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Muscle Enzyme Activities in a Deep-Sea Squaloid Shark, *Centroscyllium fabricii*, Compared With Its Shallow-Living Relative, *Squalus acanthias*

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ABSTRACT The activities of several enzymes of energy metabolism were measured in the heart, red muscle, and white muscle of a deep and a shallow living squaloid shark, *Centroscyllium fabricii* and *Squalus acanthias*, respectively. The phylogenetic closeness of these species, combined with their active predatory nature, similar body form, and size makes them well matched for comparison. This is the first time such a comparison has been made involving a deep-sea elasmobranch. Enzyme activities were similar in the heart, but generally lower in the red muscle of *C. fabricii*. Paralleling the trend seen in deep-sea teleosts, the white muscle of *C. fabricii* had substantially lower activities of key glycolytic enzymes, pyruvate kinase and lactate dehydrogenase, relative to *S. acanthias* or other shallow living elasmobranchs. Unexpectedly, between the squaloid sharks examined, creatine phosphokinase activity was higher in all tissues of the deep living *C. fabricii*. Low white muscle glycolytic enzyme activities in the deep-sea species coupled with high creatine phosphokinase activity suggests that the capacity for short burst swimming is likely limited once creatine phosphate supplies have been exhausted. *J. Exp. Zool. 300A:133*–139, 2003.

INTRODUCTION

Aspects of the metabolic and biochemical adaptations found in deep-sea fish have received significant study (see Somero, '92; Childress, '95; Gibbs, '97 for recent reviews). While numerous data on differences between deep and shallow living teleosts are available, to our knowledge similar data on elasmobranchs are entirely lacking. Deep-sea teleosts generally have reduced metabolic rates and low levels of key enzymes of energy metabolism in white muscle compared to shallow living species. No trend between enzyme activity and depth has been observed in the heart or brain of teleosts.

With no data available on deep-sea elasmobranchs, we test the hypothesis that the activities of several enzymes of energy metabolism in the heart, red muscle, and white muscle of the deep living *Centroscyllium fabricii* differ from that of the shallow living *Squalus acanthias* as well as literature values for other elasmobranch species.

Interpretation of data on the adaptations of deep-sea fish is often difficult due to the inability to conclude that differences are due to adaptation to the deep-sea and not some other factor. For example, Sullivan and Somero ('80), as well as Siebenaller et al. ('82), have suggested interspecific differences in white muscle enzyme activities from shallow and deep living fish may be explained in part by the feeding and locomotory habits of each species. As explained by Gibbs ('97), these data are further confounded by the potential influence of phylogenetic differences.

To eliminate as many confounding factors as possible, some investigators have made efforts to utilise species of close phylogeny, with similar lifestyles, but inhabiting different depths. This is exemplified by the work on *Sebastolobus alascanus* and *S. altivelis*, the latter of which inhabits deeper water (for example, Siebenaller and Somero, '78, '79, '82).

Although *C. fabricii* and *S. acanthias* belong to different families (Etmopteridae and Squalidae, respectively) they are both basal squaloids in the

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order Squaliformes (de Carvalho, '96; Compagno, '99). Furthermore, sharks feature low genetic diversity (Smith, '86) and slow rates of genetic change across familial and ordinal taxa (Martin et al., '92). Morphologically, squaloids are 'extremely homogeneous' (Bigelow and Schroeder, '57). The chosen species share the same basic body form and swimming style (Thompson and Simanek, '77) and are of similar size, having many body and morphological characteristics in common (Bigelow and Schroeder, '48; Bass et al., '76; Compagno, '84).

The general prey categories, and trophic level, are very similar between these species (Cortez, '99) with *S. acanthias* known to be an active predator of fast-swimming prey (Bigelow and Schroeder, '48, '53; Compagno, '84). The non-overlapping tricuspidate teeth of *C. fabricii* suggest grasping fast-moving prey, an interpretation supported by stomach contents (Bigelow and Schroeder, '53; Compagno et al., '89; Ebert et al., '92). Both species appear to be active predators, and they are among the few shark species known to school (Bigelow and Schroeder, '53; Templeman, '63; Compagno, '84).

While there are many similarities between these species, *C. fabricii* is abundant at 550–1010m (Bigelow and Schroeder, '57) or 400–1340m (Templeman, '63), depending on region, whereas *S. acanthias* is uncommon below 180m (Templeman, '63).

In addition to being the first data of this kind on a deep-sea elasmobranch, we measured a greater variety of enzymes than in previous studies on teleosts and for the first time include red muscle in the tissues surveyed for enzyme activities involved with energy metabolism in a deep-sea fish.

MATERIALS AND METHODS

Animals

Specimens of *C. fabricii* were captured by otter trawl on the *CCGS Teleost* in November, 1999 off the coast of Newfoundland where bottom temperature was between 3.5 and 5°C. *C. fabricii* were caught in water with a bottom depth below 1000 m and ranged from approximately 300 to 900 grams. *S. acanthias* were caught by otter trawl in Passamaquoddy Bay, New Brunswick, in less than 100 m of water at approximately 10 to 12°C in August, 2000, and weighed between approximately 400 and 1150 grams. All animals were killed by a blow to the head and tissues were dissected out, blotted dry, and frozen with liquid nitrogen. Red muscle was taken from the lateral region between the second dorsal fin and the caudal peduncal, and white muscle was sampled from the deep dorsal region below the first dorsal fin. In the case of *C. fabricii*, only animals that were still responsive to handling were sampled. Tissues were stored at or below −60°C until analysis (between 20 and 30 months). Although

TABLE 1. Activities (μmol min^{-1} g wet tissue^{-1}) of heart enzymes in Squalus acanthias *and* Centroscyllium fabricii

Enzyme	*Squalus acanthias*	*Centroscyllium fabricii*
Oxidative		
CS	5.12 ± 0.81	3.72 ± 1.27
MDH	54.9 ± 6.00	59.3 ± 13.2
Amino acid		
AlaAT	0.56 ± 0.17	0.39 ± 0.28
AspAT	24.9 ± 5.41	12.0 ± 4.58*
GDH	0.60 ± 0.23	0.64 ± 0.23
Ketone body		
βHBDH	0.31 ± 0.29	0.20 ± 0.10
Glycolytic/anaerobic		
PK	34.3 ± 5.39	23.9 ± 6.01*
LDH	48.9 ± 5.44	40.0 ± 8.19
CPK	2.14 ± 0.89	7.23 ± 2.43*

Values are means ± SD with n=5 for all. * - sig. difference between species ($p < 0.05$, Student's t-test). Abbreviations: MDH, malate dehydrogenase; AlaAT, alanine aminotransferase; GDH, glutamate dehydrogenase otherwise see text for full enzyme names.

TABLE 2. Activities (μmol min^{-1} g wet tissue^{-1}) of red muscle enzymes in Squalus acanthias *and* Centroscyllium fabricii

Enzyme	*S. acanthias*	*C. fabricii*
Oxidative		
CS	9.92 ± 1.48	5.84 ± 0.78*
MDH	51.0 ± 5.95	75.1 ± 9.70*
Amino acid		
AlaAT	2.10 ± 0.19	0.59 ± 0.25*
AspAT	29.4 ± 5.86	18.3 ± 3.84*
GDH	5.97 ± 0.85	1.41 ± 0.23*
Ketone body		
βHBDH	2.49 ± 0.61	1.11 ± 0.63*
Glycolytic/anaerobic		
PK	29.6 ± 5.26	26.4 ± 9.04
LDH	20.1 ± 4.98	33.5 ± 7.79*
CPK	32.8 ± 6.15	56.7 ± 17.1*

Values are means ± SD with n=5 for all. * - sig. difference between species ($p < 0.05$, Student's t-test). See Table 1 for abbreviations.

TABLE 3. Activities (μmol min^{-1} g wet tissue^{-1}) of white muscle enzymes in Squalus acanthias *and* Centroscyllium fabricii

Enzyme	*S. acanthias*	*C. fabricii*
Oxidative		
CS	0.54 ± 0.18	0.24 ± 0.04*
MDH	2.39 ± 0.27	4.05 ± 1.03*
Amino acid		
AspAT	2.09 ± 0.24	0.82 ± 0.39*
Glycolytic/anaerobic		
PK	101 ± 17.3	18.4 ± 5.25*
LDH	160 ± 25.0	35.9 ± 9.75*
CPK	94.0 ± 4.43	107 ± 8.95*

Values are means ± SD with n=5 for all. * - sig. difference between species ($p < 0.05$, Student's t-test). See Table 1 for abbreviations.

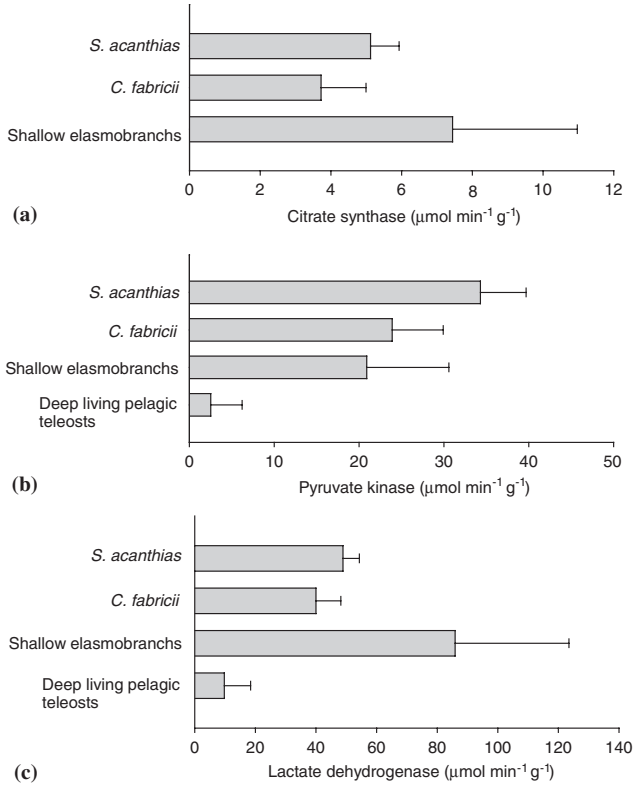

Fig. 1. Heart enzyme activities for (a) citrate synthase, (b) pyruvate kinase, and (c) lactate dehydrogenase in *S. acanthias* and *C. fabricii* compared to shallow living elasmobranchs and deep living pelagic teleosts, values are means ± SD. Shallow living elasmobranchs, n = 6 with duplicate values for *Raja erinacae* for CS and PK averaged, n=5 with duplicate values for *Raja erinacea* for LDH averaged. Deep living teleost, n=13 for all enzymes. Data for shallow living elasmobranchs: Alp et al., 1976; Zammit et al., 1978; Moon and Mommsen, 1987; Sidell et al., 1987; Dickson et al., 1993. Deep living teleosts data from Childress and Somero (1979). Where necessary, activities adjusted to 5°C using a Q_{10} of 2.

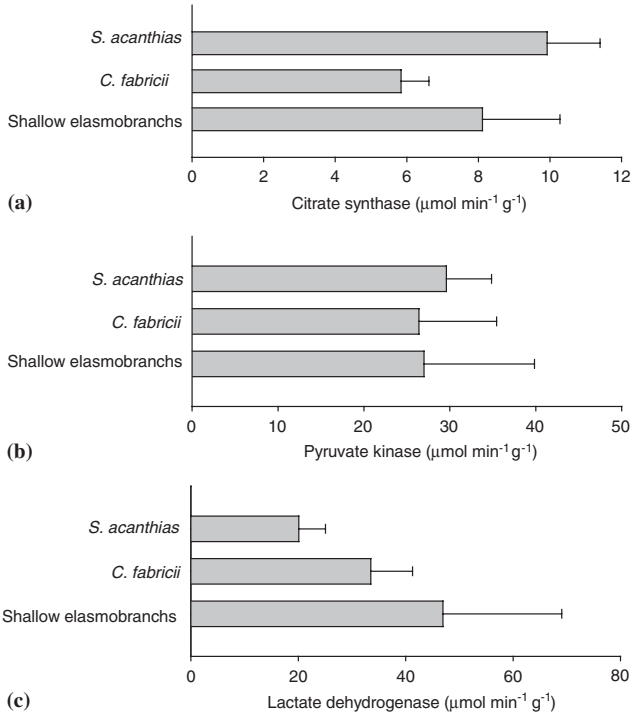

Fig. 2. Red muscle enzyme activities for (a) citrate synthase, (b) pyruvate kinase and (c) lactate dehydrogenase in *S. acanthias* and *C. fabricii* compared to shallow living elasmobranchs. Shallow living elasmobranchs, values are means ± SD, n=5 for all enzymes. Data for shallow living elasmobranchs: Crabtree and Newsholme, 1972; Alp et al., 1976; Zammit et al., 1978; Moon and Mommsen, 1987; Dickson et al., 1993. Where necessary, activities adjusted to 5°C using a Q_{10} of 2.

this storage period was quite long, all samples were stored for similar duration until analysis allowing comparisons between species and tissues. Furthermore, the observed activities of several enzymes in *S. acanthias* are similar to values from the literature (see Figs. 1–3).

Enzyme assays

Samples were weighed and homogenised in nine volumes of ice-cold buffer (50 mM imidazole at pH 7.4) with a Polytron homogenizer. Homogenates were centrifuged at 10 000 g for five minutes at 4–8°C and the supernatants used directly for enzyme assays. All enzyme activities were determined spectrophotometrically at 5°C ± 0.1°C on a Beckman DU640 spectrophotometer equipped with a jacketed cell holder connected to a thermostated water chiller. This temperature was chosen because it is near, or within, the published temperature ranges for both species, 1–4.5°C for

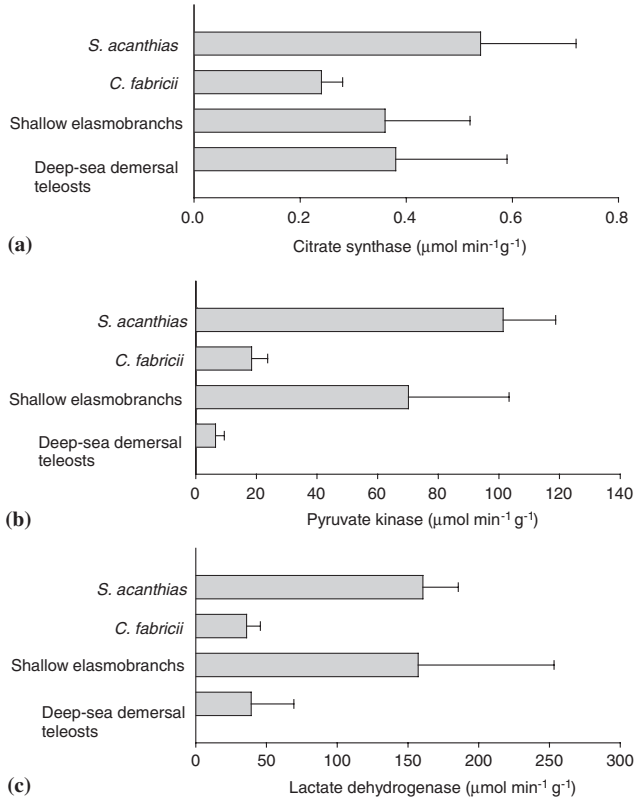

Fig. 3. White muscle enzyme activities for (a) citrate synthase, (b) pyruvate kinase and (c) lactate dehydrogenase in *S. acanthias* and *C. fabricii* compared to shallow living elasmobranchs and deep-sea demersal teleosts, values are means ± SD. Shallow living elasmobranchs, n=10 species with duplicate values for *Squalus acanthias* for CS and PK averaged, n=11 for lactate dehydrogenase. Deep-sea demersal teleosts, n=8; with duplicate values for 6 species averaged for all enzymes. Data for shallow living elasmobranchs: Alp et al., 1976; Zammit et al., 1978; Sullivan and Somero, 1980; Moon and Mommsen, 1987; Dickson et al., 1993; Battersby et al., 1996. Data for deep-sea demersal teleosts from Sullivan and Somero, 1980; Siebenaller and Somero, 1982; Siebenaller et al., 1982. Where necessary, activities adjusted to 5°C using a Q_{10} of 2.

C. fabricii (Templeman, '63) and 3–15°C for *S. acanthias* (Scott and Scott, '88) and thus is unlikely to cause thermal instability in the enzymes. Assays were based on established protocols on fish muscle enzymes (Suarez et al., '86; Moon and Mommsen, '87; Sidell et al., '87) and were measured by monitoring the oxidation or reduction of pyridine nucleotides at 340 nm with the exception of citrate synthase which followed the production of free CoA with 5,5'–dithiobis (2–nitrobenzoic acid), (DTNB) at 412 nm. Control rates were determined in the absence of substrate, and preliminary studies confirmed reaction rates were linear with time and homogenate added. All chemicals were purchased from Sigma Chemical Company.

Assay conditions were as follows: Citrate synthase (E.C. 4.1.3.7). 50 mM imidazole (pH 8.0), 0.1 mM DTNB and 0.3 mM acetyl CoA. The reaction was initiated with 0.5 mM oxaloacetate. Malate dehydrogenase (E.C 1.1.1.37). 50 mM imidazole (pH 7.4) and 0.2 mM NADH. The reaction was initiated with 1.0 mM oxaloacetate. Alanine aminotransferase (E.C. 2.6.1.2). 50 mM imidazole (pH 7.4), 200 mM L-alanine, 0.2 mM NADH, 0.05 mM pyridoxal–5–phosphate and 5.0 IU/ml lactate dehydrogenase. The reaction was initiated with 11.5 mM α-ketoglutarate (αKG). Aspartate aminotransferase (E.C. 2.6.1.1). 50 mM imidazole (pH 7.4), 30 mM aspartate, 0.2 mM NADH, 0.05 mM pyridoxal–5–phosphate and 3.0 IU/ml malate dehydrogenase. The reaction was initiated with 7.0 mM αKG. Glutamate dehydrogenase (E.C. 1.4.1.3). 50 mM imidazole (pH 7.4), 250 mM ammonium acetate, 0.25 mM EDTA, 0.1 mM NADH and 0.1 mM ADP. The reaction was initiated with 14 mM αKG. β-Hydroxybutyrate dehydrogenase (E.C. 1.1.1.30). 50 mM Imidazole (pH 8.0), 11.25 mM NAD, 50.0 mM nicotinamide, and 2.0 mM dithiothreitol. The reaction was initiated with 25 mM DL-β-hydroxybutyrate. Pyruvate kinase (E.C. 2.7.1.40). 50 mM imidazole (pH 7.4), 10.0 mM $MgCl_2$, 50.0 mM KCl, 0.15 mM NADH, 5.0 mM ADP, 0.1 mM fructose–1,6–bisphosphate and 5.0 IU/ml lactate dehydrogenase. Reaction was initiated by the addition of 5.0 mM phosphoenolpyruvate. Lactate dehydrogenase (E.C. 1.1.1.27). 50 mM imidazole (pH 7.4) and 0.2 mM NADH. The reaction was initiated by the addition of 1.0 mM pyruvate. Creatine phosphokinase (E.C. 2.7.3.2). 50 mM Imidazole (pH 7.4), 5.0 mM $MgCl_2$, 1.0 mM ADP, 0.4 mM NADP, 1.0 mM glucose, 10.0 mM AMP, 2.0 IU/ml hexokinase, and 2.0 IU/ml glucose–6–phosphate dehydrogenase. The reaction was initiated by the addition of 50.0 mM phosphocreatine.

Analysis

All data were compared by Student's t-test with $p < 0.05$ being considered significant.

RESULTS AND DISCUSSION

With enzyme activities measured in only two species, our results allow for comparison between these particular sharks, but do not allow for broad 'generalisations' to be made about deep-sea elas-

mobranch metabolism. Further, the possibility that the ambient temperature to which each species was adapted may have an effect on enzyme levels cannot be eliminated from this study. However, as explained previously, despite not being of as close phylogeny as the *Sebastolobus* species referred to above, the species used are reasonable for comparison and similar data on deep-sea elasmobranchs are entirely lacking. The present study, in conjunction with literature values for elasmobranch enzyme activities, provides a first snapshot into the metabolism of a deep-sea elasmobranch.

Enzyme activities in muscle tissues of C. fabricii and S. acanthias

Overall, the enzyme activity profiles in the heart of both species are quite similar (Table 1). Although aspartate aminotransferase (AspAT) and pyruvate kinase (PK) activities are about half those in *S. acanthias*, creatine phosphokinase (CPK) activity in *C. fabricii* is higher. This elevated CPK activity may be representative of how *C. fabricii* deals with burst activity, as outlined below.

In contrast to the heart, all measured red muscle enzyme activities, with the exception of PK, differ between *S. acanthias* and *C. fabricii* (Table 2). Low citrate synthase (CS) in the red muscle of *C. fabricii* indicates reduced overall oxidative metabolism, and implies lower rates of mitochondrial fuel utilisation compared to *S. acanthias*. Consistent with this, enzymes associated with providing oxidative substrates, amino acid metabolising enzymes, and β-hydroxybutyrate dehydrogenase (βHBDH), are lower in *C. fabricii*. Red muscle lactate dehydrogenase (LDH) activity is higher in *C. fabricii*, whereas PK is similar between species. CPK activity in the red muscle of *C. fabricii* was nearly double that of *S. acanthias*, similar to the case in heart.

The white muscle of *C. fabricii* has much lower activities for CS, AspAT, PK, and LDH than *S. acanthias* (Table 3). As in red muscle, *C. fabricii* had higher white muscle CPK activity; however, this difference between species is much less than in the heart or red muscle. Several white muscle enzymes, alanine aminotransferase (AlaAT), glutamate dehydrogenase (GDH), and βHBDH, had activities below 0.01 μmol min^{-1} g wet $tissue^{-1}$ for both species and were not included in the analysis.

Comparison with shallow living elasmobranchs and deep living teleosts

Data on muscle CS, PK, and LDH activities are available for a sufficient number of shallow living elasmobranchs and deep-sea teleosts to allow comparison with our data. To accommodate for differences in assay temperature, literature values have been adjusted to 5°C using a Q_{10} of 2 where necessary. Despite the long storage period, our enzyme values for *S. acanthias* generally agree well with literature values (see references listed in Figs. 1–3). Furthermore, in a study of several elasmobranch species, Dickson et al. ('93) found that storage from 1.5 to 44 months under similar conditions as the present study did not affect the activity of CS, PK, or LDH when expressed on a wet weight basis. Therefore, tissue dehydration was not considered to be a critical factor. Shallow living teleosts have not been included in figures 1–3 as Dickson et al. ('93) found that elasmobranchs and teleosts of similar locomotory habits have comparable muscle enzyme activities.

Both species in the present study have similar heart CS, PK, and LDH activity as other elasmobranchs (Fig. 1). Although the LDH activity appears lower in the present study, the high degree of variability in literature values makes our numbers not sufficiently low to be considered exceptional. This similarity is consistent with the notion that heart does not show a decrease in enzyme activity with increasing depth (Childress and Somero, '79); however, heart PK and LDH activity in *C. fabricii* are much higher than the deep living pelagic teleosts examined by Childress and Somero ('79). The pelagic species used by Childress and Somero were substantially smaller, up to three orders of magnitude, than the specimens used in the present study. LDH has been shown to be positively scaled with increasing body mass while PK is negatively scaled in a teleost heart (Ewart et al., '88). Thus, higher heart LDH in *C. fabricii* compared to much smaller deep-sea teleosts may be explained by scaling effects, but this does not explain the high PK values.

Red muscle CS, PK, and LDH activities show the same trend as heart (Fig. 2), with neither species appearing exceptional to shallow living elasmobranchs measured in previous studies. To our knowledge, no comparable deep-sea teleost data are available. With respect to white muscle enzymes, deep-sea elasmobranchs may show the same trend of reduced enzyme activity found in deep-sea teleosts (Fig. 3). White muscle CS activity

is similar in all groups, though *C. fabricii* is on the low end of the spectrum of the data. The enzymes PK and LDH have parallel trends, with activity in *C. fabricii* being much lower than in the shallow elasmobranchs, yet very similar to the deep-sea demersal teleosts (Fig. 3b,c).

Unlike in heart, white muscle PK and LDH activity are positively scaled with increasing body mass in teleosts (Childress and Somero, '90), and presumably in elasmobranchs. Thus, higher white muscle activities of PK and LDH in shallow living elasmobranchs relative to the generally smaller deep-sea demersal teleosts could be partially explained by scaling effects but this does not explain the substantial difference between values for shallow living elasmobranchs and *C. fabricii*. Of note, the deep-sea demersal teleosts in figure 3 are generally smaller than the *C. fabricii* used in the present work, but the size ranges have substantial overlap (teleosts ranged from approximately 50 to 800 g).

Relevance of elevated CPK activity in C. fabricii

The consistently higher CPK activity in *C. fabricii* may reflect interspecies differences in the dependence on phosphocreatine to supplement ATP during 'burst' activity, although the possibility that these results are due to thermal compensation cannot be ruled out. Newsholme et al. ('78) have demonstrated that CPK activity in fish muscle is highest in white muscle, which has a greater dependence on glycolysis than tricarboxylic acid cycle for energy metabolism.

While higher CPK activity in the deeper living species may seem in conflict with the hypothesis of reduced metabolic capacity in deep-sea fish, it is consistent with the notion that these predators spend most of their time in slow 'cruising,' but are metabolically prepared for short rapid bursts in pursuit of prey. Since anaerobic glycolytic capacity is so reduced in the white muscle of *C. fabricii*, this deep-sea fish may rely almost entirely on stored phosphocreatine to supplement ATP synthesis in the white muscle during short bursts. This would provide for rapid burst swimming until phosphocreatine is depleted, after which ATP generation would be limited to the reduced anaerobic glycolytic flux.

Sullivan and Somero ('80) hypothesise that with the lack of light in the deep-sea, with the exception of bioluminescence, fish would have little need for high glycolytic capacity for escaping predators (see also Childress, '95). Our results are consistent with this, and we add that a predator's loss of visual cues may result in a similar 'laxness' in the selection for extended burst capacity, thus limiting the ability for prolonged rapid and metabolically costly pursuit of prey.

In summary, we have shown that the trend of greatly decreased activities of PK and LDH in the white muscle of deep-sea teleosts also occurs in a deep-sea elasmobranch. With respect to the aerobic red muscle and heart, the deep-sea species had mostly lower red muscle enzyme activities whereas the heart showed little difference between species. The shallow living *S. acanthias* displays a white muscle enzyme profile typical of the utilisation of phosphocreatine stores with subsequent upregulation of anaerobic glycolysis to fuel burst swimming; however, the very low anaerobic glycolytic capacity in conjunction with high CPK activity in the white muscle of *C. fabricii* suggests that phosphocreatine stores alone may account for the majority of burst swimming capacity in *C. fabricii*.

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