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PLASTICITY OF FISH MUSCLE MITOCHONDRIA WITH THERMAL ACCLIMATION

HELGA GUDERLEY[1,2] AND IAN A. JOHNSTON[2]

[1]*Département de Biologie, Université Laval, Québec, PQ, Canada G1K 7P4* and
[2]*Gatty Marine Laboratory, University of St Andrews, St Andrews, Scotland, UK*

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Summary

Short-horned sculpin *Myoxocephalus scorpius* were acclimated to 5 and 15 °C to evaluate the impact of thermal acclimation upon maximal rates of substrate oxidation by mitochondria and upon the thermal sensitivity of their ADP affinity. Cold acclimation virtually doubled maximal rates of pyruvate oxidation at all experimental temperatures (2.5, 7.5, 12.5 and 20 °C). Rates of palmitoyl carnitine oxidation were also enhanced by cold acclimation, but to a lesser degree. At their respective acclimation temperatures, the mitochondria attained similar rates of pyruvate oxidation. For warm-acclimated sculpin, the Q_{10} values for mitochondrial pyruvate and palmitoyl carnitine oxidation were higher between 2.5 and 7.5 °C than between 7.5 and 12.5 °C or between 12.5 and 20 °C. In contrast, for cold-acclimated fish, the Q_{10} values did not differ over these thermal ranges. The Arrhenius activation energy for pyruvate oxidation was reduced by cold acclimation (from 70 to 55 kJ mol^{-1}), whereas that for palmitoyl carnitine oxidation was unchanged (approximately 75 kJ mol^{-1}). Cold acclimation did not alter the ADP affinity of mitochondria at low temperatures but markedly increased the apparent K_m for ADP ($K_{m,app}$) at 12.5 and 20 °C. At the acclimation temperatures, mitochondrial ADP $K_{m,app}$ values did not differ. The loss of ADP affinity at higher temperatures may represent a cost of the enhanced maximal oxidative capacity achieved during cold acclimation.

Key words: acclimation, skeletal muscle, mitochondria, short-horned sculpin, *Myoxocephalus scorpius*, temperature, maximal capacities, ADP affinity.

Introduction

For fish and other aquatic ectotherms, environmental temperature markedly influences the rates of physiological processes. Many temperate-zone fish show a range of phenotypic responses which serve to enhance locomotory activity during short-term cold acclimation (Johnston, 1993). Cold acclimation increases mitochondrial volume density, enhances the activities of mitochondrial enzymes and leaves glycolytic capacities largely unchanged (Sidell, 1980; Johnston *et al.* 1985; Egginton and Sidell, 1989; Johnston, 1993; Sänger, 1993), suggesting that aerobic generation of ATP becomes limiting as temperature decreases. An increase in mitochondrial volume density with cold acclimation may enhance catalytic capacities, facilitate diffusive processes (Egginton and Sidell, 1989) and facilitate metabolic regulation (Dudley *et al.* 1987).

Although the impact of cold acclimation on the activity of mitochondrial enzymes, mitochondrial volume density and the phospholipid composition of isolated mitochondria in muscle is well established (Van den Thillart and Modderkolk, 1978; Wodtke, 1981*a*; Guderley and Blier, 1988; Hazel and Williams, 1990), little is known of its impact upon the functional properties of mitochondria. Thermal acclimation modifies the apparent Arrhenius activation energies and the phospholipid composition of mitochondria isolated from red muscle of the goldfish *Carassius auratus* and the carp *Cyprinus carpio* (Van den Thillart and Modderkolk, 1978; Wodtke, 1981*a*,*b*). The increases of muscle activities of mitochondrial enzymes brought about by cold acclimation certainly reflect, in part, the higher mitochondrial volume densities (Johnston and Maitland, 1980; Tyler and Sidell, 1984; Egginton and Sidell, 1989). Nonetheless, the changes in mitochondrial phospholipid composition with cold acclimation modify the activities of certain mitochondrial enzymes (Hazel, 1972; Wodtke, 1981*b*). Homeoviscous adaptation of mitochondrial membranes during thermal acclimation is more complete than that of other subcellular fractions in green sunfish (*Lepomis cyanellus*) liver and frog (*Rana temporaria*) skin epidermis (Cossins *et al.* 1980; Lagerspetz and Laine, 1984). The expression of mitochondrial proteins may also change during thermal acclimation (Moon and Hochachka, 1971). These compositional changes may well, by extrapolation, alter the oxidative capacities and regulatory properties of mitochondria.

The short-horned sculpin *Myoxocephalus scorpius* L. is widely distributed in the northern Atlantic. It is a cold-water species that occurs in deeper cooler waters towards the southern end of its distribution (Wheeler, 1969). In the Firth

of Forth, the species experiences minimal temperatures near 5 °C and maximal temperatures near 15 °C (Beddow and Johnston, 1995). Thermal acclimation and seasonal acclimatization modify the contractile properties of its fast myotomal muscle (Johnson and Johnston, 1991; Beddow and Johnston, 1995) as well as the kinematics of fast-start swimming (Beddow *et al.* 1995). The plasticity of the contractile properties of sculpin muscle suggests that the metabolic machinery of muscle may also respond to thermal acclimation with compensatory changes. Thus, one of the aims of this study was to establish whether cold acclimation of the short-horned sculpin enhances the oxidative capacities of mitochondria isolated from its red myotomal muscle.

The little that is known about the thermal sensitivity of the regulatory properties of mitochondria from ectotherms suggests that the mitochondrial affinities for oxygen and carbon substrates are relatively independent of temperature (Bouwer and Van den Thillart, 1984; Yacoe, 1986; Blier and Guderley, 1993*a*). In contrast, Blier and Guderley (1993*b*) have shown that the ADP affinity of mitochondria isolated from rainbow trout (*Oncorhynchus mykiss*) red muscle dropped significantly between 15 and 8 °C. Given the importance of ADP as a substrate for and a regulator of oxidative phosphorylation (Brand and Murphy, 1987), such a loss of affinity could seriously hamper aerobic ATP generation and modify patterns of metabolic regulation. As shifts in phospholipid composition induced by thermal acclimation could modify the thermal sensitivity of the adenine nucleotide transporter, a likely determinant of the mitochondrial ADP affinity (Duée and Vignais, 1969; Blier and Guderley, 1993*b*), the impact of thermal acclimation upon the ADP affinity of sculpin mitochondria was also examined.

Materials and methods

Fish

Short-horned sculpin (*Myoxocephalus scorpius* L.) were caught in the Firth of Forth, Scotland, during June 1993 when seawater temperatures were 13–15 °C. Fish were acclimated for 2 months to 5 or 15 °C (12 h:12 h light:dark) in salt-water aquaria before use. The fish acclimated to 15 °C measured 25.1±6.4 cm in total length and had a mass of 350±224 g (N=11), whereas those acclimated to 5 °C measured 21.0±1.5 cm in total length and had a mass of 149.6±24.9 g (N=6; mean ± S.D.). Fish were stunned by a blow to the head and killed by pithing and transection of the spinal cord.

Isolation of mitochondria

Red fibres (1–3 g) were dissected from the lateral trunk muscle and finely diced with razor blades. Mitochondria were isolated at 4 °C in a medium containing (in mmol l^{-1}): 140, KCl; 10, EDTA; 5, $MgCl_2$; 20, Hepes; 0.5 % bovine serum albumin (BSA), pH 7.3 at 20 °C (Moyes *et al.* 1989). The diced tissue was initially very gently homogenised with a Polytron (speed 3 for 10 s) in 9 volumes of ice-cold isolation medium to eliminate most of the connective tissue. The muscle was homogenised with a motorised Potter-Elvejhem tissue grinder (two passes at 900 revs min^{-1}). The homogenate was centrifuged at 1400 ***g*** for 5 min at 4 °C and the supernatant collected and centrifuged for 7 min at 9000 ***g***. The resultant pellet was resuspended at a final concentration of 2–4 mg ml^{-1} in the assay medium (in mmol l^{-1}): 140 KCl, 20 Hepes, 5 Na_2HPO_4, pH 7.3 at 20 °C.

For protein determinations, 50 μl samples of the mitochondrial preparation were resuspended in 0.5 ml of the assay medium minus BSA and centrifuged at 9000 ***g*** at room temperature for 10 min. The supernatant was discarded and the pellet resuspended, washed and centrifuged a further two times to remove the BSA. The protein concentration of the mitochondrial pellet was determined using a modified Lowry method using 10 % deoxycholate to solubilise membranes (Maddy and Spooner, 1970).

Oxygen consumption was measured in water-jacketed respiration cells using Clarke-type electrodes (Rank Brothers Ltd, Cambridge, UK). The system was calibrated using the assay medium saturated with air at each experimental temperature (2.5, 7.5, 12.5 and 20 °C). Approximately 0.5–1.5 mg of mitochondrial protein was added to 1.5 ml of assay medium. Oxygen uptake was measured following the addition of 0.25 mmol l^{-1} malate to spark the Krebs cycle and a saturating concentration of pyruvate (1.58 mmol l^{-1}) or palmitoyl carnitine (0.03 mmol l^{-1}). The maximal (state 3) rate was obtained by adding 0.5 mmol l^{-1} ADP. The respiratory control ratio (RCR) was calculated from the ratio of the state 3 rate to the state 4 rate determined after all the ADP had been phosphorylated. Only mitochondrial preparations with RCR values above 4 were retained for study.

ADP affinity determinations

The apparent affinity of mitochondria for ADP was determined polarographically using the hexokinase reaction to maintain constant ADP levels. The assay medium was supplemented with glucose, $MgCl_2$ and hexokinase (yeast, Boehringer Mannheim Biochemicals) at final concentrations of 38 mmol l^{-1} glucose, 19 mmol l^{-1} $MgCl_2$ and 2 units ml^{-1} hexokinase. A unit of activity is the amount of enzyme required to convert 1 μmol of substrate to product per minute at 25 °C. Jacobus *et al.* (1982) indicate that hexokinase exerts no control over mitochondrial respiration when the ratio of milligrams of mitochondrial protein to units of hexokinase is less than 3. In our study, these ratios were consistently less than 0.2. As the mitochondrial preparations varied in their rates of oxygen consumption per millilitre, the excess of hexokinase varied among the experiments but similar ranges were present for the mitochondria from cold- and warm-acclimated sculpin. Pyruvate was the carbon substrate (1.58 mmol l^{-1}), with malate present at 0.25 mmol l^{-1}. Saturation curves for mitochondria were determined by sequential additions of ADP starting with the lowest concentration of ADP (approximately 10^{-6} mol l^{-1} total ADP), followed by gradual additions of ADP to attain saturating concentrations. Oxygen uptake rates were determined for at least 90 s at each ADP concentration. ADP

solutions were calibrated spectrophotometrically using the pyruvate kinase and lactate dehydrogenase reactions (Bergmeyer, 1983).

Calculations and statistical analyses

Oxygen solubility coefficients determined by coulorimetry (Johnston *et al.* 1994) were used to establish the oxygen contents of the mitochondrial media at the different experimental temperatures. Apparent K_m ADP ($K_{m,app}$) values were calculated by iterative fitting of the Michaelis–Menten equation using the program Regression (Blackwell Scientific Publications, Oxford, UK). The Arrhenius equations used to determine the apparent Arrhenius activation energies were calculated using the linear regression procedure in the program Statview (Abacus Concepts, Berkeley, CA, USA).

Statistical comparisons were carried out using Statview and Superanova (Abacus Concepts, Berkeley, CA, USA). In comparisons of the RCR, ADP/O and apparent ADP K_m values, as well as the relative rates of palmitoyl carnitine and pyruvate oxidation, two-factor analyses of variance (ANOVAs) were used in which acclimation temperature and the assay temperatures were the factors. Specific differences were detected using the Duncan new multiple range test. For a given acclimation group, the effect of assay temperature upon these variables was evaluated using one-factor repeated-measures ANOVAs, followed by the Fisher protected least squares difference (LSD) test to detect specific effects. Differences between the warm- and cold-acclimated sculpin at a given assay temperature were detected using unpaired *t*-tests. The significance threshold was 0.05.

Results

The respiratory control ratios of the isolated mitochondria (RCRs; Table 1) were significantly higher in the cold- than in the warm-acclimated sculpin ($P<0.001$), but the RCR did not vary as a function of assay temperature. The ADP/O values were not affected by either the acclimation or the assay temperature.

Table 1. *Thermal sensitivity of the respiratory control ratios (RCR) and ADP/O ratios of pyruvate-oxidising mitochondria isolated from red muscle of warm- and cold-acclimated* Myoxocephalus scorpius

	Assay temperature (°C)	RCR	ADP/O
15 °C-acclimated	2.5	5.67±0.73	3.41±0.16
N=7	7.5	4.73±0.46	3.24±0.11
	12.5	6.39±1.13	3.20±0.07
	20.0	6.70±0.82	3.44±0.06
5 °C-acclimated	2.5	12.66±3.75	3.41±0.16
N=6	7.5	12.17±4.19	3.24±0.11
	12.5	10.94±1.71	3.20±0.07
	20.0	10.77±1.12	3.44±0.06

Values are mean ± S.E.M.

Maximal oxidative capacities

The maximal oxidative capacity of isolated red muscle mitochondria, expressed per milligram of mitochondrial protein, was markedly enhanced by cold acclimation, particularly in the case of pyruvate oxidation (Fig. 1). At a given assay temperature, rates of mitochondrial substrate oxidation were approximately doubled by cold acclimation. At the respective acclimation temperatures, rates of pyruvate oxidation by the isolated mitochondria were similar. For mitochondria from cold-acclimated sculpin, palmitoyl

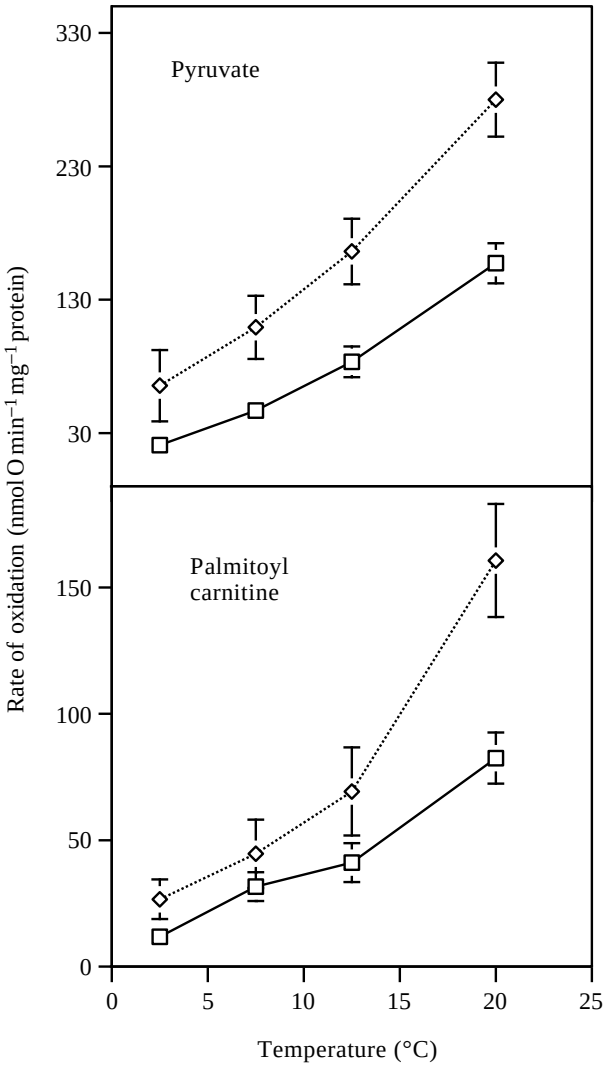

Fig. 1. Effect of thermal acclimation on maximal rates of pyruvate and palmitoyl carnitine oxidation by mitochondria isolated from the red lateral line muscle of short-horned sculpin *Myoxocephalus scorpius*. Mitochondria were isolated and assayed as described in Materials and methods. Diamonds represent the values for the cold-acclimated sculpin, whereas squares represent the values for the warm-acclimated fish. Values are means ± S.D., *N*=6 for cold-acclimated and *N*=7 for warm-acclimated sculpin.

Table 2. *Thermal sensitivity of substrate oxidation by mitochondria isolated from the red muscle of cold- and warm-acclimated sculpin* Myoxocephalus scorpius

	5 °C-acclimated	15 °C-acclimated
E_a (kJ mol^{-1})		
Pyruvate oxidation	55.44±4.78	70.19±4.24*
Palmitoyl carnitine oxidation	77.58±10.50	68.88±3.51
Q_{10} values		
Pyruvate oxidation		
2.5–7.5 °C	4.65±1.32	5.43±1.22**
7.5–12.5 °C	3.13±0.75	2.19±0.61
12.5–20 °C	2.26±0.41	2.66±0.27
Overall Q_{10} value	2.47±0.30	2.67±0.21
Palmitoyl carnitine oxidation		
2.5–7.5 °C	4.44±2.32	9.54±2.93**
7.5–12.5 °C	4.42±1.39	2.00±0.53
12.5–20 °C	3.64±0.56	2.67±0.31
Overall Q_{10} value	3.37±0.53	2.88±0.18

Values are means ± S.E.M. (N=6 for the cold-acclimated and 4–8 for the warm-acclimated fish).

*****Significant difference between the acclimation conditions (*t*-test, P<0.05).

******Significantly different from the corresponding values for the ranges 7.5–12.5 °C and 12.5–20 °C (ANOVA, P<0.02).

E_a, apparent Arrhenius activation energy.

carnitine was oxidized at approximately 42 % of the rates of pyruvate, whereas for warm-acclimated sculpin this value was approximately 57 % (P<0.002). Assay temperature did not affect the relative rates of pyruvate and palmitoyl carnitine oxidation.

Thermal sensitivity of maximal capacities

The thermal sensitivity of mitochondrial substrate oxidation was altered by thermal acclimation as reflected both by the apparent activation energies (E_a) obtained from Arrhenius plots and by the Q_{10} values over the different portions of the experimental temperature range. E_a values for pyruvate oxidation were lower for mitochondria isolated from cold- than from warm-acclimated sculpin (P<0.05; Table 2). The E_a values for palmitoyl carnitine oxidation were not modified by thermal acclimation. For mitochondria from warm-acclimated sculpin, the Q_{10} values for both pyruvate and palmitoyl carnitine oxidation were significantly higher at cold temperatures (2.5–7.5 °C) than at higher temperatures (7.5–12.5 or 12.5–20 °C; Table 2). In contrast, for mitochondria from cold-acclimated sculpin, the Q_{10} values for pyruvate and palmitoyl carnitine oxidation did not differ throughout the experimental temperature range (Table 2).

ADP affinity

Cold acclimation of sculpin did not alter the ADP affinity (apparent K_m for ADP, $K_{m,app}$) of mitochondria at low temperatures but markedly increased the $K_{m,app}$ values at 12.5 and 20 °C (Fig. 2). At the acclimation temperatures, mitochondrial ADP $K_{m,app}$ values did not differ. For warm-acclimated sculpin, assay temperature did not affect $K_{m,app}$ values for ADP (P=0.07), whereas inter-subject variability did (P<0.05). For cold-acclimated sculpin, assay temperature had a more marked effect on $K_{m,app}$ values than did inter-subject variability (P<0.005 *versus* P<0.02).

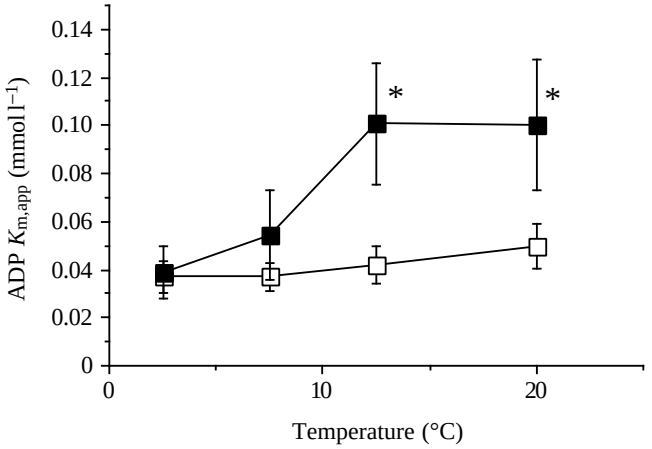

Fig. 2. Effect of thermal acclimation on the apparent affinity ($K_{m,app}$) for total ADP of mitochondria isolated from the red lateral line muscle of short-horned sculpin. ADP affinities were determined as described in Materials and methods using an ADP regenerating system. Filled symbols represent values for the cold-acclimated sculpin, whereas open symbols represent values for the warm-acclimated fish. * indicates a significant difference between values for the cold- and warm-acclimated fish ($P \leqslant 0.05$). Values are means ± S.D., N=6 for cold-acclimated and N=7 for warm-acclimated sculpin.

Discussion

The properties of mitochondria isolated from sculpin red muscle were markedly altered by thermal acclimation. Cold acclimation not only enhanced the maximal oxidative capacity but also modified the thermal sensitivities of the ADP affinity and of the maximal rates of substrate oxidation of the isolated mitochondria. Consequently, oxidation rates of mitochondria from cold-acclimated sculpin muscle at cold temperatures were comparable to rates at warm temperatures for mitochondria from warm-acclimated sculpin muscle. If these compensatory changes in mitochondrial capacity are accompanied by an increase in mitochondrial volume density, as in other fish species (Johnston and Maitland, 1980; Egginton and Sidell, 1989; Sänger, 1993), the aerobic capacity of red muscle would be higher at 5 °C in cold-acclimated sculpin than at 15 °C in warm-acclimated sculpin. Such a response is suggested by the finding that isolated red muscle fibres from cold-acclimated striped bass (*Morone saxatilis*) have higher rates of oxygen uptake at 5 °C than isolated fibres from warm-acclimated bass at 25 °C (Jones and Sidell, 1982). In sculpin, cold acclimation is normally associated with the development of gonadal tissue and potential changes in

foraging patterns and activity levels which could benefit from an increased aerobic capacity.

Mitochondria isolated from warm-acclimated sculpin had high apparent affinities for ADP at low assay temperatures. In contrast to the response of trout (*Oncorhynchus mykiss*) muscle mitochondria (Blier and Guderley, 1993*b*), the ADP affinity of sculpin muscle mitochondria did not decline at low temperatures. The thermal sensitivity of the ADP $K_{m,app}$ values of sculpin mitochondria was similar to that of the pyruvate affinity of trout muscle mitochondria (Blier and Guderley, 1993*a*) and that of the succinate affinity of iguana (*Dipsosaurus dorsalis*) liver mitochondria (Yacoe, 1986). Instead of enhancing the mitochondrial ADP affinity at low temperatures, cold-acclimation of sculpin led to a reduction of mitochondrial ADP affinity at high temperatures. Nonetheless, the RCR values remained high at high temperatures, indicating that the mitochondria remained well coupled at these temperatures. The mitochondrial ADP affinities are in the range of estimates of cytosolic ADP concentrations in muscle of carp (*Cyprinus carpio*), tilapia (*Oreochromis mossambicus*) and goldfish (*Carassius auratus*) (0.02–0.12 mmol l^{-1}; Van Waarde *et al.* 1990). These estimates of the ADP that is not complexed to macromolecules are calculated from nuclear magnetic resonance data assuming equilibrium of the creatine phosphokinase reaction. The decrease in ADP affinity at warm temperatures for mitochondria from cold-acclimated sculpin would probably perturb metabolic regulation at these temperatures. Trade-offs between the benefits and costs of temperature acclimation may be common for many traits. For example, Beddow and Johnston (1995) found that potentially beneficial adjustments in the force–velocity relationship of muscle fibres in cold-acclimated short-horned sculpin occurred at the expense of contractile failure at high temperatures.

Whereas cold acclimation led to a reduction of mitochondrial ADP affinity at high temperatures, it made the thermal sensitivity of maximal rates of substrate oxidation more uniform throughout the experimental temperature range. For mitochondria from warm-acclimated sculpin, Q_{10} values were higher at low than at high temperatures. Mitochondria from the liver of lake charr *Salvelinus namaycush* show a similar response for pyruvate oxidation (Ballantyne *et al.* 1989). For mitochondria from warm-acclimated sculpin, these shifts in thermal sensitivity occurred for both pyruvate and palmitoyl carnitine oxidation, suggesting that steps common to both pathways must be involved. As the disappearance of these shifts in thermal sensitivity with cold acclimation is correlated with the reduction of ADP affinity at high temperatures and since the adenine nucleotide transporter is probably a major determinant of the mitochondrial ADP affinity (Blier and Guderley, 1993*b*), it is possible that these changes reflect modifications of the adenine nucleotide transporter or its membrane environment.

Shifts in the phospholipid composition and membrane fluidity with thermal acclimation have been documented for muscle mitochondria isolated from goldfish *Carassius auratus* (Hazel, 1972) and carp *Cyprinus carpio* (Wodtke, 1981*a*; Van den Thillart and de Bruin, 1981; Hazel and Williams, 1990). Thermal acclimation changes the expression of mitochondrial proteins in rainbow trout *Oncorhynchus mykiss* (Moon and Hochachka, 1971). Mitochondrial lipids from cold-acclimated goldfish provide a greater activation of succinate dehydrogenase than those from warm-acclimated goldfish (Hazel, 1972). Similarly, the increases in the specific activity of cytochrome *c* oxidase during cold acclimation of carp are thought to reflect changes in the lipid composition of the mitochondrial membrane (Wodtke, 1981*b*). Cold acclimation shifts the Arrhenius break temperatures, the nature of the Arrhenius plots and the phospholipid composition of goldfish muscle mitochondria, as well as increasing the mitochondrial respiration rate per gram of muscle. However, the respiration rate increases less than the specific activity of cytochrome *c* oxidase (Van den Thillart and Modderkolk, 1978). This rise in oxidation rate per gram of tissue is likely to reflect both the changes in mitochondrial properties and an increased mitochondrial volume density (Tyler and Sidell, 1984). Although the mechanistic basis of the increased oxidative capacity of the mitochondria from cold-acclimated sculpin is not known, our data provide clear evidence that, per milligram of mitochondrial protein, the mitochondria from cold-acclimated sculpin have a higher oxidative capacity than those from warm-acclimated sculpin.

Both sculpin and goldfish modify the thermal sensitivity and functional properties of their muscle mitochondria during thermal acclimation (the present study; Van den Thillart and Modderkolk, 1978). In contrast, long-term (>12 weeks) cold acclimation of the sea bass *Dicentrarchus labrax* decreases maximal rates of glutamate oxidation at 20 °C by mitochondria from liver and heart (Trigari *et al.* 1992). No changes in membrane lipid unsaturation accompany cold acclimation in this species. The extent to which fish in different phylogenetic groups modify their mitochondrial capacities during thermal acclimation remains to be seen. Nonetheless, our data indicate that, when mitochondrial volume density is modified during acclimatory responses to temperature, the overall aerobic capacity may change more than is indicated by the shifts in mitochondrial volume density.

These data raise the question as to what sets the maximal oxidative capacities of mitochondria. Cristae density is not modified by thermal acclimation of goldfish (Tyler and Sidell, 1984) or of striped bass (Egginton and Sidell, 1989). If this proves to be a general finding, then changes in membrane lipid composition or in the properties of mitochondrial enzymes must underlie increases in capacity. Interspecific comparisons of fish living at different temperatures indicate a general relationship between maximal oxidative capacities of muscle mitochondria and habitat temperature (Johnston *et al.* 1994). Nonetheless, comparison of our data with these and other data in the literature suggests that interspecific variation in maximal rates of mitochondrial oxygen uptake is sufficiently great that similar rates are often found for species with rather different body temperatures. For example, muscle mitochondria isolated from the tilapias *Oreochromis*

andersoni and *O. niloticus* oxidize pyruvate at 113 and 100 natom O min^{-1} mg^{-1} mitochondrial protein, respectively, at 30 °C (Johnston *et al.* 1994), whereas muscle mitochondria from warm-acclimated sculpin oxidize pyruvate at 157 natom O min^{-1} mg^{-1} mitochondrial protein at 20 °C. Mitochondria from the swordfish *Xiphias gladius* heater organ oxidize pyruvate at 300 natom O min^{-1} mg^{-1} mitochondrial protein at 20 °C (Ballantyne *et al.* 1992). This interspecific variation in mitochondrial capacities is probably more related to differences in tissue aerobic power requirements than to thermal factors. Accordingly, even higher oxidative capacities are characteristic of mitochondria from insect and mammalian muscle. Mitochondria from locust flight muscle oxidize pyruvate at 600 natom O min^{-1} mg^{-1} mitochondrial protein at 30 °C (Suarez and Moyes, 1992), whereas mitochondria from rat muscle oxidize pyruvate at this rate at 37 °C (Davies *et al.* 1981). The higher cristae density of mammalian muscle mitochondria is probably critical in setting these higher capacities (Else and Hulbert, 1985). Scaling studies in endotherms suggest that organismal $\dot{V}_{O_2}$max is limited by delivery of substrates and oxygen and not by mitochondrial capacities (Weber, 1992). These considerations suggest that when delivery of substrates and oxygen to muscle fibres is enhanced by phenotypic modifications, mitochondrial capacities can increase to exploit this greater availability. There is little information on circulatory adjustments with temperature acclimation in fish, although cold acclimation is known to result in an increase in capillary density in red muscle fibres of the crucian carp (Johnston, 1982).

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