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ULTRASTRUCTURE OF AEROBIC MUSCLE IN ANTARCTIC FISHES MAY CONTRIBUTE TO MAINTENANCE OF DIFFUSIVE FLUXES

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Summary

Quantitative ultrastructural analyses were performed on red (oxidative) and white (glycolytic) skeletal muscles from two species of antarctic fish to identify features of subcellular structure that may be related to muscle metabolism at cold body temperature. *Trematomus newnesi* (Boulenger) is an active pelagic species and *Notothenia gibberifrons* (Lönnberg) is a sluggish bottom-dweller. White fibres of both species are poorly vascularized [capillary density, N_A(c,f), for *T. newnesi* is $73.9\pm11.3\,mm^{-2}$; for *N. gibberifrons* is $76.0\pm14.1\,mm^{-2}$], and have low percentages of cell volume occupied by mitochondria [volume density, V_v(mit,f), for *T. newnesi* is 0.014 ± 0.005; for *N. gibberifrons* is 0.006 ± 0.003]. Ultrastructure of oxidative fibres in both species resembles that of cold-acclimated temperate-zone fishes. Mitochondrial volume densities of red fibres reflect differences in ecotype between species [V_v(mit,f) for *T. newnesi* is 0.348 ± 0.012; for *N. gibberifrons* is 0.249 ± 0.007]. The less clustered array of mitochondria in oxidative fibres of *T. newnesi* compared with *N. gibberifrons* may support an equivalent flux of aqueous metabolites between mitochondrial and cytoplasmic compartments, despite a greater mean intracellular diffusion distance (τ_h) between these compartments in *T. newnesi* than in *N. gibberifrons* ($\tau_h=1.05\pm0.07\,\mu m$ and $0.77\pm0.06\,\mu m$, respectively). Although V_v(mit,f) is higher in red fibres of the active species, capillary supply is less extensive [capillary length density, J_v(c,f), for *T. newnesi* is $481.3\pm49.0\,mm\,mm^{-3}$; *N. gibberifrons* is $696.3\pm33.7\,mm\,mm^{-3}$] and the maximal diffusion-distance for oxygen is greater in *T. newnesi* than in *N. gibberifrons* (Krogh's radius, $R=26.3\pm1.64\,\mu m$ and $21.5\pm0.51\,\mu m$, respectively). A mismatch appears to exist between oxygen supply [J_v(c,f)] and oxygen demand [V_v(mit,f)] in *T. newnesi* red fibres in view of published data for other fishes. The twofold higher volume density of lipid [V_v(lip,f)] in *T. newnesi* compared with *N. gibberifrons* may resolve this paradox [V_v(lip,f) is 0.026 ± 0.002 and 0.012 ± 0.004, respectively]. Because oxygen is at least four times more soluble in lipid than in aqueous cytoplasm, lipid may enhance oxygen flux through oxidative muscle and play a role similar to myoglobin in these myoglobin-poor fishes.

Key words: ultrastructure, diffusion, antarctic fish, aerobic muscle, stereology.

Introduction

Several physiological adaptations that permit normal cell function near the freezing point of sea water are expressed in antarctic fishes, including antifreeze glycoproteins, maintenance of cell membrane fluidity (homeoviscous adaptation) and metabolic cold adaptation (see DeVries and Eastman, 1981; Macdonald *et al.* 1987, for reviews). Intracellular processes that may be limited by diffusion at cold body temperature, however, have received relatively little attention. The high thermal dependence of diffusive processes may have profound effects on the delivery of oxygen and nutrients to skeletal muscle in cold-bodied fishes (Sidell and Hazel, 1987).

Sidell and Hazel (1987) measured diffusion coefficients ($\boldsymbol{D}$) of intracellular metabolite analogues through fish muscle cytosol at 5 and 25°C. Their reported values for $\boldsymbol{D}$ were significantly lower in the 5°C preparations, suggesting that exchange of intracellular metabolites may be diffusion-limited at low body temperatures. However, ultrastructural adaptations may offset low diffusion coefficients. Several authors have reported a substantial increase in the percentage of cell volume occupied by mitochondria [mitochondrial volume density, V_v(mit,f)] in fish muscle upon cold-acclimation (reviewed in Sidell, 1988). Tyler and Sidell (1984) and Egginton and Sidell (1989) reported that this increase in V_v(mit,f) significantly reduced the mean diffusion distance between mitochondrial and cytoplasmic compartments, thus offsetting low values of $\boldsymbol{D}$. If low body temperature and thus low values of $\boldsymbol{D}$ influence the ultrastructural composition of fish muscle, antarctic fishes should have subcellular architectures similar to those of cold-acclimated fishes.

Krogh (1919) first modelled oxygen delivery to skeletal muscle as non-overlapping cylinders of oxygenated tissue surrounding each capillary. Mean radii of the cylinders is estimated from the number of capillary profiles per area of muscle fibre [N_A(c,f)] or length of capillaries per unit volume of muscle fibre [J_v(c,f)], providing a morphological indicator of oxygen supply. In the same manner, volume density of mitochondria provides an estimate of oxygen demand. Researchers working primarily with mammalian systems have used this morphological approach to test the match between O_2 supply and demand in several types of skeletal muscle tissues (Hoppeler *et al.* 1987*a*; Kayar *et al.* 1988). A strong positive correlation is found between capillary densities and mitochondrial volume densities of the tissues (Conley *et al.* 1987; Hoppeler and Kayar, 1988). Many cold-bodied fishes, however, have high volume densities of mitochondria and low capillary densities. Thus, an apparent mismatch between O_2 supply and demand is observed in these fishes (Sidell, 1988). Recently, it has been proposed that intracellular lipid may facilitate the matching of O_2 supply and demand (Sidell, 1988; Egginton and Sidell, 1989).

The present study employed stereological techniques (Weibel, 1979) to quantify morphological parameters that estimate potential exchange of metabolites between cellular compartments and capacities for oxygen supply and demand in oxidative skeletal muscles from two phylogenetically related, yet ecotypically

dissimilar, antarctic fishes. *Trematomus newnesi* is an active pelagic species and *Notothenia gibberifrons* a sluggish benthic species. Both are labriform swimmers and use their pectoral adductor muscles (composed exclusively of slow oxidative-type fibres) to power their primary mode of locomotion. The specific objectives of our study were (1) to ascertain whether antarctic fishes have muscle ultrastructures similar to those of cold-acclimated temperate-zone fishes, (2) to identify the ultrastructural characteristics most probably influenced by differences in ecotype, and (3) to assess the match between structural indicators of oxygen supply and demand.

Our results indicate that the muscle ultrastructure of *T. newnesi* and *N. gibberifrons* resembles that of cold-acclimated temperate-zone fishes. Mitochondrial volume densities of red fibres reflect differences in activity level between species. Simple models suggest that the potential flux of metabolites between mitochondrial and cytoplasmic compartments and oxygen flux through Krogh's tissue cylinder are equivalent in the oxidative muscles of both species. Furthermore, the results are consistent with a major role for intracellular lipid in enhancing oxygen diffusion through oxidative muscles of cold-bodied fishes.

Materials and methods

Animals

Notothenia gibberifrons (32–37 cm total length, 309–467 g) and *Trematomus newnesi* (15.5–16.5 cm, 48–75 g) were collected by otter trawl off the Antarctic Peninsula in February and March 1987 from *R/V Polar Duke*. Fishes were transported to the US Antarctic research station, Palmer Station, where they were maintained in running-seawater tanks at 0–1.5 °C. Twice a week, each species was fed items similar to those of their natural diet.

Tissue preparation

Ten fish of each species were killed by sharp blows to the head, and dissections were performed on a chilled stage. Fast glycolytic (white) muscle was sampled from a region just posterior to the first dorsal fin and deep within the myotome. Slow oxidative (red) muscle was dissected from deep within the pectoral adductor. Tissues were fixed by immersion in an ice-cold solution of 4 % (w/v) formaldehyde, 1 % (w/v) glutaraldehyde, 10 % (w/v) sucrose and 2 mmol l^{-1} $CaCl_2$ in 0.1 mol l^{-1} Hepes buffer (adjusted to pH 7.4). Fresh fixative was substituted after 24 h and samples were kept refrigerated until post-fixation. After storage of less than 1 month, samples were rinsed in 0.1 mol l^{-1} Hepes buffer, post-fixed in 0.1 mol l^{-1} Hepes-buffered 1 % OsO_4, rinsed in distilled water and dehydrated in an ethanol series (to 100 %). Tissues were then cleared in propylene oxide and embedded in Epon/Araldite under vacuum.

Sampling design

Capillary supply and dimensions

Blocks were chosen randomly to form two sets; one set had the cutting face oriented transverse to the longitudinal axis of the muscle fibres and the other had the cutting face oriented parallel to the longitudinal axis of the muscle fibres (each set consisted of one block from each of five fish). Thick sections (1.5–2.0 μm) were cut from each transversely oriented block on a Sorvall JB-4 microtome and stained with 0.5 % Toluidine Blue in 0.5 % $Na_2B_4O_7$ at 60 °C on a hot plate. Sections were viewed at 400× magnification on a Zeiss microscope fitted with a drawing tube and were analyzed, using point-count analysis, by superimposing a square-lattice grid pattern on the viewing area. Four randomly selected fields of 0.046 mm^2 each were sampled per section per fish to determine the following parameters: mean cross-sectional area of fibres, $\bar{A}$(f); number of capillaries per unit of fibre area (i.e. excluding connective tissue), N_A(c,f); volume density of nuclei, V_v(nuc); and numerical capillary-to-fibre ratio (C:F). Care was taken to sample only from those areas of the section that displayed an intact matrix of fibres and connective tissue. Thin sections (approx. 80 nm) were cut with a diamond knife on a Sorvall MT2-B ultramicrotome and placed on thin-bar, 300 hexagonal mesh copper grids. These were doubly stained with 2 % aqueous uranyl acetate and 0.5 % lead citrate, then viewed on a Philips EM201 transmission electron microscope fitted with a rotating grid holder in a goniometer stage. Precise magnification was calculated for each set of micrographs using a calibration grid (Pella, Inc.). One section from each block was subsampled for each fish. Micrographs of randomly selected capillaries (10 000–10 500× final magnification) were digitized to estimate mean capillary cross-sectional area, $\bar{A}$(c), and mean capillary boundary length, $\bar{B}$(c). Forty capillaries (eight per fish) in oxidative muscle and 10 capillaries (two per fish) in glycolytic muscle were measured in each species.

Vertical sections

Vertical sectioning (Baddeley *et al.* 1986) is a sampling technique designed to obtain an unbiased estimate of surface density for anisotropically distributed organelles, such as those found in striated muscle. In this muscle, vertical sections are longitudinal sections; therefore, parameters sensitive to error introduced by anisotropic distribution (surface density) or those insensitive to orientation [volume density, diffusion distance between mitochondrial and cytoplasmic compartments (τ_h)] were measured from longitudinal sections. In the same manner, parameters sensitive to error introduced by longitudinal sections [N_A(c,f), $\bar{A}$(f), C:F, $\bar{A}$(c), $\bar{B}$(c)] were measured from transverse sections.

Computation of stereological parameters

Thin sections from longitudinally oriented blocks were subsampled with eight micrographs per section (10 000–10 500× final magnification) by the method of systematic area-weighted quadrats (Cruz-Orive and Weibel, 1981). Micrograph

were analyzed using point-count analysis (Weibel, 1979). In this technique, each intersection in the sampling grid represents a unit area, and parameters are computed by comparing total intersections falling upon organelles of interest (hit points) to total intersections in the reference area. This technique was semi-automated by digitizing the irregular shape of the reference area, then converting this area value to the appropriate number of reference points so that x reference points/digitized area=known reference points/known area. Reference areas were digitized (Summasketch MM1201 tablet) and hit points with organelles of interest tallied with Sigma-Scan software (Jandel Scientific). Data were then transferred to a computerized spreadsheet for computation of stereological parameters. Volume density was calculated as the percentage of total cell volume and surface density was calculated as the unit of outer surface area per unit of fibre volume, according to the procedure for vertical sections outlined in Baddeley *et al.* (1986).

Intracellular diffusion distances of aqueous metabolites within red muscle fibres were estimated using linear analysis techniques as described by Tyler and Sidell (1984). τ_h was computed as two-thirds of the harmonic mean of half the distance between groups of mitochondria (Weibel, 1979). Distances between groups of mitochondria were measured transverse to the longitudinal axis of the muscle fibre. 'Groups' of mitochondria were defined as aggregations of mitochondria bordered by myofibrils, whereas 'clusters' of mitochondria were defined as mitochondria whose outer membranes were in contact with other mitochondria (i.e. there may have been clusters within groups).

Radial distribution of mitochondria

Radial distribution of mitochondria around capillaries in red muscle was estimated from electron micrographs of transverse sections, as described in detail by R. L. Londraville and B. D. Sidell (in preparation). Briefly, volume density of mitochondria was measured using a test pattern of concentric rings centred over a randomly selected capillary. Each annulus of the test pattern delineated a sampling zone of equal area but of increasing radial distance from the capillary such that the outer annuli included tissue supplied by adjacent capillaries. The volume density of mitochondria was computed independently for each annulus, V_v(mit,ann,f), and provided an estimate of the change in mitochondrial volume density with increasing radial distance from the capillary. Plots of V_v(mit,ann,f) *vs* total area between the ring and the centre point of the test pattern were fitted to a second-order polynomial. Assuming that V_v(mit,ann,f) reflects P_{O_2} at any position in the muscle fibre (as proposed by Weibel, 1984, and Kayar *et al.* 1988), the minimum in the parabola will correspond to the area of a circle whose radius is the maximal diffusion distance for oxygen (Krogh's radius; Krogh, 1919; R. L. Londraville and B. D. Sidell, in preparation). Knowing Krogh's radius, R, one can determine capillary length density [J_v(c,f)] using the following relationship:

$$J_v(c,f) \approx 1/[R^2\pi] \quad (1)$$

(modified from Weibel, 1984). The degree of tortuosity of the capillary bed, estimated by the tortuosity constant $c(k,0)$, can be determined from capillary numerical density $N_A(c,f)$ using the following relationship:

$$c(k,0) = J_v(c,f)/N_A(c,f) \quad (2)$$

(modified from Hoppeler and Kayar, 1988).

Statistics

The number of samples per species was treated as five (one value per fish, $N=5$) for all parameters with the exception of capillary cross-sectional area, $\bar{A}(c)$, and capillary boundary length, $\bar{B}(c)$, where measurements of individual capillaries were treated as independent measurements. Sample data were tested for normality of distribution and non-normally distributed data sets were transformed as indicated on Tables 1–4. Sample means between species were compared by Student's *t*-test.

Results

Ultrastructural differences between Trematomus newnesi *and* Notothenia gibberifrons *muscle*

Volume densities of all organelles measured in red muscle are significantly different between the two species. Cross-sectional area of oxidative fibres, however, is equivalent in *T. newnesi* and *N. gibberifrons* (Table 1). Oxidative fibres of the active *T. newnesi* have a higher volume density of mitochondria and a lower volume density of myofibrils than those from the sluggish *N. gibberifrons*

Table 1. *Muscle fibre size and capillarity in two antarctic fishes*

	Oxidative fibres (red)		Glycolytic fibres (white)	
Parameter	*T. newnesi*	*N. gibberifrons*	*T. newnesi*	*N. gibberifrons*
$\bar{A}(f)$ (μm^2)	2153.4±215.3	2102.8±193.7	16974±2283	10192±1456
C:F	0.62±0.66	0.91±0.07*	1.20±0.20	0.71±0.10
$N_A(c,f)$ (mm^{-2})	295.79±29.69	437.59±8.72**	73.93±11.33	76.08±14.10
$\bar{A}(c)$ (μm^2)	39.62±5.17	53.29±5.70*	21.16±3.60	21.48±5.17
$\bar{B}(c)$ (μm)	28.35±1.70	30.64±2.48	23.02±2.85	24.94±2.76

Values are means±S.E.M., $N=5$ per species; * $P<0.05$, ** $P<0.01$ for between-species comparisons of homologous fibre type.

An arcsine transformation was performed on the $N_A(c,f)$ oxidative-fibre data set.

A logarithmic transformation was performed on the $\bar{B}(c)$ data set for both fibre types.

$\bar{A}(c)$ between oxidative fibres of the two species was previously reported as not significant (Londraville, 1988). Logarithmic transformation of the data set into a normal distribution revealed a significant difference.

$\bar{A}(f)$, mean cross-sectional area of fibres; C:F, numerical capillary-to-fibre ratio; $N_A(c,f)$, numerical capillary density per unit fibre area; $\bar{A}(c)$, mean cross-sectional area of individual capillaries; $\bar{B}(c)$, mean boundary length (perimeter) of capillaries.

Table 2. *Ultrastructure of muscle fibres from two antarctic fishes*

	Oxidative fibres (red)		Glycolytic fibres (white)	
Parameter	*T. newnesi*	*N. gibberifrons*	*T. newnesi*	*N. gibberifrons*
$\bar{A}$(sam,in) (μm^2)	2705.2±26.3	2527.9±7.17	2520.7±94.6	2403.1±109.7
V_v(nuc,f)	0.002±0.0002	0.0008±0.00006***	0.0002±0.00009	0.0003±0.00009
V_v(sarc,f)	0.172±0.015	0.186±0.030	0.187±0.007	0.106±0.010***
V_v(myf,f)	0.451±0.019	0.549±0.028*	0.798±0.011	0.888±0.011***
V_v(mit,f)	0.348±0.012	0.249±0.007***	0.014±0.005	0.006±0.003
V_v(lip,f)	0.026±0.002	0.012±0.004**	ND	ND
S_v(lip,f) (μm^{-1})	0.212±0.023	0.114±0.037	ND	ND
τ_h (μm)	1.05±0.07	0.778±0.06*	ND	ND

Values are means±S.E.M., N=5 for each species; * $P<0.05$, ** $P<0.01$, *** $P<0.001$ for between-species comparisons of homologous fibre type; ND, not determined.

$\bar{A}$(sam,in) and S_v(lip,f) data sets for oxidative fibres were arcsine transformed.

$\bar{A}$(sam,in), area sampled per individual; V_v, percentage of muscle fibre volume (volume density) occupied by (nuc,f) nuclei, (sarc,f) sarcoplasm, (myf,f) myofibrils, (mit,f) mitochondria, and (lip,f) lipid; S_v(lip,f), the surface area of lipid droplets per unit of fibre volume (surface density); τ_h, the mean intracellular diffusion distance for small molecules between groups of mitochondria.

(Table 2, Figs 1 and 2). Oxidative fibres from *T. newnesi* also have a 1.9-fold higher volume density of lipid droplets than *N. gibberifrons* red fibres. Although red muscle of the active species has a higher volume density of mitochondria than that of the sluggish species, mean intracellular diffusion distance (τ_h) is greater in *T. newnesi* than in *N. gibberifrons* (Table 2).

Differences in ultrastructure of white muscle fibres between the two species generally parallel those seen in red muscle. Volume density of myofibrils is higher in *N. gibberifrons* glycolytic fibres and, although not statistically significant, volume density of mitochondria tends to be higher in *T. newnesi* ($P>0.1$; Table 2).

Arrangement and dimensions of oxidative muscle mitochondria

Red muscle fibres in *T. newnesi* have more outer mitochondrial membrane exposed to sarcoplasm than red muscle fibres in *N. gibberifrons* [S_v(mit,cl); Table 3]. This effect is due both to a higher volume density of mitochondria and to a lower degree of mitochondrial clustering in *T. newnesi* relative to *N. gibberifrons*. The ratio of surface density of individual mitochondria [including *all* mitochondrial outer membrane, S_v(mit,indiv)] to surface density of mitochondrial clusters [including all mitochondrial outer membrane *except* that in contact with adjacent mitochondria, S_v(mit,cl)] is an indicator of the degree of mitochondrial clustering within a species. The lowest value of this ratio, 1, describes no mitochondrial clustering. Therefore, a lower ratio indicates a smaller degree of mitochondrial clustering. The mitochondrial clustering ratio in *T. newnesi* red fibres is 1.27±0.043 and in *N. gibberifrons* is 1.65±0.136 (P=0.0215). The specific-

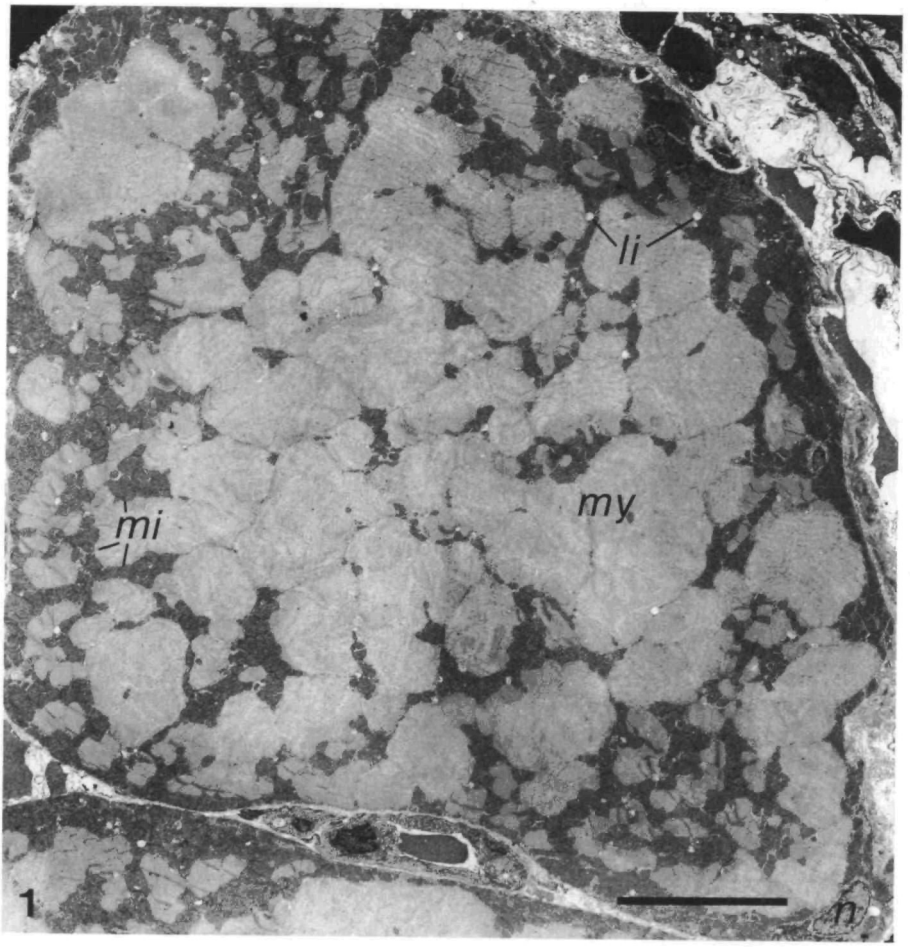

Fig. 1. Transverse section of oxidative muscle in *Notothenia gibberifrons*. Muscle cells are shown in cross-section for illustrative purposes only; some parameters were estimated from longitudinal sections. *my*, myofibrils; *mi*, mitochondria; *li*, lipid droplets; *n*, nucleus. Scale bar, 10 μm.

surface ratio of mitochondria measured as individuals [S/V(mit,indiv)] is equivalent in red fibres of the two species (Table 3).

Capillarity and maximal oxygen diffusion distance

Capillary supply is significantly different between *T. newnesi* and *N. gibberifrons* red fibres. *N. gibberifrons* has a higher numerical capillary density per square millimetre of fibre [N_A(c,f)], a higher numerical capillary-to-fibre ratio (C:F; Table 1), and a higher length density of capillaries [J_v(c,f); Table 4]. Maximal oxygen diffusion distance (mean radius of Krogh's oxygenated tissue cylinder) is larger in *T. newnesi* than in *N. gibberifrons* (Table 4). A greater cross-sectional

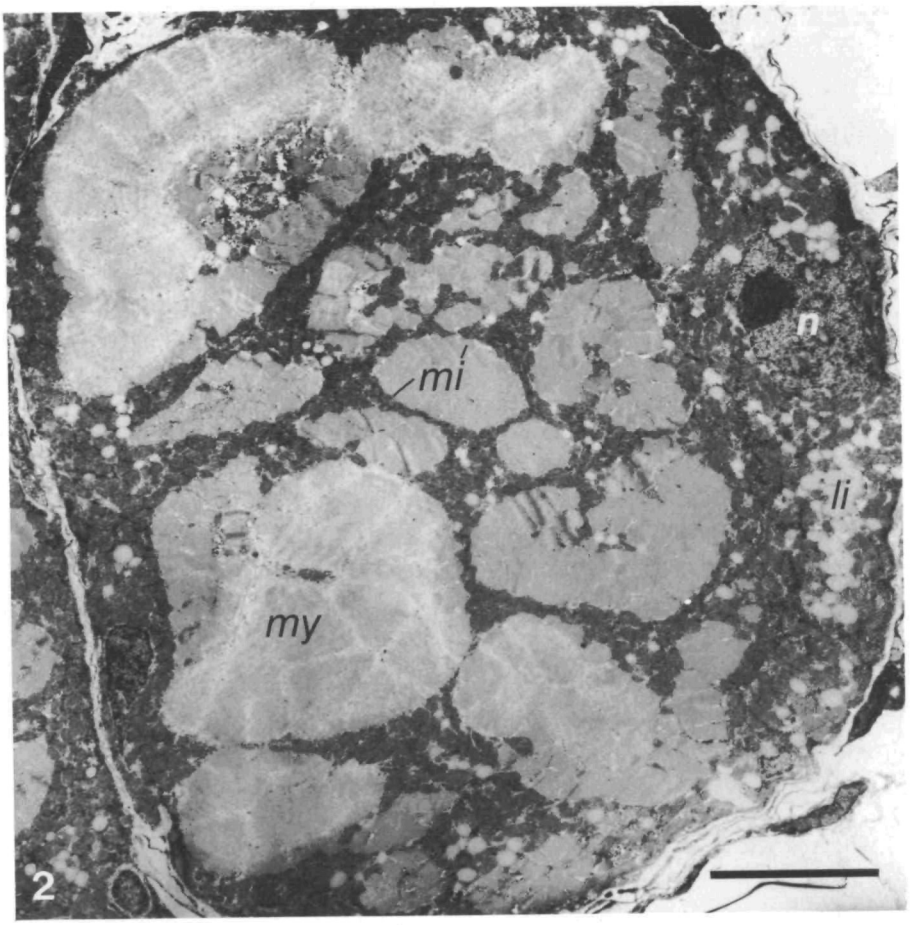

Fig. 2. Transverse section of oxidative muscle in *Trematomus newnesi*. Note differences in content of mitochondria and lipid droplets compared with *N. gibberifrons* (Fig. 1). *my*, myofibrils; *mi*, mitochondria; *li*, lipid droplets; *n*, nucleus. Scale bar, 10 μm.

area of individual capillaries, $\bar{A}$(c), is found in *N. gibberifrons* than in *T. newnesi*. Boundary length of individual capillaries, $\bar{B}$(c), is equivalent in oxidative fibres of the two species (Table 1). The degree of tortuosity of the capillary bed is not significantly different between the two species.

White fibres are five-and eightfold larger in cross-sectional area than red fibres in *N. gibberifrons* and *T. newnesi*, respectively, and capillary supply to glycolytic fibres is equivalent in the two species (Table 1). Because cross-sectional areas of individual capillaries in glycolytic fibres are approximately half those of red fibres and boundary lengths are similar in the two fibre types, cross-sectional areas measured for glycolytic fibre capillaries may be underestimated (i.e. capillaries in white fibres may have partially collapsed during fixation).

Table 3. *Geometric arrangement and surface area of oxidative (red) muscle mitochondria*

Parameter	*T. newnesi*	*N. gibberifrons*
S_v(mit,cl) (μm^{-1})	1.26±0.072	0.816±0.060**
S_v(mit,indiv) (μm^{-1})	1.60±0.010	1.31±0.006*
S/V(mit,indiv) (μm^{-1})	4.63±0.306	5.29±0.865
S_v(mit,indiv)/S_v(mit,cl)	1.27±0.043	1.65±0.136*

Values are means±S.E.M., N=5 for each species; * P<0.05, ** P<0.01 for between-species comparisons.

S_v(mit,indiv)/S_v(mit,cl) data were log-transformed.

S_v is the surface area of mitochondrial membrane per unit of fibre volume (surface density) for (mit,cl) mitochondria with contiguous membrane boundaries (clustered), and (mit,indiv) individual mitochondria; S/V(mit,indiv) is the specific-surface ratio for individual mitochondria; S_v(mit,indiv)/S_v(mit,cl) is an indicator of the degree of mitochondrial clustering; the closer this ratio is to 1, the lesser the degree of clustering (see text for details).

Table 4. *Dimensions of the capillary bed in oxidative fibres of two antarctic fishes*

Parameter	*T. newnesi*	*N. gibberifrons*
Krogh's R (μm)	26.3±1.64	21.5±0.51*
J_v(c,f) (mm mm^{-3})	481.3±49.0	696.3±33.7*
c(k,0)	1.71±0.25	1.59±0.06

Values are means±S.E.M., N=5 for each species; * P<0.05 for between-species comparisons.

Krogh's R data were transformed to normality using the following equation: transformed variable=$1/[R^2\pi]$.

Krogh's R, radius of Krogh's tissue cylinder; J_v(c,f), length density of capillaries per unit of fibre volume; c(k,0), tortuosity constant of the capillary bed.

All parameters were derived from distributional analysis of mitochondria: see text for details.

Discussion

Ultrastructural changes induced by environmental stress include responses to temperature acclimation (Tyler and Sidell, 1984; Egginton and Sidell, 1989), hypoxia (Johnston *et al.* 1983) and exercise (Hoppeler and Lindstedt, 1985). The present study compares two ectothermic species that have identical body temperatures due to the extremely narrow range of water temperatures of their environment. Hypoxia is probably rarely encountered because the entire antarctic water column is generally saturated with oxygen (Everson, 1984). Because of the equivalent thermal histories and similar labriform locomotory patterns of the two species examined, differences in the ultrastructure of their muscle fibres should reflect differences in overall workloads placed upon their muscles and, thus, differences in ecotype. In this sense, our study is similar in approach to those comparing mammals of differing oxidative capacity (see Hoppeler *et al.* 1987*b*).

Although scaling effects may contribute to the difference in subcellular structure between species, scaling effects alone cannot account for the differences reported here for animals with body masses that differ by only one order of magnitude (see Mathieu *et al.* 1981). Furthermore, comparison of *T. newnesi* and *N. gibberifrons* is not hampered by phylogenetic constraints. Both are members of the family Nototheniidae which dominates the suborder Notothenioidei. Notothenioids constitute 53 % of the species and 90 % of the individual fishes in the Antarctic. All notothenioids are thought to be derived from the same ancestral benthic stock (Dewitt, 1971).

Although our study reports a more complete ultrastructural analysis for *N. gibberifrons* skeletal muscle, Johnston (1987) has previously reported mitochondrial and myofibrillar volume density values for red fibres in this species. Our results agree with those of Johnston (1987) with the exception of our value for V_v(lip,f).

Solutions to diffusion problems at low body temperatures – intracellular metabolites

Antarctic fishes face a double challenge to the maintenance of aqueous metabolite diffusion in their muscle cells. First, the cells themselves are very large (Johnston, 1987; Johnston and Camm, 1987). For example, *T. newnesi* oxidative fibres are almost identical in cross-sectional area (and capillarity) to striped bass (*Morone saxatilis*) glycolytic fibres (Egginton and Sidell, 1989). Second, diffusion coefficients of aqueous metabolites are reduced significantly at low temperatures (Sidell and Hazel, 1987). Cold-bodied fishes may compensate for these limitations through ultrastructural adaptation. In fact, the high volume densities of mitochondria in red fibres from antarctic fishes are similar to those of cold-acclimated fishes (Tyler and Sidell, 1984; Johnston *et al.* 1988; this study). High mitochondrial densities in oxidative fibres of antarctic and cold-acclimated temperate-zone fishes may serve to decrease mean intracellular diffusion distance (Tyler and Sidell, 1984; Johnston *et al.* 1988; Egginton and Sidell, 1989). Differences in V_v(mit,f) of oxidative fibres between the species we studied appear to be due to greater numbers of mitochondria rather than to larger mitochondria, because specific-surface ratios (i.e. surface-to-volume ratios) for individual mitochondrial profiles are equivalent in both species. Comparison of specific-surface ratios alone, however, is only indicative of individual shape, not conclusive (i.e. small, bean-shaped mitochondria and larger, spherical mitochondria may have the same specific-surface ratio).

Diffusive flux between cytoplasmic and mitochondrial compartments can be approximated by the one-dimensional diffusion equation:

$$\mathrm{d}n/\mathrm{d}t = -D_n A(\Delta C/X) \qquad (3)$$

(Tyler and Sidell, 1984), where $\mathrm{d}n/\mathrm{d}t$ is the flux of substance n that is diffusing, D is the diffusion coefficient of n, A is the area through which diffusion occurs, and ΔC is the concentration gradient of n over pathlength X. Stereological parameters

can be substituted into this equation to obtain an estimate of dn/dt between the cytoplasmic and mitochondrial compartments (Tyler and Sidell, 1984). Specifically, the surface density of mitochondrial membrane exposed to the cytoplasm [S_v(mit,cl)] can be substituted for the A term, and the mean intracellular diffusion pathlength (τ_h) can be substituted for the X term. Cytoplasmic D should be nearly identical between the species because of their identical body temperatures. ΔC is unknown and, for the purposes of this model, is assumed to be equivalent between species. Substituting measured values into equation 5, one finds that *T. newnesi* (dn/dt=1.74) potentially may support a higher flux of metabolites between mitochondrial and cytoplasmic compartments than *N. gibberifrons* (dn/dt=1.26). This, in turn, should support higher rates of aerobic respiration in *T. newnesi*, providing that oxygen is not limiting.

Morphological indicators of O_2 supply and demand in skeletal muscle

Despite its simplicity, Krogh's original model for the oxygenated tissue cylinder still dominates the literature as a basis for more modern treatments of oxygen delivery to muscle cells (Kreuzer, 1982; Weibel, 1984). These models conventionally interpret capillary density as an indicator of oxygen supply; similarly, mitochondrial densities are viewed as an indicator of oxygen demand (aerobic capacity).

Glycolytic (white) fibres of both species have extremely low capillary density and mitochondrial volume density (Tables 1 and 2). By comparison, white fibres from a temperate-zone striped bass have threefold greater capillary density and mitochondrial volume density than those of *T. newnesi* (Egginton and Sidell, 1989). White fibres of the polar species are also comparatively large, being five- to eightfold the cross-sectional area of the already large red fibres (Table 2). The very low capillarity and mitochondrial content of these large fibres suggest that white fibres of the notothenioid species studied have a very low oxygen demand and a correspondingly low oxygen supply. Indeed, enzymic markers of aerobic capacity are expressed at consonantly low activities in white muscle of both species (Crockett and Sidell, 1989), and activity of white muscle in antarctic fishes appears to be supported predominantly by anaerobic discharge of the tissue's substantial creatine phosphate pool (Dunn *et al.* 1989).

Apparent mismatch of oxygen supply and demand in oxidative fibres

Red fibres of *T. newnesi* and *N. gibberifrons* are equivalent in cross-sectional area. This allows an uncomplicated comparison of ultrastructural features because both species have, in effect, the same reference frame. *T. newnesi* has a higher mitochondrial volume density in its red muscle fibres than *N. gibberifrons*, and yet this greater population of mitochondria is supported by a lower density of capillaries. *T. newnesi* does not compensate for this difference by having capillaries with larger bores or more convoluted boundaries. Even after correcting for shrinkage induced by fixation [by assuming a circular profile area with a

boundary length as measured (Conley *et al.* 1987)], *N. gibberifrons* has larger-bore capillaries.

Capillary density [N_A(c,f)], however, may not be an accurate estimator of the animal's ability to deliver oxygen to the tissue, for it assumes complete anisotropy of the capillary bed. Because capillary length density [J_v(c,f)] accounts for deviations from anisotropy, it is generally used to describe density of the capillary bed (Conley *et al.* 1987; Hoppeler and Kayar, 1988). J_v(c,f) and Krogh's radius, R, both depend upon a capillary tortuosity constant, c(k,0) (equations 2 and 1, respectively). c(k,0) is not significantly different between the two species in this study. Therefore, it is unlikely that differences in Krogh's radius and J_v(c,f) are artefacts introduced by differing degrees of contraction during immersion fixation (see Mathieu-Costello, 1987).

Because mitochondrial volume densities are indicative of the aerobic capacity of a muscle and J_v(c,f) and V_v(mit,f) are positively correlated in a wide variety of species, there appears to be a mismatch between the oxygen supply and the oxygen sink in one of the two species we studied. Either *N. gibberifrons* is oversupplied with capillaries or *T. newnesi* is undersupplied with them. In view of the mass of data suggesting that animals do not maintain structures above those necessary for metabolism, the latter case seems more probable (Conley *et al.* 1987; Hoppeler *et al.* 1987*a*,*b*). Higher rates of blood flow in *T. newnesi*'s capillaries could compensate for this apparent shortfall in oxygen supply. However, this seems unlikely because decreasing the dimensions of Krogh's radius [i.e. increasing N_A(c,f)] is a more effective means of raising tissue P_{O_2} than increasing perfusion rate (Tenney, 1974). This consideration may be of particular importance in cold-bodied fishes because the higher viscosity of blood at cold temperatures will make pumping it faster even more energetically expensive.

O_2 delivery to *T. newnesi*'s oxidative muscles at low tissue perfusion rates could also be enhanced by a high haematocrit or intracellular concentration of myoglobin (relative to *N. gibberifrons*). Published values indicate, however, that *N. gibberifrons* has a haemoglobin concentration that is twice that of *T. newnesi* (Everson and Ralph, 1968; Wells *et al.* 1980). Myoglobin is not likely to be an important contributor to oxygen flux in antarctic fishes because the pigment is expressed either in very low concentrations or is absent (as in channichthyid icefishes) (Everson and Ralph, 1968). Myoglobin concentrations are less than 15 nmol g^{-1} in both the species we studied (R. L. Londraville, unpublished data). Also, myoglobin does not function optimally at low temperatures because of its increased affinity for O_2 and dramatically lengthened off-constant (Stevens and Carey, 1981). How, then, does the red muscle in *T. newnesi* ensure an oxygen flux of sufficient magnitude to support the high mitochondrial density of its pectoral muscle?

Lipid may facilitate O_2 diffusion

Krogh's diffusion constant for oxygen, K_{O_2}, is at least 4.4-fold higher in lipid than in aqueous cytoplasm (Sidell, 1988). This is due to an oil:water partition

coefficient for O_2 of 4.4 (Battino *et al.* 1968) and equivalent D_{O_2} in the two phases (Windrem and Plachy, 1980). Could the higher volume density of lipid in *T. newnesi* compensate for its less extensive capillary bed?

Sidell (1988) modified Mahler's (1978) one-dimensional diffusion equation so that stereological parameters could be used to estimate the contribution of lipid to oxygen flux. Using this approach, it is possible to construct a simple model to estimate dO_2/dt to mitochondria at the end of Krogh's radius, accounting for the effects of lipid on K_{O_2} (see also Egginton and Sidell, 1989). Results of our study suggest that *T. newnesi* can support an equivalent dO_2/dt to the periphery of its oxygenated tissue cylinder, despite significantly larger O_2 diffusion pathlengths relative to *N. gibberifrons* (*T. newnesi* $dO_2/dt=0.056\pm0.001$; *N. gibberifrons* $dO_2/dt=0.057\pm0.003$, $P>0.9$). Although admittedly simplified, this model illustrates the power for increased O_2 flux by virtue of increased volume density of intracellular lipid.

A growing body of evidence supports the hypothesis that intracellular lipid may facilitate oxygen diffusion. Ellsworth and Pittman (1984) reported oxygen diffusion coefficients through striated muscles of different fibre-type composition. Although their values are reported as diffusion coefficients (D_{O_2}), their experimental protocol suggests that data reflect mass transfer of O_2 through muscle tissues and thus diffusion constants (K_{O_2}). They found that K_{O_2} was positively correlated with the percentage of oxidative fibres in the muscle and proposed that this was due to a higher percentage of lipid in oxidative than in glycolytic fibres. Longmuir *et al.* (1980), measuring solubility coefficients for O_2 in a variety of tissues, found that high oxygen solubilities were found in lipid-rich cells or tissues while low oxygen solubilities were found in tissues/cells composed primarily of aqueous cytoplasm. Egginton and Sidell (1989) proposed that the proliferation of lipid droplets in striped bass oxidative fibres upon cold acclimation induced an increased flux of O_2 through those fibres. Finally, all fishes that deviate from the positive linear correlation between N_A(c,f) and V_v(mit,f) [i.e. those having a high V_v(mit,f) and a low N_A(c,f)] also have a high volume percentage of lipid droplets (Egginton and Sidell, 1989; this study).

It has long been recognized that aerobic muscle tissues from antarctic fishes are rich in lipid. Several functions have been proposed for this high lipid content, including increasing the buoyancy of these swim-bladderless fishes (DeVries and Eastman, 1981) and serving as an important metabolic fuel for aerobic respiration (Crockett and Sidell, 1989). Regardless of other functions, the substantial lipid phase of antarctic fish muscles has chemical properties that may increase O_2 flux through the cell. Indeed, this last function may be crucial to antarctic fishes that are either without myoglobin or express the pigment in very low concentrations.

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