

Review

Muscle metabolism and growth in Antarctic fishes (suborder Notothenioidei): evolution in a cold environment[☆]

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Abstract

The radiation of notothenioid fishes (order Perciformes) in the Southern Ocean provides a model system for investigating evolution and adaptation to a low temperature environment. The Notothenioid fishes comprising eight families, 43 genera and 122 species dominate the fish fauna in Antarctica. The diversification of the clade probably began 15–20 million years ago after the formation of the Antarctic Polar Front. The radiation was, therefore, associated with climatic cooling down to the present day temperature of -1.86 °C. Origins and Evolution of the Antarctic Biota Geological Society Special Publication No. 47, Geological Society of London, pp. 253–268). The success of the group has been closely linked with the evolution of glycopeptide and peptide antifreezes, which are amongst the most abundant proteins in blood and interstitial fluid. The radiation of the clade has been associated with disadaptation (evolutionary loss of function) and recovery. For example, it is thought that the icefishes (Channichthyidae) lost haemoglobin through a single mutational event leading to the deletion of the entire β -globin gene and the 5' end of the linked α -globin gene, resulting in compensatory adaptations of the cardiovascular system. Phylogenetically based statistical methods also indicate a progressive and dramatic reduction in the number of skeletal muscle fibres (FN_{max}) at the end of the recruitment phase of growth in basal compared to derived families. The reduction in FN_{max} is associated with a compensatory increase in the maximum fibre diameter, which can reach 100 μm in slow and 600 μm in fast muscle fibres. At -1 to 0 °C, the oxygen consumption of isolated mitochondria per mg mitochondrial protein shows no evidence of up-regulation relative to mitochondria from temperate and tropical Perciform fishes. The mitochondria content of slow muscle fibres in Antarctic notothenioids is towards the upper end of the range reported for teleosts with similar lifestyles, reaching 50% in Channichthyids. High mitochondrial densities facilitate ATP production and oxygen diffusion through the membrane lipid compartment of the fibre. Modelling studies suggest that adequate oxygen flux in the large diameter muscle fibres of notothenioids is possible because of the reduced metabolic demand and enhanced solubility of oxygen associated with low temperature. At the whole animal level size-corrected resting metabolic rate fits on the same temperature relationship as for Perciformes from warmer climates. It seems likely that the additional energetic costs associated with antifreeze synthesis and high mitochondrial densities are compensated for by reductions in other energy requiring processes: a hypothesis that could be tested with detailed energy budget studies. One plausible candidate is a reduction in membrane leak pathways linked to the loss of muscle fibres, which would serve to minimise the cost of maintaining ionic gradients.

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1. Introduction

A series of tectonic and oceanographic events at the Oligocene/Miocene boundary 25 Ma resulted in the breakup of Gondwana and the isolation of Antarctic from other landmasses. The subsequent opening of the Drake Passage and formation of the Antarctic Polar Front (APF) is thought to have initiated a long period of climatic cooling persisting to the present day (Eastman, 1993). Analysis of oxygen isotope ratios from ocean drilling cores indicates a general decrease in seawater temperature throughout the Cenozoic, with periods of rapid cooling and intermittent warming (Fig. 1). Periods of glaciation followed by ice-sheet retreat probably resulted in the loss and creation of near shore and coastal habitats and this is thought to have been a major factor in the evolution of the present day fish fauna (Clarke and Johnston, 1996). The lack of competing taxa and the increasing productivity of the Southern Ocean beginning approximately 22 Ma probably also contributed to the extensive radiation of the Notothenioid species flock which has come to dominate the modern ichthyofauna (Eastman, 1993, 1995; Clarke and Crame, 1989). Although the Southern Ocean represents 10% of the world's ocean it contains only 1% of the recorded species, with approximately 88% of them endemic to Antarctica (Andriashev, 1987). The restricted taxonomic diversity of the Southern Ocean may reflect the lack of estuaries, reefs, intertidal zones and shallow continental shelf relative to temperate and tropical seas (Eastman, 1995). Overall approximately 35% of all Antarctic fish belong to the Perciform sub-order Notothenioidei, and this figure rises to 55% in the subzero shelf and continental slope waters (Eastman, 1995). In the eastern and

southern Weddell Sea more than 90% of fish that were caught in a fishing survey belonged to a single family, the Nototheniidae (Hubold, 1991). The extant notothenioid fishes comprise eight families, 43 genera and 122 species, of which six families are found south of the Antarctic Polar Front (Balushkin, 2000; Eastman and Eakin, 2000). It has been suggested that the key evolutionary novelty accounting for the success of the radiation of the core Antarctic notothenioids was the evolution of glycopeptide antifreezes (Cheng and DeVries, 1991) from a trypsinogen-like protease (Cheng and Chen, 1999). Anti-freeze proteins bind ice nuclei and depress the freezing point by a non-colligative absorption inhibition mechanism (Fletcher et al., 2001).

The ancestor of the notothenioids was probably a relatively small bottom living fish lacking a swim bladder (Eastman, 1993). The notothenioids have undergone substantial ecological diversification in relation to depth distribution involving changes in body shape, colour and the attainment of near neutral buoyancy through decreased mineralisation of the skeleton and the accumulation of lipid (Eastman, 1995; Klingenberg and Ekau, 1996). In some species, such as *Pleuragramma antarcticum* secondary pelagicism is associated with the retention of larval characteristics and the notochord persists in adult stages (Eastman, 1997).

A particular feature of the radiation of the Antarctic notothenioids is a loss of characters or evolutionary function (disadaptation) followed by subsequent adaptive recovery (Montgomery and Clements, 2000). For example, the family Channichthyidae (icefishes) is notable for the loss of haemoglobin, which is thought to have resulted from a single mutational event that deleted the

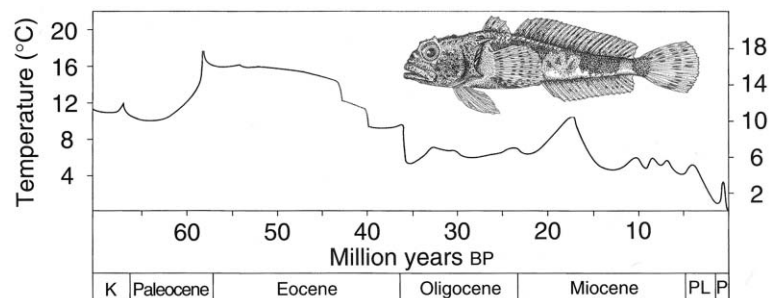


Fig. 1. A schematic representation of surface water temperatures for southern high latitudes from palaeoclimate data. Based on Clarke and Johnston (1996) and original references cited therein. The insert shows *H. antarcticus*.

entire β -globin gene and the 5' end of the linked α -globin gene (Cocca et al., 1995). There have been significant compensatory changes to the circulatory system including a high blood volume (Acierno et al., 1995) and a large heart ventricle relative to typical teleosts (Johnston et al., 1993), which operate at low frequencies and pressures with a high stroke volume (Tota et al., 1997). Myoglobin is not expressed in the ventricle of 6 of the 15 species of icefishes (Moylan and Sidell, 2000), and at least three independent mechanisms for its loss have been identified including a 5-nucleotide insertion leading to premature termination in *Champscephalus gunnari*, an aberrant polyadenylation signal in *Pagetopsis macropterus* (Vayda et al., 1997), and a duplicated TATAAAA sequence that interferes with transcription in *Chaenocephalus aceratus* (Small et al., 2003).

The fossil record for notothenioids is sparse and, therefore, their evolutionary history has largely been inferred from studies on present day species (Eastman, 1993). Cladistic analysis of morphological characters has been able to resolve the higher family relationships (Iwami, 1985). There have been several molecular phylogenies constructed based on the nucleotide sequencing of mitochondrial 12S and 16S rRNA genes (Bargelloni et al., 1994, 2000; Ritchie et al., 1997) and nuclear rRNA (Lecointre et al., 1997). These studies indicate extensive paraphyly, especially in the Bovichtidae and Nototheniidae whilst the Harpagiferidae and Channichthyidae are monophyletic.

Four families and 27 species of Notothenioid are found north of the AFP in the Beagle Channel, Patagonian Shelf, along the Pacific Coast of South America, and in the sub-Antarctic waters of New Zealand (Eastman, 1993). Some sub-Antarctic species are from basal families such as the Bovichtidae that are not represented in the Antarctic and lack antifreezes (Eastman, 1993). The phylogenetic evidence suggests that other sub-Antarctic notothenioids are of relatively recent origin and have diverged subsequent to the main radiation of the core Antarctic species, perhaps over the last 1–7 million years (Bargelloni et al., 2000; Stan-kovic et al., 2001). The presence of antifreezes in many of the sub-Antarctic species is consistent with their relatively recent origin (Cheng, 2000). Closely related species are found on either side of the APF (Fisher and Hureau, 1985) reflecting either passive vicariance and/or episodes of 'jump dispersal' associated with episodes of climate

change (Bargelloni et al., 2000). Extant notothenioids in the Beagle Channel experience a seasonal temperature range of 5–11 °C, compared to a near constant –1.86 °C in the coastal waters of high Antarctic latitudes such as McMurdo Sound.

The aim of the present paper is to review current knowledge on the metabolism and growth of skeletal muscle in notothenioids in relation to the thermal tolerance and evolution of the group and make suggestions for future research.

2. Thermal tolerance

The seasonal range of sea surface temperature varies from less than 0.2 °C at high Antarctic latitudes to approximately 2.5 °C close to the APF (Eastman, 1993). The marine environment in Antarctica is characterised by large variations in day length, seasonal ice cover and associated trophic factors such as feeding opportunity. There have been few systematic studies on the thermal tolerance of the Antarctic notothenioids. Wilson et al. (2001) found that fast-start behaviour could be elicited in several notothenioid species from McMurdo Sound (*Trematomus bernachii*; *T. centronotus* and *Pagothenia borchgrevinki*) over the range –1 to 10 °C, indicating a surprisingly broad acute temperature tolerance. It has been suggested that the proximal cause of heat death is a mismatch between oxygen supply and demand to the tissues (Pörtner, 2002a,b). In the zoarcid, *Pachycara brachycephalum*, ventilatory effort was found to increase up to the lethal temperature of 13 °C, whereas blood flow levelled off at 7 °C, indicating a cardiovascular capacity limitation (Mark et al., 2002). Aerobic scope was found to decline at approximately 6 °C associated with a breakdown in the alpha-stat pattern of acid base regulation, lending support to the idea that thermal tolerance is related to an oxygen limitation (Pörtner, 2002a,b; Mark et al., 2002). The upper lethal limit for different physiological functions is likely to vary with the time course of acclimation. In particular, the upper limit for growth and reproduction would be expected to be less than for acute survival (Pörtner, 2002a,b).

3. Mechanisms and patterns of muscle growth

Three main phases of muscle growth have been described in teleosts and these are illustrated in Fig. 2a. Initially muscle fibres are produced from

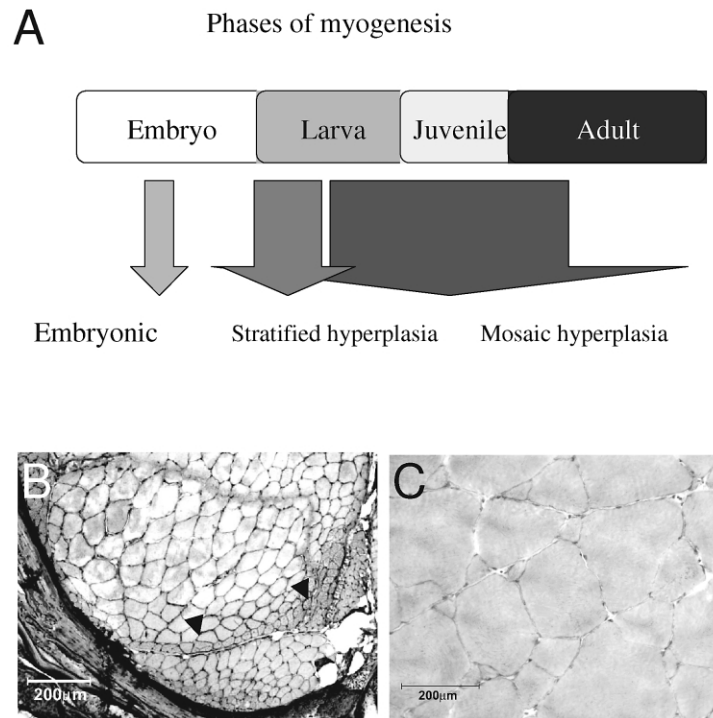


Fig. 2. (a) The phases of myotomal muscle growth in teleost fish (b) Stratified hyperplasia (arrowhead) in a juvenile *Notothenia coriiceps* 6 cm standard length (SL) and (c) mosaic hyperplasia in an *Eleginops maclovinus* 37 cm SL.

discrete germinal zones, a process that has been termed stratified hyperplasia (Rowlerson and Veggetti, 2001) (illustrated in Fig. 2b). Later, new fibres are produced through the activation and proliferation of myogenic precursor cells that are more widely scattered through the myotome (Rowlerson et al., 1995; Johnston et al., 1999) (illustrated in Fig. 3a). Myogenic cells withdraw from the cell cycle and fuse to form myotubes on the surface of existing muscle fibres (Fig. 3a). Because of the characteristic mosaic pattern of large and small diameter fibres this produces this process is usually termed mosaic hyperplasia (Rowlerson and Veggetti, 2001) (see Fig. 2c). Other nuclei are absorbed into muscle fibres as they expand in diameter during the process of hypertrophic growth (Fig. 3a). Mature muscle fibres contain several thousand nuclei per cm fibre length (Fig. 3b).

Myogenic progenitor cells express the cell surface protein c-met, which is the receptor for Hepatocyte Growth Factor thought to be involved in their activation (Cornelison and Wold, 1997). We measured the cell cycle and S-phase duration of myogenic cells in the fast myotomal muscle of

two closely related *Harpagifer* species by cumulative S-phase labelling with bromo-deoxyuridine (BrdU) identifying myogenic cells by their expression of c-met (Table 1) (Brodeur et al., 2003). *Harpagifer antarcticus* was obtained from the Antarctic Peninsula (-1.86 to $+1$ °C) whereas the *Harpagifer bispinis* studied were a eurythermal species from the Beagle Channel, Tierra del Fuego, experiencing $+4$ °C in winter and up to 11 °C in summer. Cell cycle time in vivo is regulated by numerous transcription factors and growth factors and varies with growth rate. For fish that had growth rates not significantly different from zero 75% of the myogenic progenitor cells were in a proliferative state in both species. Cell cycle time was 150 h at 5 °C and 81.3 h at 10 °C in *H. bispinis* ($Q_{10}=3.4$). Since these fish were not growing the myogenic cells studied were probably involved in nuclear turnover. Cell cycle duration was 35% shorter in *H. antarcticus* at 0 °C (111 h) than in *H. bispinis* at 5 °C. The predicted cell cycle time for *H. bispinis* at 0 °C (based on the Q_{10} relationship) was 277 h, which was more than double that measured for the Antarctic species at this temperature. The results are consistent with an

adjustment of cell cycle time for function at low temperature in *H. antarcticus*. However, the measured cell cycle times were considerably longer than the 32 h reported for muscle satellite cells in the rat (Schultz, 1996)

Several authors have reported that Antarctic notothenioids have unusually large diameter fibres, which can reach 100 μm in slow muscle and 600 μm in fast muscle (Smialowska and Kilariski, 1981; Dunn et al., 1989; Battram and Johnston, 1991). Johnston et al. (2003) used phylogenetically based statistical methods to test the hypothesis

Table 1

Total cell cycle time and S-phase time estimated by repeated S-phase labelling with BrdU in *H. antarcticus* and *H. bispinis*. From Brodeur et al. (2003)

Species	Temperature (°C)	Cell cycle time (h)	S-phase time (h)
<i>H. antarcticus</i>	0	111	37.3
<i>H. bispinis</i>	5	150	40
<i>H. bispinis</i>	10	81.3	20.9

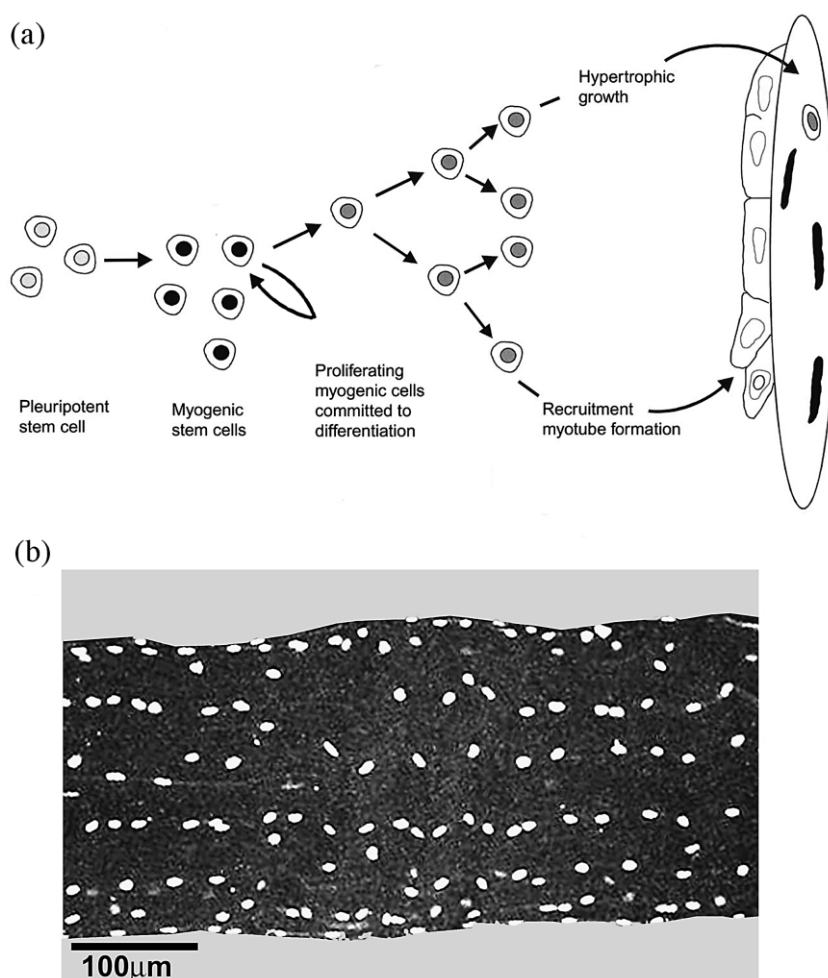


Fig. 3. (a) Working hypothesis concerning the mechanism of muscle fibre expansion in juvenile and adult fish. Pleuripotent stem cells become committed to a muscle fate and then undergo an asymmetric division to produce a daughter cell committed to terminal differentiation. These cells divide for a limited number of times providing a source of cells for myotube formation (fibre recruitment) and a source of nuclei for hypertrophic growth. (b) Muscle nuclei in an isolated fast fibre segment from the sub-Antarctic notothenioid, *E. maclovinus* stained for Sytox GreenTM (Molecular Probes, Leiden) to visualise the myonuclei. The image represents a superimposed Z-series of 1 μm optical sections taken with a BioRad 2000 laser scanning confocal microscope. I.A. Johnston and M. Abercromby, unpublished results.

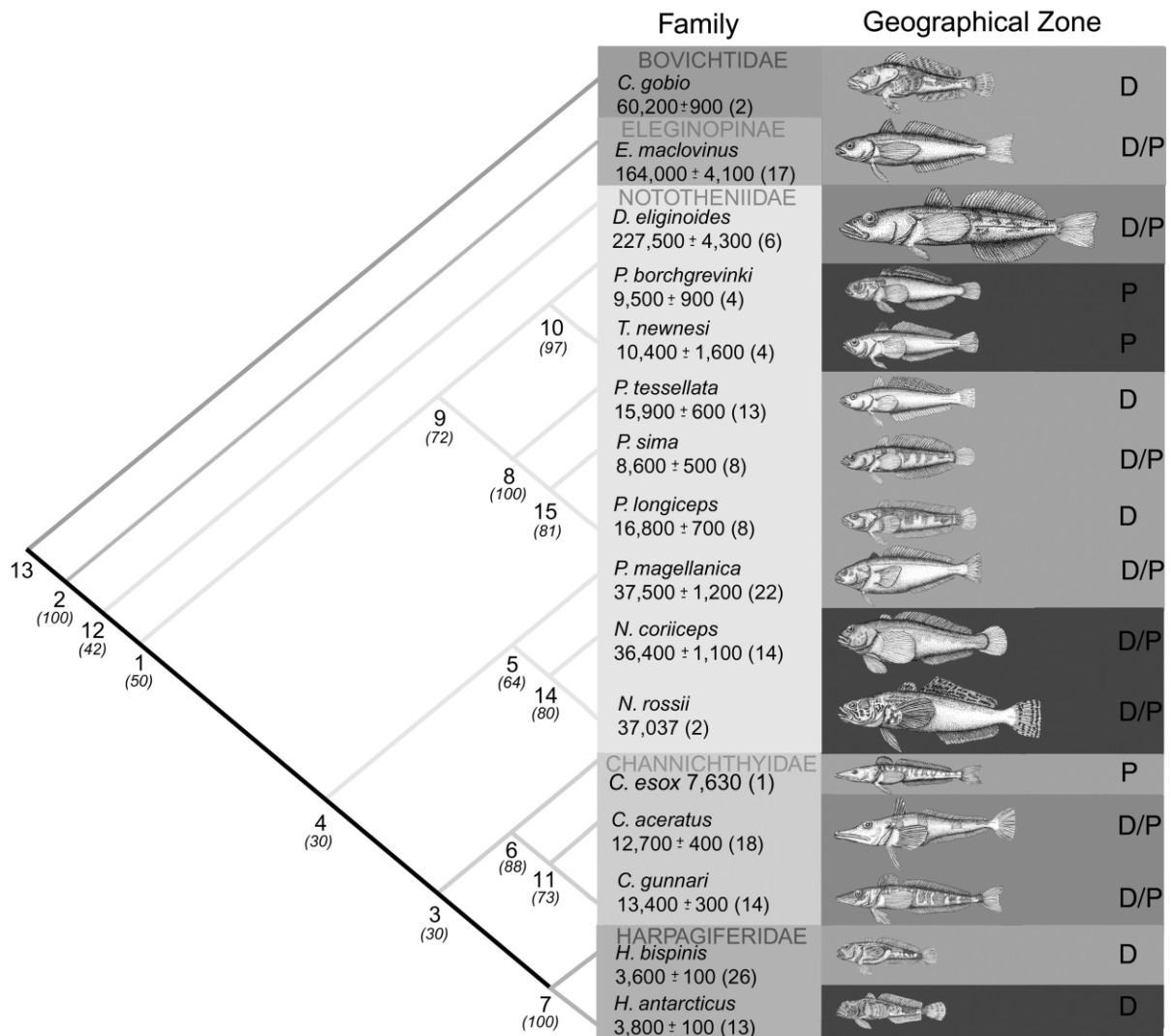


Fig. 4. The final number of fast muscle fibres (FN_{max} : presented as mean \pm S.E., number of individuals) for notothenioid fishes mapped onto a maximum likelihood phylogenetic tree estimated from 12S mitochondrial rRNA gene sequences from Johnston et al. (2003). Bootstrap support values for the nodes are presented and branch length of the tree are given in the original publication. The size of the fish gives some indication of their relative sizes, but they are not drawn to scale. The locomotory habit of each species is also shown D: demersal; DP: demersal-pelagic and P: pelagic. Geographical zone: black, Adelaide Island, Antarctic Peninsula; dark grey, Shag Rocks, South Georgia; light grey, Beagle Channel, Tierra del Fuego.

that in fast myotomal muscle a high maximum fibre diameter (FD_{max}) was related to a reduction in the number of muscle fibres present at the end of the recruitment phase of growth (no fibres less than 10 μ m diameter). Sixteen species were studied from the Beagle Channel, Tierra del Fuego (5–11 °C), Shag Rocks, South Georgia (0.5–4 °C), and Adelaide Island, Antarctic Peninsula (–1.5 to 0.5 °C) and independent molecular phylogeny was constructed using DNA sequences from

12S mitochondrial rRNA genes (Fig. 4). Other hypotheses tested were that the maximum fibre number (FN_{max}) would be negatively related to body size, and that both body size and size-corrected FN_{max} would show phylogenetic signal (tendency for related species to resemble each other) (see Blomberg and Garland, 2002).

The maximum standard body length (SL_{max}) recorded for each species accounted for 70% of the variation in FN_{max} , i.e. larger species tended

to have a higher fibre number. The allometric scaling of SL_{max} and FN_{max} was, therefore, determined using the method of phylogenetically independent contrasts, which takes into account the topology of the phylogenetic tree for the species studied (Blomberg and Garland, 2002). The amount of phylogenetic signal was assessed using the K statistic developed by Blomberg et al. (2003). A K statistic of unity represents the amount of signal expected under Brownian motion evolution along the specified topology and branch lengths, whereas a K greater or less than unity indicates more or less phylogenetic signal than expected. The K statistic for size-corrected FN_{max} was 2.81, which is higher than reported for any of the 35 morphological traits studied by Blomberg et al. (2003). There is, therefore, a strong tendency for closely related species to have a similar fibre number (Johnston et al., 2003). Estimates of trait values at nodes of the maximum likelihood phylogenetic tree were consistent with a progressive reduction in fibre number during the notothenioid radiation (Fig. 4). The 95% CI for the estimated ancestral value of $\log_{10} SL_{max}$ at node two, representing the ancestor at the base of the notothenioid radiation, overlapped with node six, representing the ancestor of the Channichthyidae, one of the more derived families (Fig. 4). This suggests that there has been no general trend for a reduction in body size during the radiation of the group. Indeed, the ancestral condition is generally thought to have been a small benthic species (Eastman, 1993). In contrast, 95% CI for \log_{10} size-corrected FN_{max} for node two did not overlap with node six, consistent with a general trend for reduced fibre number in the more derived species leading to the Channichthyidae. For example, FN_{max} in *C. aceratus* (2700 ± 300 , Mean \pm S.E., $n=11$) was only 7.7% of the value found in *Eleginops maclovinus* ($164\,000 \pm 4100$, $n=16$), which reaches a similar maximum length (84 cm) (Johnston et al., 2003) (Fig. 4).

No evidence for the mosaic hyperplasia phase of growth was found in two of the most derived families (Channichthyidae and Harpagiferidae), suggesting post-embryonic growth is largely or exclusively based on stratified hyperplasia as occurs in species that only grow to a few cm in body length (Johnston et al., 2002, 2003). In the largest specimens of *Notothenia coriiceps*, *Patagonotothen tessellata*, and *P. longipes* spp. there was evidence for fibre splitting, although this

increased FN_{max} by less than 3% (Johnston et al., 2003). The appearance of these sub-divided fibres, which were surrounded by a network of collagen fibrils, was quite distinct from the pattern of fibre diameters observed following either stratified or mosaic hyperplasia. Sub-division was not restricted to fibres that had reached the maximum diameter. Further investigation is required to determine whether 'fibre splitting' is a normal growth process or related to some pathological phenomenon.

The divergence of the notothenioids in Antarctica after the formation of the Antarctic Polar Front and more recent dispersal north would explain the high maximum diameter and low fibre number in the derived sub-Antarctic notothenioids since they traits would have evolved in an earlier colder environment. Modelling studies indicate that a low fibre number and high maximum fibre diameter does not limit adequate oxygen flux at the body temperatures normally encountered by these species (4–10 °C) (Egginton et al., 2002). However, a high FD_{max} may well mean that sub-Antarctic notothenioids are operating at close to their upper thermal limit in the summer, particularly in the case of the icefish *Champscephalus esox* that lack respiratory pigments (Egginton et al., 2002).

4. Aerobic capacity of skeletal muscles

Notothenioids exhibit a range of activity patterns and styles of swimming related to their position in the water column and stage of development (Eastman, 1993; Johnston, 1989). Many notothenioids swim at low speeds using their large fan-shaped pectoral fins in a drag-based labriform mode of locomotion with contractions of the slow myotomal muscle primarily employed for steering (Archer and Johnston, 1989). However, in the early stages of many species (Dunn et al., 1989) and in a few adult fish, including *P. antarcticum*, a sub-carangiform mode of swimming is employed involving recruitment of the slow myotomal muscle layer (Johnston et al., 1988). The factorial aerobic scope (i.e. maximum/routine energy expenditure) for the slow-swimming cryopelagic Antarctic fish *P. borchgrevinki* (Forster et al., 1987) and the demersal species, *N. coriiceps* (Johnston et al., 1991) was within the range of 4–7. Both of these species use a labriform mode of locomotion at sustainable speeds.

The distribution, relative proportions, morphology, contractile properties and metabolic charac-

teristics of muscle fibre types in the myotomes and major pectoral fin muscles has been described in several Antarctic and sub-Antarctic notothenioids (Harrison et al., 1987; Fernandez et al., 2000). It is well established that there is a trade-off in the relative proportions of the major organelles in skeletal muscle (mitochondria, myofibrils and sarcoplasmic reticulum) depending on the functional demands. Thus, in general fast swimming pelagic species, which require a high aerobic power output, have a higher volume density of mitochondria than more sedentary species (Johnston et al., 1998). However, comparative studies of demersal and moderately active demersal-pelagic fish do suggest that slow muscle fibres in Antarctic notothenioids have relatively high densities of mitochondria (Fig. 5a,c). Mitochondria are particularly abundant in some Channichthyids (haemoglobin-less icefishes) reaching 50% of fibre volume in *C. aceratus* (Johnston, 1987; O'Brien et al., 2003) (Fig. 5a,b). The rate of oxygen delivery to aerobic muscle fibres is a function of the fibre diameter and factors that affect diffusion rate (Egginton et al., 2002). The latter include temperature, the distribution of mitochondria and lipid droplets within the fibre, and overall metabolic demand (Desaulniers et al., 1996; Londraville and Sidell, 1990; Egginton et al., 2002; O'Brien et al., 2003). Mitochondria are found in the central zone of even the largest-diameter slow fibres in channichthyids (Johnston, 1987; Archer and Johnston, 1991), consistent with the maintenance of adequate tissue oxygenation at the low body temperature of these species (Egginton et al., 2002). Adequate oxygen delivery to the central zone of large-diameter muscle fibres is probably only possible because of the very low metabolic demand in notothenioids at low temperature and the evolution of relatively large bore capillaries (Egginton et al., 2002). The capillaries found in the slow muscle of *C. aceratus* are of particularly large diameter relative to other notothenioids that have been studied (Egginton et al., 2002), including another icefish *Chinodraco rastrasoinosus* (O'Brien et al., 2003).

Several authors have reported that the cristae surface density per unit mitochondrion is significantly lower in icefish than red-blooded species (Archer and Johnston, 1991; O'Brien et al., 2003). Johnston (1987) measured ADP-stimulated respiration rates in isolated skinned fibre segments from several Antarctic species including the Channi-

chthyid, *C. aceratus*. In spite of their different mitochondrial contents, slow fibres from the two Antarctic species utilized pyruvate and palmitoyl-1-carnitine at similar rates ($1.0\text{--}1.2 \mu\text{mol O}_2 \text{ g wet mass min}^{-1}$). O'Brien et al. (2003) suggested that the particularly high mitochondrial content of icefish slow muscle serves to facilitate intracellular oxygen diffusion since oxygen is more soluble in membrane lipids than the aqueous cytoplasm (Desaulniers et al., 1996).

Interestingly, the temperature-dependence of the state three respiration rate of isolated mitochondria with pyruvate as substrate was described by a single quadratic relationship for all Perciform fish studied, with no significant up-regulation of the maximum rate of oxygen uptake per mg mitochondrial protein in Antarctic and sub-Antarctic notothenioids (Fig. 5a,b). Therefore, oxygen consumption at the level of the individual mitochondrion shows no evidence of cold compensation. Whether the primary purpose of elevated mitochondrial densities is to increase aerobic power output or facilitate oxygen diffusion it seems that additional energy costs would be required for their turnover and maintenance relative to 'typical' perciform species. Studies with perfused rat skeletal muscle preparations found that the leak of protons across the inner mitochondrial membrane accounted for 50% of resting respiration rate (Rolfe and Brand, 1996). The maintenance of high mitochondrial densities to enhance maximum aerobic power or facilitate oxygen diffusion might, therefore, be expected to raise standard metabolic rate through increased proton leak activity: a hypothesis that could be tested in vitro.

5. Routine metabolic requirements

One of the most investigated aspects of the physiology of Notothenioids is the nature of their routine energy requirements. Scholander et al. (1953) reported that the routine oxygen consumption of some arctic fish at 0 °C was substantially lower than that for tropical species extrapolated to the same temperature, and this was interpreted as evidence for cold adaptation. Similar findings were reported for an Antarctic notothenioid (Wohschlag, 1960), and the concept of metabolic cold adaptation (MCA) became established in the literature, i.e. the theory that polar fish have higher routine metabolic rates than would be expected for temperate or tropical fish at the same body tempera-

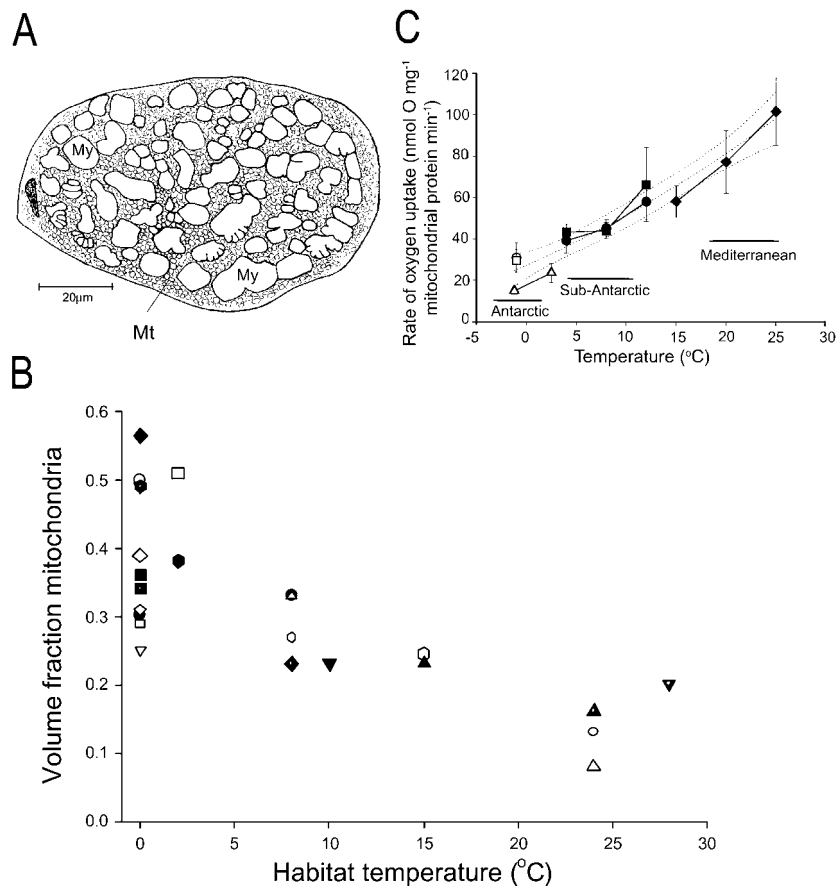


Fig. 5. (a) A camera Lucida drawing of an electron micrograph showing a transverse section of slow muscle fibre from the pectoral fin adductor muscle of the icefish, *C. esox* from the Beagle Channel (I.A. Johnston, J. Calvo and M. Abercromby, unpublished result). Abbreviations: My, myofibril; Mt, mitochondria. (b) State 3 respiration rates of isolated mitochondria with pyruvate as substrate. Values represent Mean \pm S.E. ($n=5/6$). Antarctic species, *Trematomus newnesi* (\square) and *N. coriiceps* (Δ); sub-Antarctic species, *E. maclovinus* (\bullet) and *Paranotothenia magellanica* (\blacksquare); Mediterranean species, *Serranus cabrilla* (\blacklozenge). 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 $\text{nmol O mg}^{-1} \text{ protein min}^{-1}$ and T is temperature (in $^{\circ}\text{C}$); adjusted $R^2=0.93$, $P<0.001$. The outer dotted lines are 95% confidence limits. From: Johnston et al., (1998). (c) The volume density of mitochondria in the slow muscle fibres of various species of demersal and demersal-pelagic fish. The data were taken from the following original publications: (\bullet) *patagonotothen tessallata*; (\circ) *Lithognathus mormyrus*; (\square) *Lepidonotothen nudifrons*; (\diamond) *Trematomus newnesi*; (Δ) *Eleginops maclovinus*; *Paranotothenia magellanica*; *Serranus cabrilla* (Johnston et al., 1998); *Notothenia gibberifrons*; (\blacktriangledown) *Myoxocephalus scorpus*; *Oreochromis niloticus*; *Chaenocephalus aceratus* (Johnston et al., 1998); (\blacksquare) *Notothenia coriiceps*; *Notothenia coriiceps* adult (Johnston and Camm, 1987); (\blacklozenge) *Pleurogramma antarcticum* (Johnston et al., 1998); *Conger conger* (Egginton and Johnston, 1983); (\blacktriangle) *Tinca tinca* (Johnston and Bernard, 1982); *Clarias mossambians* (Johnston et al., 1983); *Psilodraco breviceps*; (∇) *Notothenia gibberifrons*; *Chaenocephalus aceratus*; *Champocephalus gunnari* (Archer and Johnston, 1991; O'Brien et al., 2003); *Notothenia rossi* (Walesby and Johnston, 1980); *Pleuronectes platessi* (Johnston, 1981).

ture. Holeyton (1974) was amongst the first to question the theory of MCA, suggesting the high values of metabolism reported for arctic fishes was related to methodological problems associated with handling stress and uncontrolled and variable levels of activity during the experiment. Steffensen and co-workers have used automated respirometers and shown that oxygen consumption can very

several fold over a 24 h period in 'undisturbed' fish (Steffensen, 2002). Other studies have addressed the issues of the use of an appropriate scaling correction; phylogeny and the life-style of the species used for such inter-specific comparisons. Johnston et al. (1991) measured the routine metabolic rate of sit and wait predators from the Antarctic, North Sea and Indo West-Pacific Ocean

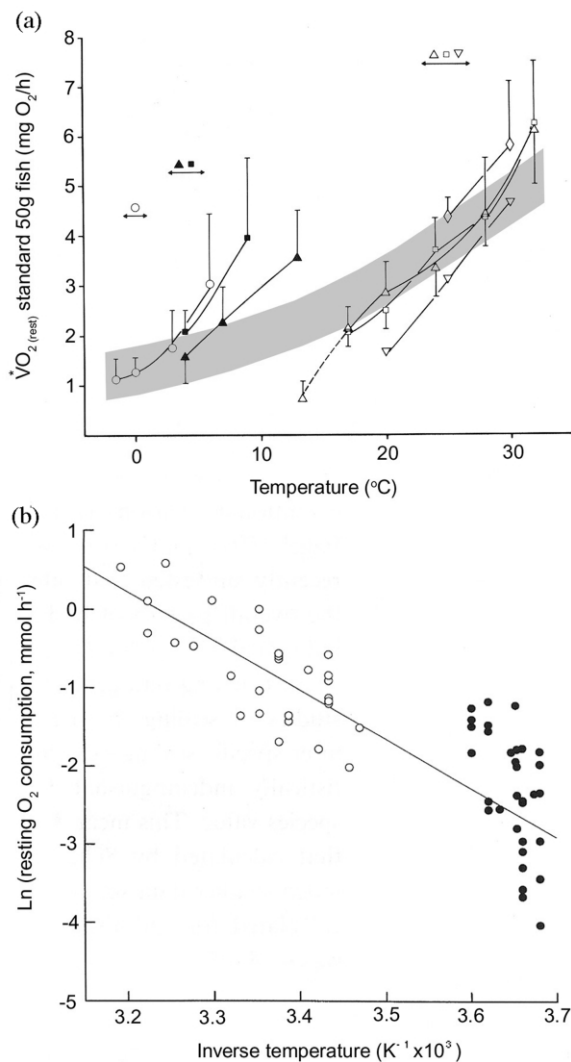


Fig. 6. (a). Effects of temperature on routine metabolic rate of sedentary fish species. Values represent Mean \pm S.D., four to nine fish, see original publication for details. Adapted from Johnston et al. (1991). (b). Literature data for the routine oxygen consumption of perciform fish, the solid circles represent Antarctic notothenioids and the open circles diverse species from warmer climates. The data are presented as an Arrhenius plot, and the line fitted to all of the non-polar data from Clarke and Johnston (1999).

(order Perciformes) (Fig. 6a). For a standard 50 g fish the routine oxygen consumption for several tropical hawk fishes at 25 $^{\circ}\text{C}$ was in the range 3.4–4.4 $\text{mg O}_2 \text{ h}^{-1}$, compared with 1.3 $\text{mg O}_2 \text{ h}^{-1}$ for the Antarctic species, *N. coriiceps* at 0 $^{\circ}\text{C}$ (Fig. 6a). Routine metabolic requirements over the normal temperature range of each species were adequately described by a single temperature-

dependent function (Fig. 6a). Clarke and Johnston (1999) reached a similar conclusion from a desktop study of data from the literature involving 69 species representing 28 families and 12 orders. The relationship between routine oxygen consumption and environmental temperature for a 50 g fish was curvilinear, and for perciform fish, which includes the Antarctic, notothenioids, there was no evidence for metabolic cold adaptation, (Fig. 6b). Although there was significant variability in the data a typical tropical species at 30 $^{\circ}\text{C}$ had a routine energy requirement, which was 6-fold higher than that for a representative Antarctic notothenioid at 0 $^{\circ}\text{C}$ (Clarke and Johnston, 1999). It has been suggested that Arctic fish species show a degree of seasonal plasticity in routine oxygen consumption rates that is lacking in the more stenothermal Antarctic notothenioids (Pörtner, 2002b).

6. Concluding remarks

At the whole animal level size-corrected resting metabolic rate fit on the same temperature relationship as for Perciformes from warmer climates. It seems likely that the additional energetic costs of antifreeze synthesis and the maintenance of high muscle mitochondrial volume densities are compensated for by reductions in other energy requiring processes: a hypothesis that could be tested with detailed energy budget studies. It has been estimated that 20–40% of routine energy expenditure is associated with the maintenance of ionic gradients across membranes in which passive leaks are compensated for by active transport processes (Jobling, 1994). Therefore, one plausible candidate for 'energy sparing' is a reduction in membrane leak pathways linked to the loss of muscle fibres, which would serve to minimise the cost of maintaining ionic gradients.

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