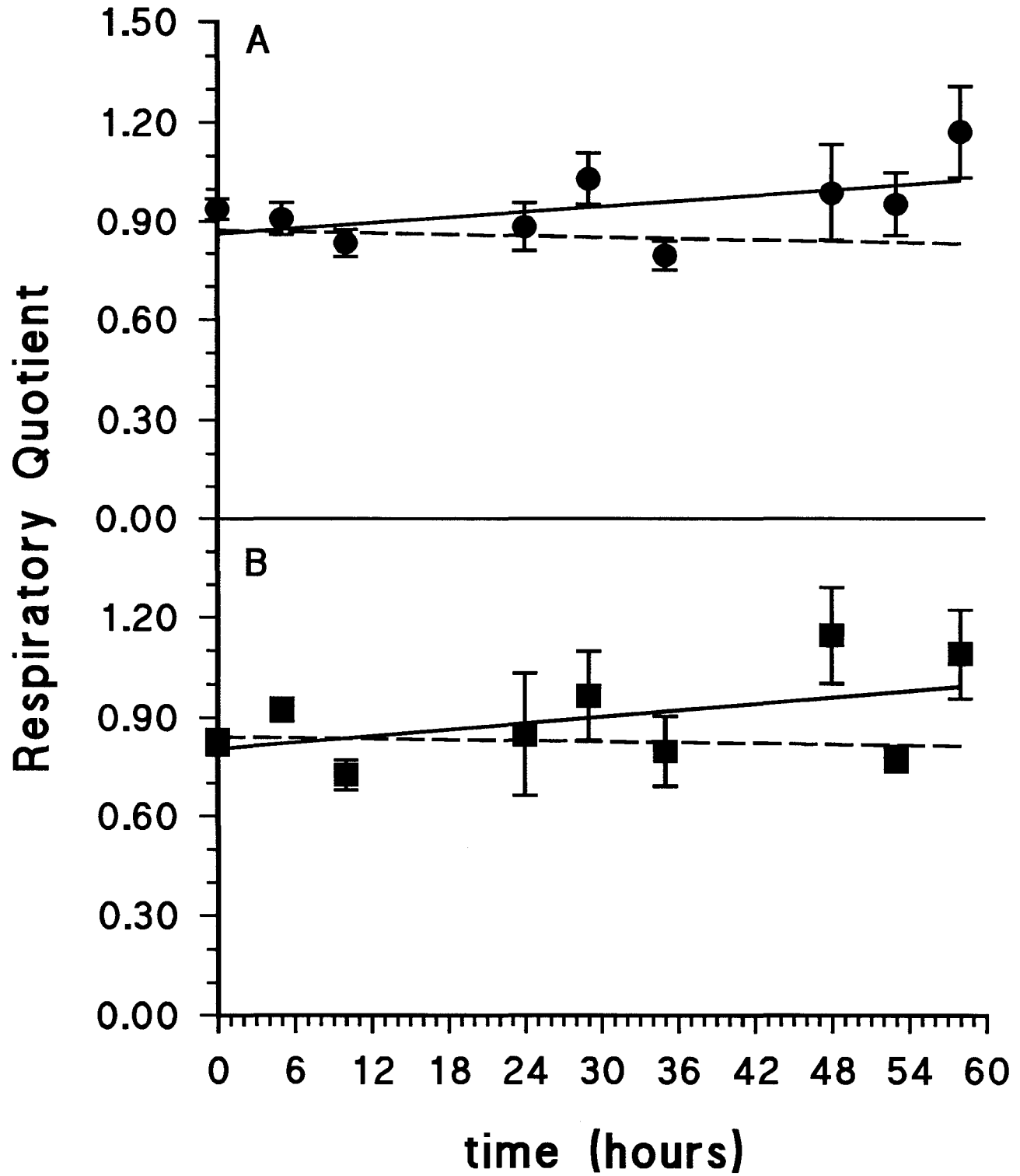
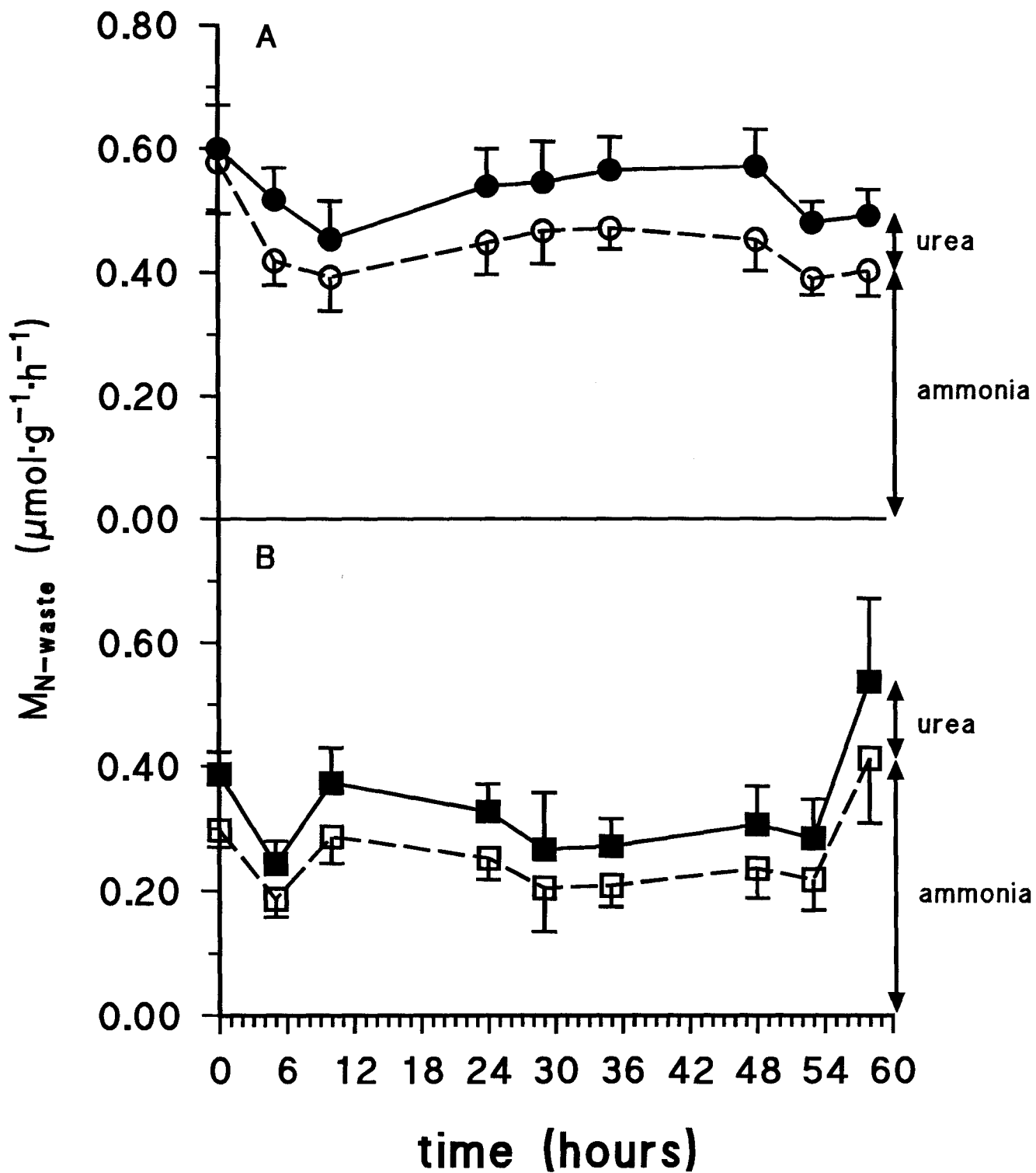


Figure 20. Total nitrogenous waste (t-Nitrogen as the sum of ammonia-N and urea-N; solid symbols) and ammonia-N (open symbols) excretion for (A) untrained (n = 10) and (B) trained (n = 5) juvenile rainbow trout swimming at  $2.1 \text{ L}\cdot\text{s}^{-1}$  over the three day test.



$T_{amm}$ ;  $p < 0.01$  for the lower rate,  $p < 0.0001$  for the difference in composition). The nitrogen quotient (NQ) remained effectively stable ( $p > 0.05$ ) at 0.04 in the trained group, but increased linearly ( $p < 0.001$ ;  $r = 0.418$ ) from 0.06 to over 0.11 in the untrained group (Fig. 21). Given that protein catabolism is proportional to the nitrogenous waste excretion, the right axis of Fig. 21 allows interpretation of this variable. The relatively stable nitrogen quotient of the trained group arose from a protein use which represented 17% of the total fuel mixture. The protein use in the untrained group was greater than the trained fish ( $p < 0.02$ ) and also rose steadily ( $p < 0.001$ ) from 22% to greater than 35% at the end of the final day.

Before the balance of the fuel use can be calculated, the theoretical maximum RQ for protein use ( $RQ_{protein}$ ) must be determined since this value is dependent on the relative proportions of  $T_{amm}$  and urea excreted (Chapter 2). For the untrained group, the  $RQ_{protein}$  was previously calculated to be 0.95 (Chapter 3), while for the trained fish, the value was calculated at 0.94. The relative contributions of the different fuels between and among groups are compared in Fig. 22. The patterns of lipid and carbohydrate were similar in the trained and untrained fish. Lipid was always responsible for greater than half the fuel used in the trained group and showed a final contribution of 60%. The contribution of lipid to the total fuel mixture in the untrained fish was consistently 10% lower than the trained group. In contrast, carbohydrate played an initially stronger role in the untrained fish,

Figure 21. Nitrogen quotients (left axis) and protein use (right axis) for untrained (circles, n = 10) and trained (squares, n = 5) juvenile rainbow trout swimming at  $2.1 \text{ L}\cdot\text{s}^{-1}$  over the three day test. Means  $\pm$  sem.



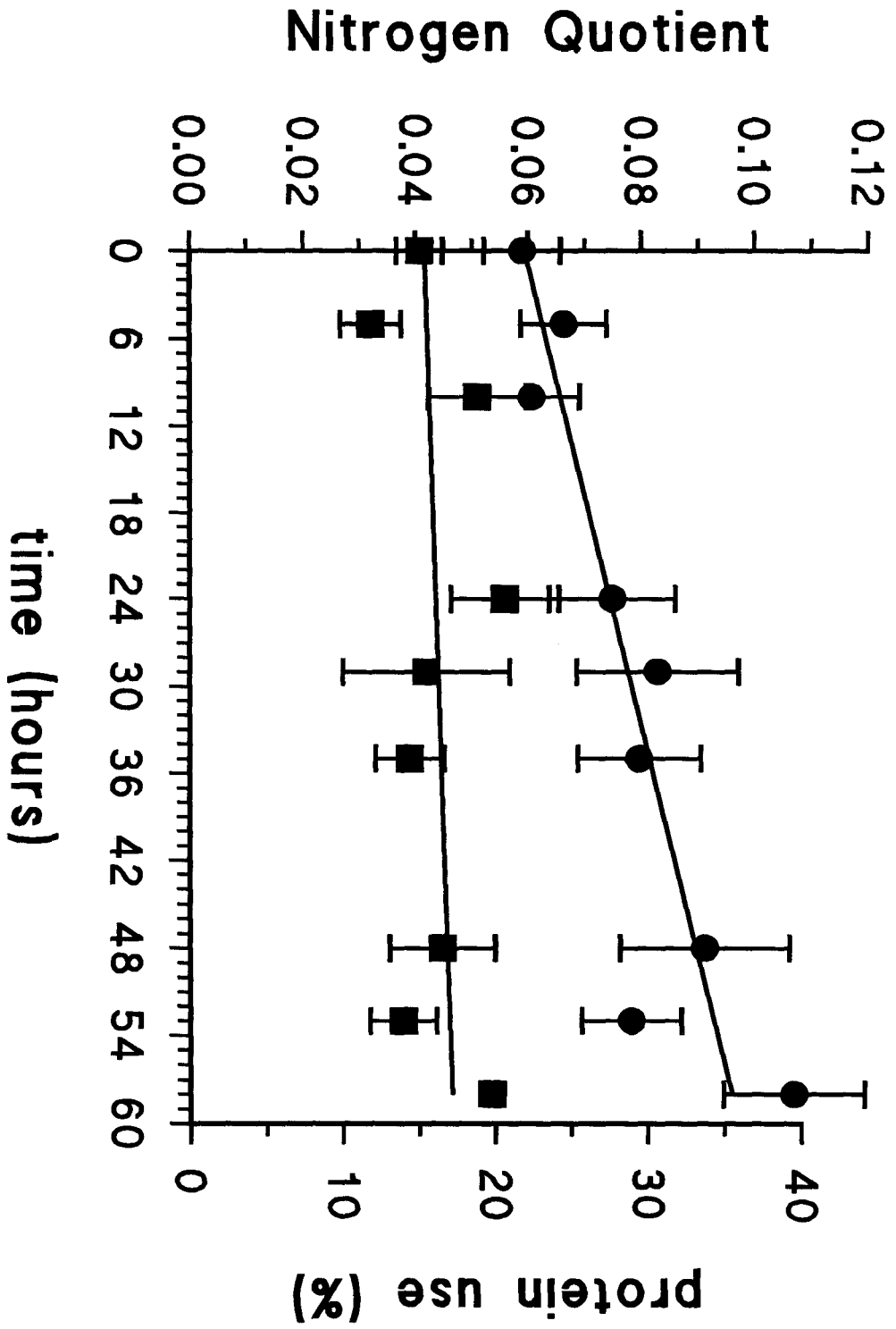
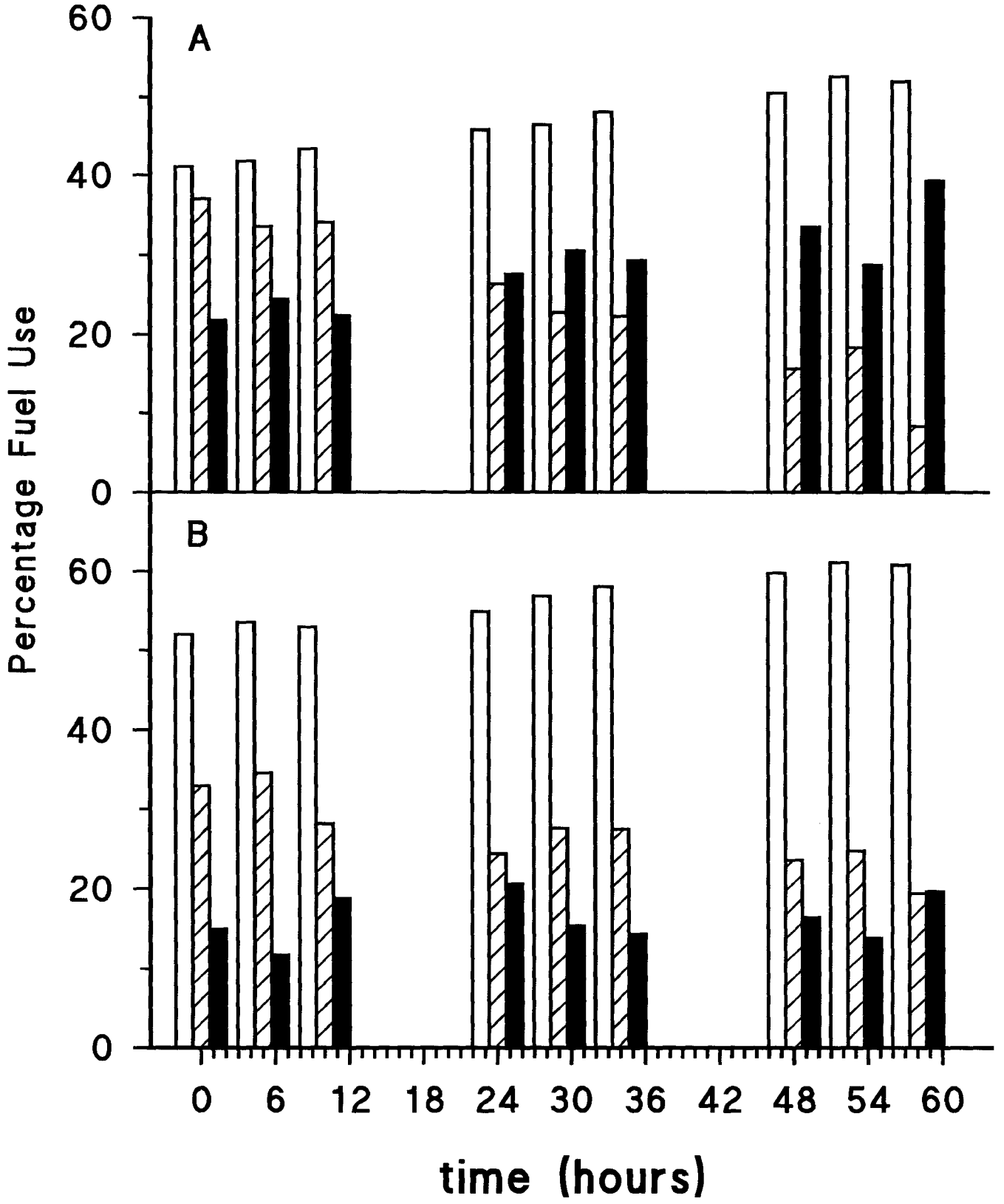


Figure 22. Percentage use of lipid (open bars), carbohydrate (hatched bars) and protein (solid bars) in (A) untrained and (B) trained juvenile rainbow trout swimming at  $2.1 \text{ L}\cdot\text{s}^{-1}$  over the three day test.



representing an average of 37% on the first day but the contribution decreased to 14% on the final day; in the trained group, carbohydrate usage also decreased over time, but with a narrower range of 34 to 21%.

In both groups, the carbon use rate from protein remained relatively constant, though the average use in the untrained fish was  $22 \mu\text{g C}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$ , whereas in the trained fish the value was  $13 \mu\text{g C}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$  (Fig. 23). Carbohydrate use decreased continuously in the untrained group from 43 to  $5 \mu\text{g C}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$ , whereas after an initial decline in the trained fish, carbohydrate use stabilized at  $20 \mu\text{g C}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$ . Finally, lipid was predominant ( $48 \mu\text{g C}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$ ) at the onset of the test period in the untrained group, but stabilized at  $38 \mu\text{g C}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$  for the duration of the experiment. In contrast, lipid was always at least  $10 \mu\text{g C}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$  greater in the trained fish, but the trend was not linear, rather carbon use from lipid decreased over the first day, but showed a general increase over the following two days.

### Body Composition

There were no significant differences in terminal concentrations of lipid, inorganics or water between the trained and untrained fish ( $p > 0.05$ , independent t-test; Table 6). Both protein and total carbohydrate were significantly higher ( $p < 0.025$  and  $0.05$ , respectively) in the trained fish than the controls.

Wet weight did drop in both groups ( $p < 0.05$ , paired t-test) though data based on the controls from the previous exercise indicated that over half of the weight loss occurred within the first two days.

**Figure 23.** The contribution of carbon from lipid (open bars), carbohydrate (hatched bars) and protein (solid bars) in (A) untrained and (B) trained juvenile rainbow trout swimming at  $2.1 \text{ L}\cdot\text{s}^{-1}$  over the three day test.

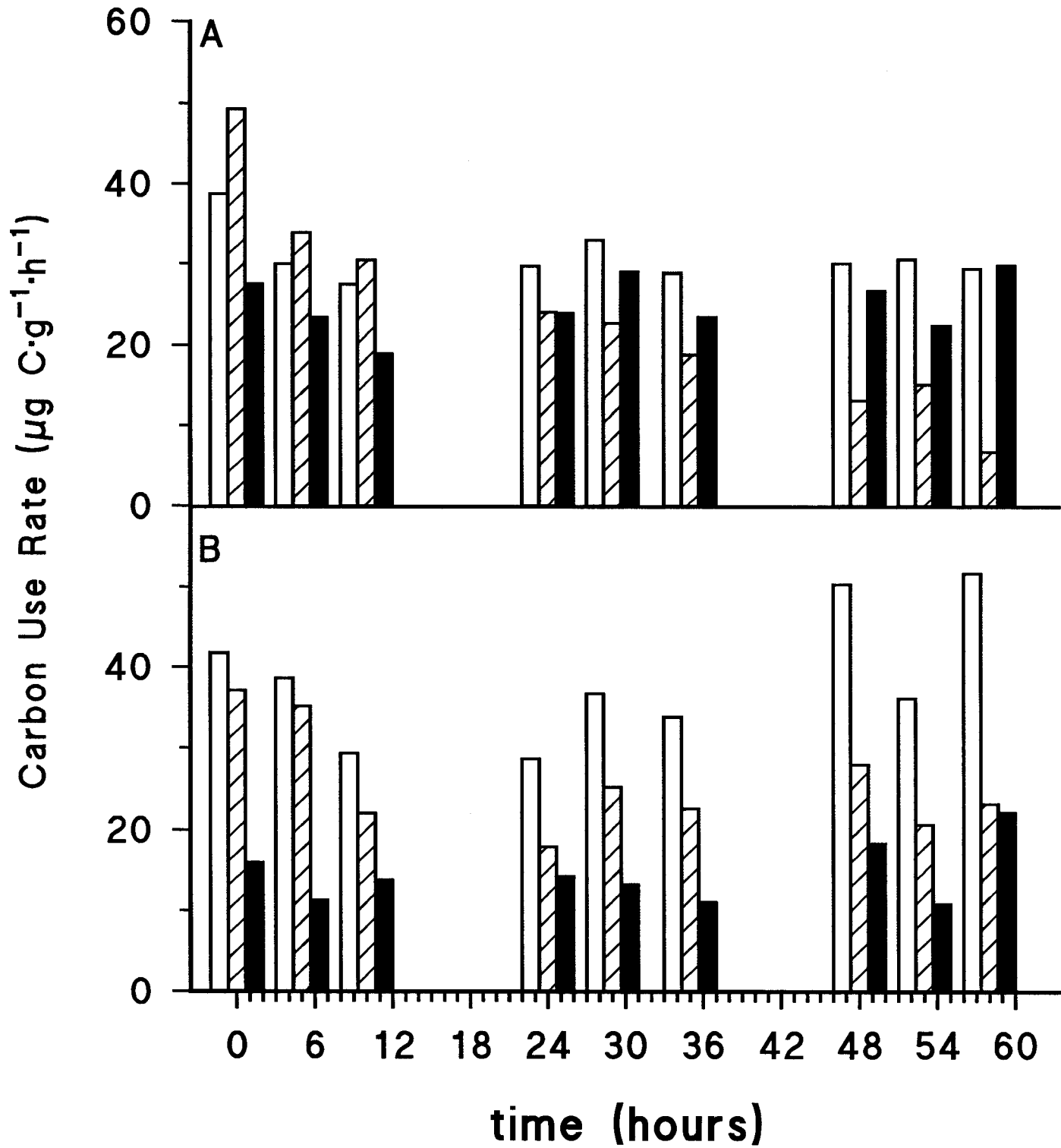


Table 6. Body compositions ( $\text{mg}\cdot 100\text{mg}^{-1}$ , wet weight) of untrained and trained juvenile rainbow trout, post-exercise. Total carbohydrate includes glucose, glycogen and lactate. \*  $p < 0.05$ , \*\*  $p < 0.025$ , indicate significant difference from untrained fish. mean  $\pm$  sem.

	lipid	total carbohydrate	protein	inorganic	water
untrained fish	5.1 $\pm$ 0.7	0.20 $\pm$ 0.02	13.2 $\pm$ 0.7	2.68 $\pm$ 0.17	77.8 $\pm$ 1.2
trained fish	5.0 $\pm$ 0.1	0.25 $\pm$ 0.02*	15.3 $\pm$ 0.4**	2.42 $\pm$ 0.11	77.5 $\pm$ 0.5

Table 6. Body compositions ( $\text{mg}\cdot 100\text{mg}^{-1}$ , wet weight) of untrained and trained juvenile rainbow trout, post-exercise. Total carbohydrate includes glucose, glycogen and lactate. \*  $p < 0.05$ , \*\*  $p < 0.025$ , indicate significant difference from untrained fish. mean  $\pm$  sem.

	lipid	total carbohydrate	protein	inorganic	water
untrained fish	5.1 $\pm$ 0.7	0.20 $\pm$ 0.02	13.2 $\pm$ 0.7	2.68 $\pm$ 0.17	77.8 $\pm$ 1.2
trained fish	5.0 $\pm$ 0.1	0.25 $\pm$ 0.02*	15.3 $\pm$ 0.4**	2.42 $\pm$ 0.11	77.5 $\pm$ 0.5



## Discussion

In this study, some of the measured parameters were strongly affected by training, whereas others were not. There was in fact, a dichotomy between the respiratory and N-waste excretion parameters. In the former, even though the trained fish did not show the decline in respiratory gas exchange that was present in the untrained group, there was no difference in absolute amount of gas exchange between groups (Fig. 18). In the latter, the decreased N-waste excretion and resulting fuel use differences were obvious (Figs. 20, 21). Since the total amount of carbon used by the trained group was not lower than that of the untrained fish, I suggest that no greater efficiency of the conversion of fuel to energy existed as a result of the training regime imposed.

The changes that did occur, were the result of lower protein catabolism (Fig. 21) which was reflected in the lower N excretion (Fig. 20). This is exactly opposite the speculation of the Introduction that the gradual increase in relative protein use by the untrained fish over the 3 day test period (Figs. 14, 21) might be the start of a training effect. Rather, it is clear that the trained fish relied to a lesser extent on protein as a fuel. Since the total amount of carbon burned was similar in the two groups (Fig. 18, CO<sub>2</sub> excretion), and the total carbon originating from protein was lower in the

trained fish (Fig. 23), then the prediction would be a simple increase in contribution of either or both of the other fuels to make up the balance. In fact, the contribution of lipid, the most efficient fuel on a per weight basis (Kleiber, 1992) was consistently higher in the trained fish. This at least, did agree with the increasing reliance on lipid exhibited by the untrained fish during their 3 days of low speed swimming (Figs. 15, 22). The contribution of carbohydrate in the trained fish was at, or below the contribution in the untrained group seen over the first day of the test period. By the third day, the untrained fish continued to decrease their reliance on carbohydrates such that the contribution fell below that of the trained fish. This observation is supported by the fact that the terminal body analyses showed higher stores of carbohydrate present in the trained fish. The reason for the drop in the contribution of carbohydrate relative to the trained counterparts is not clear.

Even though the white muscle is considered by many to function primarily in burst swimming (Weihs, 1973), it has been shown electromyographically that the mosaic fibers are indeed active at speeds as low as 35%  $U_{crit}$  in rainbow trout (Hudson, 1973). It therefore seems probable that the fish in the current study were utilizing white muscle for their swimming at 55%  $U_{crit}$ , though no study has investigated differential recruitment as a result of training. Carbohydrate is typically thought of as the fuel of the white muscle (Johnston and Goldspink, 1973; Driedzic and Hochachka, 1978a,b; Mommsen *et al.*, 1980), though Mommsen *et al.*,

(1980) and Johnston and Moon (1980) have both found lipolytic enzymes involved in  $\beta$ -oxidation to be active in the white muscle of sockeye salmon (*O. nerka*) and coalfish (*Pollachius virens*) respectively. The fish in both of those studies were wild caught and thus had been subjected to real-life training regimes. Whether the lipolytic enzymes would be (as) active in hatchery reared (untrained) fish is unknown, though undisputedly, trained fish used more lipid than their untrained counterparts (Fig. 23). The mechanism by which lipids are mobilized from muscle and other depots is ill-defined (Henderson and Tocher, 1987). A triacylglycerol lipase has been found in rainbow trout red muscle, though its mode of activation is unclear; it is known that epinephrine is not involved (Bilinski and Lau, 1969).

Protein content was significantly higher in the trained fish (Table 6), which reflected the well documented (Greer Walker and Emerson, 1978; Johnston and Moon, 1980; Davie *et al.*, 1986; Gamperl and Stevens, 1991) though not universally found (Houlihan and Laurent, 1987) hypertrophy of both muscle types during training. Depletion was also lower (Fig. 21) which would work in conjunction with the increased muscle mass to give rise to higher protein contents in trained fish. Interestingly, it was found in Chapter 3 that the high speed swimmers, the most exercised group, used the lowest percentage protein as a fuel. The trained group in this study had gone directly from a two week training regime to the swimming test and could be also described as being more exercised than their untrained counterparts.

Whatever mechanism (ie. hormonal) was responsible for mobilizing the lipid in the high speed swimmers could also have been in place in the endurance (trained) swimmers. This transition of fuel use certainly warrants more study.

Whole body carbohydrate levels were 25% higher in the trained fish in this study (Table 6). A 2-fold increase in white muscle, and a 5-fold increase in red muscle glycogen levels (dry weight) were reported by Johnston and Moon (1980) after 3 weeks of training at  $2.1 \text{ L}\cdot\text{s}^{-1}$ . Even though lipid use was higher in the trained fish (Figs. 22, 23), there was no difference between the two groups with respect to the final composition of lipid. Either the 3 day test period was too short to detect differences in depletion, or there was more lipogenesis (or higher lipid stores at the end of the training period and prior to the test) in the trained fish. Certainly, more work in this field is needed.

Houlihan *et al.*, (1986) and Houlihan and Laurent (1987) have used a  $^3\text{H}$ -phenylalanine swamping technique to measure protein turnover in several tissues (gill, heart, muscle) that has not been repeated for either lipids or carbohydrates. The conditions of those studies, were not directly comparable to the current study, though an interesting feature of the trained fish (in the latter study) was that the protein degradation and synthesis rates were only higher in trained fish that were measured while swimming; synthesis and degradation rates of nonswimming trained fish were no

different than those of untrained controls. Total protein synthesised was greater in their trained fish (as was the growth rate), especially in the muscle. This would certainly explain the elevated protein content in the trained fish in the current study (Table 6). Beyond calculating the degradation rates, no attempt was made to determine the destination of the amino acids. Certainly many were simply recycled back into proteins (the degradation rates were only slightly lower than the synthesis rates), but it would be interesting to follow the differential recruitment of amino acids into aerobic metabolism. An analogous expansion of Houlihan's approach to the current study would have been to take a group of trained fish and perform nonswimming respirometry (see Chapters 1 and 2) on them.

In summary then, the main effect of training in this study was a decrease in protein catabolism during swimming (reflected in the N excretion), with a resulting increase in lipid use, though the total amount of fuels used remained constant.

### Concluding Remarks

The results of this study indicate a rethinking of the physiology of fuel use in fish is required. The two distinct types of fuel use, *instantaneous* and *compositional*, have been differentiated and defined. Where possible, previous studies of instantaneous fuel use in fish have been compared to the current study, though out of necessity, the vast majority of fuel studies cited have used the compositional approach; these have been liberally used with the appropriate admonition.

Protein use, the factor most widely studied, was found to rarely approach even the lower end of literature values when assessed using the instantaneous approach, ie. by respirometry. Lipid was apparently the fuel of choice in most groups tested, at least over part of the temporal regime of individual experiments. The relative contribution of carbohydrate was greatest only during the latter parts of the starvation and high speed swimmer groups. Training, both before an experiment (Chapter 4) and for the low speed swimmers during an experiment (Chapter 3) gave rise to a relatively higher contribution of lipid with time.

The results of several conceptually simple experiments have formed the foundation for more research in this field, either using similar techniques or alternative ones. Radiotracer studies, which are widely used

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The results of several conceptually simple experiments have formed the foundation for more research in this field, either using similar techniques or alternative ones. Radiotracer studies, which are widely used

in all areas of biological research, have been used to some degree in fuel use studies. However, to mimic the goals of this study, radiotracers would first have to be incorporated into the tissues of the animal, presumably via the diet. Simply injecting the animal with a labelled fuel or a mixture of fuels via the bloodstream would not be sufficient. Times for mobilization and transport (both within the circulatory system and across membranes) and rates of ATP synthesis (as reviewed by Weber, 1988) would not be the same unless the metabolites were mobilized from their storage sites. Similar studies using tissue culture or mitochondrial extracts would be of preliminary value, though there is no substitution for *in vivo* work, if one wants to find out what is going on *in vivo*. In attempting either of the latter investigations, fuel mixtures, perhaps starting with the ratios determined here, should be supplied. It is of some value to know specific oxidation rates of certain amino or fatty acids (Moyes *et al.*, 1990), though substrate utilization is not analogous to harvesting a monoculture, rather it is more like shopping at the open market, ie. a wide variety of individual molecules are metabolized. This is contrary to conventional thought on the topic, but what's science without controversy?



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