

Activity levels of enzymes of energy metabolism in heart and red muscle are higher in north-temperate-zone than in Amazonian teleosts

J.L. West, J.R. Bailey, V.M.F. Almeida-Val, A.L. Val, B.D. Sidell, and W.R. Driedzic

Abstract: Fish living in the Amazon basin typically have body temperatures of about 30°C, whereas freshwater fishes of the north-temperate zone are eurythermal, with typical body temperatures of 10–20°C in summer. Enzyme activity levels in heart and red muscle of Amazonian species, which display various physiological mechanisms for dealing with hypoxic conditions, were compared with those in north-temperate-zone species. Five Amazonian species (acará-açu (*Astronotus ocellatus*), acari-bodó (*Lipossarcus pardalis*), tambaqui (*Colossoma macropomum*), tamoatá (*Hoplosternum littorale*), and pirarucu (*Arapaima gigas*)) and four north-temperate-zone species (American eel, bullhead, yellow perch, and rainbow trout) were studied. The Amazonian species included obligate and facultative air breathers. Activities of key indicator enzymes associated with carbohydrate metabolism, fatty acid metabolism, the citric acid cycle, and the electron-transport chain were determined. There was no obvious correlation between cardiac enzyme activity levels and the potential ability of fish to maintain blood oxygen levels in hypoxic water or the capacity of isolated heart preparations to survive anoxia. In heart, activity levels of hexokinase, phosphofructokinase, pyruvate kinase, lactate dehydrogenase, citrate synthase, cytochrome oxidase, and β -hydroxyacyl CoA dehydrogenase were about twice as high in north-temperate-zone species than in Amazonian species. Activities of red-muscle enzymes, especially those associated with aerobic fatty acid metabolism, were significantly higher in comparable north-temperate-zone species relative to Amazonian species. Increased enzyme activity levels in north-temperate-zone species relative to Amazonian species is considered to be an adaptation to generally lower body temperatures. This finding is consistent with earlier comparisons of Antarctic and north-temperate-zone species and with the results of studies of cold acclimation within north-temperate-zone fishes.

Résumé : Les poissons qui vivent dans l'Amazonie ont une température corporelle normale d'environ 30°C, alors que les poissons d'eau douce de la zone tempérée nord sont eurythermes et leur température normale en été se situe entre 10 et 20°C. L'activité des enzymes du cœur et celle des enzymes du muscle rouge ont été comparées chez des espèces amazoniennes, qui utilisent des mécanismes physiologiques variés en réaction à des conditions d'hypoxie, et chez des espèces de la zone tempérée du nord. Cinq espèces de l'Amazonie (Acará-açu (*Astronotus ocellatus*), Acari-bodó (*Lipossarcus pardalis*), Tambaqui (*Colossoma macropomum*), Tamoatá (*Hoplosternum littorale*) et Pirarucu (*Arapaima gigas*)) et quatre espèces de la zone tempérée du nord (Anguille d'Amérique, Barbote, Perchaude et Truite arc-en-ciel) ont été étudiées. Parmi les espèces amazoniennes, certaines avaient une respiration aérienne obligée, d'autres, une respiration aérienne facultative. L'activité de diverses enzymes indicatrices associés au métabolisme des hydrates de carbone, au métabolisme des acides gras, au cycle de Krebs et à la chaîne de transport des électrons a été mesurée. Nous n'avons pas trouvé de corrélation nette entre l'activité des enzymes du cœur et la capacité potentielle des poissons de maintenir leurs concentrations d'oxygène dans le sang en eau hypoxique ou la capacité de préparations de cœurs isolés de survivre à l'anoxie. Dans le cœur, l'activité des enzymes hexokinase, phosphofructokinase, pyruvate kinase, lactate déshydrogenase, citrate synthase, cytochrome oxydase et β -hydroxyacyl déshydrogenase était environ deux fois plus élevée chez les poissons du nord que chez ceux de l'Amazonie. L'activité des enzymes du muscle rouge, particulièrement l'activité des enzymes associées au métabolisme des acides gras, était significativement plus élevée chez les espèces tempérées comparables que chez les espèces amazoniennes. L'activité enzymatique plus élevée chez

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J.L. West, J.R. Bailey, and W.R. Driedzic.¹ Department of Biology, Mount Allison University, 63B York Street, Sackville, NB E4L 1G7, Canada.

V.M.F. Almeida-Val and A.L. Val. Laboratory for Ecophysiology and Molecular Evolution, National Institute for Amazon Research, Alameda Cosme Ferreira, 1756, 69.083-000, Manaus AM, Brazil.

B.D. Sidell. School of Marine Sciences, University of Maine, Orono, ME 04469-5741, U.S.A.

¹Author to whom all correspondence should be sent at the following address: Ocean Sciences Centre, Memorial University of Newfoundland, NF A1C 5S7, Canada (e-mail: wdriedzic@mun.ca).

les espèces tempérées est considérée comme une adaptation à des températures corporelles généralement plus basses. Cette constatation correspond aux résultats obtenus lors de comparaisons antérieures entre des poissons de l'Antarctique et des poissons de la zone tempérée du nord, de même qu'au cours d'études sur l'acclimatation au froid chez des espèces de la zone tempérée du nord.

[Traduit par la Rédaction]

Introduction

Two potential determinants of the organization of energy metabolism in heart and red muscle of fish are thermal history and oxygen availability. This paper reports the level of key indicator enzymes in selected Amazonian and north-temperate-zone fish species with the objective of assessing the importance of these environmental factors in the expression of metabolic enzymes. A companion paper (Bailey et al. 1999) addresses anaerobic heart performance.

Water temperatures in the Amazon basin typically range from about 25 to about 32°C, although under some conditions temperatures may be higher (Kramer et al. 1978). This is in marked contrast to water in the north-temperate zone, which approaches 0°C in the winter and usually does not exceed 25°C in summer. A life cycle spent at very low temperature is often associated with elevated activities of key enzymes of energy metabolism. In a broad summary of cardiac enzyme levels, once adjustments were made for assay temperature, Antarctic species typically exhibited higher activities of indicator enzymes than north-temperate-zone species (Driedzic 1992). In a comparison of pectoral muscle from ecotypically similar Antarctic and north-temperate-zone fishes, activity levels of enzymes associated with aerobic energy metabolism, when assayed at a common temperature of 1°C, were higher in the cold-adapted species (Crockett and Sidell 1990). In heart and red muscle, a suite of enzymes, including hexokinase (HK), carnitine palmitoyl transferase (CPT), β -hydroxyacyl CoA dehydrogenase (HOAD), citrate synthase (CS), and cytochrome oxidase (Cyt Ox), are often more active within north-temperate-zone species acclimated to low temperature than in those acclimated to high temperature (for instance, see Jones and Sidell 1982; Johnston et al. 1985; Way-Kleckner and Sidell 1985; Guderly and Gawlicka 1992). There is also a propensity for aerobic oxidation to be enhanced, especially the use of fatty acids to meet energy demands at low temperatures.

In the Amazon drainage basin, the lower level of the water column may be hypoxic or even anoxic (Val 1996), and in flood-plain areas, the dissolved oxygen level can approach zero at night (Kramer et al. 1978). Amazonian fishes deal with these conditions through metabolic depression, anaerobic metabolism, surface-skimming, air-breathing, or some combination of these strategies. Aerial respiration and a high anaerobic potential are not mutually exclusive, since there may be times when air-breathing is inappropriate. In contrast to Amazonian species, north-temperate-zone fishes are generally obligate water-breathers and inhabit water of higher oxygen content than Amazonian species. Our understanding of the influence of oxygen availability on the organization of energy metabolism in fish heart and red muscle is limited. Following adaptation to hypoxia, activity levels of phosphofructokinase (PFK) and pyruvate kinase (PK) did not change in heart of the small marine teleost *Zoarcetes viviparus* (Driedzic et al. 1985), nor did activity levels of HK,

PK, or lactate dehydrogenase (LDH) in red muscle of goldfish (van den Thillart and Smit 1984). Studies that relate enzyme activity levels to actual muscle performance are few. In one report, there was no obvious relationship between the ability of ventricular strips to contract under anoxic conditions and activity levels of PK or LDH in seven species of fish (Gesser and Poupa 1974). Concentrations of other potential regulatory enzymes were not measured. This study, in concert with its companion paper (Bailey et al. 1999), therefore revisits and extends the data base.

We report that there is no obvious correlation between heart enzyme activity levels and cardiac resistance to oxygen limitation. However, activity levels of selected enzymes in heart and red muscle are higher in north-temperate-zone species than in Amazonian species, implying that thermal history is an important factor in the organization of energy metabolism.

Materials and methods

Animals

Four north-temperate-zone and five Amazonian species were studied. The Amazonian species used were tambaqui (*Colossoma macropomum*) (body mass 255 ± 16 g, ventricle mass 0.093 ± 0.007 g), which surface skims with extended lips in hypoxic water; acará-açu (*Astronotus ocellatus*) (body mass 106 ± 20 g, ventricle mass 0.027 ± 0.005 g), commonly known as the oscar, a species with very good whole-animal hypoxia tolerance; two species of armoured catfish, acari-bodó (*Lipossarcus pardalis*) (body mass 240 ± 25 g, ventricle mass 0.056 ± 0.006 g) and tamoatá (*Hoplosternum littorale*) (body mass 110 ± 11 g, ventricle mass 0.058 ± 0.004 g), both of which are facultative air-breathers; and pirarucu (*Arapaima gigas*) (body mass 321 ± 73 g, ventricle mass 0.32 ± 0.07 g), an obligate air-breather. All species were purchased from a fish farm (Amazon Fish (Itacoatiara Road, AM-10, km 251, Manaus)), held in outdoor tanks at the National Institute for Amazon Research, and kept in recirculated aerated water until use. Two of the north-temperate-zone species, rainbow trout (*Oncorhynchus mykiss*) (body mass 371 ± 34 g, ventricle mass 0.64 ± 0.07 g) and yellow perch (*Perca flavescens*) (body mass 86 ± 23 g, ventricle mass 0.071 ± 0.02 g), are not hypoxia-tolerant; the other two, American eel (*Anguilla rostrata*) (body mass 515 ± 30 g, ventricle mass 0.31 ± 0.04 g) and brown bullhead (*Ictalurus punctatus*) (body mass 115 ± 12 g, ventricle mass 0.083 ± 0.02 g), are hypoxia-tolerant. All four species are obligate water-breathers. Perch, eels, and bullheads can be found in warm (20°C) water, while trout favour colder water even in summer. All animals were kept in flow-through aquaria at ambient temperature (13–15°C) at Mount Allison University until use.

Experimental protocols

After being arbitrarily selected from the holding tanks, all fish were killed by a sharp blow to the head except American eels, which were rendered torpid in ice-water and then doubly pithed. Hearts were excised and body and ventricle masses recorded. Fresh ventricles were divided in two, and one half was frozen. Red muscle was dissected from two Amazonian (tambaqui and acari-bodó) and two north-temperate-zone species (rainbow trout and bullhead)

Table 1. Enzymes of carbohydrate metabolism in hearts of Amazonian and north-temperate-zone fishes.

	HK	PFK	PK	LDH
Amazonian species				
Acará-açu	2.4±0.3 (5)	9.5±1.8 (4)	46.2±8.1 (5)	134±3 (5)
Tambaqui	3.0±0.6 (5)	40.4±5.5 (5)	73.5±6.9 (5)	573±11 (5)
Tamoatá	20.2±2.1 (5)	16.9±2.5 (5)	40.8±1.6 (5)	281±19 (5)
Acari-bodó	9.0±0.4 (5)	29.5±3.5 (5)	60.0±2.0 (5)	235±14 (5)
Pirarucu	10.1 (2)	24.6±1.7 (3)	27.3±2.8 (3)	256±30 (3)
Mean	8.9±3.2	24.2±5.3	49.6±8.0	296±73
North-temperate-zone species				
Yellow perch	23.5±2.4 (4)	52.5±3.4 (4)	158.7±9.9 (4)	255±11 (4)
Rainbow trout	25.6±1.5 (11)	55.9±6.4 (10)	71.2±2.6 (8)	307±18 (6)
American eel	14.7±0.5 (6)	33.0±6.0 (5)	90.0±0.7 (4)	726±55 (5)
Bullhead	13.2±1.4 (4)	26.7±3.0 (4)	58.8±3.6 (4)	423±12 (4)
Mean	19.3±3.1	42.0±7.2	94.5±22.1	427±11
<i>P</i> (Amazonian vs. north-temperate-zone species)	0.058	0.08	0.075	0.326

Note: Values are given as the mean ± SEM ($\mu\text{mol}\cdot\text{min}^{-1}\cdot\text{g}^{-1}$), with the sample size given in parentheses; the assay temperature was 22–25°C. Values for acará-açu and tambaqui (assay temperature 25°C) are taken from Driedzic and Val (1996).

and divided into two portions, one of which was frozen for later use. Tissues were homogenized in ground-glass homogenizers in 9 volumes of medium containing 75 mM Tris and 1 mM EDTA at pH 7.6. All assays were conducted on crude homogenates or dilutions thereof. HK and PFK were always measured in fresh tissue and CS, PK, CPT, and HOAD in frozen tissue. LDH and Cyt Ox were measured in either fresh or frozen tissue. Values presented here for enzymes of carbohydrate metabolism in acará-açu and tambaqui hearts are from Driedzic and Almeida-Val (1996). In the current experiment, preliminary remeasurement of the activity of these enzymes yielded data in the same ranges found in the previous study. However, it was necessary to reassay CS and HOAD, as we recognized that in the earlier study the mitochondria had not been adequately disrupted. Maximal enzyme activity levels were measured at 22–25°C, using either a Shimadzu UV-240 or a Gilford 2600 spectrophotometer.

Assay conditions were based on well-established protocols for fish tissues (Sidell et al. 1987). All enzyme assays had a final cuvette volume of 1 mL. HK, PFK, PK, LDH, and HOAD were measured at 340 nm, CS and CPT were measured at 412 nm, and Cyt Ox was measured at 550 nm. Composition of the assay media was as follows: HK (EC 2.7.1.1): 75 mM Tris-HCl, 7.5 mM MgCl_2 , 0.8 mM EDTA, 1.5 mM KCl, 2.5 mM ATP, 10 mM CP, 0.9 IU/mL glucose-6-phosphate dehydrogenase, and 0.4 mM NADP^+ , at pH 7.4. The reaction was initiated with 1 mM glucose; PFK (EC 2.7.1.11): 75 mM Tris, 7 mM MgCl_2 , 200 mM KCl, 1 mM KCN, 2 mM AMP, 0.15 mM NADH, 2 IU/mL aldolase, 10 IU/mL triose phosphate isomerase, and 2 IU/mL glycerol-3-phosphate dehydrogenase, at pH 8.2. The reaction was initiated with 1.0 mM ATP and 4 mM fructose-6-phosphate; PK (EC 2.7.1.40): 50 mM imidazole, 150 mM KCl, 10 mM MgSO_4 , 0.15 mM NADH, 5 mM ADP, 10 IU/mL LDH, and 1 mM KCN, at pH 6.9. The reaction was initiated with 2.5 mM phosphoenolpyruvate; LDH (EC 1.1.1.27): 50 mM imidazole, 1 mM KCN, and 0.15 mM NADH, at pH 7.4. The reaction was initiated with 1 mM pyruvate; CPT (EC 2.3.1.21): 75 mM Tris, 1.5 mM EDTA, 0.25 mM DTNB (5,5'-dithiobis-2-nitrobenzoic acid), and 0.035 mM palmitoleoyl CoA (16:1 CoA), at pH 8.0. The reaction was initiated with 1.25 mM L-carnitine; HOAD (EC 1.1.1.35): 50 mM imidazole, 1 mM EDTA, 1 mM KCN, and 0.15 mM NADH, at pH 7.5. The reaction was initiated with 0.1 mM acetoacetyl CoA; Cyt Ox (EC 1.9.3.1): 10 mM $\text{K}_2\text{HPO}_4/\text{KH}_2\text{PO}_4$ buffer at pH 7.0. Cytochrome *c* in phosphate buffer was reduced with ascorbate and run through a Bio-Rad 10DG desalting column to remove the ascorbate. The

maximum theoretical concentration of reduced cytochrome *c* in the assay cuvette was 1 mM, but actually varied between 0.5 and 0.7 mM. A 100- μL aliquot of cytochrome *c* solution was used in a total cuvette volume of 1 mL. The reaction was initiated by the addition of 5 or 10 μL of crude homogenate; CS (EC 4.1.3.7): 75 mM Tris, 0.25 mM DTNB, and 0.4 mM acetyl CoA, at pH 8.0. The reaction was initiated with 0.5 mM oxaloacetate.

Data analysis

Extinction coefficients were 6.2 for assays carried out at 340 nm, 13.6 for assays carried out at 412 nm, and 18.6 for Cyt Ox assayed at 550 nm. Enzyme activities are all expressed in micromoles of substrate converted to product per minute per gram of tissue. Heart enzyme activity levels, within either the Amazonian or the north-temperate-zone group, were analyzed with a one-way analysis of variance followed by Tukey's multiple comparisons test. Red-muscle enzyme activity levels between comparable species (i.e., rainbow trout against tambaqui and bullhead against acari-bodó) were assessed with Student's *t* test. The difference in activities of the full set of enzymes between Amazonian and north-temperate-zone species was assessed with the paired Wilcoxon's signed-rank test. A *P* value of less than 0.05 was considered to be significant. All analyses were executed with InStat 2.01 for MacIntosh.

Results

Heart enzymes

The armoured catfish tamoatá had significantly higher heart HK activity levels than the other Amazonian species, the levels being comparable to the highest activities noted in the north-temperate-zone species (Table 1). Heart HK activity levels were significantly higher in yellow perch and rainbow trout than in American eel and bullhead. The lowest HK activity levels were observed in acará-açu heart. The tambaqui heart had the highest PFK activity levels among the Amazonian species (significantly higher than tamoatá and acará-açu heart). Yellow perch and rainbow trout tended to have the highest heart PFK activity levels among the north-temperate-zone species, while acará-açu had lower heart PFK activity levels than all the other species. The highest level of heart PK activity occurred in yellow perch,

Table 2. Enzymes of fatty acid metabolism, cytochrome oxidase, and citrate synthase in hearts of Amazonian and north-temperate-zone fishes.

	HOAD	CPT	Cyt Ox	CS
Amazonian species				
Acará-açu	5.8±0.4 (6)	1.32±0.03 (4)	43.0±6.2 (5)	10.4±0.7 (6)
Tambaqui	8.7±1.0 (4)	—	13.9±1.5 (4)	13.9±3.0 (4)
Tamoatá	10.7±0.9 (5)	3.3±0.4 (4)	29.1±2.1 (5)	20.3±1.3 (5)
Acari-bodó	6.6±1.0 (5)	1.9±0.3 (4)	12.3±1.4 (5)	19.5±1.4 (5)
Pirarucu	2.4±0.4 (3)	1.0±0.07 (3)	24.5±1.3 (3)	23.0±2.0 (3)
Mean	6.8±1.4	1.9±0.51	24.6±5.9	17.4±2.3
North-temperate-zone species				
Yellow perch	7.6±0.7 (4)	1.4±0.3 (3)	48.6±7.9 (5)	26.4±2.3 (4)
Rainbow trout	17.8±2.3 (7)	2.2±0.2 (4)	81.5±11.8 (4)	47.8±3.1 (7)
American eel	10.3±0.5 (6)	2.1±0.14 (4)	30.9±3.5 (4)	38.9±3.0 (6)
Bullhead	5.6±0.1 (4)	0.8±0.2 (3)	40.9±2.6 (4)	12.5±0.31 (4)
Mean	10.3±2.7	1.6±0.3	50.5±11.0	31.4±7.7
<i>P</i> (Amazonian vs. north-temperate-zone species)	0.258	0.688	0.059	0.094

Note: Values are given as the mean ± SEM ($\mu\text{mol}\cdot\text{min}^{-1}\cdot\text{g}^{-1}$), with the sample size given in parentheses; the assay temperature was 22–25°C. CPT assays were not conducted with tambaqui heart.

Table 3. Enzymes of carbohydrate metabolism in red muscle of Amazonian and north-temperate-zone fishes.

	HK	PFK	PK	LDH
Amazonian species				
Tambaqui	0.45±0.1 (5)	15.6±1.4 (5)	99.3±7.8 (4)	115±13 (7)
Acari-bodó	Not detectable (5)	29±6.8 (5)	59.5±1.8 (5)	96±13 (5)
North-temperate-zone species				
Rainbow trout	0.26±0.1 (4)	21.8±9 (4)	106.8±11.2 (8)	227±6* (4)
Bullhead	1.49±0.1 [†] (4)	48±4.5 (4)	121±5.2 [†] (4)	337±13 [†] (4)

Note: All values are given as the mean ± SEM ($\mu\text{mol}\cdot\text{min}^{-1}\cdot\text{g}^{-1}$), with the sample size in parentheses; assay temperature was 22–25°C.

*Statistically significantly different from tambaqui.

[†]Statistically significantly different from acari-bodó.

the level was significantly higher than in all the other north-temperate-zone species. In species other than yellow perch, heart PK activity ranged from ≈ 30 to $90 \mu\text{mol}\cdot\text{min}^{-1}\cdot\text{g}^{-1}$. Heart LDH activity was highest in the American eel (significantly higher than in the other north-temperate-zone species) and lowest in the acará-açu.

Tamoatá heart had the highest HOAD activity levels among the Amazonian species (significantly higher than acará-açu and acari-bodó hearts). Rainbow trout had significantly higher heart HOAD activity levels than the other north-temperate-zone species (Table 2). Tamoatá heart had significantly higher CPT activity levels than the heart of any of the other Amazonian species. Within north-temperate-zone species, levels of CPT activity were similar in all hearts, with the exception of the bullhead heart, in which activity was significantly lower than in rainbow trout and American eel hearts. Among the Amazonian species, heart Cyt Ox activity levels were significantly higher in the acará-açu than in any of the other Amazonian species except the tamoatá. Within north-temperate-zone species, heart Cyt Ox activity levels in rainbow trout was significantly higher than in the other three species. Tamoatá, acari-bodó, and pirarucu hearts had CS activity levels around $20 \mu\text{mol}\cdot\text{min}^{-1}\cdot\text{g}^{-1}$, which tended to be higher than in tambaqui heart and were significantly higher than in acará-açu heart. In north-temperate-zone species, rainbow trout and American eel had

significantly higher heart CS activity levels than the other two species.

Red-muscle enzymes

The bullhead displayed the highest red muscle HK activity levels of the four species assayed; there was no detectable HK activity in the red muscle of acari-bodó (Table 3). Tambaqui and rainbow trout had similar levels of red muscle HK activity. Bullhead red muscle had the highest PFK activity levels. There was no significant difference in PFK activity in red muscle between rainbow trout and tambaqui or between bullhead and acari-bodó. PK activity levels in red muscle were significantly higher in bullhead than in acari-bodó. There was no difference in red-muscle PK activity levels between rainbow trout and tambaqui. There were significantly higher levels of LDH activity in red muscle of rainbow trout and bullhead than in red muscle of tambaqui and acari-bodó, respectively.

Activity levels of all the indicator enzymes directly linked to fatty acid catabolism were significantly higher in red muscle from the north-temperate-zone species than in red muscle from the Amazonian species (Table 4). That is, red muscle HOAD and CPT activity levels were higher in rainbow trout and bullhead than in tambaqui and acari-bodó, respectively. As for HOAD and CPT, Cyt Ox and CS activity levels were significantly higher in red muscle from the

Table 4. Enzymes of fatty acid metabolism, cytochrome oxidase, and citrate synthase in red muscle of Amazonian and north-temperate-zone fishes.

	HOAD	CPT	Cyt Ox	CS
Amazonian species				
Tambaqui	8.1±1.6 (7)	0.79±0.08 (4)	9.8±1.5 (5)	12.4±1.6 (7)
Acari-bodó	3.1±0.6 (5)	0.39±0.01 (3)	3.5±0.26 (5)	4.6±0.42 (5)
North-temperate-zone species				
Rainbow trout	29±3.7* (4)	3.2±0.42* (4)	35.6±5.2* (4)	41.4±3.4* (4)
Bullhead	6.7±0.45 [†] (4)	0.87±0.08 [†] (4)	16.6±0.54 [†] (4)	19.9±2.0 [†] (4)

Note: All values are given as the mean ± SEM ($\mu\text{mol}\cdot\text{min}^{-1}\cdot\text{g}^{-1}$), with the sample size given in parentheses; assay temperature was 22–25°C.

*Statistically significantly different from tambaqui.

[†]Statistically significantly different from acari-bodó.

north-temperate-zone species than in red muscle from the Amazonian species.

Discussion

Within the Amazonian species, the activity levels of enzymes required for the use of glucose and glycogen in anaerobic metabolism (i.e., PFK, PK, and LDH) do not correlate with the ability of ventricular strips to maintain force development in the face of an anoxic challenge (Bailey et al. 1999), nor are the activity levels of these enzymes any higher in the gill-breathing acará-açu and tambaqui relative to the facultative (acari-bodó and tamoatá) and obligate (pirarucu) air-breathers sampled. One potentially important point is that the activity level of HK, which catalyzes the first step in glucose breakdown, is higher in the two armoured catfish species, which show a greater capacity to maintain ventricular-strip contraction under cyanide poisoning than do the acará-açu and tambaqui, which fail (Bailey et al. 1999). High activity levels of HK would be consistent with the indirect arguments that extracellular glucose is used by the fish heart as a metabolic fuel under oxygen limitation (Bailey et al. 1991) and that enhanced uptake of glucose by American eel ventricular strips takes place under anoxia (Rodnick et al. 1997). There is no obvious correlation between the potential to obtain oxygen through air-breathing and activity levels of cardiac enzymes associated only with aerobic energy metabolism (i.e., Cyt Ox, CS, CPT, and HOAD). Paradoxically, the activity level of Cyt Ox is highest in the heart of acará-açu, a species that must tolerate anoxic water.

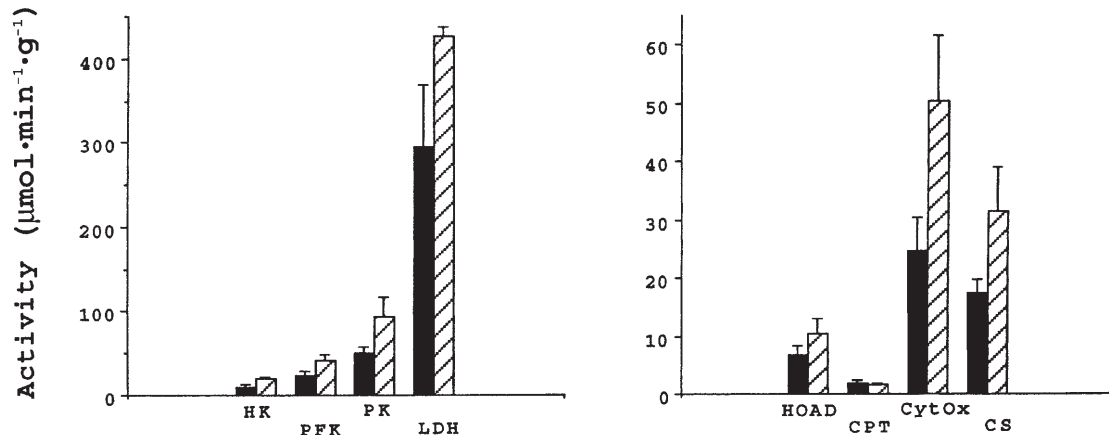
Among the north-temperate-zone species studied, as in the Amazonian species, there was no consistent pattern of enzyme activity associated with anaerobic glycolysis and either whole-animal hypoxia tolerance or the ability of isolated hearts to maintain performance under conditions of impaired oxidative phosphorylation (Bailey et al. 1999). One aspect of species-specific fine-tuning may be the extremely high level of LDH activity in American eel heart, a tissue that has high anoxia resistance. HK, PFK, and PK activity levels, however, were not remarkable in American eel heart. Trout heart consistently had the highest activity levels of enzymes involved only with aerobic energy metabolism (i.e., Cyt Ox, CS, HOAD, and CPT). This may be related to high-speed swimming with its associated high cardiovascular demand.

Since the analysis indicates that heart enzyme activity patterns are not generally associated with aerial respiration in

Amazonian fish or the anaerobic tolerance of hearts from either Amazonian or north-temperate-zone species, it is possible to compare these groups to assess whether thermal history is a critical factor in levels of enzyme activity. The life cycles of the Amazonian species occur at temperatures between about 25–35°C; the north-temperate-zone species typically live at temperatures between about 0 and 20°C on an annual basis. Tables 1 and 2 present the mean values for each of the enzymes for these two broad groups of fishes. All assays were conducted at a common temperature of about 25°C and enzyme activity is considered to reflect the amount of protein present. No single enzyme shows a significant difference between the Amazonian and north-temperate-zone species, although a number (e.g., HK, PFK, PK, Cyt Ox, and CS) approach *P* values of less than 0.05. The mean values in most cases are approximately twice as high in north-temperate-zone than in Amazonian species (Fig. 1). The one exception to this finding was CPT, activity levels of which were similar in hearts from Amazonian and north-temperate-zone species. When the indicator enzymes measured are considered as a group, a paired statistical analysis reveals significantly higher activity levels in hearts of north-temperate-zone species than in those of Amazonian species (*P* = 0.016, *n* = 8). Therefore, it appears that the thermal history due to geographic origin may be an important determinant of the enzyme framework in the hearts of these animals.

In comparative studies involving red muscle, it is necessary to select species carefully, given the range of activity patterns among fishes and the consequent demands on energy metabolism. In this experiment, information was obtained for two Amazonian and two north-temperate-zone teleosts. The acari-bodó and bullhead are very similar in body form and, at least in aquaria, have similar swimming abilities. The other pair of species, tambaqui and rainbow trout, also appear similar in body form and apparent swimming ability, although the match is less precise. As the selection of species to be compared was based on anecdotal observation, caution must be exercised in the interpretation of the data. The mean activity level for each of the enzymes, with one exception, was higher in the north-temperate-zone than in the Amazonian species (i.e., bullhead versus acari-bodó; rainbow trout versus tambaqui). The one exception to this was red-muscle HK activity, where levels in tambaqui and rainbow trout were low and similar. In most of the 16 cases, the difference in enzyme activity levels between the Amazonian and north-temperate-zone species was statisti-

Fig. 1. Activity levels of enzymes involved in energy metabolism in hearts of fishes. Values are given as the mean \pm SEM for Amazonian species (solid bars) and north-temperate-zone species (hatched bars). Data are from Tables 1 and 2.



cally significant, the exceptions being HK, PFK, and PK between rainbow trout and tambaqui and PFK between bullhead and acari-bodó ($P = 0.64$). Therefore, it appears that, generally, enzyme activity levels, especially those related to aerobic fatty acid metabolism, are greater in red muscle from the north-temperate-zone species than in red muscle from the Amazonian species. We cannot rule out the possibility that this observation is due to differences in oxygen delivery to muscle, to locomotory patterns, or to some other factor; however, it is likely that thermal history is an important determinant.

In summary, there is no obvious relationship between the overall pattern of enzyme expression and anaerobic tolerance. Levels of the suite of enzymes associated with anaerobic glycolysis are not elevated in hearts with a high resistance to anoxia. Some refinement of this concept may occur, as in the hearts of the armoured catfishes and the American eel, which have a high tolerance to anoxia in concert with high HK activity levels in the former and high LDH activity levels in the latter. However, there are general differences in enzyme activities between Amazonian and north-temperate-zone species, in that the levels are usually higher in the latter group. The most likely explanation for this, and the one that is consistent with the current literature, is the impact of thermal history. Enzyme activity levels are often higher in Antarctic species than in north-temperate-zone species (Crockett and Sidell 1990; Driedzic 1992) and within north-temperate-zone species when they have been acclimated to low as opposed to high temperatures (for instance, see Jones and Sidell 1982; Johnston et al. 1985; Way-Kleckner and Sidell 1985; Guderly and Gawlicka 1992). In the current experiment, enzyme activity levels were determined at a common assay temperature of about 25°C. This is about 10°C higher than the temperature to which the north-temperate-zone species were acclimated under laboratory conditions. Enzyme activity levels were typically 1.5- to 2.2-fold higher in hearts from north-temperate-zone species than in those from Amazonian species. In red muscle, the range was greater, a number of enzymes being 3- to 4-fold more active in the north-temperate-zone species than in the comparable Amazonian species. The larger differences in red muscle than in heart may be a function of the limited comparison and of the differing energy demands

made on skeletal muscle. The Q_{10} values for the activity of the enzymes measured here as a function of assay temperature have been determined in other studies and have been found to be highly variable with respect to enzyme and species, with typical Q_{10} values ranging from about 1.5 to 3 (Crockett and Sidell 1990; Bailey et al. 1991; Sephton and Driedzic 1991). Therefore, if the in vitro enzyme activity levels noted for the north-temperate-zone species are decreased by about 2-fold to bring them into the physiological range, the resultant activity levels would be similar in the two groups of animals. That is, the same metabolic capacity would exist in the tissues of the Amazonian and north-temperate-zone species at their respective body temperatures. This finding extends the continuum from Antarctic to temperate-zone to tropical fishes. The lower the life-cycle body temperature, the greater the expression of many of the enzymes associated with energy metabolism.

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