ARE MITOCHONDRIA SUBJECT TO EVOLUTIONARY TEMPERATURE ADAPTATION?

IAN A. JOHNSTON[1,3], HELGA GUDERLEY[1,3], CRAIG E. FRANKLIN[1],
TONY CROCKFORD[1] AND COLLINS KAMUNDE[2]

[1]*Gatty Marine Laboratory, School of Biological and Medical Sciences, University of St Andrews, St Andrews, Fife, KY16 8LB, Scotland,* [2]*Department of Veterinary Anatomy, University of Nairobi, Nairobi, PO Box 30197, Kenya* and [3]*Département de Biologie, Université Laval, Québec, Canada G1K 7P4*

Summary

Thermal tolerance and the respiratory properties of isolated red muscle mitochondria were investigated in *Oreochromis alcalicus grahami* from the alkaline hot-springs, Lake Magadi, Kenya. Populations of *O. a. grahami* were resident in pools at 42.8 °C and migrated into water reaching temperatures of 44.8 °C for short periods. The maximum respiration rates of mitochondria with pyruvate as substrate were 217 and 284 natom O mg^{-1} mitochondrial protein min^{-1} at 37 °C and 42 °C, respectively (Q_{10}=1.71). Fatty acyl carnitines (chain lengths C8, C12 and C16), malate and glutamate were oxidised at 70–80 % of the rate for pyruvate. In order to assess evolutionary temperature adaptation of maximum mitochondrial oxidative capacities, the rates of pyruvate and palmitoyl carnitine utilisation in red muscle mitochondria were measured from species living at other temperatures: *Notothenia coriiceps* from Antarctica (−1.5 to +1 °C); summer-caught *Myoxocephalus scorpius* from the North Sea (10–15 °C); and *Oreochromis andersoni* from African lakes and rivers (22–30 °C). State 3 respiration rates had Q_{10} values in the range 1.8–2.7. At the lower lethal temperature of *O. andersoni* (12.5 °C), isolated mitochondria utilised pyruvate at a similar rate to mitochondria from *N. coriiceps* at 2.5 °C (30 natom O mg^{-1} mitochondrial protein min^{-1}). Rates of pyruvate oxidation by mitochondria from *M. scorpius* and *N. coriiceps* were similar and were higher at a given temperature than for *O. andersoni*. At their normal body temperature (−1.2 °C), mitochondria from the Antarctic fish oxidised pyruvate at 5.5 % and palmitoyl-DL-carnitine at 8.8 % of the rates of mitochondria from the hot-spring species at 42 °C. The results indicate only modest evolutionary adjustments in the maximal rates of mitochondrial respiration in fish living at different temperatures.

Introduction

Within the normal thermal range of each fish species, the maximum sustained swimming speed (U_{crit}) increases with temperature to some maximum and thereafter

declines (Brett and Glass, 1973; Beamish, 1978). There is clear evidence for evolutionary adjustments in aerobic performance in fish living in different thermal environments. For example, *Pagothenia borchgrevinki*, an Antarctic fish, reaches its maximum cruising speed at $-0.8\,°C$ and is unable to swim above $2\,°C$ (Wohlschlag, 1964). In contrast, maximum aerobic performance in the temperate species *Micropterus salmoides* occurs at $25–30\,°C$, with the fish becoming torpid below $7–10\,°C$ (Beamish, 1970; Lemons and Crawshaw, 1984). Wohlschlag (1964) suggested that routine metabolic rates in Antarctic fish were adjusted upwards at low temperatures relative to those for temperate and tropical species, a phenomenon which became known as metabolic cold adaptation (MCA). Holeton (1973, 1974) subsequently found significantly lower rates of routine metabolism for both Arctic and Antarctic species than previously reported. He attributed the higher values obtained in earlier studies to a failure to account for handling stress, thereby calling into question the existence of MCA.

Most studies on MCA have concentrated on measuring respiration in Antarctic fish (Holeton, 1973, 1974; Wells, 1987) and have compared their results with data from the literature on the respiration of temperate and tropical fish. Antarctic fish are largely sluggish, bottom-living species, whereas most of the temperate and tropical fish that have been well studied are active mid-water species. As routine metabolic rates are likely to be significantly higher for continuously swimming pelagic species than for demersal species (Hubold, 1991), such comparisons are biased. Johnston *et al.* (1991) measured fasting metabolic rates ($\dot{V}_{O_2fast}$) in sluggish demersal species from the Antarctic, North Sea and Indo-West Pacific. After correcting for differences in body mass, $\dot{V}_{O_2fast}$ was 2–4 times higher in tropical species at $25\,°C$ than in a representative Antarctic species at $-1\,°C$. Factorial aerobic scope varies with swimming behaviour but is independent of adaptation temperature; therefore, a similar relationship would be expected for maximum aerobic performance (Forster *et al.* 1987: Johnston *et al.* 1991). At the lower lethal temperature of the tropical species ($12–15\,°C$), their fasting metabolic rates were similar to those of the Antarctic species at $-1\,°C$ (Johnston *et al.* 1991; Johnston and Battram, 1993). Thus, these data indicate that rates of aerobic metabolism show some temperature compensation. However, there is little hard evidence for MCA as originally formulated.

Polar fish could theoretically improve their capacity for aerobic work at low temperatures by increasing the relative mass and/or oxidative capacities of red muscle. Both are used to increase U_{crit} at low temperatures in certain eurythermal species following several weeks of acclimation to cold conditions (Fry and Hart, 1948; Johnston and Lucking, 1978; Sidell, 1980). Crockett and Sidell (1990) compared the activities of aerobic enzymes in the red swimming muscles of sedentary and pelagic fish from different latitudes. Citrate synthase and cytochrome oxidase activities per wet muscle mass were 1.5–5 times higher at $1\,°C$ in Antarctic than in temperate-zone species. Similarly, Johnston (1987) reported higher ADP-stimulated rates of oxygen uptake at $0\,°C$ in skinned muscle fibre segments isolated from Antarctic than from temperate species. However, rates of oxygen consumption per cm^3 of mitochondria showed more similarity among the species, reflecting the higher volume densities of mitochondria in the red muscles of Antarctic fish (Johnston, 1987). These data suggest only limited evolutionary adjustments of the thermal sensitivity of rates of mitochondrial respiration.

No previous studies have examined the maximum rates of oxygen uptake of isolated mitochondria from fish living at different thermal regimes. The aim of the present study was, therefore, to examine the maximum oxidative capacities of red muscle mitochondria isolated from fish adapted to a wide range of temperatures, to ascertain whether mitochondria show evidence of evolutionary temperature-adaptation. In particular, we have studied teleost species living at temperature extremes, including the tilapia *Oreochromis alcalicus grahami*, which has one of the highest temperature tolerances of any fish (40 °C) (Reite *et al.* 1974), and the Antarctic species *Notothenia coriiceps*, which requires special adaptations to avoid freezing. To evaluate the natural thermal tolerances of *O. a. grahami*, we have described its distribution as a function of temperature in a natural lagoon.

Materials and methods

Fish

Tilapia (*Oreochromis alcalicus grahami* Trewavas) for mitochondrial experiments were collected from a man-made pool in a hot-spring lagoon (35–37 °C) close to the factory of the Magadi Soda Company, Lake Magadi, Kenya (Fig. 1). *O. a. grahami* (8.3±0.7 cm total length, 7.7±1.8 g, mean ± s.D., *N*=34) were transported to the University of Nairobi and maintained in aerated Magadi water at around 35 °C for 1–5 days prior to experiments. No food was given, but the fish were observed to browse on algae growing on stones from Lake Magadi placed in the tank. Pure strain *Oreochromis andersoni* (21.2±1.4 cm total length, 129.4±40.0 g, mean ± s.D., *N*=6) were obtained from the Tilapia Reference Collection, Institute of Aquaculture, University of Stirling. *O. andersoni* were maintained in aerated fresh water at 28 °C and fed on a proprietary brand of trout pellets. Short-horned sculpin (*Myoxocephalus scorpius* L.) (25.1±6.4 cm total length; 350±224 g, mean ± s.D., *N*=11) were caught in the Firth of Forth during June 1993. They were acclimated for 2 months to 15 °C (12 h:12 h light:dark) in saltwater aquaria at the Gatty Marine Laboratory before use. *Notothenia coriiceps* Nybelin (19.0±0.9 cm total length, 76.2±12.6 g, mean ± s.D., *N*=7) were caught around Signy Island, Antarctica, and transported to the UK by the British Antarctic Survey. *N. coriiceps* were maintained at 0–1 °C at the Gatty Marine Laboratory for 3–15 months. *M. scorpius* and *N. coriiceps* were fed a varied diet of chopped squid, shrimps and fish flesh. 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 pectoral fin adductor muscle (*N. coriiceps*) or lateral trunk muscle (all other species) and finely diced with razor blades. Preparations were usually made from individual fish, with the exception of *O. a. grahami*, where it was necessary to pool the muscle from 2–4 fish. Mitochondria were isolated at 4 °C in a medium containing (in mmol l^{-1}): 140, KCl; 10, EDTA; 5, MgCl$_2$; 20, Hepes; 1 % (*O. andersoni*) or 0.5 % (all other species) bovine serum albumin (BSA), pH 7.3 at 20 °C (Moyes *et al.* 1989). In most cases, the diced tissue was initially very gently homogenised with a Polytron blender (Kinematica Gmbtt, Luzern, Switzerland) (speed 3 for 10 s) in 9
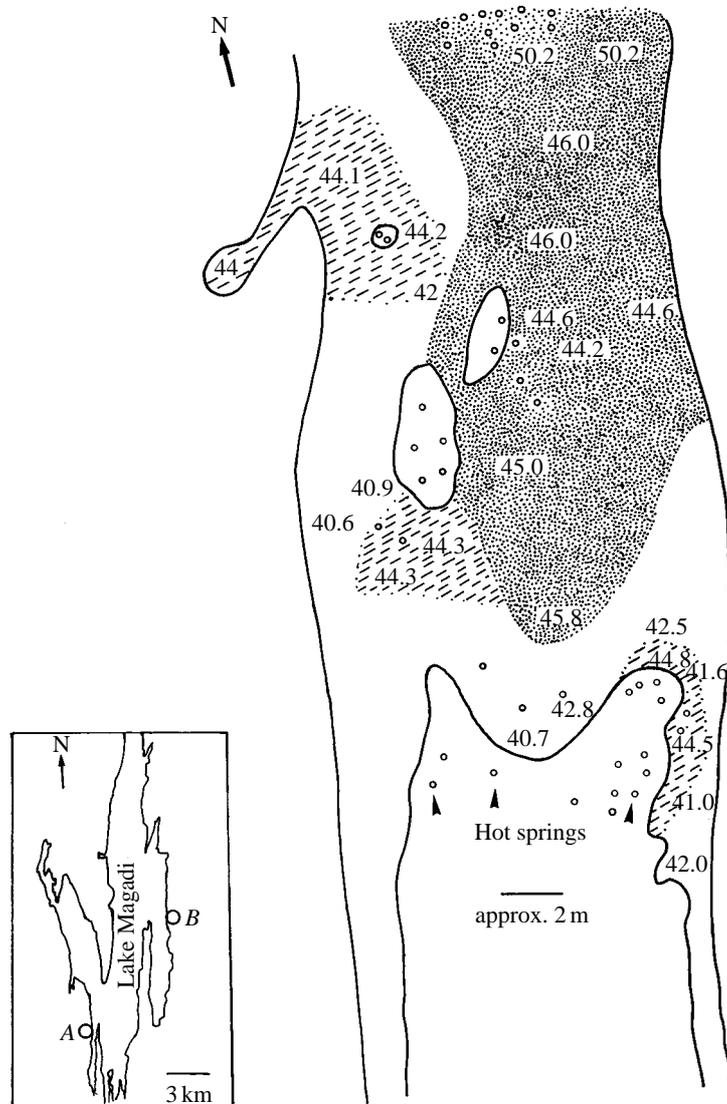
Fig. 1. Study sites at Lake Magadi in the Great Rift Valley, Kenya (latitude 1°43′–2°00′S, longitude 36°13′–36°18′E). The inset shows Lake Magadi with the South Western Fish lagoons pump-house site (A) and the Magadi Soda Company Factory (B) The main figure shows the South Western Fish lagoon with the stippled areas showing water in which no fish were present. The dashed areas show water into which fish migrated for short periods. Fish were resident in large numbers in all other areas. The numbers show the temperatures (in °C) measured in January 1993.

volumes of ice-cold isolation medium to break the muscle into small pieces. Muscle from *O. a. grahami* was further homogenised using a Dounce tissue grinder (two passes with a loose pestle followed by two passes with a tight pestle). The muscle from all other species

was further homogenised with a motorised Potter–Elvejhem tissue grinder (two passes with a loose pestle and two passes with a tight pestle, both at $900 \, \mathrm{revs \, min^{-1}}$). The homogenate was centrifuged at $1400 \, \boldsymbol{g}$ for 5 min and the supernatant collected and centrifuged for 7 min at $9000 \, \boldsymbol{g}$. The resultant pellet was resuspended at a final concentration of $2–4 \, \mathrm{mg \, ml^{-1}}$ in the respiration assay medium which contained (in $\mathrm{mmol \, l^{-1}}$): 140, KCl; 5, $\mathrm{Na_2HPO_4}$; 20, Hepes 0.5 % BSA, pH 7.3 at 20 °C. $\Delta \mathrm{pH}/\Delta t$ of the assay medium was $-0.015 \, \mathrm{pH \, units \, °C^{-1}}$.

For protein determinations, $50 \, \mu \mathrm{l}$ samples of the mitochondrial preparation were resuspended in 0.5 ml of the assay medium minus the BSA and centrifuged at $9000 \, \boldsymbol{g}$ for 10 min. The supernatant was discarded and the pellet resuspended and washed and centrifuged a further twice to remove the BSA. The protein concentration of the mitochondrial pellet was determined in duplicate with a modified Lowry method, using 10 % deoxycholate to solubilise membranes (Maddy and Spooner, 1970).

Oxygen consumption was measured in a water-jacketed respiration cell using a Clarke-type electrode (Rank Brothers Ltd, Cambridge, UK). The system was calibrated with the respiration assay medium saturated with air at a range of temperatures (see Appendix 1). Around $0.5–1.5 \, \mathrm{mg}$ mitochondrial protein was added to 1.5 ml of assay medium. The rate of oxygen uptake was measured following the addition of $0.1 \, \mathrm{mmol \, l^{-1}}$ malate to spark the Krebs cycle and a saturating concentration of substrate (see Table 1). The maximal (state 3 rate) was obtained by adding $0.5 \, \mathrm{mmol \, l^{-1}}$ ADP. The respiratory control ratio (RCR) was calculated from the ratio of the state 3 rate (+ADP) to the state 4 rate determined after all the ADP had been phosphorylated (Estabrook, 1967). All measurements were carried out within 4 h of the isolation of mitochondria and the order of assay temperatures was varied. Mitochondrial activity remained constant over the time of the experiments for all species. After a short initial period, state 3 respiration rates were approximately linear at all temperatures until ADP concentrations were exhausted. Biochemicals were obtained from Sigma Chemicals (St Louis, USA).

Results are presented as means ± S.E.M. Statistical differences were determined using a Student's *t*-test.

Results

Lake Magadi tilapia: thermal habitat

The man-made pool from which the tilapia were collected was up to 2 m deep and fed by several hot springs. Its temperature ranged from 35.7 to 37 °C, pH 10.35. Fish in the pump house pool often exceeded 10 cm total length. *O. a. grahami* were also present in large numbers in the South Western fish lagoons (approximately 5 km from the pump house) and small individuals (less than 2 cm total length) were more abundant than at the pump house, perhaps reflecting a higher predation pressure from wading birds due to the shallow depth (3–300 mm) and remoteness of the site. The temperatures measured in the lagoon in January 1993 are shown in Fig. 1. Fish were observed to be resident in water of 40.7–42.8 °C and to swim briefly into patches of water up to 44.8 °C (shown by dashed areas in Fig. 1). Larger masses of water adjacent to hot springs of 44.4–50.2 °C contained no fish and appeared to represent an effective barrier to migration (stippled areas in

Fig. 1). At about 42 °C, pH and oxygen concentration during mid-afternoon were 10.35 and 3.2 mg ml^{-1} respectively. Ventilation rates at 42 °C were observed to be extremely rapid in freshly captured fish, certainly exceeding 120 min^{-1}.

The temperature-tolerance of fish caught at the pump house was determined in the laboratory in Nairobi by raising the temperature in small tanks in increments of 2 °C h^{-1}, starting at 35 °C. All the fish studied lost territorial behaviour and exhibited escape behaviour at 42 °C, and lost equilibrium at 43 °C. Thus, fish from the pump-house pond could not tolerate the temperatures routinely experienced by fish from the South Western fish lagoon, which suggests resistance acclimatization in the different populations. It is evident that *O. a grahami* from the shallow lagoon are living very close to their upper thermal limit.

Thermal sensitivity of maximum rates of mitochondrial respiration

Mitochondria isolated from the red muscle of *O. a grahami* were highly coupled, with respiratory control ratios (RCR) values around 10 (Table 1). The highest respiration rate was observed with pyruvate (Table 1). There was no significant difference between the rates of oxidation of fatty acyl carnitines with different chain lengths (C8, C12 and C16). The state 3 respiration rate with palmitoyl-DL-carnitine was 35 % lower than with pyruvate as substrate (Table 1). Malate and glutamate were also good substrates, being oxidised at 77 % and 73 %, respectively, of the rate for pyruvate (Table 1).

Figs 2 and 3 show the effects of temperature on the mean state 3 respiration rates of mitochondria isolated from red muscle of four species of fish from diverse thermal environments. RCR values for isolated mitochondria with pyruvate as substrate were 7.0±2.4 for *N. coriiceps* at −1.2 °C, 5.8±2.8 for *M. scorpius* at 20 °C and 5.9±1.2 for *O. andersoni* at 20 °C (*N*=6–8). In general, RCR values were in the range 3–5 with palmitoyl-DL-carnitine, indicating that the mitochondria were less coupled than with

Table 1. *Respiratory control ratios (RCR) and state 3 oxygen consumption of mitochondria isolated from the red muscle of the hot-spring fish* Oreochromis alcalicus grahami *at 37 °C*

Substrate	Concentration (mmol l^{-1})	State 3 O$_2$ uptake rates (natom O mg^{-1} mitochondrial protein min^{-1})
Pyruvate	2.5+malate (0.1)	217±26 (7)
Octanoyl-DL-carnitine	0.4+malate (0.1)	178±22 (5)
Lauroyl-DL-carnitine	0.4+malate (0.1)	172±34 (5)
Palmitoyl-DL-carnitine	0.025+malate (0.1)	161±19 (7)
Malate	0.1	18±4 (7)
Malate	5.0+malate (0.1)	168±16 (5)
Glutamate	5.0+malate (0.1)	158±31 (5)

Respiratory control ratio (RCR) for pyruvate was 10.0±1.4 (7) and for palmitoyl-DL-carnitine 9.8±1.8 (7).

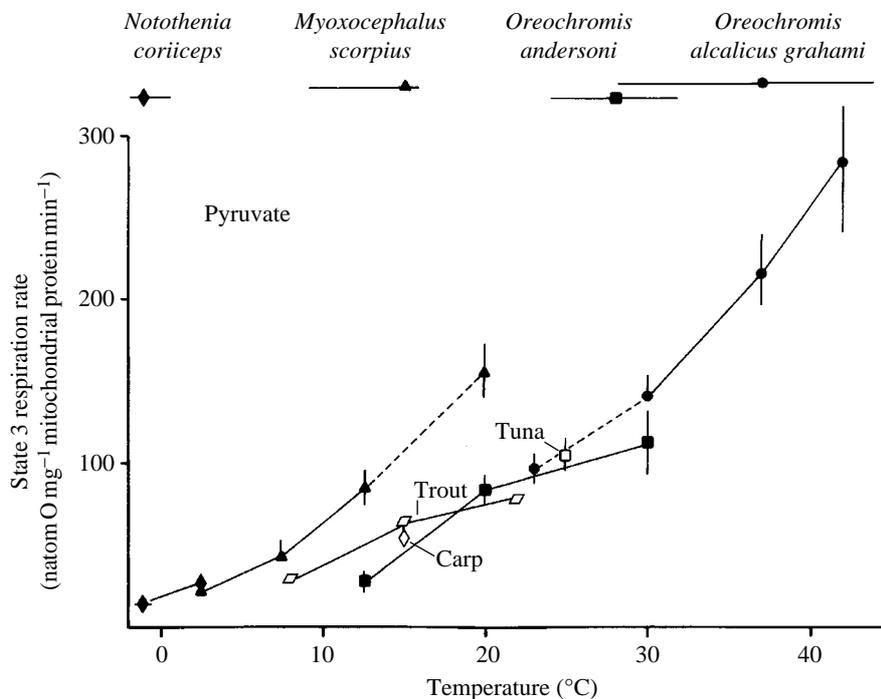Values are mean ± S.E.M. (*N*).

Fig. 2. The effects of temperature on state 3 respiration rates, with pyruvate as substrate, for isolated red muscle mitochondria from *Notothenia coriiceps* (◆) (Antarctica), *Myoxocephalus scorpius* (▲) (North Sea), rainbow trout (◇) (from Blier and Guderley, 1993), common carp (◇) (from Moyes *et al.* 1989), skipjack tuna (□) (from Moyes *et al.* 1992), *Oreochromis andersoni* (■) and *Oreochromis alcalicus grahami* (●). The normal environmental temperature range of each species is shown above with a solid line. Values are mean ± S.E.M., *N*=5–8.

pyruvate. RCR values showed no systematic variation with assay temperature over the range studied. Pyruvate and palmitoyl-DL-carnitine were equally good substrates for *N. coriiceps* and *O. andersoni* mitochondria (Figs 2 and 3). In contrast, the mitochondria from 15 °C-acclimated *M. scorpius* oxidised palmitoyl-DL-carnitine at only 49 % of the rate for pyruvate at 12.5 °C ($P<0.01$, Figs 2 and 3). The maximum rate of pyruvate oxidation was 18.3 times higher for muscle mitochondria from the hot-spring fish at 42 °C (284 natom O mg protein^{-1} min^{-1}) than for the Antarctic species at −1.2 °C (15.5 natom O mg protein^{-1} min^{-1}) ($P<0.005$; Fig. 2).

For mitochondria isolated from *O. a. grahami*, the Q_{10} for pyruvate utilisation was in the region of 1.79 between 23 and 42 °C, whereas palmitoyl-DL-carnitine oxidation had Q_{10} values of 1.43 (23–37 °C) and 1.56 (30–42 °C). Double logarithmic plots of state 3 respiration rates *versus* temperature were linear (not shown). The intercept of the double logarithmic plot for pyruvate was 1.7 °C, the temperature at which the state 3 respiration rate extrapolates to zero. There were no significant differences in the state 3 respiration rates for the two *Oreochromis* species at 30 °C with either pyruvate (Fig. 2) or
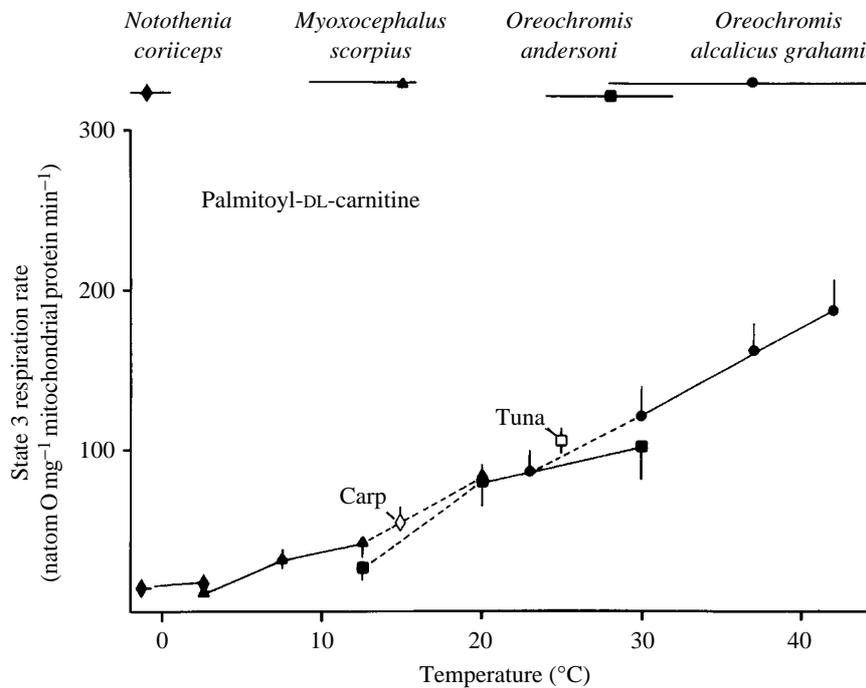
Fig. 3. The effects of temperature on state 3 respiration rates, with palmitoyl-DL-carnitine as substrate, for isolated red muscle mitochondria from *Notothenia coriiceps* (◆) (Antarctica), *Myoxocephalus scorpius* (▲) (North Sea), common carp (◇) (from Moyes *et al.* 1989), skipjack tuna (□) (from Moyes *et al.* 1992); *Oreochromis andersoni* (■) and *Oreochromis alcalicus grahami* (●). The normal environmental temperature range of each species is shown above with a solid line. Values are mean ± S.E.M., N=5–8.

palmitoyl-DL-carnitine (Fig. 3). The respiration rates for mitochondria from *N. coriiceps* and *M. scorpius* were similar to each other, but translated to the left of the curves for the two *Oreochromis* species, particularly with pyruvate as substrate (Figs 2 and 3). At 12.5 °C and 20 °C with pyruvate as substrate, respiration rates were 2.9-fold and 1.9-fold higher for mitochondria from *M. scorpius* than for those from *O. andersoni* (P<0.005; Figs 2 and 3). In contrast, the rates of palmitoyl-DL-carnitine oxidation at common temperatures were similar for both species. Over the studied temperature range, mitochondria from *M. scorpius* and *N. coriiceps* oxidised pyruvate with Q_{10} values of 2.6 and 1.9, respectively.

Discussion

Lake Magadi tilapia have been found in water ranging in temperature from 30 to 40 °C (Coe, 1966; Reite *et al.* 1974). In the present study, fish were observed to be resident in lagoons with temperatures of up to 42.8 °C (Fig. 1), which is higher than the upper temperature limit reported previously (Coe, 1966; Reite *et al.* 1974). The upper lethal

temperature of *O. a. grahami* is similar to that for the desert pupfish *Cyprinodon macularius* and represents the highest temperature at which teleosts can survive (see Brown and Feldmeth, 1971). In the present study, fish from a 37 °C pond held for several days at 35 °C were unable to tolerate 41–43 °C, temperatures at which tilapia were routinely found in the South Western fish lagoons (Fig. 1). These results suggest that acclimatisation and acclimation can extend the thermal tolerance range of *O. a. grahami*, as has been reported for numerous other ectotherms (Cossins and Bowler, 1987). In contrast to many species, Lake Magadi tilapia live very close to their upper thermal tolerance limit.

Mitochondria from *O. a. grahami* utilise fatty acyl carnitines, malate and glutamate at 70–80 % of the rate for pyruvate, which suggests that a wide range of carbohydrates, fatty acids and amino acids can be used as fuels (Table 1). The ratio of glutamate to pyruvate utilisation in red muscle mitochondria is 0.22 in skipjack tuna (Moyes *et al.* 1992), 0.60 in common carp (Moyes *et al.* 1989) and 0.73 in *O. a. grahami* (Table 1). The ability of mitochondria from Magadi tilapia to utilise glutamate at high rates may reflect the unusual pattern of nitrogen metabolism in this species (Randall *et al.* 1989; Walsh *et al.* 1993). The rate of oxidation of fatty acyl carnitines was similar for chain lengths C8–C16 (Table 1), as has been reported for carp red muscle mitochondria (Moyes *et al.* 1989). In contrast, mitochondria isolated from carp white muscle utilise C16 and C12 chain lengths at about twice the rate of octonyl-carnitine (Moyes *et al.* 1989).

At 37 °C, the maximum rate of oxygen uptake of isolated red muscle mitochondria with pyruvate was 217 natom O mg protein^{-1} min^{-1} (Table 1). This is considerably lower than the values of 300–320 natom O mg protein^{-1} min^{-1} that are typical of avian and mammalian muscle mitochondria at this temperature (Hoppeler and Lindstedt, 1985; Suarez *et al.* 1991). *In vivo*, oxygen or substrate delivery, [NADH]/[H$^+$] ratio and/or the availability of ADP or inorganic phosphate (P$_i$) could limit the maximum aerobic capacity of mitochondria. In mammals, however, there is evidence that at $\dot{V}_{O_2max}$ skeletal muscle mitochondria operate close to the maximum rates obtained with isolated mitochondria (Schwerzmann *et al.* 1989).

Red muscle mitochondria isolated from Lake Magadi tilapia oxidised pyruvate and palmitoyl carnitine with Q$_{10}$ values of 1.79 and 1.56, respectively, over the temperature range studied (Figs 2 and 3). Between 20 and 30 °C, mitochondria from *Oreochromis andersoni*, which lives in this thermal range, oxidised both substrates at similar rates to *O. a. grahami* (Figs 2 and 3). However, at 30 °C, the maximum rate of pyruvate utilisation in *O. andersoni* was 111.5±9.4 natom O mg protein^{-1} min^{-1} (mean ± S.E.M., N=4), which was similar to the rate at 20 °C, indicating a reduced thermal dependence of maximal rates of pyruvate oxidation at high temperatures.

Comparisons of the maximum aerobic capacity of muscle mitochondria are complicated by interspecific differences in substrate preferences and differences in mitochondrial purity, which would tend to increase the variance in the data. For example, red muscle mitochondria from *O. a. grahami* and *N. coriiceps* (Figs 2 and 3) utilise pyruvate and palmitoyl carnitine at equivalent rates, whereas mitochondria from summer-caught, short-horned sculpin oxidise palmitoyl carnitine at only half the rate of pyruvate (Figs 2 and 3). Fuel preferences may also be influenced by such factors as nutritional

state, developmental stage, temperature acclimation, etc. The limited data available suggest that maximum respiration rates are obtained with pyruvate, a pivotal substrate during carbohydrate and amino acid oxidation (Moyes *et al.* 1989, 1992).

The results of the present study are consistent with a limited degree of temperature compensation of maximum respiration rates. For example, state 3 respiration rates of mitochondria from Lake Magadi extrapolate to zero at around 2 °C, whereas mitochondria from *N. coriiceps* still oxidise substrates at significant rates at much lower temperatures (15.5 and 16.5 natom O mg protein^{-1} min^{-1} for pyruvate and palmitoyl carnitine, respectively, at -1.2 °C, Figs 2 and 3). Similarly, mitochondria from the short-horned sculpin oxidise pyruvate at higher rates than *O. andersoni* at 12.5 °C. In spite of potential methodological differences, the maximal rates of pyruvate oxidation obtained in other studies, e.g. with carp (Moyes *et al.* 1989), trout (Blier and Guderley, 1993) and tuna (Moyes *et al.* 1992), also fall close to the temperature–rate curve shown in Fig. 2. We therefore conclude that interspecific differences in the oxidative capacities of red muscle mitochondria are small and that at normal body temperatures mitochondrial respiration rates are much higher for warm- than for cold-water species. For example, mitochondria from the Antarctic species at -1.2 °C oxidise pyruvate at only 5.5 % of the rate of mitochondria from the hot-spring species at 42 °C (Fig. 2), indicating relatively modest evolutionary adjustments in maximum oxidative capacities.

The higher rates of oxygen consumption and cytochrome oxidase activities per milligram of mitochondrial protein that have been observed following cold-acclimation in cyprinid fish (Van den Thillart and Modderkolk, 1978) are thought largely to reflect changes in the lipid composition of mitochondrial membranes (Wodtke, 1981). The temperature compensation of mitochondrial function in Antarctic and North Sea fish could indicate adaptations in the fatty acid composition of membranes and/or associated proteins (pumps, ion channels and enzymes). Whole-animal experiments indicate a greater degree of evolutionary adjustments of the thermal sensitivity of aerobic performance than we observed for individual mitochondria (Wells, 1987; Forster *et al.* 1987; Johnston *et al.* 1991; Johnston and Battram, 1993). Improved aerobic performance at low temperatures could be achieved *via* an increase in red muscle mass, higher volume densities of muscle mitochondria and/or an increase in the oxidative capacity per unit volume of mitochondria. Srere (1985) calculated that 83 mm^2 mm^{-3} was the maximum degree of cristae packing that would allow enough matrix space for two average-size Krebs cycle enzymes in contact with an opposing membrane. The cristae surface density in hummingbird flight muscle (58 mm^2 mm^{-3}) approaches the theoretical maximum and is around twice that of the pectoralis muscle of less active bird species (Suarez *et al.* 1991). In contrast, the surface density of mitochondrial cristae in the red muscle of the fast-swimming tropical tuna is only 21.9 mm^2 mm^{-3}, compared with 32.8–37.0 mm^2 mm^{-3} in bottom-living Antarctic fish (Archer and Johnston, 1991). The relatively higher cristae density for the Antarctic fish is compatible with the slight thermal compensation of aerobic performance observed in this study.

There is a trend for higher mitochondrial densities in the red muscles of cold- than in those of warm-water fish (Johnston, 1987). The Antarctic silverfish *Pleuragramma*

antarcticum is relatively sluggish, using passive buoyancy mechanisms rather than active swimming to maintain a pelagic existence. Myofibrils in the red muscle fibres of *P. antarcticum* are arranged in columns one fibril thick surrounded by the mitochondria, which constitute 56.3 % of the fibre volume (Johnston *et al.* 1988). In contrast, mitochondria only occupy 32.4 % of the volume of red muscle fibres in skipjack tuna (Moyes *et al.* 1992) and 23 % in the tilapia *Oreochromis niloticus*, both of which are much more active species (Johnston, 1987). The denser packing of mitochondria in the muscle fibres in cold- compared with warm-water fish will also serve to reduce diffusion distances for metabolites which may be limiting at low temperature (Londraville and Sidell, 1990).

Antarctic fish probably use a combination of mechanisms to offset the adverse effects of low temperature on aerobic capacity. Compensatory increases in the aerobic capacity of mitochondria are probably secondary to increases in mitochondrial abundance in enhancing muscle aerobic capacity. In spite of these adaptations, the maximum aerobic capacity is constrained in polar fish with implications for energy budgeting, requiring more pronounced seasonal switching between reproduction and growth than occurs in temperate or tropical fish (Johnston and Battram, 1993). Jones (1971) estimated that the cost of ventilation and circulating the body fluids accounted for 15 % of the energy expenditure at U_{crit} in rainbow trout at 15 °C. The combined cost of ventilation and circulation will account for an even higher proportion of the total energy expenditure in the hot-spring fish due to the environmental conditions of Lake Magadi, placing some constraint on the maximum aerobic scope for activity.
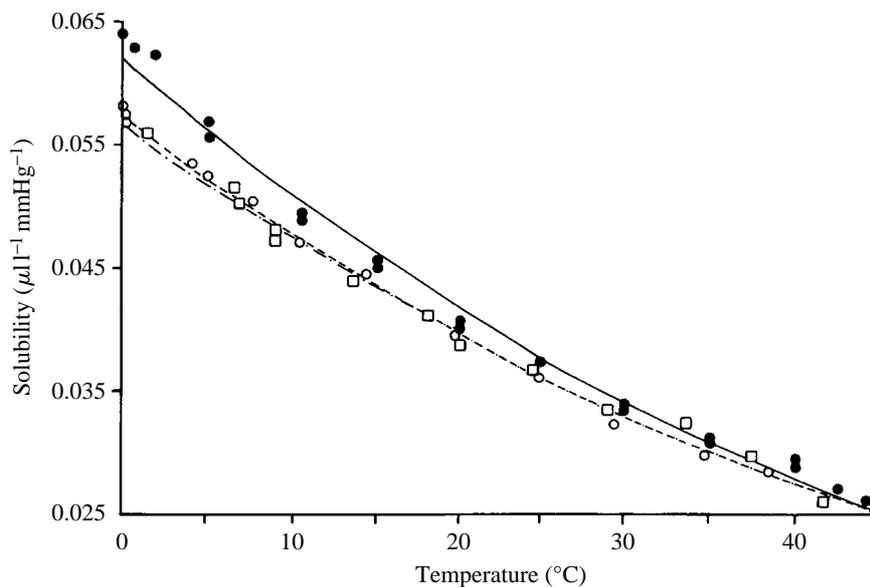

Fig. 4. The effect of temperature on the oxygen solubilities of distilled water (●), mitochondrial respiration medium without bovine serum albumin (○) and mitochondrial respiration medium with 0.5 % BSA (□).

Appendix

Solubility of oxygen in mitochondrial assay media

The precise determination of mitochondrial oxygen consumption rates is dependent, among other things, on accurate estimates of the solubility of oxygen within the assay medium at the experimental temperature. Previous estimates of oxygen solubility in assay media and Ringer's solutions have used the Winkler method to determine oxygen content from which solubilities can be calculated (Graham, 1987). However, as Graham (1987) pointed out and we have confirmed by our own experience, the presence of bovine serum albumin (BSA) in the sample medium influences the Winkler reaction, making the detection of the starch-mediated endpoint difficult to determine. To eliminate this possible source of error, couloximetry was used to determine the oxygen content of the mitochondrial assay media used in this study. This technique utilises an oxygen fuel cell that produces a current that is directly proportional to the number of oxygen molecules and functions independently of the composition of the fluid being analysed (Peck and Uglow, 1990). The oxygen content of air-saturated, distilled water, mitochondrial assay medium without BSA and assay medium with 0.5 % BSA were determined using couloximetry at temperatures ranging from 0 to 44˚C. The oxygen solubilities of the three media are shown in Fig. 4 and described by the following equations:

$$\text{Distilled water:} \quad y = 0.0622\text{e}^{-0.0200T},$$

$$\text{Mitochondrial medium (no BSA):} \quad y = 0.0575\text{e}^{-0.0184T},$$

$$\text{Mitochondrial medium}+0.5\,\%\ \text{BSA:} \quad y = 0.0565\text{e}^{-0.0178T},$$

where T is temperature (˚C) and y is oxygen solubility (μl l^{-1} mmHg^{-1}).

Graham (1987) found that the oxygen solubility of physiolgical salines decreased markedly with the addition of BSA. However, in this study using couloximetry instead of the Winkler method to determine oxygen content, the influence of 0.5 % BSA on the oxygen solubility of mitochondrial respiration medium was found to be insignificant. These differences could be attributed to the interference of BSA on the Winkler method.

References

Archer, S. D. and Johnston, I. A. (1991). Density of cristae and distribution of mitochondria in the slow muscles of Antarctic fish. *Physiol. Zool.* **64**, 242–258.

Beamish, F. W. H. (1970). Oxygen consumption of largemouth bass, *Micropterus salmoides*, in relation to swimming speed and temperature. *Can. J. Zool.* **48**, 1221–1228.

Beamish, F. W. H. (1978). Swimming capacity. In *Fish Physiology*, vol. VII (ed. W. S. Hoar and D. J. Randall), pp. 101–187. New York, San Francisco, London: Academic Press.

Blier, P. U. and Guderley, H. E. (1993). Effects of pH and temperature on the kinetics of pyruvate

oxidation by muscle mitochondria from rainbow trout (*Oncorhynchus mykiss*). *Physiol. Zool.* **66**, 474–489.

BRETT, J. R. AND GLASS, N. R. (1973). Metabolic rates and critical swimming speeds of sockeye salmon, *Oncorhynchus nerka*, in relation to size and temperature. *J. Fish. Res. Bd Can.* **30**, 379–387.

BROWN, J. H. AND FELDMETH, C. R. (1971). Evolution in constant and fluctuating environments: thermal tolerances of desert pupfish (*Cyprinodon*). *Evolution* **25**, 390–398.

COE, M. J. (1966). The biology of *Tilapia grahami* (Boulenger) in Lake Magadi, Kenya. *Acta trop.* (Basel) **23**, 146–177.

COSSINS, A. AND BOWLER, K. (1987). *Temperature Biology of Animals*. London, New York: Chapman and Hall. 339pp.

CROCKETT, E. L. AND SIDELL, B. D. (1990). Some pathways of energy metabolism are cold adapted in Antarctic fishes. *Physiol. Zool.* **63**, 472–488.

ESTABROOK, R. W. (1967). Mitochondrial respiratory control and the polarographic measurement of ADP:O ratios. *Meth. Enzymol.* **10**, 41–47.

FORSTER, M. E., FRANKLIN, C. E., TAYLOR, H. H. AND DAVISON, W. (1987). The aerobic scope of an Antarctic fish, *Pagothenia borchgrevinki*. *Polar Biol.* **8**, 155–159.

FRY, F. E. J. AND HART, J. S. (1948). Cruising speed of goldfish in relation to water temperature. *J. Fish Res. Bd Can.* **7**, 169–175.

GRAHAM, M. (1987). The solubility of oxygen in physiological salines. *Fish Physiol. Biochem.* **4**, 1–4.

HOLETON, G. F. (1973). Respiration of Arctic char (*Salvelinus alpinus*) from a high arctic lake. *J. Fish Res. Bd Can.* **30**, 717–723.

HOLETON, G. F. (1974). Metabolic cold adaptation of polar fish: fact or artifact ? *Physiol. Zool.* **47**, 137–152.

HOPPELER, H. AND LINDSTEDT, S. L. (1985). Malleability of skeletal muscle in overcoming limitations: structural elements. *J. exp. Biol.* **115**, 355–364.

HUBOLD, G. (1991). Ecology of notothenioid fish in the Weddell Sea. In *Biology of Antarctic Fish* (ed. G. di Prisco, B. Maresca and B. Tota), pp. 3–23. Berlin, Heidelberg, New York: Springer-Verlag

JOHNSTON, I. A. (1987). Respiratory characteristics of muscle fibres in a fish (*Chaenocephalus aceratus*) that lacks haem pigments. *J. exp. Biol.* **133**, 415–428.

JOHNSTON, I. A. AND BATTRAM, J. (1993). Feeding energetics and metabolism in demersal fish species from Antarctic, temperate and tropical environments. *Mar. Biol.* **115**, 7–14.

JOHNSTON, I. A., CAMM, J.-P. AND WHITE, M. (1988). Specialisations of swimming muscles in the pelagic antarctic fish *Pleuragramma antarcticum*. *Mar. Biol.* **100**, 3–12.

JOHNSTON, I. A., CLARKE, A. AND WARD, P. (1991). Temperature and metabolic rate in sedentary fish from the Antarctic, North Sea and Indo-West Pacific Ocean. *Mar. Biol.* **109**, 191–195.

JOHNSTON, I. A. AND LUCKING, M. (1978). Temperature induced variation in the distribution of different types of muscle fibe in the goldfish (*Carassius auratus*). *J. comp. Physiol.* **124**, 111–116.

JONES, D. R. (1971). The effect of hypoxia and anaemia on the swimming performance of rainbow trout (*Salmo gairdneri*). *J. exp. Biol.* **55**, 541–551.

LEMONS, D. E. AND CRAWSHAW, L. I. (1985). Behavioural and metabolic adjustments to low temperatures in the largemouth bass (*Micropterus salmoides*). *Physiol. Zool.* **58**, 175–180.

LONDRAVILLE, R. L. AND SIDELL, B. D. (1990). Ultrastructure of aerobic muscle in Antarctic fishes may contribute to maintenance of diffusive fluxes. *J. exp. Biol.* **150**, 205–220.

MADDY, A. H. AND SPOONER, R. O. (1970). The erythrocyte agglutinability. I. Variation in the membrane protein. *Vox. Sang.* **18**, 34–41.

MOYES, C. D., BUCK, L. T., HOCHACHKA, P. W. AND SUAREZ, R. K. (1989). Oxidative properties of carp red and white muscle. *J. exp. Biol.* **143**, 321–331.

MOYES, C. D., MATHIEU-COSTELLO, O. A., BRILL, R. W. AND HOCHACHKA, P. W. (1992). Mitochondrial metabolism of cardiac and skeletal muscles from a fast (*Katsuwonus pelamis*) and a slow (*Cyprinus carpio*) fish. *Can. J. Zool.* **70**, 1246–1253.

PECK, L. S. AND UGLOW, R. F. (1990). Two methods for the assessment of the oxygen content of small volumes of seawater. *J. exp. mar. Biol. Ecol.* **141**, 53–62.

RANDALL, D. J., WOOD, C. M., PERRY, S. F., BERGMAN, H., MALOIY, G. M. O., MOMMSEN, T. P. AND WRIGHT, P. A. (1989). Ureotelism in a completely aquatic teleost fish: a strategy for survival in an extremely alkaline environment. *Nature* **337**, 165–166.

REITE, O. B., MALOIY, G. M. O. AND AASEHAUG, B. (1974). pH, salinity and temperature tolerance of Lake Magadi *Tilapia*. *Nature* **247**, 315.

SCHWERZMANN, K., HOPPELER, H., KAYAR, S. R. AND WEIBEL, E. (1989). Oxidative capacity of muscle and mitochondria: correlation of physiological, biochemical and morphometric characteristics. *Proc. natn Acad. Sci. U.S.A.* **86**, 1583–1587.

SIDELL, B. D. (1980). Response of goldfish (*Carassius auratus* L.) muscle to acclimation temperature: alterations in biochemistry and proportions of different fibre types. *Physiol. Zool.* **53**, 98–107.

SRERE, P. A. (1985). Organisation of proteins within the mitochondrion. In *Organised Multienzyme Systems* (ed. G. R. Welch), pp. 1–66. New York: Academic Press.

SUAREZ, R. K., LIGHTON, J. R. B., BROWN, G. S. AND MATHIEU-COSTELLO, O. (1991). Mitochondrial respiration in hummingbird flight muscles. *Proc. natn. Acad. Sci. U.S.A.* **88**, 4870–4873.

VAN DEN THILLART, G. AND MODDERKOLK, J. (1978). The effect of temperature on state 3 respiration and on the unsaturation of membrane lipids of goldfish mitochondria. *Biochim. biophys. Acta* **510**, 38–51.

WALSH, P. J., BERGMAN, H. L., NARAHARA, A., WOOD, C. M., WRIGHT, P. A., RANDALL, R. J., MAINA, J. N. AND LAURENT, P. (1993). Effects of ammonia on survival, swimming and actiovities of enzymes of nitrogen metabolism in the Lake Magadi tilapia *Oerochromis alcalicus grahami*. *J. exp. Biol.* **180**, 323–327.

WELLS, R. M. G. (1987). Respiration of antarctic fish from McMurdo Sound. *Comp. Biochem. Physiol.* **88**A, 417–424.

WODTKE, E. (1981). Temperature adaptation of biological membranes. Compensation of the molar activity of cytochrome c oxidase in the mitochondrial energy-transducing membrane during thermal acclimation of the carp (*Cyprinus carpio* L.). *Biochim. biophys. Acta* **640**, 710–720.

WOHLSCLAG, D. E. (1964). Respiratory metabolism and ecological characteristics of some fishes in McMurdo Sound, Antarctica. *Antarct. Res. Ser.* **1**, 33–62.