PROTEIN METABOLISM IN FISH: COMPARATIVE TURNOVER RATES

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Introduction

When we look at an animal growing we are seeing the balance between the animal's rates of protein synthesis and protein degradation. Therefore, in order to understand what is controlling growth rates we need measurements of the rates of protein synthesis and growth; if we wish to go further and relate protein growth and protein synthesis rates to, for example, the amount of amino acid ingested, we need to know in the same individuals, rates of food consumption and digestive efficiency. As measurements of protein growth and protein synthesis are normally carried out on individuals, increasingly protein metabolism studies are being preceeded by measurements of protein and energy absorption of individual fish which may have been held in groups (e.g. Carter *et al.* 1993; McCarthy *et al.* 1994).

Food consumption

Rates of food consumption of the individual fish or of the group as a whole and the dynamics of the distribution of food between individuals are turning out to be key measurements in understanding food conversion efficiency. If we take a group of Atlantic salmon being fed a reduced ration as an example, at one extreme, the dominant animals, although gaining the highest proportion of the available food may also be expending energy at a high rate in order to maintain their dominant position. The most subordinate animals will be eating less and may be forced by hunger to occasionally compete for food with more dominant animals and may suffer fin damage as a result. However, the reduced activity levels of the subordinate fish may promote increased conversion eficiency of the food they acquire. We are only just beginning to be able to understand social hierarchies in groups of fish and the likely trade offs between dominance, food acquisiton, energy expenditure, growth rates and possible stress and disease resistance.

Protein synthesis methodology

The majority of studies that have measured rates of protein synthesis in fish have used a single flooding dose injection (Garlick *et al.* 1980) of a radiolabelled amino acid, commonly ^{3}H-phenylalanine, and have measured the incorporation of radiolabel into body protein over a known incubation time. The methodology, necessary validation criteria and a thorough review of the available data is provided by Houlihan *et al.* (1995a, b & c). Recently our attention has turned

to measurements of protein synthesis using stable isotopes, principally ^{15}N, which has been used extensively in the study of protein synthesis in mammals (reviewed in Houlihan *et al.* 1995a & b). Our approach has been to utilise ^{15}N-phenylalanine to make single terminal measurements of protein synthesis using the flooding dose technique (Owen *et al.* unpublished data, 1995) and to use make non-invasive measurements of protein synthesis using ^{15}N protein.

We have adapted the stochastic end-point model to measure rates of protein synthesis in fish following ingestion of ^{15}N protein (Carter *et al.* 1994), using the simple single pool model of Waterlow *et al.* (1978). The theoretical assumptions of the model and the methodology employed have been described in detail in Carter *et al.* (1994) and Houlihan *et al.* (1995b). Initial results from our laboratory on the protein metabolism of rainbow trout indicate that protein synthesis rates obtained from feeding ^{15}N-enriched protein and collecting ammonia are similar to those obtained with radiolabelled amino acids (Table 1). Carter *et al.* (1994) reported a mean fractional rate of synthesis of 2.30 ± 0.67 %.day^{-1} in 100g rainbow trout measured over the first 24 h following a ^{15}N-labelled meal. Following a flooding dose injection of L-[2,6-^{3}H]-phenylalanine to measure protein synthesis in fish of a similar size, the relationship between fractional rates of protein consumption (k_c, gramme of protein consumed per gramme of fish protein expressed as a percent per day) and synthesis (k_s) for feeding rainbow trout (40-100g:14°C) was described by $k_s = 1.69 + 0.45\ k_c$ (McCarthy *et al.* 1994). This relationship predicted a mean ks of 2.60 %.day^{-1} for the trout used in the ^{15}N experiment which was similar to that calculated from the stable isotope analysis. Similar rates of protein synthesis were also found in the white muscle of rainbow trout of a similar size (Table 1). These synthesis rates were obtained following a flooding dose injection of ^{3}H-phenylalanine (incubation period of 3 to 6 hours) and from a terminal measurement of the incorporation of ^{15}N-protein into white muscle protein 48 hours after a meal. The similarity between whole-animal and white muscle rates of protein synthesis measured over 2 days using a stochastic end-point model and over a few hours, as by the flooding dose method, provides confidence in the results obtained using the two techniques. Also, the similarity in measuring rates of protein synthesis using the two techniques further supports the validity of calculating rates of protein degradation as the difference between synthesis and growth (Millward *et al.* 1975) and therefore, the estimates of protein turnover made for fish (*e.g.* Carter *et al.* 1993; Houlihan *et al.* 1994; MCarthy *et al.* 1994).

Feeding ^{15}N enriched protein and collecting the excreted ammonia in laboratory studies is advantageous because it allows non-invasive and non-destructive measurements of protein synthesis which can be repeated on the same animal (Owen *et al.* 1995; Carter *et al.* unpublished data 1993). Therefore the use of stable isotopes should be used increasingly in the study of protein synthesis in fish. This would enable us to answer questions such as, how do changes in environmental conditions affect the protein turnover of the same fish? Furthermore, being able to 'track' the same fish provides the opportunity to measure ontogenic changes in protein synthesis and construct synthesis/weight relationships for the same fish; the only assumption that we need to make is on the protein-nitrogen content of the fish. The application of stable isotopes to measure rates of protein synthesis in the field is discussed below.

DIURNAL CYCLING

Studies with individual animals have shown that the postprandial increase in oxygen consumpion is accompanied by a stimulation in the rates of protein synthesis and an increase in ammonia excretion. Results from our laboratory with ^{15}N suggest that the ammonia that initially appears after a meal is not derived from the amino acids that were ingested (Owen *et al.*, unpublished data 1995); there may be an obligatory amino acid oxidation of currently held amino acid to supply energy for protein synthesis before the arrival in the tissues of the absorbed amino acids.

Table 1. A comparison of whole body and white muscle fractional rates of protein synthesis (k_s, %.day^{-1}) in rainbow trout, *Oncorhynchus mykiss*, measured by different methods. Wt = body weight (g) and T°C = water temperature (°C)

k_s (%.day^{-1})	Method	Wt	T°C	Ref
a) Whole body				
2.3 ± 1.9[a]	Caudal vein infusion (L-U[^{14}C]-Leu)	80g	10°C	1
2.5 ± 1.0	Flooding dose injection (L-[2,6 3H]-phe)	100g	10°C	2, 3
2.3 ± 0.7	Fed ^{15}N-protein	117g	12°C	4, 5
b) White muscle				
1.5 ± 1.0	Flooding dose Injection (L-[2,6 ^{3}H]-phe)	100	10°C	3
2.5 ± 0.6*	Fed ^{15}N-protein	117g	12°C	5

a = synthesis rates calculated using the specific radioactivity of the plasma as an estimate of the free pool specific radioactivity
* = synthesis rates calculated from killing fish 48 h after feeding and measuring the incorporation of ^{15}N-protein into muscle protein.
(1 = Fauconneau & Arnal 1985; 2 = McCarthy *et al.* 1994; 3 = McCarthy, unpublished data, 1991; 4 = Carter *et al.* 1994; 5 = Owen unpublished data, 1993)

Efficiency of retention of synthesised proteins

The efficiency of retention of synthesised protein (protein growth * 100/protein synthesis) seems to be a key indicator of strategies of protein metabolism (Table 2). High efficiencies of retention of synthesised proteins indicate reduced protein degradation rates and hence low turnover rates in growing animals. At first sight it looks as though invertebrate animals have higher efficiencies of retention of synthesised proteins compared with most fish and with the mammalian examples cited in Table 2. Further studies will reveal whether invertebrates have minimized protein turnover in order to maximise growth rates but presumably with the loss of the advantages that protein turnover brings.

In a recent review of protein metabolism in fish larvae we concluded that although weight-specific growth rates are high, rates of protein synthesis, amounts of free amino acids and RNA concentrations are not exceptional considering the small size of the organisms and in fact fish larvae seem to be following scaling relationships established for larger fish (Houlihan *et al.* 1995c). There was little evidence that rapidly growing fish larvae or juvenile fish sacrifice protein turnover in order to maximize retention efficiencies of synthesized protein; i.e. fish larvae are not exhibiting the invertebrate strategy described above. However, it would be surprising if examples of reduced turnover growth were not found in some rapidly growing

Table 2. The efficiency of retention of synthesized protein (k_g/k_s, %, whole-body protein growth *100/whole-body protein synthesis) for various endotherms and ectotherms. The sources of these data are provided in Houlihan *et al.* (1995b).

Species	k_g/k_s	Weight
a) Endotherms		
Pig	15	32 kg
Lamb	26	12.2 kg
Rat	20	211 g
Chicken	24	138 g
b) Ectotherms		
Mussel	92	10 g
	70	10 g
Octopus	95	150 g
Brown Tiger Prawn	48 - 93	5 g
Herring larvae	50	0.05 g
Nase	50	0.045 g
Rainbow Trout	35 - 69	0.2 g
Mossmbique Tilapia	30 - 40	0.0 g
	30 - 40	0.1 g
	30 - 40	1.0 g
	30 - 40	10 g
Sea bass	30 - 60	2.3 g
	47	3.5 g
	77	8 g
Goldfish	69	14 g
Grass carp	54	23 g
Turbot	33	50 g
Common carp	25	30-60 g
Rainbow Trout	45	51 g
	53	51 g
	35	70 g
Plaice	51	60 g
Dab	43	250 g
Salmon	32	180 g
Cod	42	300 g

species. In the remainder of the fish species in Table 2, retention efficiencies are mainly between 30 and 50 percent. At the moment it is not possible to clearly relate these different efficiencies to different growth rates, environmental conditions, temperature etc. However, the value of protein degradation may permit both rapid adaptation to environmental perturbations as well as the removal of damaged or abnormal proteins which may be harmful (Kirkwood 1981).

ENERGY COST OF PROTEIN SYNTHESIS

Although it is recognied that making proteins consumes energy there is still a a degree of uncertainty as to the contribution that protein synthesis makes to resting oxygen consumption. Polypeptide synthesis proceeds through three stages - the formation of the initiation complex that contains two ribosomal subunits, secondly the process of peptide chain elongation and finally the process of termination. From ATP utilization in these processes it can be estimated that 40 mmole of ATP are needed for the synthesis of each gram of protein. In place of the conventional energy budget where the emphasis has been on the components such as energy used in maintenance, specific dynamic action, activity, growth and reproduction it is now possible to calculate at least one of the costs of living - the proportion of the ingested energy that is used for the synthesis of proteins. In inactive animals it seems that protein synthesis represents between 20 and 40% of the total oxygen consumption. In ectotherms where 55% of the absorbed energy is used for respiration (Jobling 1994) protein synthesis may account for 15% of the total energy ingested (calculated from Carter *et al.* 1993).

BIOCHEMICAL CORRELATES OF PROTEIN SYNTHESIS AND GROWTH

One important question in protein metabolism in fish concerns the extent of turnover in naturally occuring populations. The data thus far discussed have been drawn from laboratory studies: how can these techniques and results be applied to fish in the wild?. One strategy that has been used is to estimate the growth rates of wild fish populations is to compare growth correlates from laboratory studies with measurement of these correlates from wild animals. In this approach laboratory growth studies are conducted under experimental conditions that seek to mimic the environmental conditions experienced by the fish. Through manipulation of the amount of food offered, a range of individual growth rates are generated for the species of interest: tissue samples are then taken for measurement of the likely growth correlates. Samples from the wild population are compared with those generated from the laboratory and likely growth rates of the wild animals are estimated (reviewed in Houlihan *et al.* 1993). We have used this approach in our laboratory to estimate the growth rates of saithe, *Pollachius virens* L. from the wild (Mathers *et al.* 1992a & b) using muscle RNA concentrations (RNA to protein; RNA to DNA and RNA/g wet weight) and aerobic enzyme activities (Citrate synthase; Cytochrome c oxidase and Lactate dehydrogenase). Recent work has also shown a correlation between epaxial muscle ornithine decarboxylase activity and growth rate (Benfey *et al.* 1994): measurement of this enzyme activity in wild fish, compared to laboratory-reared animals may also be a suitable correlate of recent growth rates in the wild. This leaves the question of the rates of protein synthesis in wild populations. To our knowledge there have been no attempts to estimate rates of protein synthesis of fish from the wild using laboratory-derived calibrations. Studies from our laboratory have shown that tissue and whole-animal RNA to protein ratios (μg RNA/mg protein) are correlated with both rates of protein synthesis and growth for a number of fish species (e.g. Houlihan *et al.* 1989, 1993, 1994). We would suggest that measurement of RNA to protein ratios in wild animals, together with careful laboratory calibration, may provide valuable estimates of both growth and protein synthesis in wild fish. It has also been suggested that measurement of muscle ornithine decarboxylase activity may also be a possible biochemical correlate of protein synthesis (Arndt *et al.* 1994).

STABLE ISOTOPES: LINKING LABORATORY AND FIELD?

We have recently turned our attention to the use of stable isotopes to estimate rates of protein synthesis in the field and we would suggest that there are two possible approaches. Firstly, the use of ^{15}N-phenylalanine, administered as a single flooding dose, would allow wild-caught fish to be injected and held in net pens for a known incubation time. The use of the stable isotope-labelled amino acid removes the danger of radioactive contamination in the field and allows the use of an established technique to measure rates of protein synthesis. However, it is possible that the synthesis rates obtained may not be valid due to the stress of capture, injection and confinement. A second approach may be to use seasonal changes in tissue $^{15}N/^{14}N$ ratios to estimate tissue turnover rates and hence protein synthesis in wild fish. We have recently examined seasonal changes in the $^{15}N/^{14}N$ composition of Flounder, *Platichthys flesus* L., and its major prey items in the Ythan estuary (Aberdeenshire, N.E. Scotland). We are currently examining the possibility of using this data to model rates of protein synthesis in wild fish populations.

We are grateful to the Natural Environment Research Council and the Biotechnology and Biological Sciences Research Council for funding.

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