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SCALING OF ATP-SUPPLYING ENZYMES, MYOFIBRILLAR PROTEINS AND BUFFERING CAPACITY IN FISH MUSCLE: RELATIONSHIP TO LOCOMOTORY HABIT

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Summary

Pelagic fishes with an ability to swim in strong bursts have previously been shown to have large size-dependent increases (positive allometric scaling exponents) in the activities of glycolytic enzymes in white skeletal muscle. This scaling of glycolytic activity has been hypothesized to provide the anaerobic power supporting the size-independence of relative burst swimming speeds (body lengths s^{-1}) in these fishes. This paper presents tests of several predictions of this hypothesis, using different-sized individuals of two pelagic teleosts, the kelp bass (*Paralabrax clathratus*) and the freshwater rainbow trout (*Salmo gairdneri*), and a flatfish, the Dover sole (*Microstomus pacificus*). In the two pelagic species, an increase in body size was accompanied by an increase in activities in white muscle (i.u. g wet mass of muscle^{-1}) of lactate dehydrogenase (LDH), an indicator of potential for anaerobic glycolysis, and creatine phosphokinase (CPK), an enzyme that helps maintain stable ATP concentration during muscular activity. Activities of citrate synthase (CS), an indicator of the potential for aerobic metabolism, decreased with size. In the flatfish, activities of all enzymes in white muscle decreased with body size, a trend proposed to reflect lack of adaptive value of strong burst swimming ability in this benthic fish. Activities of LDH and CS were size-independent in brain of flatfish, indicating that the scaling patterns observed in the muscle of this species were related to muscle function, not to common, organism-wide changes with size. In white muscle of *P. clathratus*, total protein and soluble protein concentrations and buffering capacity increased with body size in parallel, but myofibrillar protein was size-independent. These results suggest that the capacity for anaerobically powered work and the maximal potential to generate force scale only modestly in relation to total body mass and therefore do not appear to be functionally related to the pattern of glycolytic scaling. Thus, these data support the hypothesis that the functional role of the strongly positive scaling of glycolytic enzymes in the white muscle of pelagic fish is to provide increased power during burst swimming in larger-sized fishes.

Key words: glycolytic enzymes, locomotion, scaling, subcarangiform locomotion.

Introduction

The study of size-dependent properties of organisms, i.e. scaling, has frequently focused on aerobic processes and the underlying structures supporting them (Schmidt-Nielsen, 1984). The equation describing aerobic metabolism as a function of body size, $y=aM^b$, where y is total oxygen consumption, M is body mass, and a is a constant, almost invariably has a scaling exponent (b value) of less than unity (Schmidt-Nielsen, 1984). For mass-specific scaling (oxygen consumption g^{-1}), b values are normally negative, and often lie near -0.25 (Schmidt-Nielsen, 1984). Anaerobic metabolic processes, which have received relatively little study in the context of scaling relationships, may exhibit very different scaling patterns from those characterizing aerobic metabolism in the same species (Childress and Somero, 1990; Hochachka *et al.* 1987; Somero and Childress, 1980). In pelagic teleost fishes, for example, the activities (i.u. g wet mass^{-1}) of enzymes responsible for ATP generation in white muscle under conditions of oxygen limitation increase in larger individuals of a species, although the activities of aerobically adapted enzymes show the decrease in activity with size typical of aerobic processes. The increases with size in anaerobic metabolic potential per gram of white skeletal muscle in pelagic fishes have been interpreted as providing these species with size-independent capacities for high-speed (burst) swimming (Somero and Childress, 1980, 1985). The increases in anaerobic metabolic power with body size also show that the scaling of aerobic metabolism cannot be explained by engineering principles involving skeletal strength (McMahan, 1984) because anaerobic power in fish muscle scales inversely with aerobic power and vastly exceeds it (Childress and Somero, 1990).

To understand more fully the scaling of proteins in white skeletal muscle of fishes, we have tested predictions based on two aspects of the proposed function of glycolytic scaling in pelagic species. First, we have been concerned with confirming the adaptive relationship between strong positive scaling of glycolytic enzymes and a pelagic (continuously free in the water column) lifestyle in fishes. Since all previous studies on this topic have been on marine fishes, we have measured the scaling of enzymes involved in anaerobic and aerobic ATP supply in muscle in a freshwater pelagic fish [*Salmo gairdneri*; Salmonidae; Richardson, 1836 (=*Oncorhynchus mykiss*; Walbaum, 1792)] to determine if it follows the same pattern. Previous studies have suggested that fishes that are denser than water and spend most of their time as adults resting on the bottom or other supporting substratum (benthic species) show much lower scaling coefficients for glycolytic enzymes than do pelagic species (Childress and Somero, 1990). Since the three previously studied benthic species (Somero and Childress, 1980; Siebenaller and Somero, 1982) are fusiform in body configuration, and therefore not especially cryptic in their habitats, we have measured enzymatic scaling in the more cryptic benthic fish, the Dover sole (*Microstomous pacificus*; Pleuronectidae; Lockington, 1879), to test the hypothesis that the lower glycolytic scaling relationships of benthic species reflect a greater degree of refuge available in their habitat and therefore different predator–prey relationships from pelagic species. The scaling of brain

enzymes in the sole was measured to test the assertion that the scaling patterns observed in muscle were specifically related to muscle function, and not to organism-wide size effects.

Second, we were concerned with testing the proposed function of the positive scaling of glycolytic enzymes (increased anaerobic power to support burst swimming) by examining the scaling of other biochemical properties of muscle in pelagic species. We measured the activities of creatine phosphokinase (CPK), an enzyme of importance in stabilizing ATP concentration during muscular activity, in *S. gairdneri* and *Paralabrax clathratus* (Serranidae; Girard, 1854) to examine scaling in another class of enzyme that would be predicted to exhibit positive scaling exponents if the function of the glycolytic scaling were to provide greater muscle power at larger fish sizes. Since the capacity of a fish to buffer acidic end products like lactic acid should limit the capacity of the fish to generate ATP anaerobically, if substrate is not limiting, the buffering capacity can provide an indication of the total anaerobic work which a fish can generate. We measured buffering capacity in different-sized individuals of *P. clathratus* to determine if buffering increases concomitantly with the capacity for greater rates of anaerobic generation of ATP. The protein content of muscle would also be expected to show changes with size if the scaling of glycolytic enzymes is of functional importance. If the phenomenon is as important as is suggested, one would expect to see both the total and the soluble protein fractions of muscle scale positively, reflecting increases in concentration of many ATP-generating enzymes. In contrast, one would expect the myofibrillar protein to scale positively only if there is an increase in maximal force as well as glycolytic power with increasing size. We have measured the concentrations of total protein, soluble protein and myofibrillar protein in different-sized individuals of *P. clathratus* to discriminate scaling patterns in different classes of muscle proteins.

Materials and methods

Specimen collection

Paralabrax clathratus were collected by netting and SCUBA fishing in coastal waters near Santa Barbara and San Diego. *Microstomus pacificus* were collected by trawling in the Santa Barbara Channel. *Salmo gairdneri* were purchased from the Lopez Lake Trout Farm in Arroyo Grande, CA. Fish were killed by a blow to the head and frozen immediately in a freezer at −80°C. We observed no loss of enzymatic activity during storage for periods up to several months.

Enzyme assays

Muscle and brain tissues were dissected from frozen specimens and homogenized in 10 mmol l^{-1} Tris/HCl buffer, pH 7.5 at 10°C. White muscle samples were taken from the epaxial musculature immediately behind the operculum and well above the mid-lateral red muscle band. The homogenates were centrifuged for 10 min at 2500 *g*, and the supernatants were collected, stored on ice, and used

immediately for the activity assays. All assays were performed in thermostatted cuvettes at 10±0.10°C, using substrate concentrations giving maximal velocities.

The activity of L-lactate dehydrogenase (LDH, EC 1.1.1.27; L-lactate: NAD^+ oxidoreductase) was assayed according to the procedure of Somero and Childress (1980). The assay medium contained, in a total volume of 2.0 ml, 80 mmol l^{-1} Tris/HCl buffer (pH 7.5 at 10°C), 2 mmol l^{-1} sodium pyruvate, 150 μmol l^{-1} NADH and 100 mmol l^{-1} KCl. The reaction was initiated by addition of supernatant.

Citrate synthase [CS, EC 4.1.3.7; citrate: oxaloacetate-lyase (CoA-acetylating)] activity was measured following the procedure of Somero and Childress (1980). Activity was assayed in a medium containing 50 mmol l^{-1} imidazole/HCl buffer (pH 8.0 at 10°C), 0.5 mmol l^{-1} oxaloacetate, 0.2 mmol l^{-1} acetyl-CoA, 0.1 mmol l^{-1} 5,5-dithiobis(2-nitrobenzoic)acid (DTNB) and 1.5 mmol l^{-1} $MgCl_2$. The absorbance was monitored at 412 nm. The absorbance increase in the presence of supernatant, but without oxaloacetate, was first recorded, and the complete reaction was then initiated by addition of oxaloacetate. The blank (no oxaloacetate) was subtracted from the total activity.

Creatine phosphokinase (CPK, EC 2.7.3.2.; ATP: creatine phosphotransferase) was assayed using the protocol of Dawson and Eppenberger (1970). For 10 2.0-ml assays, 19.8 ml of stock solution A [83 mmol l^{-1} Tris/HCl buffer (pH 7.5 at 10°C), 3.3 mmol l^{-1} glucose, 3.3 mmol l^{-1} $MgCl_2$, 5 mmol l^{-1} ADP and 3.3 mmol l^{-1} creatine phosphate] was mixed with 0.2 ml of stock solution B (0.5 mg hexokinase, 0.5 mg glucose-6-phosphate dehydrogenase and 1% bovine serum albumin in 100 mmol l^{-1} Tris/HCl buffer, pH 7.5). NADPH was added as a dry powder to a final concentration of 150 μmol l^{-1}. The reaction was initiated by addition of supernatant.

Buffering capacity

Non-bicarbonate buffering capacity was measured following the method of Castellini and Somero (1981). Frozen muscle (0.5 g) was homogenized in 10.0 ml of normal saline (0.9% NaCl). The homogenate was stirred continuously at room temperature (approx. 22°C) and titrated with 0.2 mol l^{-1} NaOH between pH values of approximately 6.5 and 7.5. Buffering capacity was defined as the amount (μmol) of NaOH needed to change the pH of the homogenate by 1 pH unit.

Separation of soluble and myofibrillar proteins

White skeletal muscle was separated into sarcoplasmic and myofibrillar fractions following the method of Bates and Millward (1983). White muscle (0.3 g) was added to 3.0 ml of low-salt buffer (LSB, 50 mmol l^{-1} K_3PO_4, pH 7.0) and 0.3 ml of 1% Triton X-100 and homogenized in a ground-glass-surfaced homogenizer. A sample (0.3 ml) was saved for measurement of total protein. The homogenate was centrifuged in a Fisher Microfuge for 3 min. The supernatant and the pellet were saved. The pellet was suspended by vortex mixing and rinsed twice by centrifugation with 1 ml of LSB. The three supernatant fractions were combined (soluble

fraction). To solubilize the pellet fraction containing the myofibrillar proteins, the washed pellet was resuspended by vortexing in 0.3 ml of LSB and 1.1 ml of 10 mol l^{-1} urea and 40 μl of glacial acetic acid were added to the suspension. To solubilize the total protein of the unfractionated homogenate, 1.16 ml of 10 mol l^{-1} urea and 40 μl of glacial acetic acid were added to 0.3 ml of homogenate.

Measurement of protein concentrations

Protein concentrations were measured using the bicinchoninic acid method of Smith *et al.* (1985), with bovine serum albumin as the standard. The myofibrillar fraction was diluted an additional 40-fold, and the unfractionated homogenate and supernatant fraction an additional 20-fold with LSB. Urea and Triton X-100 at the low concentrations present in the diluted fractions did not interfere with the assay. Blanks contained LSB at concentrations equal to those in the samples. Dipeptides, e.g. histidine-containing dipeptide buffers, are not detected by this method.

Statistical analysis and data presentation

Linear regressions of log-transformed data were carried out using the Statview SE+ program (Abacus Concepts, Berkeley, CA). $P \leq 0.05$ was taken as the criterion for significance of the regressions. All regressions were examined as scattergrams to ensure that the relationship being tested was not obviously nonlinear. The *b* values for the concentrations of parameters as functions of fork length (the distance from the tip of the nose to the end of the caudal fin) of *P. clathratus* in Table 1 can be converted to *b* values for the scaling of the total of the parameter in the fish's body as a function of fish length by adding 3.2 (the *b* value for total white epaxial muscle mass as a function of length in this species (Somero and Childress, 1980) to the concentration *vs* length *b* value in the table.

Results

Interspecific differences: pelagic versus *benthic species*

The scaling relationships for white muscle enzymatic activities of these species are presented in Table 1. Scaling coefficients were computed on the basis of both body mass and fork length. The former coefficient is the conventional mass-specific *b* value determined in most studies, whereas the length-specific *b* value is of particular interest for comparing locomotory capacities of fishes, where relative swimming speed (body lengths swum per unit time) is the variable of interest (see Discussion).

For the two subcarangiform-swimming pelagic species, the activities of LDH and CPK increased in larger-sized individuals (Fig. 1, Table 1). Thus, the capacities for anaerobic ATP generation, as indexed by LDH activity, and the ability to stabilize ATP concentration through exploitation of phosphocreatine, as indexed by CPK activity, were enhanced in larger-sized individuals. CS activity, an indication of aerobic metabolic capacity, decreased significantly in larger-sized individuals (Fig. 1C, Table 1). In both species white muscle CPK and LDH were

Table 1. *The regression equations relating wet mass and fork length to enzyme activities, protein fractions and buffering capacity in white muscle and brain tissue of some teleost fishes*

Species / Tissue	Parameter	x=wet body mass (g)				x=fork length (mm)			
		a	b±95 % CI	r^2	N	a	b±95 % CI	r^2	N
Paralabrax clathratus									
White muscle	LDH	29.09	0.414±0.078	0.83	25	0.64	1.089±0.258	0.77	25
White muscle	CS	2.17	−0.171±0.051	0.75	16	10.35	−0.445±0.148	0.75	16
White muscle	CPK	80.91	0.102±0.056	0.52	16	28.63	0.287±0.144	0.57	16
White muscle	Myofibrillar protein			0.00	20			0.00	20
White muscle	Soluble protein	50.46	0.056±0.023	0.41	20	28.21	0.161±0.095	0.41	20
White muscle	Total protein	148.40	0.045±0.030	0.36	20	91.90	0.131±0.083	0.37	20
White muscle	Buffering	42.19	0.064±0.023	0.75	14	23.41	0.170±0.067	0.72	14
Salmo gairdneri									
White muscle	LDH	93.74	0.400±0.111	0.81	16	0.70	1.269±0.358	0.81	16
White muscle	CS	2.79	−0.0612±0.0611	0.25	16	5.88	−0.194±0.192	0.25	16
White muscle	CPK	115.17	0.146±0.104	0.49	12	15.74	0.466±0.334	0.49	12
Microstomus pacificus									
White muscle	LDH	236.75	−0.435±0.310	0.39	16	50312.1	−1.366±0.934	0.41	16
White muscle	CS	3.83	−0.682±0.395	0.49	16	12395.6	−2.083±1.214	0.49	16
Brain	LDH			0.01	8				
Brain	CS			0.11	8				

The equation used is of the general form $y=ax^b$.
y is the enzyme activity in i.u. g wet mass^{-1}.
Protein content is in mg protein g wet mass^{-1}.
Buffering values are expressed in μmol pH unit^{-1} g wet mass^{-1}.

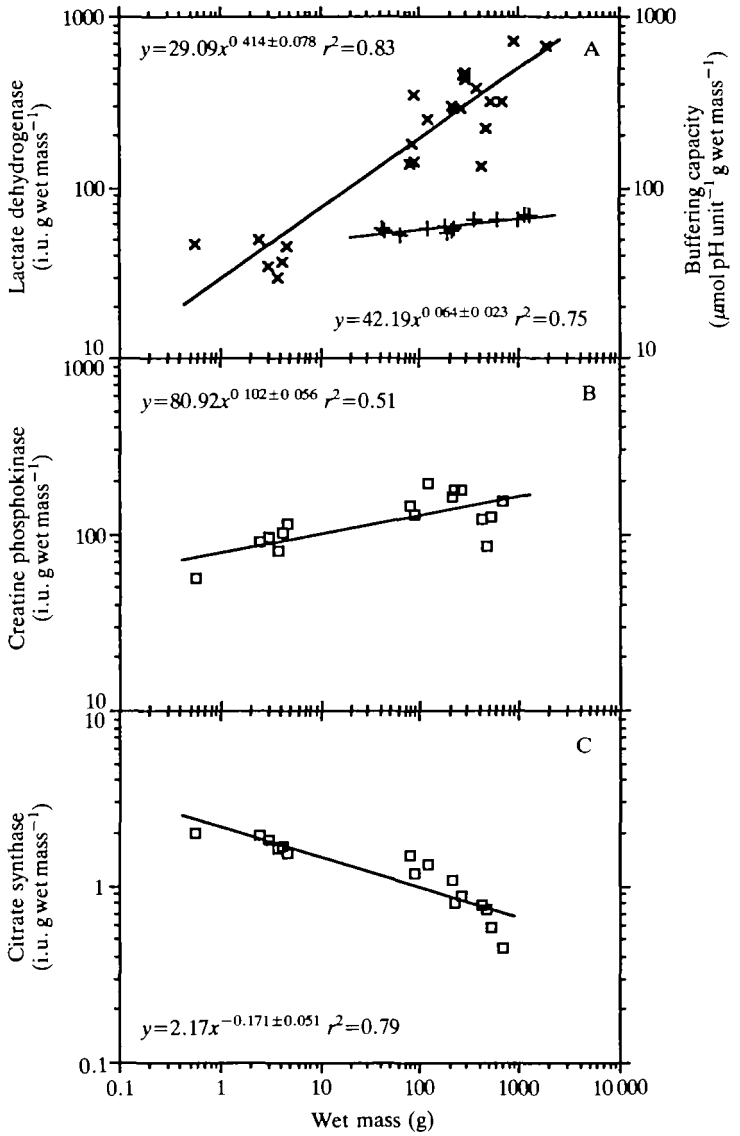

Fig. 1. The scaling of enzyme activities and buffering capacity in the white epaxial muscle of the kelp bass (*Paralabrax clathratus*). (A) LDH (×) and buffering capacity (+) as a function of wet mass. (B) CPK as a function of wet mass. (C) CS as a function of wet mass.

significantly positively related. While LDH was significantly negatively related to CS in *P. clathratus*, in *S. gairdneri* there was no significant relationship between these enzymes.

In the Dover sole the activities of LDH and CS in white skeletal muscle decreased strongly in larger-sized individuals (Fig. 2, Table 1). The LDH scaling coefficient was the most negative one yet recorded (−0.435), showing that the

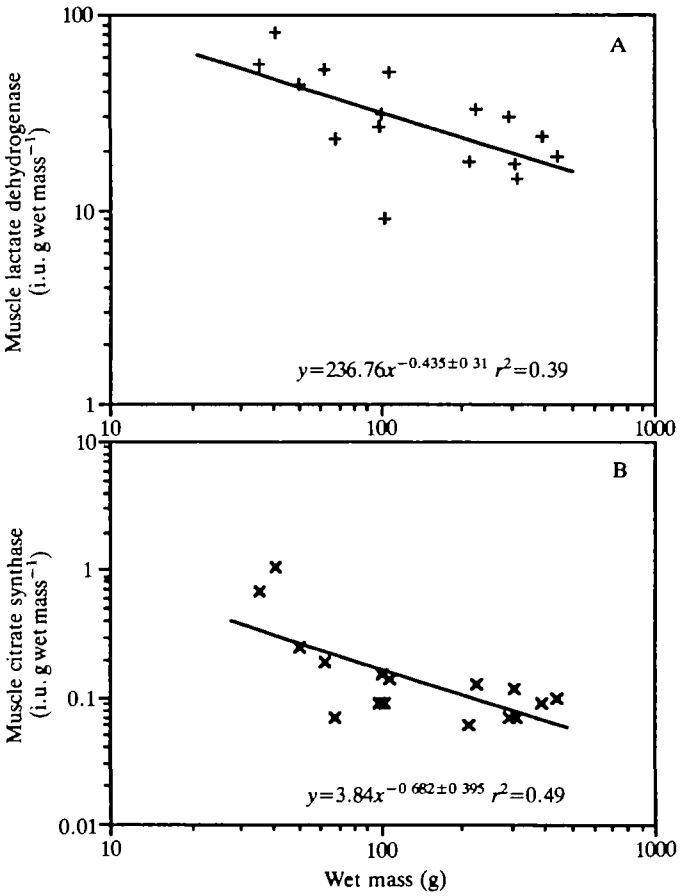

Fig. 2. The scaling of enzyme activities in the white epaxial muscle of the Dover sole (*Microstomus pacificus*). (A) LDH as a function of wet mass. (B) CS as a function of wet mass.

scaling pattern for LDH characteristic of pelagic subcarangiform swimmers is not found in a sluggish, benthic species. In fact, the white muscle LDH and CS activities in this species were significantly positively related, indicating that aerobic and anaerobic power change in parallel in this species, unlike in pelagic ones. LDH and CS activities in white muscle were also much lower in *M. pacificus* than in the two pelagic species, which is further evidence for poorer swimming ability in the flatfish. In brain, however, both LDH and CS activity were quite constant and showed no significant relationship to body mass or length (Fig. 3, Table 1). The LDH and CS activities and scaling of brain activities in *M. pacificus* are similar to those of pelagic species (Childress and Somero, 1979; Somero and Childress, 1980; Sullivan and Somero, 1980), indicating that the differences found in enzymatic scaling and activities of muscle enzymes of pelagic and benthic species reflec

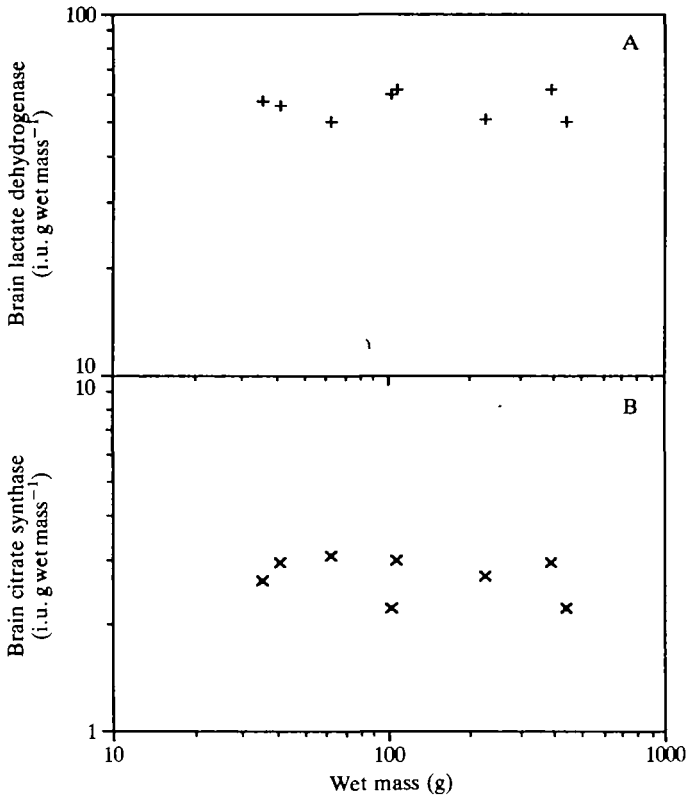

Fig. 3. The scaling of enzyme activities in the brain tissue of the Dover sole (*Microstomus pacificus*). (A) LDH as a function of wet mass. (B) CS as a function of mass.

locomotory differences and not common, size-related changes in all tissues and organs.

Buffering capacity and protein concentration

The buffering capacity of white muscle tissue in *P. clathratus* increased significantly with size (Table 1; Fig. 1A). The concentration of soluble proteins, which would include the glycolytic enzymes and much of the CPK (see Discussion), also increased significantly with size with a slope not significantly different from that of the buffering capacity (Table 1; Fig. 4B). This coincidence in slopes suggests that the scaling of buffering capacity is the result of the scaling of the soluble proteins and is not due to changes in non-protein buffering compounds in the muscle tissue. The total protein also scaled with a similar slope, which probably results entirely from changes in soluble protein (Table 1, Fig. 4C). In contrast to these other parameters, the concentration of myofibrillar protein showed no significant size-dependence (Table 1; Fig. 4A).

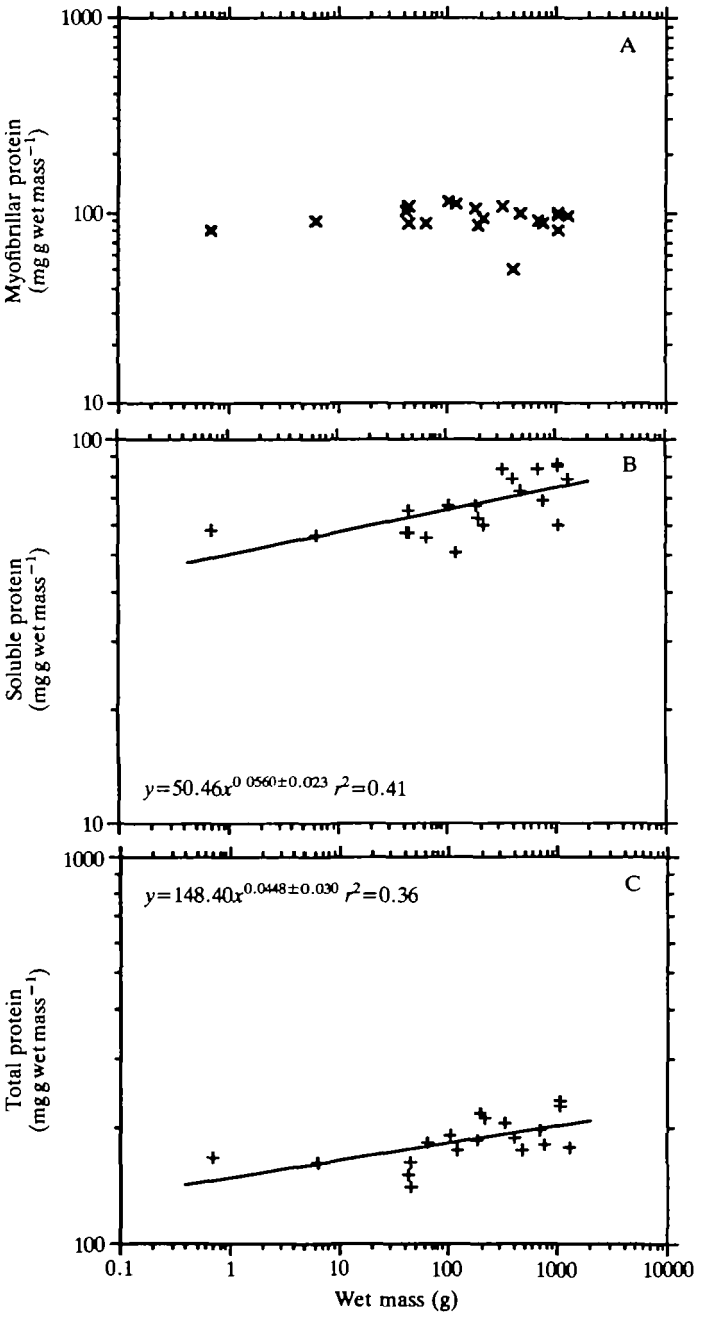

Fig. 4. The scaling of protein fractions in the white epaxial muscle of the kelp bass (*Paralabrax clathratus*). (A) Myofibrillar protein as a function of wet mass. (B) Soluble protein as a function of wet mass. (C) Total protein as a function of wet mass.

Discussion

The data presented here provide new insight into the adaptive significance as well as the functional significance of the strongly positive scaling of glycolytic enzymic activity previously observed in pelagic, subcarangiform swimming fishes (Childress and Somero, 1990; Siebenaller *et al.* 1982; Somero and Childress, 1980). We have previously proposed that the functional significance of this scaling is that it can provide the anaerobic glycolytic power required to support size-independent burst swimming speeds among different-sized individuals of the same species (Somero and Childress, 1980). Calculations of the scaling of power (ATP-generating) requirements to enable different-sized individuals of a species to maintain the same relative swimming speeds (body lengths s^{-1}) predict that anaerobic ATP-generating power, as indexed by LDH activity per gram of muscle, should increase with a mass-specific b value of approximately 0.33–0.53, depending upon the hydrodynamic model used and the degree of turbulent *versus* laminar flow (Somero and Childress, 1980). For *P. clathratus* and *S. gairdneri*, the b values for LDH are near 0.4 (Table 1). For CPK, the b values for the pelagic species are again positive and of similar magnitude, although smaller, indicating that this essential link in the supply of ATP to the contractile apparatus scales appropriately to support the proposed locomotory function of the LDH scaling. The exact timing of the roles played by these two enzymes is somewhat in question since, although Stevens and Black (1966) measured a 30% depletion of white muscle glycogen stores after 15 s of maximal activity in rainbow trout, Dobson *et al.* (1987) measured no glycogen depletion and about 50% phosphocreatine depletion after 10 s of sprint swimming by individuals of the same species. The smaller b value for CPK could reflect the dual role played by this protein in skeletal muscle. In addition to generating ATP from phosphocreatine, the enzyme is the M-line protein of muscle (Walliman *et al.* 1978). Because no scaling was observed in the myofibrillar fraction of muscle, the M-line fraction of CPK may not increase with body size.

The scaling of buffering capacity did not differ from the scaling of total protein or soluble protein concentrations in *P. clathratus* (Table 1). This suggests that the increase in buffering capacity with size was not due to increases in concentrations of non-protein buffering substances, like histidine-containing dipeptides, but was a fortuitous result of the change in protein concentration. Interspecific differences in buffering capacity in fishes are largely due to differences in the concentrations of low-molecular-weight, non-protein buffers (Dickson and Somero, 1987). In *P. clathratus*, low-molecular-weight buffers account for approximately two-thirds of white muscle buffering capacity (Dickson and Somero, 1987), yet these quantitatively dominant contributors to buffering capacity do not appear to be subject to size-dependent regulation.

The functional significance of the increased buffering capacity in larger-sized *P. clathratus* is not entirely clear. If sufficient substrate, e.g. glycogen, were available in a muscle, intracellular buffering capacity could limit the total accumulation of the acidic end products of anaerobic metabolism, that is the total

energy expended (work) during a single bout of burst swimming, since these products are retained within the muscle during burst swimming (Milligan and Wood, 1986). Thus, the positive scaling of white muscle buffering capacity may allow for some increase in capacity for work in larger-sized fishes. The relationship of buffering capacity to power generation in burst swimming is less clearly of functional significance. However, higher buffering capacity might allow more rapid removal of protons during a bout of burst swimming, thereby reducing inhibition by low pH of pH-sensitive enzymes, like phosphofructokinase, which are of critical importance in sustaining glycolysis.

The total protein concentration per gram of white muscle increased with size in *P. clathratus*. This increase is interpreted to be due to soluble proteins which, because of the extraction methods used, include the majority of the enzymes involved in ATP generation. This increase provides yet another indicator that there are major shifts in the muscle proteins involved in anaerobic ATP generation as a function of size in pelagic fishes. In contrast, the concentration of the myofibrillar fraction showed no significant change with size. This implies that the maximal force which a fish can generate should only show scaling to the extent that the total amount of muscle relative to the size of the fish increases with increasing fish size (Goldspink, 1985). For *P. clathratus* and most pelagic fishes the white epaxial muscle does increase somewhat faster than the body mass in general with increasing size (total wet mass$=2.08\times10^{-5}L^{2.9}$, epaxial muscle mass$=18.19\times10^{-7}L^{3.2}$ for *P. clathratus*, where L is the body length, Somero and Childress, 1980). However, since the same scaling factors will also apply to glycolytic enzymes which are increasing in concentration with size, it is apparent that the total anaerobic power increases much more rapidly (total LDH scales as $L^{4.3}$ in *P. clathratus*, Somero and Childress, 1980) than does maximal force in the whole fish. Thus, the scaling of glycolytic power appears to be well in excess of any requirements related to maximal force generation and therefore not functionally related to such needs.

The absence of comparable scaling of myofibrillar protein suggests that the significance of the positive glycolytic scaling is not related to the acceleration from rest to high speed (fast-start phase of burst swimming). The failure of muscle buffering capacity to increase as rapidly as LDH indicates that the function of the increased glycolytic capacity is not primarily to provide a greater capacity for anaerobic work. Thus, the increased glycolytic capacity is apt to have its most important expression during burst swimming in the period following the fast-start and preceding exhaustion. During this brief period the higher glycolytic power of larger fish should enable them to maintain greater speeds than would otherwise be possible. The depletion of phosphocreatine during sprint swimming (Dobson *et al.* 1987) suggests that the positive CPK scaling which we observed is of primary importance during the first few tail flips after the initial acceleration and should again allow larger fishes to exhibit greater performance.

The data presented here also increase our insight into the adaptive significance of the positive scaling of the activity of glycolytic enzymes in pelagic fishes in th

context of locomotory demands in predator–prey interactions. We originally suggested that the observed scaling was adaptive because visual and other modes of predator–prey detection scale with a proportionality greater than $L^{0.5}$, the scaling of sustained aerobic swimming. Therefore size-independent (L^1) burst swimming would reduce this disadvantage at the cost of requiring greatly increased anaerobic power (Somero and Childress, 1980). Weihs and Webb (1983) have modeled the chase aspect of predator–prey interactions among fishes and concluded that, to favor prey capture, swimming speed should scale as body length (L) with an exponent ≥ 0.5. These analyses were based upon pelagic species and did not consider the possibility of habitat and habit differences in the scaling of predator–prey detection. More recently, data have become available on two fishes of the genus *Sebastolobus* which sit on the bottom most of the time (Siebenaller, 1984) and have lower LDH scaling coefficients (mass-specific $b=0.20$ and -0.04, calculated from the original data used in the paper) than those of actively swimming pelagic species. We have suggested that the association of a fish with the bottom provides a degree of refuge which substantially changes the scaling of predator–prey detection, reducing the reliance on rapid swimming as a tactic in these interactions (Childress and Somero, 1990). The strongly negative scaling of LDH activity and the low level of activity in the Dover sole, a fish that takes refuge on the bottom, emphasize the different locomotor demands of the pelagic and benthic habitats. These data also support our hypothesis concerning the adaptive significance of the positive glycolytic scaling, since they show that this scaling is not found in a fish that relies on refuge on the bottom rather than active swimming as its principal tactic in predator–prey interactions. The size- and habit-independence of LDH activity in brain tissue of all fishes studied (Table 1; Siebenaller and Somero, 1982; Siebenaller *et al.* 1982; Somero and Childress, 1980; Sullivan and Somero, 1980) are further evidence that the size-dependent changes in anaerobic potential noted in locomotory muscle reflect adaptations for size-related locomotory costs and not some general, organism-wide, size-related adaptation.

The scaling of aerobic metabolism does, however, show consistent patterns among different organs, as evidenced by the decreases in activities of aerobically adapted ATP-generating enzymes in different organs of fishes and tetrapods (Emmett and Hochachka, 1981; Ewart *et al.* 1988; Hochachka *et al.* 1987; Siebenaller *et al.* 1982; Somero and Childress, 1980, 1985; Torres and Somero, 1988). The differences between scaling of anaerobic and aerobic metabolic capacities reflect the different roles of inter-organ interactions for these two forms of metabolism (Childress and Somero, 1990). Aerobic metabolism is sustained by the transport of oxygen and substrates through the respiratory and circulatory systems. The scaling of aerobic metabolism may, therefore, be an organism-wide reflection of the geometric characteristics of organisms, e.g. surface to volume ratios which may impose constraints on oxygen uptake and transport, on the one hand, while reducing costs, e.g. those associated with surface localized ion and water transport, on the other hand. The precise factors that 'explain' aerobic

scaling remain a matter of debate (Calder, 1987; Childress and Somero, 1990; Heusner, 1987; McMahan, 1984; Schmidt-Nielsen, 1984).

For scaling of anaerobic power of locomotory muscle it is not necessary to consider inter-organ interactions because of the short periods over which anaerobically powered burst locomotion occurs, times which are too short to permit transport of substrate and oxygen into the muscle. Thus, different constraints and opportunities for size-dependent activity apply than for aerobic metabolism (Childress and Somero, 1990). The dependence of burst locomotion, lasting for periods of only fractions of a minute, on oxygen is minimal. The supplies of the substrates (glycogen, phosphocreatine) are localized within the muscle, as are the end products produced during the course of burst locomotion (Milligan and Wood, 1986; Dobson and Hochachka, 1987). Thus, the scaling of anaerobic muscle power is not constrained by supply or waste-elimination problems like those that may face, and explain the scaling of, aerobic metabolism. For the scaling of anaerobic power, the primary factors in addition to the contractile apparatus itself are the occurrence of adequate levels of substrate, of enzymes able to generate ATP during anoxia, and of sufficient buffering ability to neutralize the protons generated during bursts of anaerobic glycolysis. Although we have not measured substrate concentrations in our study species, we have demonstrated that in pelagic fishes appropriate scaling of other biochemical parameters occurs as predicted.

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