LATITUDINAL VARIATION IN THE ABUNDANCE AND OXIDATIVE CAPACITIES OF MUSCLE MITOCHONDRIA IN PERCIFORM FISHES

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Summary

The abundance, distribution and oxidative capacities of mitochondria have been investigated in the red pectoral fin adductor muscles of fish (Order Perciformes) that use a predominantly labriform style of swimming. Mediterranean Sea species from the families Labridae, Serranidae, Sparidae and Antarctic Nototheniidae and non-Antarctic Nototheniidae and Channichthyidae were studied. Sub-Antarctic species from the Beagle Channel, Tierra del Fuego, included the pelagic haemoglobin-less icefish (*Champsocephalus esox*) and the róbalo (*Eleginops maclovinus*), which occurs as far north as 35°S. In *Champsocephalus esox*, the mitochondrial volume density of red muscle was 0.51 and mitochondrial cristae surface density ($43.9\,\mu m^2\,\mu m^{-3}$) was higher than reported for Antarctic icefishes. In the red-blooded, active pelagic or semi-pelagic species, mitochondrial volume density was within the range 0.27–0.33 regardless of habitat temperature. Amongst less active demersal species, mitochondrial volume density ranged from 0.29–0.33 in polar species to 0.08–0.13 in Mediterranean species. In Antarctic species and *Champsocephalus esox*, myofibrils occurred in ribbons or clusters one fibril thick entirely surrounded by mitochondria. The volume density of intracellular lipid droplets was not correlated with activity patterns or habitat temperature. In a comparison of *Eleginops maclovinus* caught in summer (approximately 10 °C) and winter (approximately 4 °C), mitochondrial volume density did not differ, whereas the surface density of mitochondrial clusters was higher in summer fish. The temperature-dependence of the state 3 respiration rate of isolated mitochondria with pyruvate as substrate was described by a single quadratic relationship for all species, indicating no significant up-regulation of the maximum rate of oxygen uptake per milligram mitochondrial protein in Antarctic species. Our results support the conclusion that increasing the volume and surface density of mitochondrial clusters is the primary mechanism for enhancing the aerobic capacity of muscle in cold-water fish.

Key words: mitochondria, metabolism, temperature, Antarctic fish, sub-Antarctic fish, skeletal muscle, Notothenioidei.

Introduction

Antarctic fish are archetypal stenotherms restricted to a narrow range of temperatures (−1.86 °C to +2 °C) that would be lethal for temperate and tropical species. There is a long history of studies on the mechanisms underlying cold survival and on the ecological consequences of low temperature for patterns of activity, growth and reproduction in polar fish (for recent reviews, see Johnston, 1989; Hubold, 1991; Clarke and Johnston, 1996). Ege and Krogh (1914) predicted that cold-water ectotherms would have higher metabolic rates then temperate species after making a suitable correction for differences in body temperature. Early experimental work appeared to confirm this prediction for Arctic (Scholander *et al.* 1953) and Antarctic (Wohlschlag, 1960, 1962) fish, and the concept of 'metabolic cold adaptation' (MCA) became established in the literature. Holeton (1973, 1974) subsequently reported much lower routine metabolic rates for Arctic fish, pointing out that handling stress may have contributed to the elevated levels of metabolism reported by early workers. However, the existence of metabolic cold-adaptation in polar fish continues to be the subject of investigation and controversy (Johnston *et al.* 1991; Steffensen *et al.* 1994) and has been offered support by some studies (Wells, 1987; Torres and Somero 1988). Unfortunately, the MCA hypothesis cannot be tested experimentally since the metabolism of warm-water species cannot be determined at polar temperatures. In an attempt to overcome this problem, Crockett and Sidell (1990) measured mitochondrial enzyme activities in homogenates in order to compare temperate and

tropical species directly. At an assay temperature of 1 °C, they found that citrate synthase and cytochrome oxidase activities in red muscle were 1.5- to fivefold higher in Antarctic than in tropical species, which is consistent with some expansion of aerobic metabolism. More recently, only modest adjustments in the maximal rates of mitochondrial respiration per milligram protein were reported in fish living at different temperatures. For example, the rates of pyruvate and palmitoyl carnitine oxidation at −1 °C for red muscle mitochondria from the Antarctic fish *Notothenia coriiceps* were similar to the estimated rates at this temperature for muscle mitochondria from a thermophilic species living at 42 °C (Johnston *et al.* 1994).

A common response to cold-acclimation in eurythermal fish is an almost twofold increase in the abundance of muscle mitochondria (Johnston and Maitland, 1980; Egginton and Sidell, 1989; Sänger, 1993). Various adaptive explanations include the hypotheses that increases in mitochondrial volume density partially compensate for the reduced catalytic capacity at low temperatures (Johnston, 1982*a*; Egginton and Sidell, 1989) or alternatively offset the reduced diffusion coefficients of cytosolic metabolites (Tyler and Sidell, 1984; Sidell and Hazel, 1987). Red muscle fibres in some polar fish habitually living at cold temperatures also have very abundant mitochondria, and similar adaptive explanations have been offered (Johnston, 1987; Londraville and Sidell, 1990). Indeed, mitochondrial volume density in the red myotomal muscle of the Antarctic silverfish *Pleuragramma antarcticum* (0.56) is amongst the highest recorded for vertebrates (Johnston *et al.* 1988).

Studies on aerobic metabolism and body temperature have often compared unrelated species with diverse lifestyles and modes of locomotion. Systematic variations in aerobic metabolism at the whole animal, organelle or enzyme level are probably to be expected both between distantly related genera and among species with different locomotory habits (Garland and Carter, 1994). The Order Perciformes show an extensive radiation, inhabiting a wide latitudinal range and both marine and freshwater environments. Species from the sub-order Notothenioidei are dominant components of the icthyofauna of the Southern Ocean, the Patagonian shelf, the Beagle Channel and the coastal zones of Argentina and Chile (Eastman, 1993; Pequeño, 1989). Notothenioidei species in the Beagle Channel, Tierra del Fuego, include *Champsocephalus esox*, the only haemoglobin-less icefish found outside the Antarctic, and *Eleginops maclovinus*, the single representative of the sub-family Eleginopinae which is absent from the Antarctic and distributed as far north as Uruguay (35°S) (Eastman, 1993). *Eleginops* is thought to have separated early during the radiation of nototheniid fishes and to be a phylogenetically primitive form related to *Dissostichus* (Balushkin, 1984). Its present-day occurrence may reflect a historical distribution on part of the Gondwana shelf that did not become associated with the margins of the Antarctic plate (Eastman, 1993). Thus, the properties of this species are of particular interest as it would have been relatively little influenced by large-scale tectonic

movements or the subsequent cooling of the Southern Ocean during the Tertiary period.

The aim of the present study was to use appropriate species comparisons to test the hypothesis that increased mitochondrial abundance provides the main mechanism for adjusting muscle aerobic capacity in cold environments. We therefore determined the abundance and oxidative capacity of red muscle mitochondria in perciform species from the families Labridae, Serranidae, Sparidae, Channichthyidae and Nototheniidae that use a similar labriform style of swimming at sustainable speeds, encompassing demersal, semi-pelagic and pelagic species.

Materials and methods
Fish

All the fish species studied belonged to the Order Perciformes. Further taxonomic information and details on the habit, number, standard length and body mass of the fish used are given in Figs 1 and 2. The Antarctic fish *Lepidonotothen nudifrons* (Lönnberg) and *Trematomus newnesi* (Boulenger) were caught around the South Orkney Islands (60°43′S, 45°36′W) during austral summer 1994–95 (sea temperature −1.0 to 0 °C). Fish were transported to the UK by the British Antarctic Survey and maintained in a purpose-built cold-aquarium at the Gatty Marine Laboratory (St Andrews, Scotland) for several months at approximately 0 °C. Antarctic fish were fed on a diet of krill and squid. The sub-Antarctic fish studied were caught using trammel nets and traps in the Beagle channel in the vicinity of Ushuaia, Argentina, during 1994, 1995 and 1996. The temperature records for the fishing sites used in the study are shown in Fig. 3. Róbalo (*Eleginops maclovinus* Valenciennes) was caught in February (summer) and August (winter). Dorado (*Paranotothenia magellanica* Forster), lorcho (*Patagonotothen tessellata* Richardson) and the haemoglobin-less pickerel icefish *Champsocephalus esox* Günther (family Channichthydiae) were sampled in February. All fish were used within 48 h of capture. *Coris julius* L., *Lithognathus mormyrus* L. and *Serranus cabrilla* L. were caught in the Bay of Naples, Mediterranean Sea, during the autumn of 1996 (temperature 18–20 °C). All the fish were sampled within a few days of capture. A further five *Serranus cabrilla* were obtained for experiments on mitochondrial oxygen consumption in the spring of 1997. They were transported to the Gatty Marine Laboratory and were maintained at 20 °C in a recirculating aquarium for 2 weeks and fed a diet of chopped squid and shrimps.

Tissue sampling

The pectoral fin adductor muscle of the species studied is composed of a mixture of muscle fibre types. All species contained a large region of homogeneous red muscle fibres adjacent to the pelvic girdle (J. Calvo, I. A. Johnston, D. Fernandez and C. E. Franklin, in preparation). For ultrastructural analyses, small bundles of red muscle fibres were isolated from the red muscle zone and pinned *via* their

ANTARCTIC SPECIES (−1.86 to +2°C)
Order Perciformes, sub-order Notothenioidei
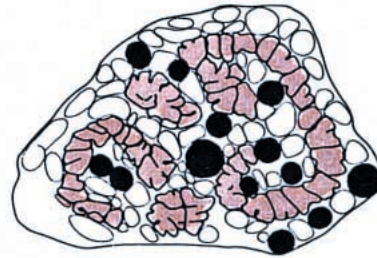Family Nototheniidae; sub-family Nototheniinae

A

12.4–14.0 cm SL
29.5–50.1 g (5)
Habit: demersal/pelagic, moderately active

B

9.2–11.5 cm SL (4)
5.4–24.1 g (5)
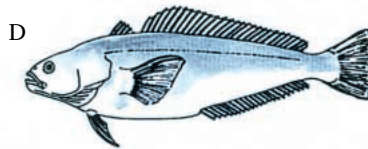Habit: pelagic/active
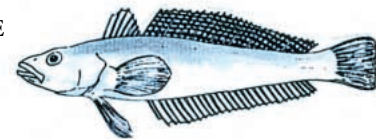
Lepidonotothen nudifrons

Trematomus newnesi

SUB-ANTARCTIC SPECIES (4–11°C)
Order Perciformes, sub-order Notothenioidei
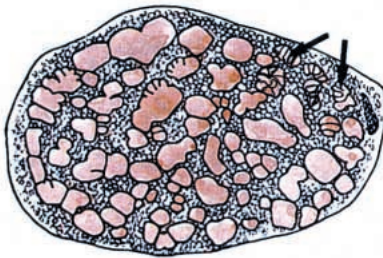Family Nototheniidae; sub-family Nototheniinae

C

22.5–25.0 cm SL
74.7–90.0 g (2)
Habit: pelagic/active

D

13.4–15.7 cm SL
36.5–69.0 g (5)
Habit: pelagic/active

E

16.7–20.4 cm SL
69.4–102.5 g (5)
Habit: demersal/sluggish

Champsocephalus esox

Paranotothenia magellanica

Patagonotothen tessellata

Fig. 1. (A–E) Antarctic and sub-Antarctic perciform fishes studied with information on their standard length (SL), body mass, locomotory habit and environmental temperature. The number of individual fish sampled is shown in parentheses. Also illustrated are *camera lucida* drawings of representative transverse sections of red muscle fibres isolated from the pectoral fin adductor muscle. Myofibrils are coloured brown, mitochondria are the white circles surrounding them and lipid droplets are coloured black. Scale bars, 10 μm.
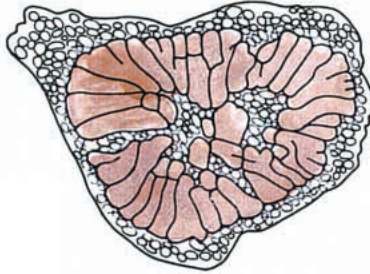
SUB-ANTARCTIC SPECIES
Order Perciformes, sub-order Notothenioidei
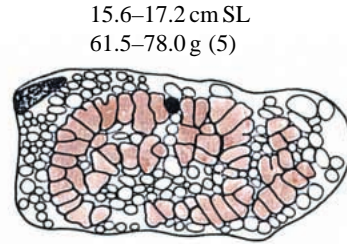Family Nototheniidae; sub-family Eleginopiinae

A

14.4–17.0 cm SL
38.8–75.7 g (5)

Habit: pelagic/active

15.6–17.2 cm SL
61.5–78.0 g (5)

Eleginops maclovinus
(winter ≈4°C)

Eleginops maclovinus
(summer ≈11°C)

MEDITERRANEAN SPECIES (16–25°C)
Order Perciformes

Family Sparidae

Family Labridae

Family Serranidae

B

C

D
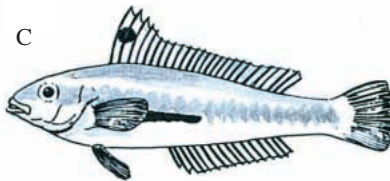
11.8–15.3 cm SL
38.3–68.5 g (5)
Habit: pelagic/moderately
active
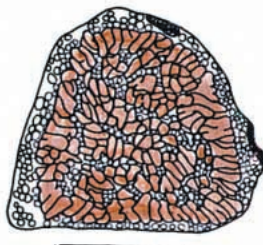
9.3–13.7 cm SL
5.8–23.2 g (4)
Habit: pelagic/active
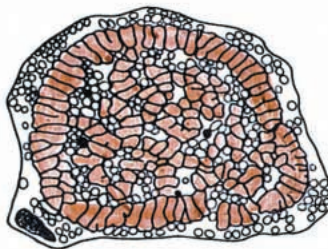
6.9–11.4 cm SL
5.7–23.9 g (9)
Habit: pelagic/sedentary

Lithognathus mormyrus

Coris julius
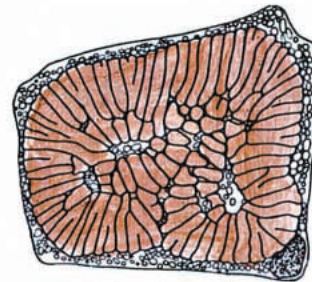
Serranus cabrilla

Fig. 2. (A–D) A non-Antarctic and the Mediterranean perciform fish studied with information on their standard length (SL), body mass, locomotory habit and environmental temperature. The number of individual fish sampled is shown in parentheses. Also illustrated are *camera lucida* drawings of representative transverse sections of red muscle fibres isolated from the pectoral fin adductor muscle. Scale bars, 10 μm. For further details, see Fig. 1.

insertions to cork or Sylgard strips and immersion-fixed in 2.5 % glutaraldehyde, 0.15 mmol l^{-1} phosphate buffer, pH 7.4, at 4 °C. Samples were subsequently cut into 3–5 small pieces, post-fixed in phosphate-buffered 1 % (m/v) osmium tetroxide, rinsed, dehydrated and embedded in Araldite CY212 resin (EM-scope, Ashford, England).

Quantitative ultrastructural analysis

Blocks were orientated and transverse 80 nm sections cut on a Reichart OM-U2 ultramicrotome. Sections were mounted on 100-mesh pyroxyline-coated copper grids and doubled-stained with 2 % (m/v) uranyl acetate and lead citrate. Micrographs of whole red muscle fibres were taken using a Philips 301 transmission electron microscope at 60 kV (magnification ×1900). For each species, approximately 20 muscle fibres obtained from two randomly selected blocks per individual were analysed. The micrographs were projected using a photographic enlarger (magnification ×2.8), and the outlines of the fibre and organelles were traced onto white paper. The cross-sectional areas of muscle fibres and the cross-sectional areas and perimeters of mitochondrial clusters, lipid droplets and myofibrils were measured using a digital planimeter (Kontron Image Analysis System, Kontron, Basel, Switzerland). The volume density (V_v) (summed cross-sectional areas of organelles/cross-sectional area of muscle fibre) and surface density (S_v) (summed perimeter of organelles/cross-sectional area of muscle fibre) ($\mu m^2 \mu m^{-3}$) of mitochondrial clusters (mt), myofibrils (my) and lipid droplets (l) were determined for each muscle fibre. From these measurements, mitochondrial mean free spacing λ_a was calculated as follows:

$$\lambda_a = 4(1 - V_v)/S_v \qquad (1)$$

(Weibel, 1980). Micrographs (approximately 20 per species) of intermyofibrillar mitochondria were chosen at random and photographed at a magnification of ×20 000. The micrographs were enlarged using a photographic enlarger (×2.8), and the mitochondria and mitochondrial cristae were traced to obtain a good black-and-white image. The sum of the perimeters of the internal membranes and the cross-sectional areas of the outer mitochondrial membrane were determined using image analysis software in order to calculate the surface density of cristae $S_v(cr,mt)$ ($\mu m^2 \mu m^{-3}$). No correction was made for section thickness.

Isolation of mitochondria

Mitochondria were isolated at 4 °C from the pooled red portion of the pectoral fin adductor muscle dissected from both sides of the body. Mitochondria were isolated as described in Johnston *et al.* (1994) in 8 volumes of a medium containing (in mmol l^{-1}): 140, KCl; 10, EDTA; 5, MgCl$_2$; 20, Hepes; 0.5 % (m/v) bovine serum albumin (BSA), pH 7.4 at 4 °C. Tissue was homogenized with a motorized Potter–Elvejhem tissue grinder (two passes with a loose pestle and two passes with a tight pestle, both at 900 revs min^{-1}). Mitochondria were prepared from the homogenate by differential centrifugation

(1400 g for 5 min and 9000 g for 7 min). The pellet was resuspended in the respiration medium, which contained (in mmol l^{-1}): 140, KCl; 5, Na$_2$HPO$_4$; 20, Hepes, 0.5 % (m/v) BSA, pH 7.4 at 4 °C. The temperature-dependence of the pH of the assay medium was −0.015 pH units °C^{-1}. A sample of the mitochondria was resuspended in the assay medium minus BSA, washed by centrifugation (9000 g for 10 min) and resuspension, and the protein concentration determined in duplicate using a modified Lowry method, using 10 % (m/v) deoxycholate to solubilise membranes. Biochemicals were obtained from Sigma Chemicals (St Louis, USA).

Oxygen consumption measurements

The rate of oxygen consumption of mitochondria was measured at a range of temperatures using a water-jacketed respiration cell fitted with a Clarke-type electrode (Rank Brothers Ltd, Cambridge, UK). The system was calibrated with the respiration medium saturated with air over the same temperature range using a couloximeter (Johnston *et al.* 1994). Approximately 0.5 mg of mitochondrial protein was added to 1 or 1.5 ml of assay medium. The rate of oxygen uptake was measured following the addition of 0.1 mmol l^{-1} malate to spark the Krebs cycle and either 2.5 mmol l^{-1} pyruvate or 0.025 mmol l^{-1} palmitoyl-DL-carnitine. The maximal state 3 rate was obtained by adding 0.5 mmol l^{-1} ADP. After a short initial period, state 3 respiration rates were approximately linear until the ADP was exhausted. 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).

Statistics

All results are expressed as means ± S.E.M. Ultrastructural data were compared using three-way analyses of variance (ANOVAs) with activity pattern and geographical zone as between-subject factors and species as a nested factor with geographical zones. The species were classified *a priori* as either highly active (*T. newnesi*, *Champsocephalus esox*, *E. maclovinus*, *Paranotothenia magellanica* and *Coris julius*) or moderately active/sluggish (*Lepidonotothen nudifrons*, *Patagonotothen tessellata*, *Lithognathus mormyrus* and *S. cabrilla*). Further *post-hoc* Fisher's protected least significant difference tests were carried out. A second-order polynomial was used to regress the rate of oxygen uptake of isolated mitochondria *versus* assay temperature.

Results

Muscle ultrastructure

The quantitative ultrastructural measurements of red pectoral fin adductor muscle fibres are shown in Table 1. Most species had red muscle fibres with mean diameters of 17–23 μm regardless of body size or habit (Table 1; Figs 1A–E, 2A–D). Larger mean fibre diameters were found in *Champsocephalus esox* (56 μm), *Patagonotothen tessellata* (41 μm) and *Lepidonotothen nudifrons* (37 μm). The total

volume density of mitochondria, $V_v(\text{mt,f})$, was 0.51 for *Champsocephalus esox* and between 0.27 and 0.33 for all the red-blooded sub-Antarctic and Antarctic species studied (Table 1). No consistent differences were noted among the mitochondrial volume densities of active mid-water species (*Trematomus newsnesi*, 0.31; *Eleginops maclovinus*, 0.31–0.33; *Paranotothenia magellanica*, 0.27) and a relatively sedentary demersal species *Patagonotothen tessellata* (0.33) (Table 1). Of the Mediterranean species studied, only red muscle fibres in *Coris julius* had comparable mitochondrial volume densities to the polar species, with significantly lower values found in *Lithognathus mormyrus* (0.13) and *Serranus cabrilla* (0.08) (Table 1). The three-way ANOVA revealed highly significant main effects of activity pattern ($F_{(1,178)}=50.2$; $P<0.0001$) and species (geographical zone) ($F_{(5,178)}=26.8$; $P<0.0001$) on mitochondrial volume density. The percentage of the total mitochondria found in the sub-sarcolemmal area varied from 37.9 % in *Champsocephalus esox* to 76.4 % in *Lithognathus mormyrus* (Table 1) and showed no consistent trend with geographical region (zone) or activity patterns. There were significant differences in the surface density of mitochondrial clusters between activity patterns ($F_{(1,178)}=35.5$; $P<0.0001$) and between species (geographical zone) ($F_{(5,178)}=6.17$; $P<0.0001$), but not amongst geographical zones. The volume density of intermyofibrillar mitochondria in *Champsocephalus esox* (0.31) was 2–3 times higher than that of the red-blooded Antarctic and sub-Antarctic species (0.094–0.17), and more than 10 times higher that in *L. mormyrus* (0.028) and *S. cabrilla* (0.018). The mean free spacing of mitochondria decreased from 9.5 µm in *S. cabrilla* to 3.1 µm in *Champsocephalus esox* (Table 1). The three-way ANOVA revealed highly significant main effects of activity pattern ($F_{(1,178)}=42.2$; $P<0.0001$) and species (geographical zone) ($F_{(5,178)}=11.0$; $P<0.0001$) on mitochondrial free spacing.

The volume density of myofibrils, $V_v(\text{my,f})$, showed considerable inter-specific variation and was approximately 0.4 in *T. newnesi*, *L. nudifrons*, *C. esox*, *E. maclovinus*, *P. magellanica* and *P. tessellata*, 0.52 in *Coris julius* and highest in *L. mormyrus* (0.74) and *S. cabrilla* (0.80). There were significant effects of activity ($F_{(1,178)}=18.5$; $P<0.001$), geographical zone ($F_{(2,178)}=8.29$; $P<0.05$) and species (geographical zone) ($F_{(5,178)}=15.0$; $P<0.001$) on the volume density of myofibrils. A Fisher's protected least significant difference test revealed that the Mediterranean species have a significantly higher volume density of myofibrils than the Antarctic and sub-Antarctic species ($P<0.05$). The spatial organization of myofibrils varied considerably among the species. *Champsocephalus esox* showed the most distinctive organisation, with single myofibrils surrounded by

Table 1. *Ultrastructural characteristics of slow muscle fibres from the pectoral fin adductor muscles of Antarctic, sub-Antarctic and Mediterranean perciform fish*

Species	Geographical zone	Muscle fibre diameter (µm)	Total mito-chondrial volume density	% Mito-chondria in sub-sarcolemmal zone	Mito-chondrial mean free spacing (µm)	Surface density of mito-chondrial clusters ($\mu m^2\,\mu m^{-3}$)	Myofibril volume density	Lipid droplet volume density
Lepidonotothen nudifrons (20)	Antarctic	36.6±2.8	0.29±0.024	48.6±1.6	4.1±0.6	0.87±0.08	0.40±0.021	0.015±0.05
Trematomus newnesi (20)	Antarctic	17.4±2.6	0.31±0.019	67.8±2.6	2.8±0.2	1.08±0.07	0.36±0.019	0.03±0.007
Champsocephalus esox (20)	Sub-Antarctic	55.6±8.8	0.51±0.026	37.9±5.5	3.1±0.3	0.69±0.06	0.38±0.026	0
Eleginops maclovinus (summer) (20)	Sub-Antarctic	19.2±3.8	0.33±0.073	60.1±3.3	2.1±0.1	1.4±0.08	0.45±0.019	0.003±0.01
Eleginops maclovinus (winter) (20)	Sub-Antarctic	20.4±3.2	0.31±0.014	69.9±3.1	3.0±0.2	0.96±0.05	0.45±0.021	0.001±0.01
Paranotothenia magellanica (summer) (20)	Sub-Antarctic	19.2±3.6	0.27±0.015	65.5±2.3	3.1±0.3	1.06±0.08	0.46±0.017	0.004±0.002
Patagonotothen tessellata (20)	Sub-Antarctic	41.4±6.4	0.33±0.028	53.1±4.4	3.8±0.4	0.76±0.07	0.37±0.022	0.03±0.012
Coris julius (20)	Mediterranean	22.8±3.0	0.32±0.015	63.3±2.8	2.7±0.3	1.16±0.07	0.52±0.035	0.002±0.004
Lithognathus mormyrus (20)	Mediterranean	20.4±2.6	0.13±0.004	76.4±1.7	6.1±0.7	0.7±0.007	0.74±0.015	0
Serranus cabrilla (20)	Mediterranean	21.4±2.6	0.08±0.007	75.6±2.3	9.5±1.0	0.47±0.04	0.80±0.004	0

Values represent means ± S.E.M. The number of fibres analysed is given in parentheses.

mitochondria (Figs 1C, 4A). In muscle fibres of the other species, myofibrils were arranged in ribbons of which the thickness varied among the biogeographical zones (Fig. 1). In the Antarctic species, the ribbons were one fibril thick (Fig. 1A,B), whereas in the sub-Antarctic species they formed thicker clusters (Figs 1D,E, 2A). In the Mediterranean species, the ribbons of myofibrils were more densely packed (Fig. 2B–D) with those closest to the sarcolemma typically flattened in cross-section, resembling the spokes in a bicycle wheel (Fig. 2D). The volume density of intracellular lipid droplets was not correlated with biogeographical zone or locomotory habit and the maximum value was 0.03 (Table 1).

The volume densities of mitochondria and myofibrils in the red muscle fibres of *E. maclovinus* were similar in summer- and winter-caught fish (Table 1). However, the surface density of mitochondrial clusters was significantly higher ($P<0.01$) and the mean free spacing of mitochondrial clusters was significantly lower ($P<0.01$) in summer than winter fish (one-way ANOVA) (Table 1).

Mitochondrial cristae surface densities were similar among the species examined in this study, ranging from 36 to $50\,\mu m^2\,\mu m^{-3}$ (Table 2, Fig. 4A–C). Mitochondria from the sub-Antarctic ice-fish *Champsocephalus esox* showed a considerably higher cristae surface density ($43.9\,\mu m^2\,\mu m^{-3}$, Fig. 4B) than previously found for two species of Antarctic icefish (25–$28\,\mu m^2\,\mu m^{-3}$) (Archer and Johnston, 1991; Table 2). The surface area of mitochondrial cristae per unit fibre volume was approximately twice as high in the pelagic sub-Antarctic channichthyid *Champsocephalus esox* as in either Antarctic icefishes or pelagic red-blooded sub-Antarctic species (Table 2). Among the Mediterranean species studied, the surface area of mitochondrial cristae per unit fibre volume was three times higher in *Coris julius* than in *Lithognathus mormyrus* (Table 2).

Mitochondrial respiration

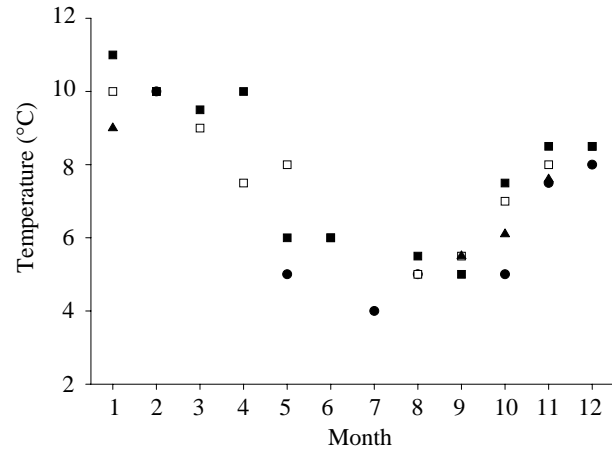The state 3 respiration rates of mitochondria with pyruvate

Fig. 3. Temperature records at the fishing sites in the Beagle Channel, Tierra Del Fuego, Argentina. Seasonal records for sea surface temperature in 1994 (circles, Bahia Ushuaia), 1995 (open squares, Conejo Island; filled squares Bahia Chica) and 1996 (triangles, Bahia Ushuaia).

as substrate are shown in Fig. 5. RCR values ranged from 4.2 to 10.0 for preparations from Antarctic species, from 4.0 to 7.7 for the sub-Antarctic species and were approximately 5 for *S. cabrilla*, showing that the mitochondria were coupled. The rate of mitochondrial oxygen consumption increased with temperature for each species with a Q_{10} of approximately 2.0. Measured at their respective habitat temperatures, the maximal rate of oxygen consumption was twice a high in pelagic and semi-pelagic sub-Antarctic as in Antarctic species. State 3 respiration rates of isolated mitochondria from *Lepidonotothen nudifrons* and *T. newnesi* at $-1\,^{\circ}C$ were only 37.5 % of that for *S. cabrilla* at $20\,^{\circ}C$ (Fig. 5). The combined data for these perciform species could be best described by a single quadratic relationship:

$$\text{oxygen consumption} = 27.21 + 2.16T + 0.028T^2, \qquad (2)$$

Table 2. *Surface density of cristae in mitochondria from the red pectoral fin adductor muscle of perciform fish*

Biogeographical zone	Species	Surface density of mitochondrial cristae ($\mu m^2\,\mu m^{-3}$)	Surface area of mitochondrial cristae per unit fibre volume ($\mu m^2\,\mu m^{-3}$)
Antarctic	*Champsocephalus gunnari**	25.2±1.5	12.3
	*Chaenocephalus aceratus**	28.2±0.8	14.1
	*Notothenia gibberifrons**	37.0±1.0	11.1
	Trematomus newnesi	35.8±8.2	11.1
Sub-Antarctic	*Champsocephalus esox*	43.9±2.1	22.4
	Eleginops maclovinus (summer)	39.2±3.9	12.9
	Paranotothenia magellanica (summer)	45.1±4.7	12.2
Mediterranean	*Lithognathus mormyrus*	39.2±3.9	5.1
	Coris julius	49.8±4.7	15.9

*Data from Archer and Johnston (1991) and Johnston (1987).

Twenty mitochondria were analysed from five fish.
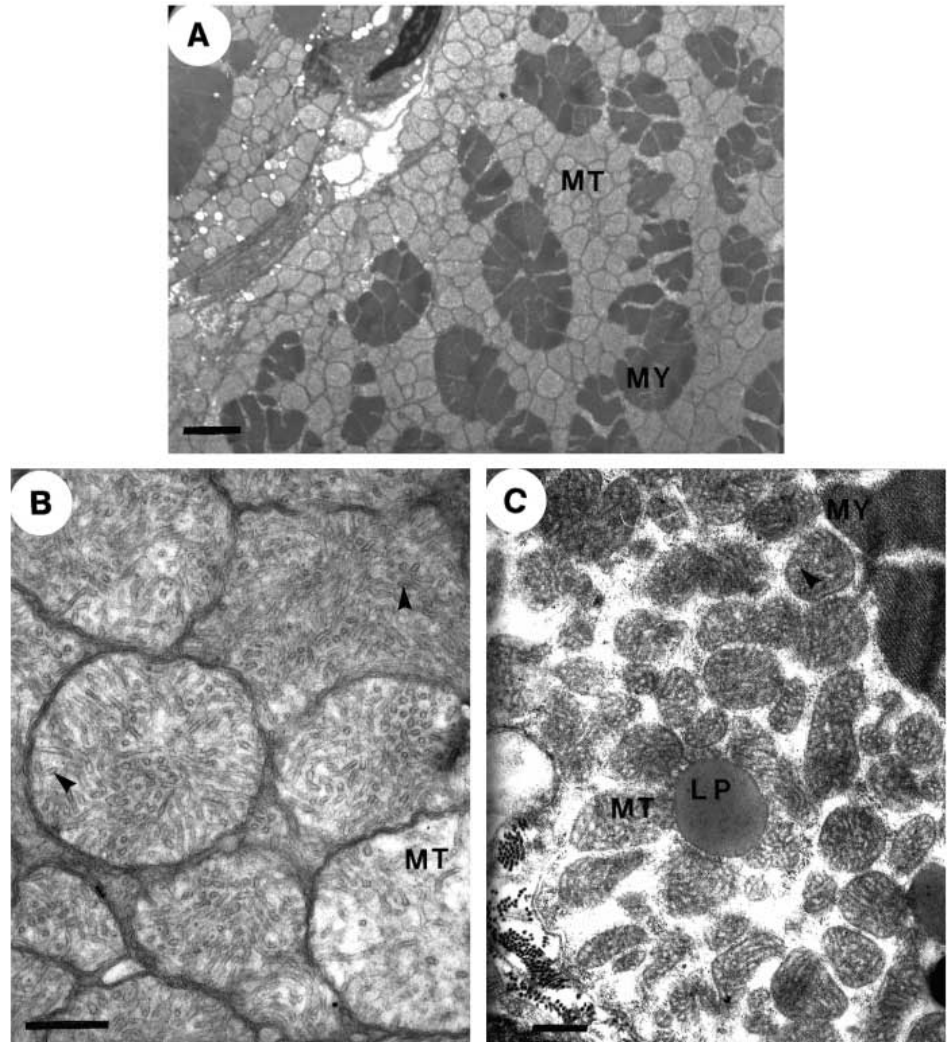
Values represent mean ± S.E.M.

Fig. 4. (A) Transmission electron micrograph of a red fibre from the pectoral fin adductor muscle of the icefish *Champsocephalus esox*. Scale bar, 2 μm. (B) Mitochondia from icefish red muscle. Scale bar, 0.5 μm. (C) Mitochondria from red muscle of *Lithognathus mormyrus*. Scale bar, 0.5 μm. The arrowheads illustrate internal cristae. MT, mitochondria; MY, myofibrils; LP, lipid droplet.

(r^2=0.93), where T is temperature (in degrees) and oxygen consumption is nmol O mg^{-1} mitochondrial protein^{-1} min^{-1}. With the exception of the data for *N. coriiceps* (taken from Johnston *et al.* 1994), the mean values at physiological temperatures fell within the 95 % confidence limits of this relationship. The mean rate of oxygen consumption per milligram mitochondrial protein at 1 °C for all the perciform species was 29.4 nmol O mg^{-1} min^{-1} compared with 37.9 nmol O mg^{-1} min^{-1} if the calculation was made with the Antarctic species omitted from the regression. A few measurements were also made with palmitoyl carnitine as substrate, giving state 3 respiration rates of 23.7±12.1 nmol O mg^{-1} min^{-1} (*N*=5) at −1 °C for *Lepidonotothen nudifrons* and 48.3±7.1, 50.6±7.4 and 72.5± 11.8 nmol O mg^{-1} min^{-1}, respectively, for *E. maclovinus* at 4, 8 and 12 °C (*N*=6) (means ± S.E.M.).

Discussion

To a first approximation, in the perciform species studied, the maximum rate of oxygen consumption per milligram of isolated red muscle mitochondrial protein was a direct function of assay temperature (Fig. 5). Pyruvate, the carbon substrate

that these mitochondria were oxidizing, generally yields maximum rates of state 3 respiration in fish mitochondria (Guderley and St Pierre, 1996). Similar results, although with more variance in the data, have been reported for mitochondria isolated from several perciform, salmoniform and cypriniform families (Johnston *et al.* 1994; Guderley and St Pierre, 1996). In the present study, the oxidative capacities of red muscle mitochondria isolated from the Antarctic species were either within or slightly below the 95 % confidence limits of the relationship between assay temperature and mitochondrial oxygen uptake for the overall data set. The predicted rate of oxygen consumption at 1 °C was slightly higher if the Antarctic species were excluded from the regression. The results therefore clearly indicate that little significant up-regulation of maximum oxidative capacity per milligram mitochondrial protein has occurred in the Antarctic perciforms.

Although insufficient information is available to make a quantitative phylogenetic analysis, it is interesting to note that the two sub-Antarctic species have similar mitochondrial respiration rates and yet the dorado (*Patagonotothen magellanica*) is thought to be more distantly related to the róbalo, *Eleginops maclovinus* than to the Antarctic
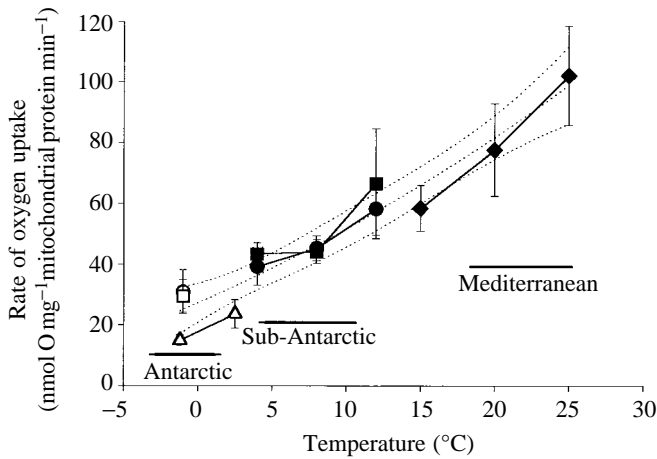
Fig. 5. State 3 respiration rates of isolated mitochondria with pyruvate as substrate. Values represent means ± S.E.M. (N=5 or 6). Antarctic species, *Trematomus newnesi* (open circles), *Lepidonotothen nudifrons* (open squares) and *Notothenia coriiceps* (open triangles) (from Johnston *et al.* 1994); sub-Antarctic species, *Eleginops maclovinus* (filled circles) and *Paranototothenia magellanica* (filled squares); Mediterranean species, *Serranus cabrilla* (filled diamonds). The central dotted line represents a second-order polynomial fitted to the data: state 3 respiration rate=27.21+2.16T+0.028T^2, where state 3 respiration rate is nmol O mg^{-1} protein min^{-1} and T is temperature (in °C); adjusted r^2=0.93, P=<0.001. The outer dotted lines are 95 % confidence intervals.

Nototheniidae *Trematomus newnesi* and *Lepidonothen nudifrons*. The oxidative capacities of mitochondria from *T. newnesi* and *Lepidonotothen nudifrons* were significantly higher than we reported previously for mitochondria from another Antarctic species *Notothenia coriiceps* (P<0.05; Mann–Whitney U-test) (Fig. 5; Johnston *et al.* 1994). The available information on the natural history, morphology and behaviour of these fish suggests that *N. coriiceps* is much less active than the other species (Eastman, 1993; North, 1996). Field observations of an individual *N. coriiceps* with an underwater television camera showed that it stayed within 3 m of a small cave for over 6 days, spending only 1.2 % of the time swimming, usually for short periods at less than 2 body lengths s^{-1} (North, 1996). The lower oxidative capacity of the isolated mitochondria of this species may be related to this more sedentary behaviour.

An enhanced mitochondrial cristae surface density, at constant mitochondrial densities, could expand tissue aerobic capacity in the absence of increases in oxidative capacity per milligram mitochondrial protein. However, as with the latter, cristae density showed no evidence of up-regulation in Antarctic species (Table 2). Interestingly, the mitochondrial cristae surface density for the pelagic sub-Antarctic icefish *Champsocephalus esox* was considerably higher than that of its Antarctic counterparts, as was the cristae surface area per unit fibre volume (Table 2). The cristae surface densities we report here are similar to or higher than values reported for mitochondria from a wide variety of mammalian skeletal muscle (20–40 µm^2 µm^{-3}) (Hoppeler and
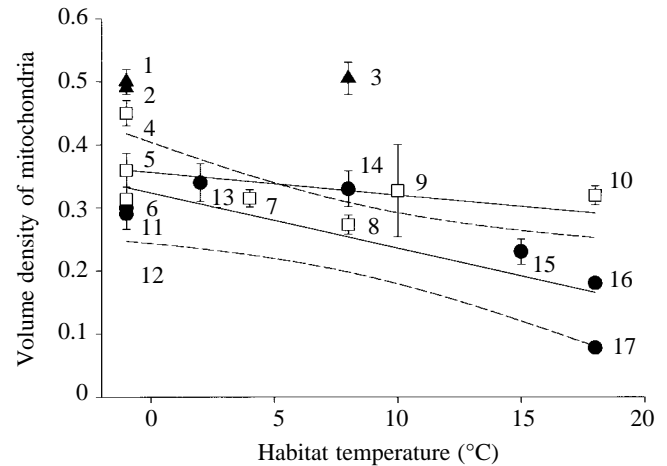

Fig. 6. Relationship between the volume density of mitochondria and habitat temperature for red pectoral fin adductor muscle fibres in perciform fish. Filled triangles are Channichthyidae; open squares are active pelagic species, and filled circles are a combination of demersal or only moderately active mid-water species. Values represent means ± S.E.M. (see Table 1 and original references for the numbers of muscle fibres per fish examined; in most cases, this was a minimum of 20 fibres from five or six fish). 1, *Chaenocephalus aceratus*; 2, *Champsocephalus gunnari* (Archer and Johnston, 1991); 3, *Champsocephalus esox* (this study); 4, *Psilodraco breviceps* (Archer and Johnston, 1991); 5, *Notothenia coriiceps* (pelagic blue phase fingerling) (Johnston and Camm, 1987); 6, *Trematomus newnesi* (this study); 7, 8, respectively, winter- and summer-caught *Eleginops maclovinus* (this study); 9, *Paranototothenia magellanica* (this study); 10, *Coris julius* (this study); 11, *Notothenia gibberifrons* (Londraville and Sidell, 1990); 12, *Lepidonotothen nudifrons* (this study); 13, *Notothenia rossii* from South Georgia (Walesby and Johnston, 1980); 14, *Patagonotothen tessellata* (this study); 15, *Callionymus lyra* from the West Coast of Scotland in summer (Archer and Johnston, 1991); 16, *Lithognathus mormyrus* (this study); 17, *Serranus cabrilla* (this study). The lines represent linear regressions and 95 % confidence limits. For the species classified as less active (solid line with dashed lines representing the 95 % confidence limits), the equation was V_v(mt)=0.33−0.0088T, where V_v(mt) is the volume density of mitochondria and T is habitat temperature; adjusted r^2=0.64. P=0.03. There was no significant relationship between mitochondrial volume density and habitat temperature for the species classified as active/pelagic, the equation was V_v(mt)=0.35−0.0036T; adjusted r^2=0.22, P=0.31.

Lindstedt, 1985; Schwerzmann *et al.* 1989), and mammalian and reptilian heart muscle (35–60 µm^2 µm^{-3}) (Else and Hulbert, 1985), but are lower than those reported for hummingbird flight muscle (58 µm^2 µm^{-3}) (Suarez *et al.* 1991) or tuna red muscle (63–70 µm^2 µm^{-3}) (Moyes *et al.* 1992). Srere (1985) calculated that, in order to have sufficient matrix space for two average-sized Krebs cycle enzymes, the maximum possible cristae surface density would be approximately 83 µm^2 µm^{-3}. The cristae surface densities of mitochondria from hummingbird flight muscle and tuna red myotomal muscle approach this value.

Mitochondrial cristae surface density is not a particularly good predictor of mitochondrial oxidative capacity corrected

for the influence of temperature. Within the perciforms, the oxidative capacities of isolated mitochondria from *E. maclovinus* and *Paranotothenia magellanica* were identical, whereas their mitochondrial cristae surface densities differed (39 *versus* 45; Fig. 5; Table 2). Whereas mitochondria from tuna muscle possess cristae surface densities of 63–70 $\mu m^2 \mu m^{-3}$, their oxidative capacities at 25 °C (106 nmol O mg^{-1} mitochondrial protein min^{-1}) (Moyes *et al.* 1992) are no higher than the rates expected at 25 °C for the perciform mitochondria we studied (Fig. 5), for which cristae surface densities ranged from 36 to 50 $\mu m^2 \mu m^{-3}$. These data suggest that, if the packing limits of cristae and matrix proteins are respected, oxidation rates per milligram mitochondrial protein at a common temperature change little. Further, these interspecific comparisons suggest a fairly constant protein content of the mitochondrial inner membrane. Nonetheless, the fourfold higher oxidation rates found at 25 °C for tuna heart mitochondria (400 nmol O mg^{-1} mitochondrial protein min^{-1}), which have a cristae surface density of 56 $\mu m^2 \mu m^{-3}$ (Moyes *et al.* 1992), suggest that oxidative capacity may be increased by other mechanisms, perhaps membrane lipid composition or specific activities of mitochondrial proteins.

The mitochondrial volume density of red muscle fibres was significantly higher in the pelagic haemoglobin-less icefish *Champsocephalus esox* than in any of the red-blooded fish (Table 1). The contractile filaments in this species were arranged as individual myofibrils or as small clusters of myofibrils entirely surrounded by mitochondria, accounting for approximately 50 % of muscle fibre volume, as was previously reported for the Antarctic icefishes *Chaenocephalus aceratus* (Johnston, 1987) and *Champsocephalus gunnari* (Archer and Johnston, 1991). Interestingly, the cristae surface density of mitochondria (43.9 $\mu m^2 \mu m^{-3}$) in *Champsocephalus esox* is comparable with that of the red-blooded species (Table 2) and was considerably higher than those reported for *Chaenocephalus aceratus* (28.2 $\mu m^2 \mu m^{-3}$) and *Champsocephalus gunnari* (25.2 $\mu m^2 \mu m^{-3}$) (Archer and Johnston, 1991). Mitochondrial packing in icefishes may approach the theoretical maximum for a locomotory muscle beyond which there is insufficient space to accommodate sufficient myofibrils to generate the required stress. The maximum stress exerted by the myofibrils is thought to be relatively constant between species with different body temperatures (Johnston, 1985). Higher values for V_v(mt,f) of 0.63 have been reported in the swordfish *Xiphus gladius* for the heater organ, which is formed from a highly modified extraoccular muscle (Block, 1994). However, in this case, myofibrils are absent, although the sarcoplasmic reticulum is more extensive than in any fish locomotory muscle, a feature thought to be related to the futile cycling of Ca^{2+} to activate thermogenesis (Block, 1994).

Among the relatively active pelagic or semi-pelagic red-blooded species studied (Figs 1, 2), the volume density of mitochondria was between 0.27 and 0.33 (Table 1). Examination of pectoral fin adductor muscles in a wider range of Perciformes that use a predominantly labriform style of

swimming suggests that there is no significant relationship between V_v(mt,f) and habitat temperature (Fig. 6). Although this conclusion relies heavily on the data point for *Coris julius* (point 10 in Fig. 6), it is supported by measurements of mitochondrial volume density in red myotomal fibres from other active pelagic species, e.g. 0.46 in the European anchovy (*Engraulis encrasicolus*), a filter feeder, living at 18–25 °C (Johnston, 1982*b*) and 0.29 in the skipjack tuna (*Katsuwonus pelamis*) (Mathieu-Costello *et al.* 1992; Moyes *et al.* 1992). The latter is a tropical species exhibiting regional endothermy and it can maintain elevated red muscle temperatures of up to 31 °C during bouts of active swimming (Stevens and Neill, 1978).

In contrast, among the demersal species and moderately active mid-water fish, there was a highly significant inverse correlation between mitochondrial volume density and the habitat temperature at which the fish were captured (adjusted r^2=0.64, P=0.03) (Fig. 6). It has been suggested that the proliferation of mitochondria with cold-acclimation represents a mechanism to compensate for the reduction in the diffusion coefficients of cytostolic metabolites by decreasing the mean free spacing between mitochondria (Tyler and Sidell, 1994). However, Hubley *et al.* (1997) recently utilised pulsed-field gradient ^{31}P nuclear magnetic resonance spectroscopy and a mathematical reaction–diffusion model to calculate profiles of phosphocreatine and ADP concentration in muscle fibres of goldfish *Carassius auratus* acclimated to either 5 °C or 25 °C. Their results showed constant metabolite gradients within the red muscle fibres over time regardless of temperature, thermal acclimation state or mitochondrial volume density. The results clearly indicate that the proximal stimulus for temperature-induced changes in mitochondrial volume density is not a disruption of intracellular diffusion of high-energy phosphates. Nonetheless, limitations in the diffusion of other metabolites, specifically carbon substrates, may provide such a stimulus. Mean free spacing of mitochondria is lower in active pelagic fish and at high temperatures; however, this may well represent a secondary consequence rather than a primary aim of the increase in mitochondrial volume density.

The total aerobic capacity is likely to be some function of the number and functional capacity of the mitochondria. However, quantitative predictions concerning the maximal rate of oxygen consumption *in vivo* are difficult since this will depend among other things on intracellular pH (Moyes *et al.* 1988), the availability of ADP and NADH (Brand and Murphy, 1987) and the delivery of oxygen and fuel by the capillary circulation (Mathieu-Costello *et al.* 1992). Nevertheless, for active pelagic species, our results indicate that the maximum aerobic capacity of red muscle is substantially lower in polar than in tropical fish. From the regression line in Fig. 6, the volume density of mitochondria in the red muscle of relatively inactive fish decreased approximately twofold between −1 and 22 °C, whereas the maximal (state 3) rate of oxygen uptake ($\dot{V}_{O_2max}$) of the isolated mitochondria increased almost fourfold (Fig. 5). Thus, for less-active swimmers, the proliferation of mitochondria in polar relative to Mediterranean species would

serve to compensate partially for the reduced mitochondrial oxidative capacity per milligram protein at low temperatures. There is evidence that the factorial aerobic scope ($\dot{V}_{O_2max}/\dot{V}_{O_2rest}$) for activity is approximately 5–7 for non-athletic fish species (Forster *et al.* 1987; Gordon *et al.* 1989; Johnston *et al.* 1991). In a study of sedentary demersal fish with similar lifestyles, it was found that, after correcting for differences in body mass, resting metabolic rate was three- to fourfold lower in Antarctic than in Indo-West Pacific species (Johnston *et al.* 1991). If a fairly constant relationship between resting and maximum aerobic metabolism (Wieser, 1985) pertains to these species, a low resting rate suggests a low maximal rate. The results of our current study are therefore consistent with the relatively low aerobic scope for activity that has been measured in polar species (Forster *et al.* 1987; Johnston *et al.* 1991; Bushnell *et al.* 1994). Although exploitation of cold seas is associated with extensive modifications in membrane lipid composition (Hazel and Williams, 1990) and the ligand-binding properties of enzymes (Somero *et al.* 1996), and such modifications are certain to have been required to permit mitochondrial activity at sub-zero temperatures, maximum rates of mitochondrial substrate oxidation (per milligram mitochondrial protein) have not been up-regulated to attain values similar to those found in species specialised for life in warm seas.

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