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H.G. Kawall · J.J. Torres · B.D. Sidell · G.N. Somero

Metabolic cold adaptation in Antarctic fishes: evidence from enzymatic activities of brain

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Abstract To evaluate the concept of metabolic cold adaptation (MCA) in fishes, we compared – in brain, red muscle, and white muscle of Antarctic notothenioid fishes and tropical/subtropical fishes – the activities of two enzymes of ATP-generating pathways, citrate synthase (CS), an indicator of citric acid cycle activity (aerobic metabolism), and lactate dehydrogenase (LDH), an indicator of potential for ATP production through anaerobic glycolysis. Brain was chosen because, unlike locomotory muscle, its metabolic activity is not likely to be influenced by a species' level of activity or nutritional status, so MCA should be readily observed if present. CS and LDH activities in brain exhibited a high level of MCA, but compensation to temperature was not complete (48% for CS; 46% for LDH). CS and LDH activities in red and white muscle varied widely among species, according to the general level of locomotory activity. The 'mode of life'-related enzymatic activities in locomotory muscle show that study of MCA at the level of whole organism metabolism is fraught with difficulties and experimental ambiguities. In contrast, the low variation among species within each group in enzymatic activities in brain, and the large differences between groups in CS and LDH activity, show that brain is an excellent study system for evaluating metabolic compensation to temperature.

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H.G. Kawall · J.J. Torres (✉)
College of Marine Science, University of South Florida,
140 Seventh Avenue South, St. Petersburg, FL 33701, USA

E-mail: jtorres@seas.marine.usf.edu
Tel.: +1-727-5531169
Fax: +1-727-5531189

B.D. Sidell
School of Marine Sciences, University of Maine,
5741 Libby Hall, Orono, ME 04469-5741, USA

G.N. Somero
Hopkins Marine Station, Stanford University,
Department of Biological Sciences, Pacific Grove,
CA 93950-3094, USA

Introduction

Metabolic cold adaptation (MCA), a concept stating that polar, temperate, and tropical ectotherms exhibit similar metabolic rates at normal habitat temperature, was first proposed by Krogh (1914) and later demonstrated in a classic paper by Scholander et al. (1953) that compared the metabolic rates of terrestrial and aquatic poikilotherms from the Arctic with those of tropical species. They found significantly higher metabolic rates in polar species than in tropical and temperate species when all rates were extrapolated to the low habitat temperature of polar species. MCA, as its name implies, thus regards the metabolic rates of polar species as having undergone an upward adjustment or compensation to offset the effects of low temperature.

MCA received considerable support through a series of studies on whole organism oxygen consumption rates of fishes (Wohlschlag 1960, 1963, 1964; Ralph and Everson 1968; Hemmingsen and Douglas 1970; Morris and North 1984; Torres and Somero 1988a, b) and on metabolism *in vitro* of isolated tissues of Antarctic and temperate fishes (Somero et al. 1968). Despite evidence for MCA in fishes, however, the concept was called into question by Holeton (1974), who questioned the validity of the concept on the basis of both methods and philosophy. Briefly, his arguments focused on the acute experimental methods of Scholander et al. (1953), which in all likelihood resulted in artifactually elevated rates in the polar species they studied, and on the use of Krogh's 'normal curve' for extrapolation of the rates of tropical species to polar temperatures, which would exaggerate the effect of cold adaptation. A further objection brought forth by Holeton (1974) concerned the logic of regarding an elevated standard metabolism as advantageous to cold-water species, when it would only serve to remove energy from other processes such as growth and reproduction. Holeton's arguments have been developed further by Clarke (1983, 1991, 1998) in a series of thoughtful reviews. Clarke, too, conjectured that

attempts to document MCA, especially through studies using whole organism respiration, are likely to fail. Recent work by Zimmermann and Hubold (1996) has carefully analyzed the influence of mode of life, especially a fish's activity level, on respiration rate. Their analyses, too, speak of the difficulty of quantifying MCA in species having different activity levels.

Despite the methodological and philosophical criticisms of the concept of MCA, the fact remains that polar fishes perform all of the functions requisite to life at temperatures lethal to tropical and many temperate species. Although some of the adaptations to low temperature found in polar fishes may be strictly concerned with survival per se at low temperatures, for instance, antifreeze thermal hysteresis proteins (Cheng 1998), other adaptations do appear to be related to thermal compensation of metabolic activity, for instance, enzymes with exceptionally high catalytic activities (k_{cat} values) in Antarctic notothenioid fishes (Fields and Somero 1998). Crockett and Sidell (1990) also report significantly higher activities of enzymes from pathways of aerobic and fatty acid metabolism in tissues of Antarctic fishes than ecotypically similar Temperate Zone species. However, even though there is strong evidence that temperature compensatory adaptations exist at the level of biochemical function, controversy remains as to whether polar species exhibit an overall metabolism higher than would be expected when the rates of temperate and tropical species are extrapolated to colder temperatures.

The present study was designed to shed light on the MCA controversy and on thermal compensation in general by following an experimental strategy that avoided the major pitfalls of much past work on this topic. Rather than examining whole organism metabolism as done in most past studies, we used the activities of enzymes associated with major ATP-generating pathways as a proxy for metabolism of individual tissues. Use of enzymatic activities eliminates effects of (1) the handling stress of direct respiratory measurements, (2) overshoot reactions of fish to abrupt changes in temperature, (3) much of the variation due to differences in nutritional state, and (4) variations in reproductive condition of the experimental animals.

The design of our experiment involved decisions related to appropriate species, enzymes, and tissues to study. We chose to examine a suite of highly cold-adapted Antarctic fishes and warm-adapted tropical/subtropical species to provide a wide range of adaptation temperatures and modes of life (locomotory habit, general activity level, and foraging strategy; see Zimmermann and Hubold 1996). To estimate metabolic activity, we used two enzymes from major ATP-generating pathways: citrate synthase (CS), an indicator of citric acid cycle activity and, therefore, of aerobic ATP-generating potential, and lactate dehydrogenase (LDH), the terminal enzyme in anaerobic glycolysis, whose activity is a strong indicator of locomotory potential (Childress and Somero 1979).

Choice of tissues/organs was especially important for avoiding the pitfalls that might preclude evaluating the extent of MCA in Antarctic fishes. Brain tissue was chosen for studying MCA because it performs the same function in all fishes and its metabolism is less likely to be influenced by the mode of life of a fish. Similar enzymatic activities have been reported in the brains of temperate fishes with diverse locomotory habits (Somero and Childress 1980; Sullivan and Somero 1980; Siebenaller and Somero 1982), which suggests that, at a common adaptation temperature, only minimal variation in the ATP-generating capacity of the brain exists among fishes. Brain enzymatic activity, unlike that of muscle, is also not influenced by a fish's nutritional condition (Yang and Somero 1992). We also investigated CS and LDH activity in red and white locomotory muscle because these two tissues, white muscle in particular, constitute a large fraction of a fish's mass, and therefore contribute importantly to whole organism metabolic rate. In summary, by measuring metabolic potential in the brain and muscle tissues of fishes from widely different thermal regimes and having different modes of life, our experimental design allows a rigorous test of the reality of MCA while, at the same time, enabling us to biochemically evaluate the major criticisms by Holeton, Clarke, and others of studies of whole organism MCA.

Materials and methods

Experimental organisms

Eight Antarctic species were studied, all belonging to the suborder Notothenioidei (Table 1). Five of the eight species were in the family Nototheniidae, including the active cryopelagic hunter *Pagothenia borchgrevinki*, and the less active benthic ambush predators *Trematomus bernacchii*, *Trematomus hansoni*, *Trematomus newnesi*, and *Trematomus pennelli*. Three Antarctic icefish (Channichthyidae) were also investigated, two of them benthic, *Chaenocephalus aceratus* and *Chionodraco rastrospinosus*, and one pelagic, *Champsocephalus gunnari*. Tropical/subtropical species examined were the active reef-associated damselfish *Pomacentrus dorsopunicans*, the wrasses *Halichoeres bivittatus* and *Halichoeres radiatus*, the chub *Kyphosus* sp., the lie-in-wait predator *Centropomus undecimalis*, and the sluggish freshwater *Oreochromis* sp.

Collection of specimens

Antarctic fishes were collected in McMurdo Sound during December 1994 and January 1995, and in the Antarctic Peninsula region in January 1996. Specimens of *Oreochromis* sp. were obtained from a fish market in San Jose, Calif. Tropical/subtropical species were collected on the west coast of Florida and in the Florida Keys in May or June of 1995 and 1996. They were caught with rectangular nets, trawls, or fish traps, or by using hook and line. Fishes were either frozen immediately in liquid nitrogen or brought alive to the laboratory where they were frozen in a cryogenic freezer (–80 °C). All fishes were stored at –80 °C. Loss of enzymatic activity was not observed between fishes immediately analyzed (McMurdo Station, 1995) and fishes stored for 6–12 months and analyzed in the United States (Hopkins Marine Station, Calif., 1996).

Table 1 Enzyme activities (international units g WM^{-1}) at 10 °C in brain, red muscle and white muscle of fishes from Antarctic and tropical regions. Species (number of replicates), *WM* wet mass (range), *TL* total length (range), enzyme activity [means(±SD)], *CS* citrate synthase, *LDH* lactate dehydrogenase

Antarctic species (8)	WM (g)	TL (mm)	CS Brain	CS Red muscle	CS White muscle	LDH Brain	LDH Red muscle	LDH White muscle
Pagothenia borchgrevinki (8)	49.8–128.0	180–280	4.78 (0.49)	50.57 (14.63)	1.30 (0.36)	44.51 (12.11)	57.61 (6.09)	99.53 (6.54)
Trematomus bernacchii (12)	24.4–185.0	150–220	4.43 (0.49)	37.29 (9.83)	1.46 (0.85)	42.63 (3.06)	40.85 (20.59)	89.63 (38.52)
Trematomus hansoni (2)	63.3–83.2	160–170	4.54 (0.06)	39.25 (4.74)	1.35 (0.18)	43.30 (6.51)	51.50 (17.68)	45.25 (5.44)
Trematomus newnesi (1)	41.0	158	5.17	44.50	2.31	48.70	43.90	47.50
Trematomus pennellii (2)	18.8–30.2	120–132	5.39 (0.39)	26.05 (12.52)	1.24 (0.18)	56.50 (2.40)	53.10 (15.84)	64.55 (38.54)
Chaenocephalus aceratus (4)	754.0–1,895.0	460–550	5.17 (0.12)	40.02 (12.46)	1.30 (0.52)	63.13 (3.64)	163.50 (63.59)	150.83 (50.93)
Champsocephalus gunnari (4)	528.0–839.0	430–490	5.08 (.067)	35.43 (2.25)	1.72 (0.21)	54.33 (7.13)	84.28 (10.38)	220.63 (69.76)
Chionodraco rastrospinosus (4)	594.0–774.0	400–475	5.22 (0.43)	32.89 (2.78)	2.33 (0.58)	56.60 (6.53)	110.18 (23.90)	196.13 (75.22)
Mean (±SD)			4.97 (0.35)	38.25 (7.38)	1.63 (0.45)	51.59 (8.24)	75.62 (42.46)	114.26 (67.47)
Tropical species (7)								
Kyphosus sp (6)	107.0–198.0	170–215	2.87 (0.62)	10.99 (5.05)	1.63 (0.51)	18.98 (2.78)	129.29 (30.36)	230.04 (53.52)
Pomocentrus dorsopunicans (8)	9.4–24.0	78–105	3.28 (1.27)	13.57 (3.00)	2.87 (0.56)	18.20 (6.18)	78.74 (22.77)	45.41 (15.34)
Halichoeres bivittatus (8)	8.8–50.0	93–155	3.66 (1.45)	19.09 (3.51)	1.67 (0.47)	12.09 (2.66)	77.78 (9.36)	75.16 (9.68)
Halichoeres radiatus (2)	36.5–54.0	150–175	2.41 (0.28)	16.66 (1.39)	2.21	11.70 (1.27)	74.00 (9.91)	54.50 (13.43)
Oreochromis sp. (4)	420.0–529.0	270–290	2.01 (0.42)	7.14 (1.75)	0.28 (0.09)	23.70 (7.50)	240.50	137.80 (15.91)
Centropomus undecimalis (4)	645.8–1,592.0	500–657	2.25 (1.20)	8.41 (3.38)	0.66 (0.22)	19.97 (2.78)	169.19 (66.17)	172.08 (36.37)
Mean (±SD)			2.75 (0.26)	12.28 (4.65)	1.55 (0.96)	17.44 (4.69)	128.25 (66.66)	119.17 (30.00)

Enzymatic assays

The entire brain was removed from the fishes and then homogenized at a ratio of 9:1 [volume buffer: wet mass (WM) tissue] in 20 mM Tris/HCl (pH = 7.2 at 20 °C) using a conical glass homogenizer (Duall model; Kontes, Vineland, N.J.). White epaxial muscle was removed from directly behind the head and red muscle was removed from the pectoral fin region (pectoral fin adductor) in all species. In the notothenioids, which lack myoglobin in oxidative skeletal muscles, the physiological equivalent to normal red muscle was located by its position in the pectoral girdle and by its typical yellow color (cf. Eastman 1993). Muscle and brain tissue were otherwise treated identically. Throughout the process, tissues and homogenates were kept ice-cold. Uncentrifuged homogenates were used in all assays to avoid loss of CS activity in pelleted mitochondria. Enzymatic activities were measured at 10 °C in a thermostatted Perkin-Elmer Lambda 3B spectrophotometer. LDH activities were measured following the methods of Torres and Somero (1988a). CS activities were also assayed according to Torres and Somero (1988a), but with the medium containing a higher concentration of DTNB [1.0 mM 5,5-dithiobis (2 nitrobenzoic acid)]. Assays were performed in duplicate and activities averaged. Units for activity are micromoles of substrate converted to product min^{-1} g WM^{-1} of tissue (international units g WM^{-1}).

Statistical analysis

Mean enzymatic activities were calculated for each species (Table 1). Subsequently, comparisons of enzymatic activities were performed between fishes from the two climatic zones: Antarctic, and tropical/subtropical. Two methods were used. First, species were weighted equally by testing the suite of species means for differences between zones by using one-way analysis of variance (ANOVA). Second, the entire (raw) data set was used to test for differences between zones, also using ANOVA, which weighted most heavily those species with the highest number of replicates. Using the entire data set also enabled us to eliminate any influence of mass on the trends in enzymatic activity between climatic zones by allowing us to use mass as a covariate in the analysis (analysis of covariance: ANCOVA). Results using species means and the entire data set with mass as a covariate differed in only one case, which is reported below. Otherwise, only the results from the ANOVAs using species means are reported. In either case, when variances were not homogeneous, the non-parametric Kruskal-Wallis test was substituted for ANOVA, or in cases where mass needed to be eliminated as a factor, data were log-transformed and ANOVA run with mass as a covariate.

Within climatic zones the influence of mass on variability in the data was first examined using correlation analysis. If correlation analysis showed significant interactions between mass and enzymatic activity, regressions were performed to determine the extent of the interaction. All means were reported ±standard deviation (±SD).

Q_{10} estimations

To convert rates of enzymatic activity from 10 °C to a species' habitat temperature, we determined CS and LDH activities in the brains of Antarctic and tropical/subtropical species at 5, 10, 20, and 30 °C, and the Q_{10}s were then calculated for each 10 °C interval (Table 2). Due to the difficulty of working at 0 °C, for the 0–10 °C interval the Q_{10} was calculated using activities determined between 5 °C and 10 °C, with the value obtained (Q_5) then multiplied by 2.

Results

Enzymatic activity: brain

Table 1 shows CS and LDH activities in the brains of Antarctic and tropical/subtropical fishes measured at

10 °C. Activity of CS in the brains of Antarctic fishes was 1.9 times higher than that in tropical/subtropical species, and this difference between the two groups was highly significant (ANOVA: $df = 13$, $F = 70.77$, $P < 0.0001$). At the common measurement temperature of 10 °C, the difference in LDH activity between Antarctic and tropical/subtropical fishes was even larger than that observed in CS: 2.5-fold. This inter-group difference too was highly significant (ANOVA: $df = 13$, $F = 81.91$, $P < 0.0001$).

Mean Q_{10}s of CS activities ranged from 1.49 in the brains of tropical/subtropical species between 20 °C and 30 °C, to 1.84 in the brains of tropical fishes between 0 °C and 10 °C (Table 2). When Q_{10} values for CS activity were averaged for all species in all three temperature intervals, the global value obtained was 1.65 (± 0.11, $n = 6$). For LDH activities, mean Q_{10}s varied from 1.81 in Antarctic brain tissue between 20 °C and 30 °C, to 2.58 in tropical brain tissue in the 10–20 °C range (Table 2). The mean Q_{10} for LDH activity was 2.14 (± 0.33, $n = 6$).

Enzymatic activity: red muscle

Citrate synthase activity in the red muscle of Antarctic and tropical/subtropical species was significantly different ($df = 13$, $F = 54.95$, $P < 0.0001$), with the values for CS activity in Antarctic species over three times those observed in tropical/subtropical species when measured at the same temperature (10 °C, Table 1). In contrast, when species means were tested for differences between climatic zones using ANOVA, LDH activities showed no significant differences between the two climatic groups ($df = 13$, $F = 3.27$, $P = 0.10$). However, when the raw data were tested with mass as a covariate, LDH showed a significantly higher activity in tropical species ($df = 57$, $F = 38.306$, $P < 0.0001$).

Enzymatic activity: white muscle

White muscle showed no significant inter-group differences in the activity of either CS or LDH when Antarctic and tropical/subtropical species were compared (CS: Kruskal-Wallis, $P \gg 0.05$; LDH: $df = 13$, $F = 0.017$, $P = 0.900$; Table 1). In fact, mean activity values for both enzymes were virtually identical in the Antarctic and tropical species.

Table 2 Estimated $Q_{10\ \text{values}}$ for CS and LDH activities in the brains of fishes from Antarctic and tropical/subtropical climatic regimes. Values are means ($\pm$SD, number of individuals). n = Number of treatments

Climatic regime	Temperature interval (°C)		
Enzyme	0–10	10–20	20–30
Antarctic			
CS	1.62 (0.26, 3)	1.65 (0.25, 5)	1.63 (0.08, 2)
LDH	2.09 (0.11, 2)	1.97 (0.25, 2)	1.81 (0.23, 2)
Tropical/subtropical			
CS	1.84 (0.06, 2)	1.65 (0.34, 4)	1.49 (0.22, 3)
LDH	2.51 (0.74, 2)	2.58 (0.59, 3)	1.89 (0.42, 3)
Global means			
CS	1.65 (0.11, $n = 6$)		
LDH	2.14 (0.32, $n = 6$)		

Sources of variability within climatic groups: Antarctic

CS activity among the eight Antarctic species showed a maximum to minimum ratio of 1.22 in brain tissue, 1.94 in red muscle, and 1.88 in white muscle. LDH showed a higher variability, with a ratio of 1.48 for brain, 3.72 in red muscle and 4.88 in white muscle. Some of the variability in activity was potentially attributable to the range in mass among Antarctic species (Table 1). No significant relationships between mass and enzymatic activity were observed in CS activity for Antarctic species. However, LDH showed significant positive scaling for enzymatic activity (Y) vs mass (X) in all tissues, with slopes (b) for the equation $Y = aX^b$ ranging from 0.10 in brain to 0.36 in red muscle (Table 3).

Another potential source of variability was the fact that three of the Antarctic species were members of the hemoglobin-less family Channichthyidae. Here, it was imperative to eliminate mass as a factor when testing for differences between the two families because the channichthyids were an order of magnitude higher in average mass than the nototheniids (Table 1). In a series of ANCOVAs testing for differences in enzymatic activity between the nototheniids and channichthyids, no significant differences between the two families were observed in CS activity, nor were any differences observed in the activity of LDH in brain and red muscle. However, significantly higher LDH activity was observed in the white muscle of channichthyids. ($df = 28$, $F = 14.239$, $P < 0.001$).

'Mode of life' differences within the suite of Antarctic species were limited to comparing the two pelagic species, *Pagothenia borchgrevinki* and *Champsocephalus gunnari*, versus the remainder of the species, which were all classified as benthic or demersal. No significant differences related to mode of life were detected in any tissue ($P > 0.05$, ANOVA).

Sources of variability within climatic groups: tropical/subtropical

Variability (maximum:minimum ratio) in CS activity was low in brain (1.63) and red muscle (2.67), but high in

Table 3 LDH activity (y: units g WM^{-1}) vs mass (x) in Antarctic species; a and b values are from the equation: $y = aX^b$

Tissue	a	b	P	F	n	r^2
Brain	0.53	0.10	< 0.001	55.84	36	36.47
Red muscle	0.36	0.36	< 0.001	42.43	32	58.58
White muscle	0.46	0.34	< 0.001	30.57	31	51.32

white muscle (10.25). Variability of LDH in tropical species showed similar trends to the Antarctic group. Brain LDH activity had a range of 1.70, red muscle 3.09, and white muscle 4.22. Some of the observed variability could be attributed to mass, which varied over a 170-fold range in the tropical/subtropical group. Significant regressions between enzymatic activity and mass were observed in all tissues for both enzymes (Table 4). In the case of CS, negative scaling occurred, i.e., CS activities were lower in larger specimens. For the equation $Y = aX^b$, where Y is enzymatic activity and X is mass, b values for CS ranged from –0.12 in brain to –0.38 in white muscle. LDH scaled positively with mass, with b values of 0.10 in brain, 0.21 in red muscle, and 0.32 in white muscle.

Differences in enzymatic activity that could be attributed to differences in mode of life among tropical/subtropical species were tested using ANCOVA with mass as a covariate, which allowed us to subtract the influence of mass on the comparison. All of the smaller species, including the chub *Kyphosus*, the damselfish *Pomacentrus*, and the wrasses, are active reef fishes and were grouped into an 'active reef fish' mode of life category. The two larger species include the snook, *Centropomus undecimalis*, a lie-in-wait ambush predator, and *Oreochromis*, a sluggish freshwater omnivore, which were grouped together in an 'ambush' lifestyle category. Significant differences were only observed between the active reef fishes and the ambush lifestyle in CS activity of red muscle ($df = 30$, $F = 7.75$, $P < 0.001$) and CS activity of white muscle ($df = 29$, $F = 11.28$, $P < 0.01$).

Enzymatic activity at normal environmental temperatures: brain

Figures 1 and 2 show the changes in CS and LDH activities, respectively, with temperature in brain tissue of fishes in the two groups. Graphs were generated using the mean Q_{10} values found in this study, 1.7 for CS, and 2.1 for LDH, to adjust enzymatic activities measured at 10 °C to the temperatures encountered by the species within their geographic distributions (approximately 0 °C for Antarctic fishes and 25 °C for tropical/subtropical species).

To obtain an index of temperature compensation, the following computation was performed. The percent of enzymatic activity in Antarctic fish brains

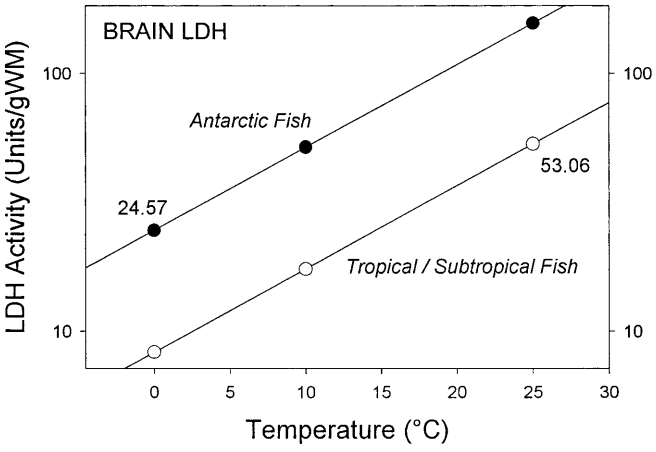

Fig. 1 Citrate synthase (CS) activities (international units g WM^{-1}) in brains of fishes from Antarctic and tropical/subtropical climatic zones in relation to environmental temperature. Curves were generated using the mean CS activities obtained at 10 °C, and adjusted using the experimentally determined Q_{10} of 1.7. Enzyme activities at 0 °C, 10 °C, and 25 °C are denoted by *filled circles* for Antarctic fish and *open circles* for tropical species. Activities at the approximate habitat temperatures of the two groups are given numerically next to the relevant symbols

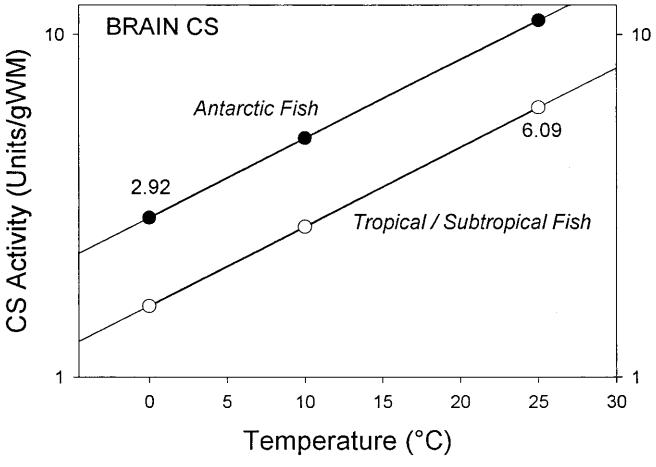

Fig. 2 Lactate dehydrogenase (LDH) activities (international units g WM^{-1}) in brains of fishes from Antarctic and tropical/subtropical climatic zones in relation to environmental temperature. Curves were generated using the mean LDH activities obtained at 10 °C, and adjusted using the experimentally determined Q_{10} of 2.1. Enzyme activities at 0 °C, 10 °C, and 25 °C are denoted by *filled circles* for Antarctic fish and *open circles* for tropical species. Activities at the approximate habitat temperatures of the two groups are given numerically next to the relevant symbols

Table 4 Enzyme activity (y: units gWM^{-1}) vs mass (X) in tropical-subtropical species; a and b values are from the equation: $y = aX^b$

Enzyme	Tissue	a	b	P	F	n	r^2
CS	Brain	0.18	–0.12	0.009	7.86	31	21.33
CS	Red muscle	0.51	–0.18	< 0.001	14.52	33	31.90
CS	White muscle	0.29	–0.38	< 0.001	47.76	32	61.42
LDH	Brain	0.37	0.10	0.019	6.27	28	19.44
LDH	Red muscle	0.57	0.21	< 0.001	38.69	28	59.61
LDH	White muscle	0.50	0.32	< 0.001	29.39	28	53.06

relative to that of tropical species was calculated by dividing the mean Antarctic brain enzymatic activity at 0 °C by the mean activity in the brain of tropical fishes at 25 °C. The resulting fractions were multiplied by 100 to estimate percent change in rate, or level of temperature compensation. CS activity in the brains of Antarctic fishes at 0 °C was 48% of that in tropical species at 25 °C. LDH activity in Antarctic species was 46% of that in tropical species at normal habitat temperatures.

Enzymatic activity at normal environmental temperatures: muscle

Differences in muscle enzymatic activity between the two groups were far less straightforward than differences observed in brain. Significant differences between Antarctic and tropical species were only observed in red muscle CS activity. An equivalent analysis to that described above for brain tissue shows that Antarctic red muscle CS activity was 58% of that in tropical species at normal habitat temperatures.

Discussion

Enzymatic activities in brain

By studying the activities of ATP-generating enzymes in brain, we were able to examine MCA without confounding effects arising from differences in mode of life, activity level in particular, or nutritional condition. The significant degree of temperature compensation in activities of CS (Fig. 1) and LDH (Fig. 2) provides what we feel is one of the stronger lines of evidence offered to date in favor of the concept of MCA. *In vitro* oxygen consumption of brain tissue of the Antarctic notothenid *Trematomus bernacchii* also exhibited a high level of MCA (Somero et al. 1968), in agreement with the enzymatic data of this study.

Despite the displacement of the enzymatic activity versus temperature curves shown in Figs. 1 and 2, compensation of activities is not complete for either enzyme. Rather, CS and LDH activities at approximate habitat temperatures (0 °C for Antarctic fish and 25 °C for tropical/subtropical species) are only about half as high in the Antarctic fishes as in the warm-adapted species. Thus, we conclude that MCA is present, but not fully compensatory to temperature. The reasons why MCA is not complete remain conjectural.

Enzymatic activities of red and white muscle

Much as the measurements of enzymatic activity in brain allow a strong test of the concept of MCA, measurements of activity in locomotory muscle speak clearly to the criticisms of MCA raised by Holeton, Clarke, and others. Because white skeletal muscle typically represents 50–60% of a fish's mass and, therefore, accounts for a substantial percentage of metabolic rate, interspecific differences in locomotory activity levels are likely to mask MCA, unless the comparison species are virtually identical in their locomotory activities. Selection of species with identical modes of life has proven to be a major stumbling block to studies of MCA, and an 'apples to oranges' artifact is often present in such studies. However, when congeneric species from temperate and Antarctic waters have been compared, MCA in whole organism respiration rate and in muscle CS and LDH activities has been observed (Torres and Somero 1988a, b). Crockett and Sidell (1990) found evidence for compensation of aerobic ATP-generating pathways in a comparison of enzymatic activities (CS, cytochrome *c* oxidase, and enzymes of fatty acid oxidation) of heart and locomotory muscle in ecotypically similar Antarctic and temperate marine fishes. CS and cytochrome *c* oxidase activities were from 1.5- to 5-fold higher, and fatty acid oxidation activities were from 1.3- to 27-fold higher in Antarctic species. In contrast, enzymes linked to aerobic and anaerobic carbohydrate metabolism were generally lower in Antarctic species, suggesting that energy metabolism of their muscles is more poised towards oxidation of fatty acids than carbohydrates. These data, while supporting MCA in Antarctic fishes, also indicate the complexity of comparative studies employing muscle tissue. In muscle, unlike brain, ATP generation may be reliant on different energy sources and enzymatic pathways in species with different modes of life. Down-regulation of one ATP-generating pathway and up-regulation of another pathway, as seen by Crockett and Sidell (1990), could make an overall assessment of MCA difficult.

The species examined in the present study represent a number of modes of life (Zimmerman and Hubold 1996). Thus, it is not surprising that wide variation in enzymatic activity among species was observed in both red and white locomotory muscles. To a large extent, these differences reflect the swimming mode of the species. For instance, the nototheniids belonging to the genera *Trematomus* and *Pagothenia* are labriform swimmers that rely on the propulsion provided by their large pectoral fins. The red muscle powering these fins has an exceptionally high level of CS activity, in accordance with its high aerobic potential for sustained locomotion. However, it would be erroneous to regard these high levels of CS activity as an indication of a high degree of MCA at the whole organism level. Red muscle represents an extremely minor fraction of body mass, and although high, the amount of CS activity in red muscles of these species is unlikely to play a dominant role in setting whole organism metabolic rate. The CS activities of white muscle are lower in nototheniids than in all of the tropical/subtropical species studied, and the LDH activities in white muscle of the nototheniids are

relatively low compared to most of the tropical/subtropical species studied. Thus, the metabolic potential of the dominant contributor to body mass shows no evidence for MCA. Our analysis therefore is consistent with the criticisms of the MCA concept at the level of whole organism metabolism, but strongly supports the concept in the context of a highly conserved organ, brain, that carries out identical functions in all species and whose metabolism is not governed by mode of life or nutritional status.

Mechanistic basis of temperature-compensated enzymatic activities

The mechanistic basis for the higher enzymatic activities in brains of Antarctic fishes could be due to (1) higher concentrations of enzymes, (2) more efficient enzymes, or (3) some combination of these two types of adaptation. In the case of LDH, it is likely that differences in catalytic efficiency account for most, if not all, of the approximately 2.5-fold difference in activity observed at 10 °C (Table 1). Fields and Somero (1998) found that the A_4-LDHs of four Antarctic notothenioids had catalytic rate constants (k_{cat}) averaging approximately 250 s^{-1} at 4 °C, whereas orthologs of warm-temperate and tropical fishes had k_{cat} at 0 °C that averaged approximately 125 s^{-1}. Fields et al (2001) also showed that activation energy values of A_4-LDHs of Antarctic notothenioids were lower than those for A_4-LDH of a warm-temperate fish, consistent with the differences in Q_{10} values reported in Table 2. These differences in k_{cat} and activation energy suggest that concentrations of LDH do not differ markedly between Antarctic and tropical fishes, and that the partial temperature compensation observed in brain LDH activity is due largely to variation in k_{cat} values. Temperature compensation of LDH activityis thus likely accomplished by production of a 'better' enzyme rather than production of higher concentrations of enzymes with the lower efficiencies characteristic of warm-adapted A_4-LDHs. There are no comparable data on k_{cat} values of CS, but, because of the widespread occurrence of k_{cat} adaptations to temperature among different classes of enzymes (see Somero 1995 ; Hochachka and Somero, in press), it is reasonable to conjecture that CS, like LDH, may be more catalytically efficient in the Antarctic species. We also know, however, that oxidative tissues of Antarctic fishes contain extremely dense populations of mitochondria, the intracellular location of CS (Londraville and Sidell 1990; O'Brien and Sidell 2000). If mitochondrial density is correlated with CS concentration in these tissues, they may also contain greater concentrations of this enzyme than are found in homologous tissues of warmer-bodied species.

In summary, we have used enzymatic indices of ATP-generating potential to evaluate the extent to which metabolism of Antarctic fishes has compensated for the effects of low temperatures. CS and LDH activities in brain exhibit substantial, yet incomplete, MCA that may be due to higher catalytic activities of cold-adapted orthologs and/or higher concentrations of enzymes. Studies of enzymatic activity in locomotory muscle reveal the difficulties in discerning the effects of MCA from effects due to species' different modes of life, their levels of activity in particular. Our study has thus achieved a two-fold purpose, demonstrating the reality of MCA in polar fishes (brain), while supporting the arguments of critics of the MCA concept, who regard studies of MCA at the level of whole organism metabolism to be plagued with artifacts due to the difficulty of finding ecotypically similar species to compare from different thermal habitats.

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