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HOW SHOULD ENZYME ACTIVITIES BE USED IN FISH GROWTH STUDIES?

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Summary

The activity of glycolytic and oxidative enzymes was monitored in the white muscle of Atlantic cod *Gadus morhua* experiencing different growth rates. A strong positive relationship between the activity of two glycolytic enzymes and individual growth rate was observed regardless of whether the enzyme activity was expressed as units per gram wet mass, units per gram dry mass or with respect to muscle protein and DNA content. The most sensitive response to growth rate was observed when pyruvate kinase and lactate dehydrogenase activities were expressed as units per microgram DNA, and this may be useful as an indicator of growth rate in wild fish. In contrast, no relationship between the activities of oxidative enzymes and growth rate was observed when cytochrome *c* oxidase and citrate synthase activities were expressed as units per gram protein. Apparently, the aerobic capacity of white muscle in cod is not specifically increased to match growth rate.

Key words: fish, enzyme activity, muscle, growth rate, Atlantic cod, *Gadus morhua*.

Introduction

Biologists studying fish in their natural habitats estimate growth rate using bony structures and mark/recapture studies. These methods do not estimate short-term changes in growth rate. A close link between the activity of some of the enzymes of energy metabolism and food availability has been shown for several fish species (Sullivan and Somero, 1983; Kiessling *et al.* 1989; Yang and Somero, 1993). Given the strong relationship between food intake and growth rate (Houlihan, 1991), the activity of these enzymes is likely to be correlated with growth rates. Pelletier *et al.* (1993*a*) have shown a strong positive correlation between growth rate and the activity of the glycolytic enzymes lactate dehydrogenase (LDH), pyruvate kinase (PK) and phosphofructokinase (PFK) in white muscle of Atlantic cod *Gadus morhua*. The activities of white muscle cytochrome *c* oxidase (CCO) and citrate synthase (CS), two mitochondrial enzymes, also reflected growth rates in two gadoid species, Atlantic cod and saithe *Pollachius virens* (Mathers *et al.* 1992; Foster *et al.* 1993; Pelletier *et al.* 1993*b*). These studies, however, did not examine whether changes in enzyme activities were specific responses of enzymes or part of a general response of proteins to changes in growth rate. Enzymes are proteins, and protein concentration in muscle is closely correlated with the amount of food eaten and the growth rate (Houlihan, 1991). This study examines whether glycolytic or mitochondrial enzymes are the most reliable biochemical indicators of growth rate in fish. We also investigate how enzyme activities should be expressed to reflect the specific responses of metabolic pathways to growth rate, rather than a general response of protein synthesis, and to determine which manner of expressing enzyme activities gives the most sensitive response to variations in growth rates. Enzyme activities in the muscle, presented as units per gram wet mass, are compared with those expressed as units per gram dry mass, units per gram muscle protein content and units per microgram DNA.

Materials and methods

Wild Atlantic cod *Gadus morhua* (L.) (approximately 4 years old) were captured in the St Lawrence estuary, near Matane (Québec, Canada) in June 1990. They were kept in seawater tanks (average salinity 28 g l^{-1}) under their natural photoperiod of 15 h:9 h L:D and acclimated to laboratory conditions for a maximum of 6 months prior to the experiments. During this period, they were fed a maintenance ration of capelin *Mallotus villosus* (Müller). At the beginning of the experiment, 70 cod with a mean mass (± S.E.M.) of 737±123 g and length of 46.0±2.9 cm were separated into seven groups of ten individuals. They were acclimated to 4 °C (two groups), 10 °C (three groups) or 13 °C (two groups) in 1.5 m^3 circular fibreglass tanks using a partially recirculating, temperature-controlled system. There was no significant difference in the initial mass and length of cod among the groups. To achieve a broad range of growth rates at each temperature, each group of fish was fed a different ration of moist pellets (Pelletier *et al.* 1993*a*) three times a week for 8

weeks. Floy tags were used to identify each fish and the relative growth rate (% body mass per day) of each individual was calculated using the following equation:

$$\text{Growth rate} = 100[(W_2 - W_1)/W_1]/t,$$

where W_1 and W_2 represent the initial and final mass (in g), and t is the growth period (in days).

At the end of the experiment, the cod were killed by a blow to the head, weighed and two small pieces of the white muscle (1–2 g) were taken from a point below the first dorsal fin and well above the lateral line. These muscle samples were immediately frozen in liquid nitrogen and stored at −80 °C until analysed. The activities of four enzymes were measured. Glycolysis was represented by pyruvate kinase (PK) and lactate dehydrogenase (LDH), and aerobic metabolism by cytochrome *c* oxidase (CCO) and citrate synthase (CS). Measurements of enzyme activity (units in μmol min^{-1}) were performed at 10 °C as described by Pelletier *et al.* (1993*a*,*b*). Enzyme activities are expressed as units g^{-1} wet mass, units g^{-1} dry mass, units g^{-1} protein or units μg^{-1} DNA.

DNA and protein assays were carried out on the muscle samples as described by Pelletier *et al.* (1994). The water content of white muscle was determined by drying approximately 2 g of tissue for 24 h at 80 °C.

All data are illustrated as scatter diagrams, except for LDH and CCO activities, for which data are presented in a table. Linear relationships were tested using regression analysis.

Results

Kruskal–Wallis tests showed no significant differences (P<0.05) in enzyme activities among temperature treatments. Water and protein content in cod white muscle were both significantly affected by growth rate (Fig. 1A,B). Relative water content in muscle varied inversely with specific growth rate, from a maximum measured value of 85.6 % muscle at a growth rate of −2.5 % day^{-1} (loss of mass) to a minimum of 79.8 % muscle at a growth rate of 0.8 % day^{-1}. Conversely, muscle protein concentration (mg g^{-1} muscle) increased with growth rate. When muscle protein concentration was expressed per microgram DNA, the relationship with growth rate was stronger (r^2 increased from 0.22 to 0.46; Fig. 1B,C). There was a strong positive relationship between PK (Fig. 2A–D) and LDH activity and individual growth rate whether activities were expressed as units per gram wet mass, units per gram dry mass or with respect to muscle protein or DNA content (Fig. 2A–D; Table 1). The activities of the two glycolytic enzymes tested were more than three times higher for fish which had higher growth rates than for those that lost mass. The activity of CS increased significantly with growth rate when activities were expressed per gram muscle mass or per microgram DNA (Fig. 2E,H). However, no significant relationships were found between CS activity and growth rate when it was expressed per gram dry mass or per gram protein (Fig. 2F,G). No relationship was found between CCO activity and growth rate (Table 1).

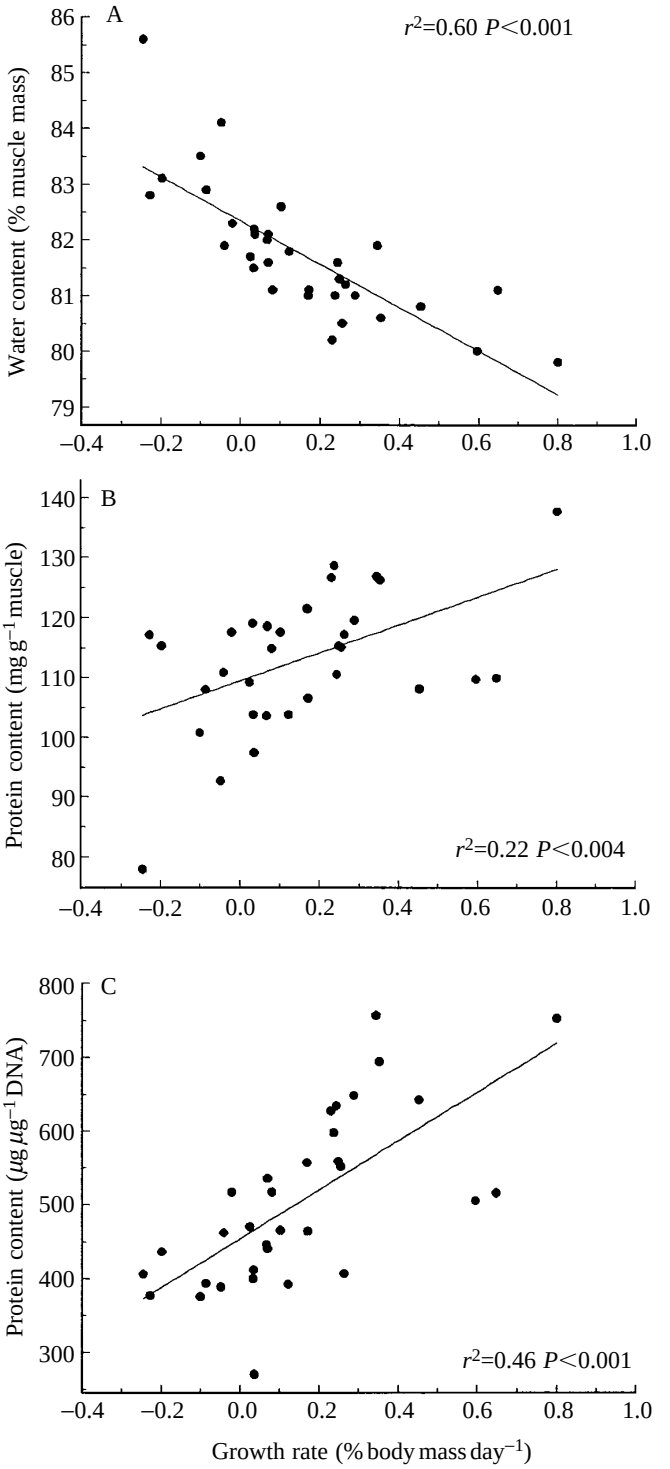

Fig. 1. Influence of growth rate on (A) water content, (B) protein content (mg g^{-1} muscle) and (C) protein content (μg μg^{-1} DNA) in white muscle of Atlantic cod. Each point represents a single individual.

Discussion

The relationships between glycolytic enzyme activities in white muscle and growth rate are more consistent than those found for the two mitochondrial enzymes. The increases in PK

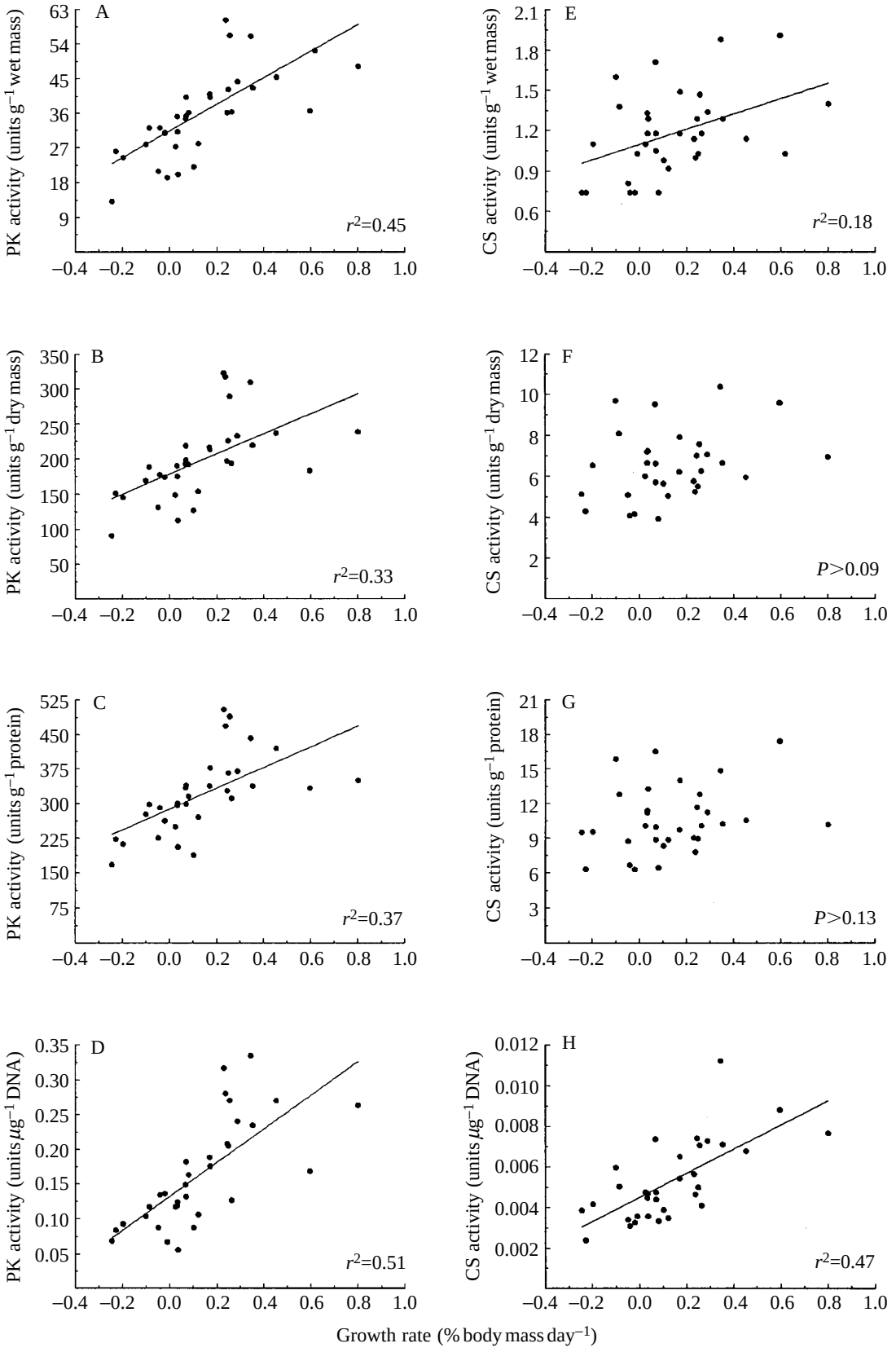

Fig. 2. Relationships between individual growth rate and activities of (A–D) pyruvate kinase (PK) and (E–H) citrate synthase (CS) in the white muscle of Atlantic cod expressed in four different ways.

Table 1. *The regression equations relating enzyme activities in white muscle of Atlantic cod to growth rate*

	Activity (units g^{-1} wet mass)			Activity (units g^{-1} dry mass)			Activity (units g^{-1} protein)			Activity (units μg^{-1} DNA)		
Enzyme	Equation	r^2	P	Equation	r^2	P	Equation	r^2	P	Equation	r^2	P
PK	y=34.6x+31.4	0.45	<0.001	y=1.79x+1.43	0.33	<0.001	y=0.23x+0.29	0.37	<0.001	y=24.3x+13.2	0.51	<0.001
LDH	y=138.1x+89.6	0.51	<0.001	y=5.07x+6.30	0.44	<0.001	y=1.06x+0.81	0.42	<0.001	y=85.6x+37.7	0.59	<0.001
CS	y=0.57x+1.10	0.18	<0.02	–	0.09	NS	–	0.07	NS	y=0.59x+0.44	0.47	<0.001
CCO	–	0.00	NS	–	0.03	NS	–	0.03	NS	–	0.10	NS

PK, pyruvate kinase; LDH, lactate dehydrogenase; CS, citrate synthase; CCO, cytochrome *c* oxidase.
y, enzyme activity; *x*, growth rate (% body mass per day).
NS, not significant.

and LDH activities with growth rate provide further support for earlier suggestions that the correlation between glycolytic enzymes and growth rate may enhance swimming capacity once food becomes available (Sullivan and Somero, 1983; Yang and Somero, 1993; Pelletier *et al.* 1993*a*).

Levels of mitochondrial enzymes, in contrast, did not increase consistently with growth rate, as only CS activity when expressed per gram muscle or per microgram DNA was positively correlated with growth rate. Our results do not agree with recent studies on cod and saithe in which CCO and CS activities, expressed per gram wet mass or per gram dry mass, increased with growth rate (Mathers *et al.* 1992; Foster *et al.* 1993; Pelletier *et al.* 1993*b*). Interestingly, CS and CCO, which are both mitochondrial enzymes, do not respond similarly to growth rate. CS is a soluble enzyme of the mitochondrial matrix and is thus dependent on mitochondrial volume, whereas CCO is an inner membrane enzyme complex and is dependent upon the total membrane surface. The CCO/CS ratio observed in response to growth rate could be associated with a change in the surface/volume ratio of mitochondria and could, consequently, reflect variations in the size and shape of mitochondria.

In the present study, the water and protein contents of the white muscle of the cod were affected by growth rate. Protein concentration increased slightly with growth rate, while the protein/DNA ratio (an index of cell size; Foster *et al.* 1993) increased markedly. This suggests that muscle cell size increased in growing fish, a result also suggested by our observation that DNA/muscle mass ratio (an index of cell number) decreased (data not shown). Given the inverse relationship between cell size and DNA concentration in growing cod, enzyme activities, in particular that of CS, obviously show stronger relationships with growth rates when expressed per microgram DNA. However, a change in enzyme activities expressed per microgram DNA does not necessarily reflect a metabolic adjustment. Instead, it could reflect a general change of protein content per cell. This could be associated with a passive response in the enzyme activity per cell caused by a significant decrease in the number of cells per volume of muscle. Considering the relative changes in cod muscle water, protein and DNA content in relation to growth rate, it is most likely that differences observed in CS and CCO activity in the different studies may be largely due to variation in muscle composition. Since no relationship was observed when the activities of oxidative enzymes were expressed per gram protein, an increase in growth rate may have increased the specific activities of these enzymes through a general enhancement of protein synthesis. Thus, cod do not appear to increase the white muscle's aerobic capacity specifically to match their growth rates.

This study has shown that the levels of cod muscle glycolytic enzymes are directly correlated with growth rates because the specific increase in glycolytic enzyme activity with growth occurs independently of muscle protein content. It has previously been demonstrated in several species that the activities of glycolytic enzymes tend to increase with body size (Somero and Childress, 1980). The correlation observed in our study might be size-dependent because fish with higher growth rates attained the largest size at the end of the experiment. However, this is unlikely, since glycolytic enzyme activities in cod white muscle have been shown to be size-independent for a larger size range than that used in our study (Pelletier *et al.* 1993*a*). Thus, we believe that growth rate, rather than body size, is the most important factor accounting for changes in glycolytic enzyme activities. PK and LDH activity expressed as units per microgram DNA gave the most sensitive response to growth rate and may be useful for assessing growth rate in wild fish. Conversely, the expression of CS and CCO activities relative to muscle protein content eliminated the correlations between growth rate and muscle-specific activity. The present data do not agree with the results of Mathers *et al.* (1992), who suggested that an increase in aerobic capacity is necessary to support enhanced rates of protein synthesis. Mathers *et al.* (1992) only obtained a weak correlation between muscle CCO or CS activities and growth rates and did not examine whether this response was specific to mitochondrial enzymes or reflected a generalized enhancement of muscle protein content. Furthermore, no effect of growth rate on white muscle CCO or CS activities has been observed in species other than gadoids (Pelletier *et al.* 1994). We suggest that the correlation reflects the general response of protein concentration rather than a specific adaptive response of the aerobic pathway to meet the energy demand of protein synthesis.

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