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Anatomical adaptations for peripheral oxygen transport at high and low temperatures

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On acute cold exposure most animals show a reduction in cardiac output and increased blood viscosity that may seriously impair oxygen supply to peripheral tissue. The apparent increase in capillarisation in response to chronic low temperature may be accompanied by significant muscle atrophy in hibernating mammals, and a rise in capillary density (CD) may then simply reflect a reduced fibre diameter. When hamsters were exposed to reduced temperature and photoperiod, capillary to fibre ratio (C:F) of the tibialis anterior muscle was unchanged (at 2.7) and CD increased by 30% (to 1538 mm^{-2}) following a similar decrease in fibre size. A measure of local C:F decreased around glycolytic fibres, but increased around both oxidative fibre types. When corrected for changes in fibre size, local CD increased around all fibre types in the cold. Fishes are particularly sensitive to fluctuations in environmental temperature, but in contrast to mammals often maintain activity and undergo muscle hypertrophy. Slow (aerobic) muscle from striped bass and goldfish held for eight weeks at seasonal extremes of 5 and 25°C had a higher C:F in cold vs. warm animals. However, increased fibre size meant that the functional capacity of the microcirculation was maintained rather than increased. Seasonal acclimatisation of trout induced an inverse relationship between environmental temperature and C:F, increasing 40% from summer (18°C) to winter (4°C). However, extensive hypertrophy at 4°C meant that the size of the capillary bed was maximal at 11°C, corresponding to the point of highest muscle blood flow and greatest scope for aerobic swimming, reflecting an optimised aerobic performance at intermediate temperatures. The consequence of altered fibre composition and capillarisation is calculated as an increase in mean fibre PO_2 from 1.9 to 4.6 kPa, and in minimum PO_2 from 0.6 to 4.2 kPa in 25° v. 5°C-acclimated striped bass, respectively. However, the most dramatic effect on intracellular oxygenation results from an altered capillary supply with the capillary supply becoming increasingly inadequate at high temperatures.

Introduction

A major problem confronting the cardiovascular system in cold environments is the increased viscosity of body fluids, which will reduce both extracellular and intracellular diffusion rates. Plasma viscosity increases by around 3% per °C fall in temperature, paralleling the change in viscosity of water (Harkness & Whittington 1970). Perfusion of the blood vascular system, and hence delivery of oxygen and other nutrients to peripheral tissue, will therefore be impaired unless either the driving force (blood pressure) is raised or the resistance to flow (blood viscosity, total vessel area) is lowered. Reports of cardiovascular adaptations to low temperature have often stressed the importance of a reduced red cell count or haematocrit in avoiding an excessive afterload on the heart, but the consequential reduction in oxygen delivery means this is a far from simple relationship (see Egginton 1996, and references therein). Other factors that may influence oxygen delivery include the ease with which red blood cells deform on passage through capillaries (Lecklin, Nash & Egginton 1995; Lecklin, Egginton & Nash 1996). This is strongly influenced by temperature in ectotherms, while endotherms are much less sensitive (Figure 1). At high temperatures fluid viscosity poses few problems, and the challenge is one of adequate delivery to supply the progressive increase in metabolic rate ($\dot{V}O_2$ has a Q_{10} of around 2). In general, endotherms with their high core temperature adopt a high pressure, high resistance cardiovascular system (CVS), while the low heart rate of cold water ectotherms leads to a low pressure, low resistance CVS. As one way of maintaining a low peripheral resistance is to perfuse a large number of vessels, it is perhaps not surprising that the capillary density in slow muscle of fishes is similar to that found in mammalian myocardium or humming bird flight muscle, even though the $\dot{V}O_2$ may be an order of magnitude lower. It is the anatomical adaptability of the capillary supply that this article will concentrate on.

The physiological importance of capillaries in providing an adequate nutrient delivery to working muscle is well established (Hoppeler, Mathieu, Weibel, Krauer, Lindstedt & Taylor 1981). However, any detailed examination of the adaptive response of the microcirculation and muscle phenotype quickly reveals an incomplete understanding of this relationship, prompting many quantitative analyses of the anatomical capillary supply. These have been reviewed in detail elsewhere (Egginton 1990). This is in fact a very old area of study: in the 17th century Malpighi and van Leuwenhoek studied the microcirculation by direct observation, and a qualitative picture of microvascular anatomy has been available for over a century (Ranvier in 1874 and Spälteholz in 1888). Although many different indices of capillary supply are in use, it is clear that none provide an adequate description of either the physical dimensions or functional capacity of the capillary bed. August Krogh (1919) was the first to consider this relationship in a quantitative manner, suggesting there was a critical density of capillaries below which some portion of the tissue must become hypoxic. Although still widely adhered to, experiments in recent years have shown this concept to be an over-simplification in that capillaries perform functions other than oxygen delivery, e.g. waste removal in some tissues may be of prime importance, while changes in other structural elements within the oxygen cascade from environment to mitochondria may be complimentary to

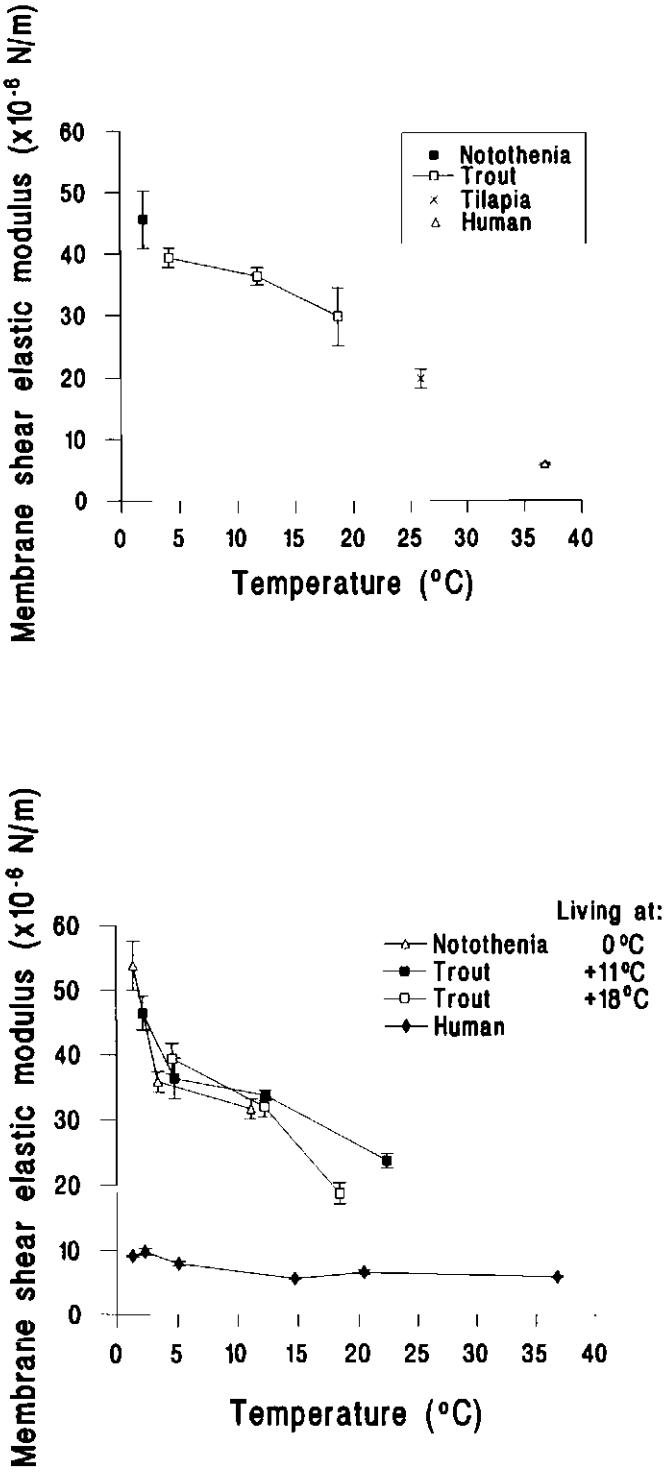


Figure 1 Membrane shear elastic modulus (μN m^{-1}) of red blood cells harvested from fish and humans. (A) Values measured at normal core body temperatures: *Notothenia coriiceps* (Antarctica, 0°C); rainbow trout (*Oncorhynchus mykiss*) seasonally acclimatised to 4°, 11° and 18°C; tilapia (*T. mossambicus*) at 25°C; human at 37°C. (B) Effect of acute changes in measurement temperature on shear elastic modulus. Data taken from Lecklin *et al.* 1995, 1996).

changes in capillarisation. In particular, an increased capillary density within skeletal or cardiac muscle does not necessarily result in an improved muscle performance, in terms of resistance to fatigue (Hudlická, Egginton & Brown 1988). In part this reflects temporal variations in capillary blood flow, heterogeneity of capillary spacing across the muscle, and the three-dimensional topology of the capillary bed.

The growth of capillaries (angiogenesis) in response to the often conflicting demands of the tissue they are supplying is subject to many influences (Hudlická, Brown & Egginton 1992). For our purposes, though, one of the most important is the influence of scaling, i.e. how the capillary bed responds to a change in fibre size. During normal development there is growth of both capillaries and fibres leading to a nearly linear increase in capillary to fibre ratio (C:F), although the rate of angiogenesis is less than that of muscle hypertrophy such that capillary density (CD, mm^{-2}) shows an approximately exponential decline (Figure 2). We will examine how this relationship affects the adaptive response of endotherm (mammals) and ectotherm (fishes) skeletal muscle to prolonged cold exposure.

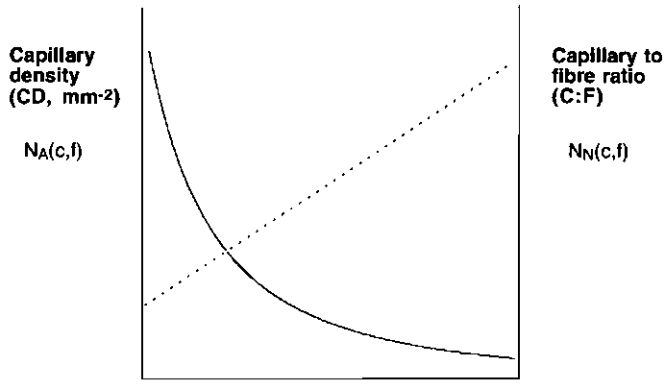


Figure 2 Changes in capillary supply with respect to fibre size in skeletal muscle determined by the use of species of different sizes, and stages of development (Hudlická *et al.* 1992). The adaptive response of the microcirculation to altered functional demand is assumed to parallel those seen during ontogeny. A similar relationship, over a limited range of fibre size, was observed after cold exposure (Banchero *et al.* 1985).

Mammals

Influence of fibre size

On acute cold exposure most mammals show a reduction in cardiac output (largely due to a pronounced bradycardia) and increased blood viscosity (in addition to direct thermal effects there may be significant haemoconcentration as a result of cold-induced vasoconstriction and subsequent diuresis). These factors are likely to seriously impair oxygen delivery to peripheral tissue such as locomotory limb muscles. The adaptive response induced by prolonged exposure to low temperatures (the process of cold acclimation) has previously been shown to involve skeletal muscle atrophy in non-hibernators, and some studies have reported an apparent increase in capillary density under these conditions (Heroux & St Pierre 1957; Wickler 1981; Banchero, Kayar & Lechner 1985). However, the extent to which cold exposure may induce a true angiogenesis (growth of new vessels) is unclear as changes in capillarisation may simply mirror a reduced fibre diameter, or even reflect an improved perfusion of existing capillaries (Figure 3).

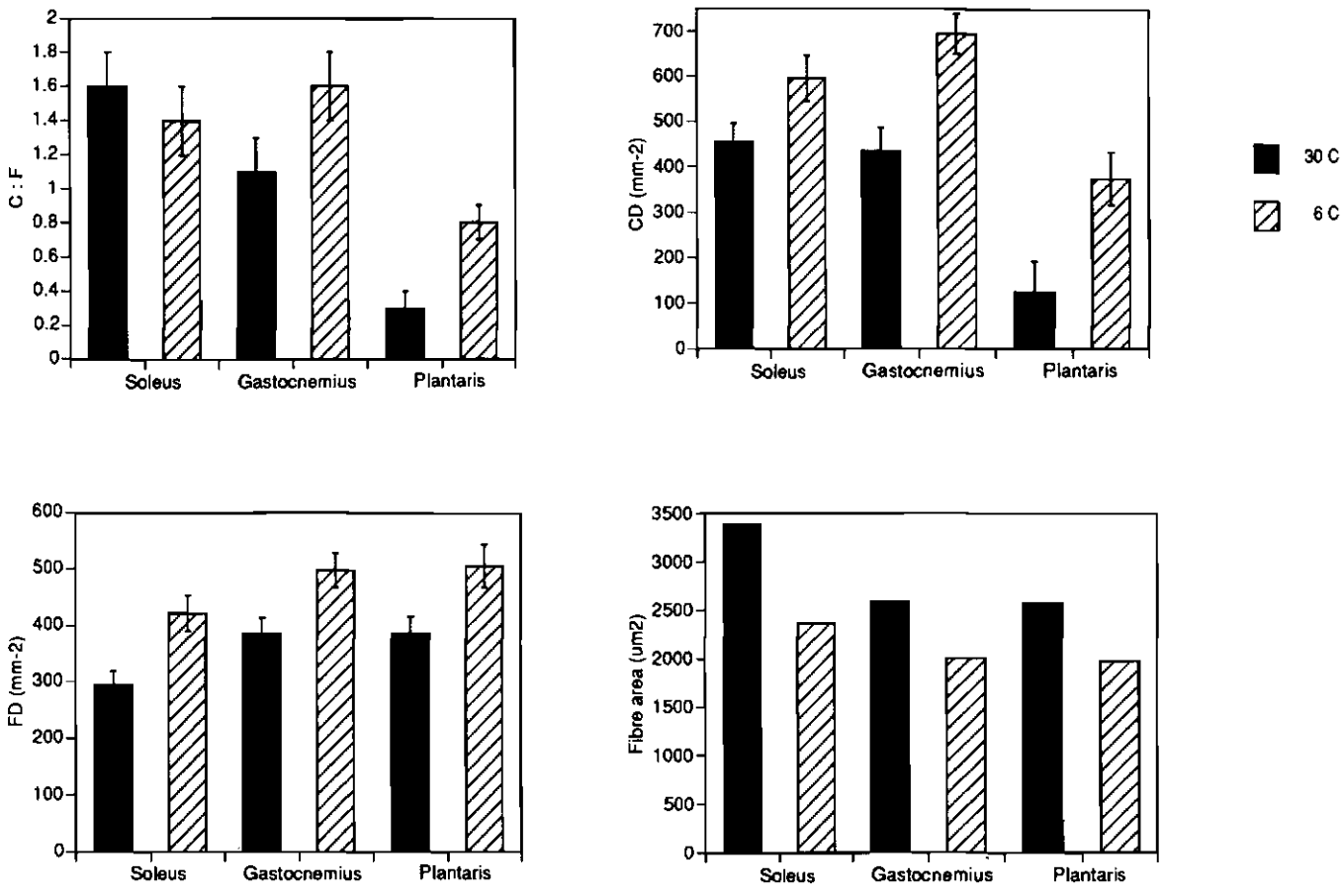


Figure 3 Effect of cold acclimation on capillary supply and fibre size in various skeletal muscles of white rats acclimated to 30°C (hatched bars) or 6°C (filled bars). Note that the apparent increase in capillary density reflects both a higher capillary to fibre ratio (C:F) and lower fibre area (calculated from fibre density, FD). Data from Heroux & St. Pierre (1957) who only counted capillaries that contained red blood cells, and hence may have quantified the extent of muscle perfusion rather than anatomical capillary supply *per se*.

In order to examine the basis of the apparent change in capillary supply, and its functional consequences for muscle performance, we are currently examining the blood flow and endurance capacity of rodent hindlimb skeletal muscles, in addition to performing morphometric analysis. Cold acclimation was induced by keeping animals in an environmental chamber for 8–10 weeks, during which time both temperature and photoperiod were progressively reduced to mimic the onset of winter (to a minimum of 4°C and 1:23 L:D). This enables the indirect effects of cold, e.g. seasonal changes in hormone status, to be incorporated into the observed response. Control animals came from the same cohort, but were held under ambient environmental conditions (21°C, 12:12 L:D). Two species were compared, a non-hibernator (Wistar rat) that defends its core temperature by behavioural and metabolic thermogenesis, and a hibernator (Syrian hamster) that can reset its hypothalamic thermoregulatory set point and allow its core temperature to drop in the face of adverse environmental conditions. Thus, following cold exposure rectal temperatures were *c.* 1°C vs. 10°C lower than control rats and hamsters, respectively. In contrast to previous reports showing muscle atrophy in non-hibernators, the m. tibialis anterior (TA) shows little change in fibre size or capillary density in rats (Bratcher & Egginton 1995). There is also no change in C:F of hamster TA, but this is countered by a 30% higher CD which parallels the significantly lower mean fibre size (Fairney & Egginton 1994). Cold exposure therefore reveals a major influence of fibre size on muscle capillarity.

Growth was not impaired in rats (final body weights were similar among groups) suggesting that the response to cold exposure is more one of avoidance than adaptation in this species. Whether the small changes found reflect an attenuated cold acclimation or different activity pattern is at present unclear, although preliminary data from telemetry experiments indicate an increased level of activity in cold-acclimated rats (D. Deveci, unpublished data). This modest 'training response' may be sufficient to avoid any significant muscle atrophy, and may also induce other adaptations that make more efficient usage of an unaltered capillary bed. For example, there is a limited conversion of glycolytic Type IIb to oxidative Type IIa fibres (Bratcher & Egginton 1995) that is paralleled by an increase in fibre mitochondrial content (Buser, Kopp, Gehr, Weibel & Hoppeler 1982), which in turn will enable greater oxygen extraction. This may explain the reduced fatigueability during repetitive activity, despite there being little or no change in muscle blood flow (Deveci & Egginton 1997). Fibre type conversion was more extensive in the hamster, with the proportion of Type IIa fibres rising from 39 to 51%, while there was a differential effect of cold acclimation on fibre size which resulted in a significant atrophy of Type IIb but not IIa fibres (Fairney & Egginton 1994). In this

case, disuse atrophy may in part explain the response of muscle, while the capillary bed may be responding to altered hormonal status. The functional consequences are as yet unclear, but hibernators such as the hamster display vascular adaptations that moderate changes in blood pressure and total peripheral resistance at low temperatures (Brown, Geal & Egginton 1993), suggesting that acclimation is more extensive than in the rat and may lead to greater endurance capacity of the reduced muscle mass.

Influence of muscle phenotype

The TA in rodents may be divided into two regions with differing metabolic characteristics — an oxidative core and less oxidative, more glycolytic cortex. This presents an opportunity to determine whether muscle phenotype has any gross influence on the adaptive response to cold exposure within one muscle, thereby avoiding the problems inherent in inter-tissue comparisons (Figure 4). No evidence for such a response is found in the rat, although in the hamster this reveals regional capillary growth (in the core) and capillary rarefaction (in the cortex). As far as the author is aware this is the first example of concurrent angiogenesis and regression within the same muscle, possibly reflecting the diversion of blood away from the inactive cortex. Impressive though this might be, it still cannot adequately explain the response in terms of individual fibre types.

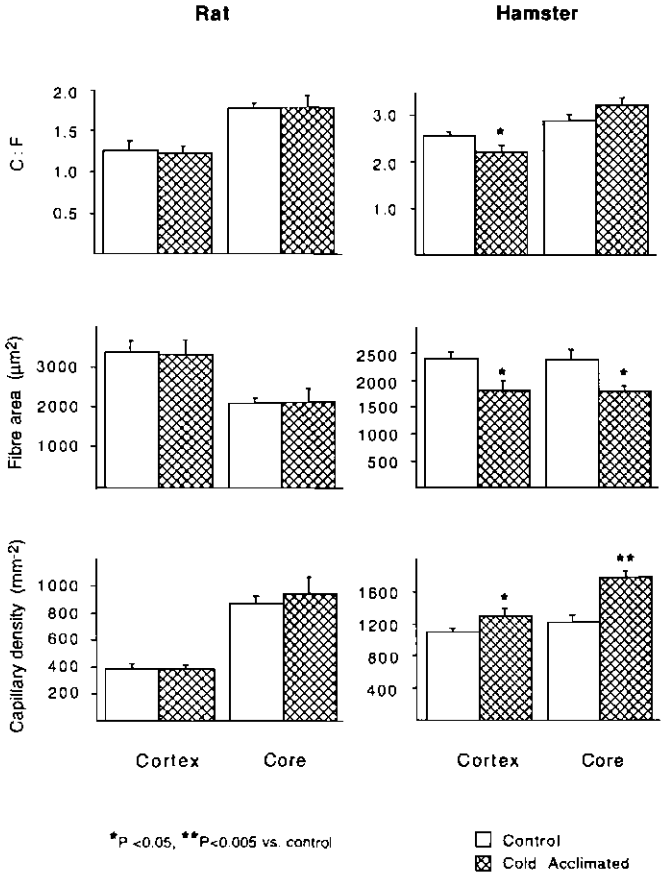


Figure 4 Effect of muscle phenotype on capillary supply. The rodent TA may be divided into an oxidative core and less oxidative cortex. This division has little effect in rats, but reveals regional capillary growth (core) and rarefaction (cortex) in the hamster. Data from Fairney & Egginton (1994) and Bratcher & Egginton (1995).

Simple counts of capillaries may give an erroneous picture of the potential oxygen transport to tissue, owing to heterogeneities in both local supply and demand (Egginton 1990). This is especially problematic with mixed mammalian muscles, where a single capillary may be surrounded by up to three different fibre types, each of differing size and oxygen consumption (Figure 5). Analysis of regional differences in capillary supply may still not offer sufficient resolution, and so methods have been developed to analyse the local capillary supply to individual fibres. These include partitioning the number of capillaries around each fibre according to the number of adjacent fibres (Plyley & Groom 1975; Gray & Renkin 1978). Differences in size among fibre types may be accommodated in the analysis by calculating the supply (or domain) area for each capillary, with the overlap of domains and muscle fibre profiles giving an index of local capillary supply (local capillary to fibre ratio, LCFR) in terms of 'capillary equivalents' of supply (Egginton & Ross 1989, 1992). This may be corrected for differences in fibre size to provide a scale-independent index, the local capillary density (LCD = LCFR/fibre area).

Hoofd, Turek, Kubat, Ringnald & Kasda (1985) introduced the analysis of capillary domains in myocardium as a means of assessing capillary spacing, a procedure that also proved useful with sparse networks in skeletal muscle (Egginton Ross & Sidell 1988; Ahmed, Egginton, Jakeman, Mannion & Ross 1997). For capillaries relatively far apart (long intercapillary distances) the domains will be larger than for those of capillaries lying close together, and the distribution of individual domain areas will therefore reflect the heterogeneity of capillary supply. Applying this analysis to hamster TA showed that cold acclimation had a differential effect on fibre size (significant atrophy of Type IIb but not Type I fibres) and capillary supply (LCFR decreased around glycolytic, but increased around oxidative fibres). When capillary supply is corrected for changes in fibre size, local capillary density increased around all fibre types in the cold (Fairney & Egginton 1994).

However, in order to examine the effects of cold exposure

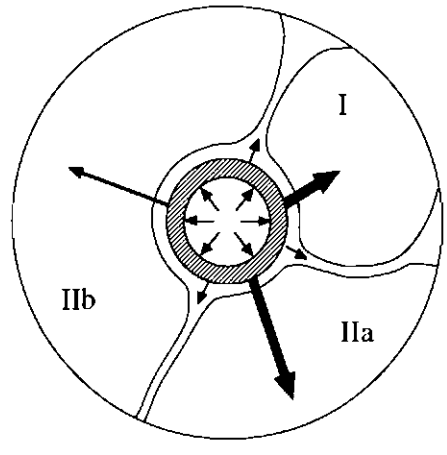


Figure 5 Partitioning of oxygen supply by an individual capillary to the surrounding tissue is complicated by local demands. While O_2 consumption in the interstitium is low and may be disregarded, the variable $\dot{V}O_2$ and permeability among fibre types leads to local differences in O_2 flux density (thickness of arrow) and magnitude (length of arrow).

per se, without the compounding effects of greatly altered activity levels, and extend the range of intramuscular temperatures investigated, we need to observe what happens in ectotherms.

Fishes

When faced with seasonal fluctuations in environmental temperature ectotherms display a range of physiological and anatomical adaptations in order to compensate for the thermal sensitivity of biological rate functions, which typically have Q_{10}s of between 1 and 3 (Johnston 1990). Fishes are of particular interest because, with few exceptions, efficient branchial heat exchange means that core temperature lies within a few tenths of a degree of the environment. This means that for temperate zone species seasonal excursions in cell temperature of up to 20°C may be found. Despite this profound metabolic challenge, complimentary adjustments preserve locomotory performance at both low (Sidell & Moerland 1989) and high (Guderley 1990; Taylor, Egginton, Taylor & Butler 1997) temperatures. The increase in cytoplasmic viscosity at low temperatures will result in a significant reduction in the rate of O_2 diffusion, although this will be to some extent balanced by a reduction in $\dot{V}O_2$, while as with mammals growth of capillaries may be expected to compensate for impaired perfusion. For example, Johnston (1982) reported an impressive increase in C:F of carp slow muscle from 2.2 at 28°C to 4.8 at 2°C. Do carp show an adaptive, or excessive angiogenic response to cold exposure? The response may be atypical, perhaps reflecting the polyploid nature of cyprinids, as much less dramatic changes in capillary supply have now been documented in more active species.

Influence of fibre size

Apart from the more extreme range of temperature that may be compared, fishes offer another significant advantage over mammals for our purpose in having discrete anatomical separation of fibre types, allowing pure samples of Type I fibres from the lateral slow muscle strip, and of Type IIb from deep within the trunk. As with mammals, cold exposure of a variety of fish species has been shown to induce an increase in mitochondrial content of slow muscle which, as an index of the potential $\dot{V}O_2$, might be expected to be accompanied by growth of capillaries. Acclimation of juvenile eels to 10° and 29°C, however, elicited a proliferation of mitochondria (1.5-fold higher at 10°C) in the absence of any adjustment in capillarisation (Egginton & Johnston 1984). It is possible that the small fibre cross-sectional area obviates any need to increase O_2 delivery, and that intramuscular diffusion distances play a limiting role in larger animals.

Although the increase in mitochondrial content and apparent capillary supply in cold-exposed fishes resemble the adaptations seen in mammals, in other ways the response is quite different. In particular, many fishes maintain locomotory activity at low core temperatures and show a paradoxical hypertrophy of slow muscle fibres in the cold. As diffusion rates decrease one might expect that an increase in the distance over which diffusion has to occur would be a counter-productive response, although this may in part be explained by changes in muscle composition (see later). The influence of fibre size on the capillary supply, though, ought to be most evident under these conditions.

Capillary supply in carp changes in parallel with increases in mitochondrial content, which increased 2-fold between 28° and 2°C (Johnston 1982). Proliferation of mitochondria in striped bass was more modest (50% increase in 5° v. 25°C fish) with little or no change in capillary density (Egginton & Sidell 1989). However, C:F increased from 1.0 to 1.4 while fibre size was 30% greater in cold-acclimated fishes. If a simple scaling response were involved then a decrease in CD would be expected (Figure 2); that bass maintain a given CD across a range of temperature represents a positive acclimatory response (Figure 6). If capillary supply to fish muscle really is scale-dependent (Egginton 1992), then cold acclimation of a small species like goldfish, with short intramuscular diffusion distances, should have a much reduced effect similar to that seen in juvenile eels. Indeed, a 20°C difference in acclimation temperature induced little or no difference in either capillary supply or fibre size. Moreover, it is unlikely that the very short intercapillary distances are physiologically relevant — this sluggish species has at least a 5-fold greater CD than the much more active bass or trout (Figure 6), with values more usually found in the mammalian myocardium.

Dramatic though these effects of scaling may be, there remains the question of how much the experimental design influences the response. For example, most studies on thermal acclimation use a two-point comparison, with the implicit assumption of a linear response between the two extremes. In an attempt to isolate the effect of temperature *per se* these are mainly laboratory-based experiments, avoiding other changes that occur during seasonal acclimatisation to different environmental temperatures in the wild (e.g. activity, reproduction, feeding, hormonal status). In order to accommodate these influences we sampled rainbow trout held in outdoor raceways at the seasonably appropriate temperatures of 18°C (summer), 11°C (autumn) and 4°C (winter). There was a linear increase in C:F of slow muscle with declining environmental temperature, but fibre hypertrophy was only evident in the winter fish (Egginton & Cordiner 1997). This meant that the initial angiogenesis was not countered by any increase in fibre size, such that CD was maximal at the intermediate temperature (Figure 6). This corresponds to the point of maximal cardiac output, muscle blood flow and aerobic swimming capacity (Taylor, Egginton & Taylor 1996), and neatly illustrates the integrative nature of acclimatisation. It also prompts the question whether all elements of the cardiorespiratory system are similarly adapted, or whether examining one aspect such as capillary supply or mitochondrial content in isolation will lead to erroneous conclusions. In addition, consideration needs to be given to the range of temperatures which are being compared, as the extent of thermal acclimation may depend on the inter-specific variations in the optimal temperature for growth (Guderley 1990).

Influence of muscle composition

One way of compensating for diffusional limitations at low temperatures is to adjust fibre ultrastructure, particularly with regard to the surface area over which diffusive exchange occurs and the diffusion pathlengths along which O_2 or metabolites must travel. Cold exposure usually increases activity of enzymes of intermediary metabolism, presumably

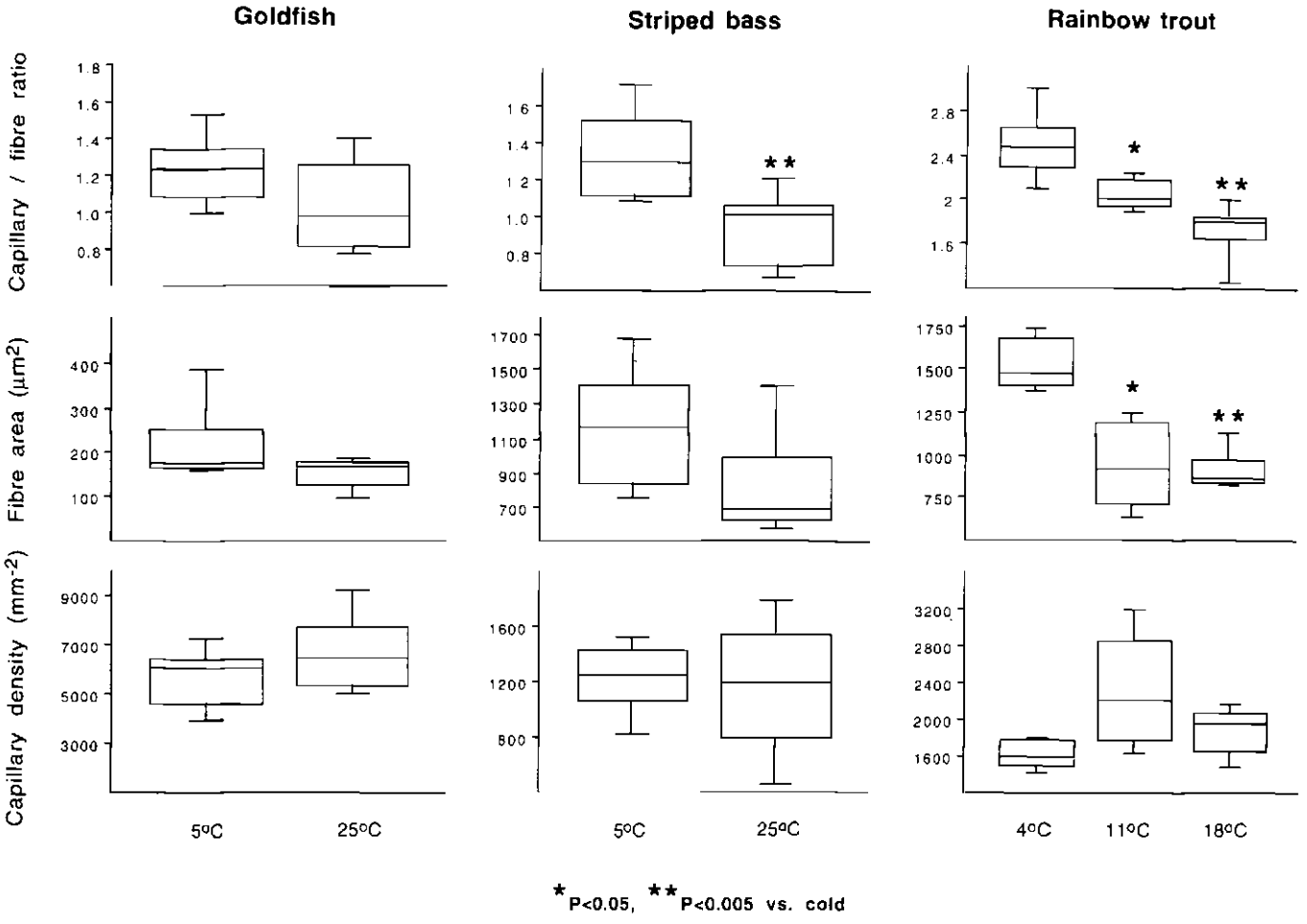


Figure 6 Effect of fibre size on capillary supply. In striped bass (middle panel) the increase in C:F is countered by fibre hypertrophy to maintain a similar CD at high and low acclimation temperatures (recalculated from Egginton & Sidell 1989). When fibre size is much less, as found in goldfish (left panel), cold acclimation has little or no effect on either capillary supply or fibre size (unpublished data). Seasonal acclimatisation of rainbow trout reveals an inverse relationship between C:F and temperature, but a non-linear hypertrophy of muscle, leading to maximal CD at the intermediate temperature (data from Egginton & Cordiner 1997). Data shown as box plots (percentile distributions).

to allow ATP synthesis at rates approaching that at higher temperatures, and this is reflected in an increase in mitochondrial content of muscle fibres (Johnston & Lucking 1978; Sidell & Moerland 1989). While this is a response common to mammals and fishes, the magnitude of the response is much greater in the latter group of animals. Stereological analysis of mitochondrial separation (Figure 7), and calculation of harmonic mean diffusion distances, T_h (Egginton *et al.* 1988), raises some important issues. In goldfish, cold-induced mitochondrial proliferation decreased T_h in proportion to the reduction in diffusivity of metabolites (Tyler & Sidell 1984; Sidell & Hazel 1987). Interestingly, while the stimulus of cold exposure was adequate to induce mitochondrial profileration, it was inadequate to cause angiogenesis. This may be due to the already high CD, the choice of a rather sluggish species, or that the temperature was not low enough. In striped bass (an active species with a lower CD, but acclimated to the same temperatures) a 60% increase in volume of mitochondria produced only a 10% rise in surface area available for diffusive exchange owing to the formation of mitochrondria clusters, suggesting that the acclimatory response is not solely to overcome catalytic limitations imposed on biochemical reactions by low temperature (Egginton & Sidell 1989). There was, however, a nearly 2-fold reduction in T_h for slow fibres at 5°C, which will ameliorate reduced metabolite diffusivity and help preserve cellular metabolism. Note that this response is found in a species that shows no change in extracellular diffusion distances (i.e. CD).

We may use this data to reassess the influence of scaling on local oxygen supply. C:F values for warm acclimated striped bass did not change with mean fibre size and consequently CD fell, whereas cold exposure produced a positive scaling of C:F that minimises the change in CD with fibre size (Figure 8). The inverse, though less dramatic, situation is found with mitochondrial separation where a positive scaling response is found only in warm acclimated bass (Figure 8). These data show that low temperature may induce angiogenesis and organelle proliferation which minimises both inter-capillary and inter-mitochondrial separation, suggesting that there is strong selection pressure to reduce both extra- and intracellular diffusion distances; neither appear to pose a serious problem at higher temperatures.

Other adaptations include a greater reliance on lipid-based metabolism at low temperatures (Sidell 1983). This may lead to an accumulation of intracellular lipid deposits in larger species (Egginton & Sidell 1989), or extracellular deposits

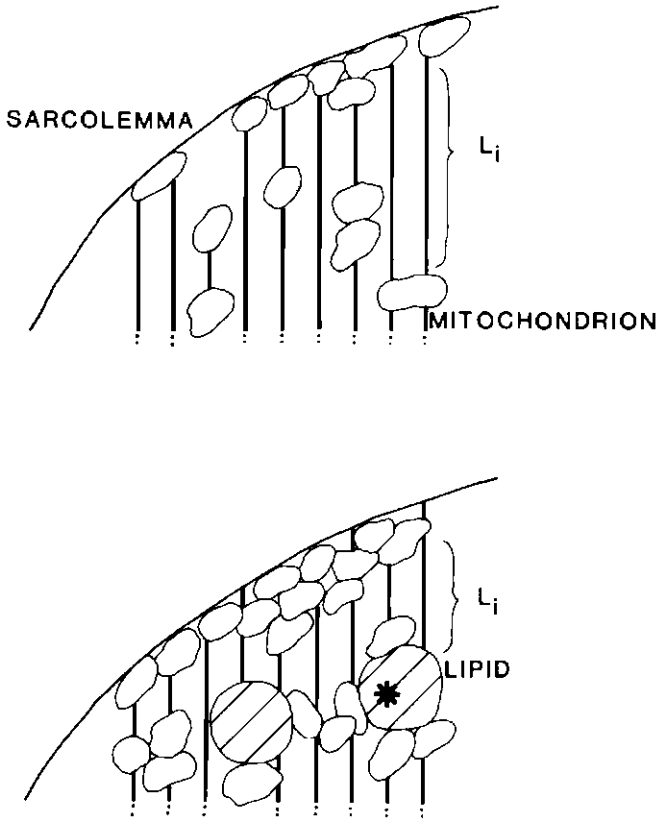


Figure 7 Estimates of mitochondrial spacing (linear intercept length, L_i) are shown as bold lines. In warm fish (top panel) these distances are large compared with cold fish (bottom panel). Intracellular lipid (asterisk) is ignored when calculating mean free sarcoplasmic spacing for diffusion of aqueous substances, though it is very important for calculating effective O_2 diffusion distances.

where fibre size is small (Figure 9). Because of the greater solubility and roughly equivalent diffusion coefficient of O_2 in lipid vs. aqueous compartments, increased intracellular lipid content may accelerate O_2 flux and also provide a significant O_2 store in cold-acclimated animals (Egginton & Sidell 1989). Measurements of O_2 diffusion through muscle show that such adaptations are effective (Desauliniers, Moerland & Sidell 1996), while NMR experiments suggest that metabolite diffusion may not be limiting, either (Hubley, Locke & Moerland 1997). The key to what drives intracellular remodelling remains elusive.

Modelling peripheral oxygen transport

Cold acclimation induces skeletal muscle fibre hypertrophy in fishes, which will increase diffusion distances from the nearest capillary and may be expected to further impair oxygen delivery to mitochondria. Other aspects of the adaptive response include modifying the fibre composition, particularly with respect to the amount of mitochondria and lipid. If we assume that PO_2 is the driving force for such adaptation, we can model the effect of changes in those elements which determine the rate of peripheral oxygen transport, namely:

(1) extent of the capillary supply (responsible for the bulk, convective delivery of oxygen and the local, diffusive source of oxygen)

(2) changes in fibre size (the area over which diffusion must take place)

(3) composition of the tissue (reflecting the permeability of oxygen in different compartments)

(4) the demand for O_2 (by which mitochondria establish the PO_2 gradient).

Because oxygen has a greater solubility in lipid than aqueous compartments, and facilitated diffusion by myoglobin is impaired at low temperatures, an increase in intracellular lipid may enhance transcellular O_2 flux in cold acclimated fishes and limit the diffusional problems associated with fibre hypertrophy (Egginton & Sidell 1989; Desaulniers, Moerland & Sidell 1996). However, testing this prediction required

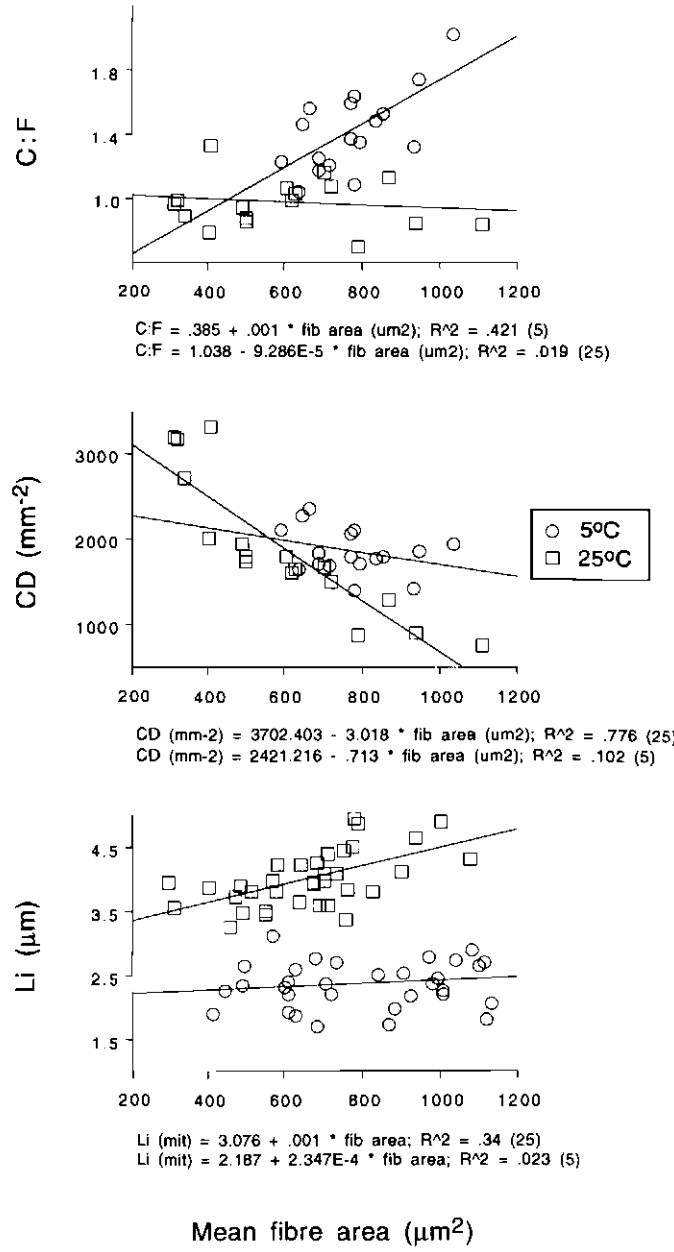


Figure 8 Scaling of extracellular and intracellular diffusion distances in thermally acclimated striped bass. Values for capillary to fibre ratio (C:F), capillary density (CD) and mitochondrial separation (L_i) are plotted against mean fibre cross-sectional area for individual sample fields of slow muscle. Note the adaptive response to minimise scaling influences on CD and L_i in 5°C acclimated fish (unpublished data; Egginton *et al.* 1988; Egginton & Sidell 1989).

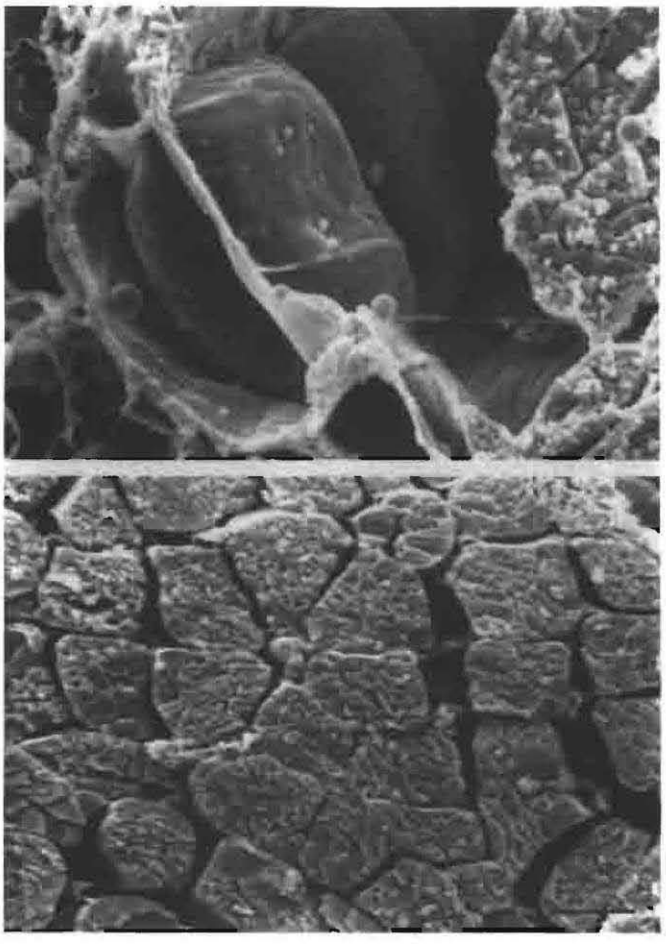
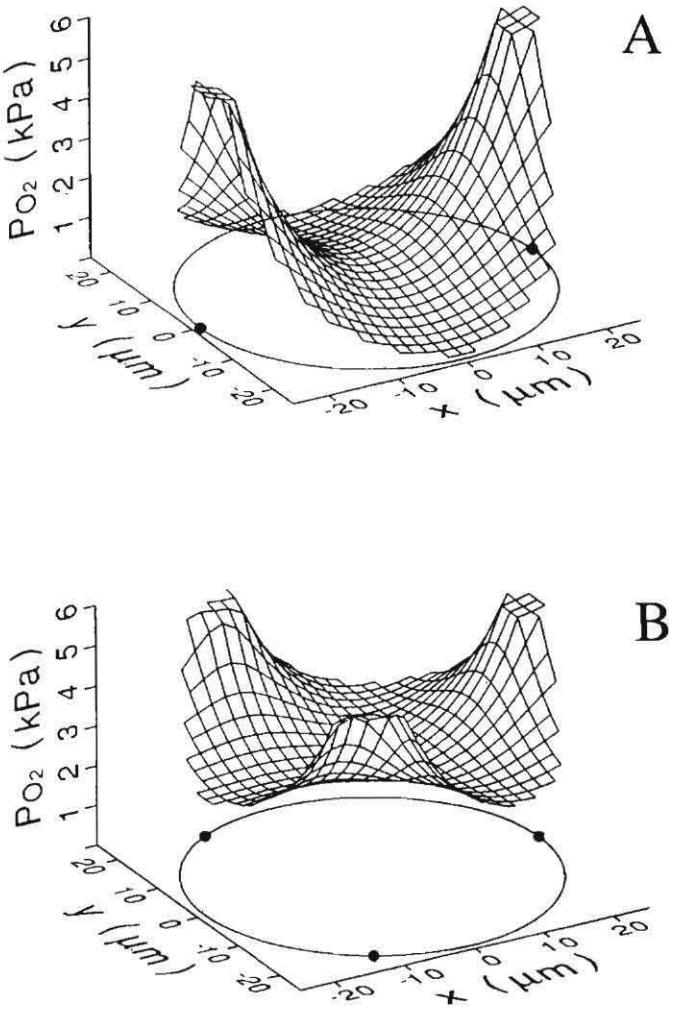


Figure 9 Scanning electron micrographs of juvenile eel slow muscle (top panel) and fast muscle (bottom panel). Intracellular lipid as well as small interstitial depots are found within slow (top panel), but not fast muscles (bottom). In small fish the majority of lipid stores are extracellular (in this case subcutaneous, asterisk, surrounded by abundant connective tissue). Note the relative size of adipocytes and muscle fibres. Cryofracture specimens; scale bar = 5 μm (top panel) and 10 μm (bottom panel).

development of a model which incorporated heterogeneities in both O_2 permeability and consumption (Hoofd, Turek & Egginton 1990). The model combines a source term (capillary) and two field terms (outer and inner zone), with the resultant O_2 tension at any point in the fibre calculated from differential equations allowing for the spatial location of sources and different characteristics of the field terms. Oxygen consumption in the outer (subsarcolemmal) and inner (intermyofibrillar) zones of fibres was scaled to mitochondrial content (volume density) with appropriate Q_{10} correction. Similarly, O_2 permeability (the product of solubility and diffusion coefficient) reflected lipid content, assuming a 5-fold greater Krogh diffusion coefficient (K) than for aqueous sarcoplasm. Facilitation pressure (maximum effect of myoglobin expressed as an O_2 driving pressure difference) was low in both groups of fish, and was omitted from subsequent calculations (Hoofd & Egginton 1997).

Striped bass acclimated to their seasonal extremes of 5° and 25°C were used to examine the combined effect of low temperature and fibre hypertrophy. While cold acclimation induced a modest (13%) increase in overall intracellular diffusion distance, $\dot{V}O_2$ fell proportionately more (60%). The increase in capillary supply (50%) aids peripheral O_2 transport, while the presence of intracellular lipid effectively reverses the cold-induced decrease in K (+ 7%). The combined effect is to increase mean fibre PO_2 by over 250%. For both groups of animals the average myofibrillar volume per fibre (product of volume density and fibre area) was similar; 385 vs. 320 μm^2 for summer vs. winter bass, hence adaptation to reduced temperature appears to be one of increasing the metabolic supply to a stable mass of contractile machinery. From these calculations the increase in mean PO_2 of slow muscle fibres is seen to reflect both an increased capillary supply and altered fibre composition. However, increased



Summer (25°C-acclimated) Striped bass

A) mean PO_2 = 1.9 kPa, min. PO_2 = 0.6 kPa
B) mean PO_2 = 3.8 kPa, min. PO_2 = 3.1 kPa

Figure 10 Effect of capillary supply on tissue oxygenation. Calculated intracellular O_2 tension in slow muscle fibres of summer (25°C-acclimated) striped bass. Open circle represents the mean fibre cross section, filled circles the capillaries, and calculated PO_2 values shown as a wire frame plot. (A) Average size, composition and capillary supply results in a mean PO_2 of 1.9 kPa and a minimum PO_2 of 0.6 kPa. (B) Increasing C:F from 1.0 to 1.5 has a dramatic effect on oxygenation, mean and minimum PO_2 are now 3.8 and 3.1 kPa, respectively (Hoofd & Egginton 1997).

capillary supply is the most effective means of increasing O_2 delivery (Figure 10), while changing composition results in only a modest increase in mean PO_2. Interestingly, the magnitude of the change would seem to be in excess of that required to maintain locomotory activity, and raises the possibility that there is some regulatory factor other than PO_2, or that cold acclimation also affects the specific $\dot{V}O_2$ of mitochondria (Johnston *et al.*, 1994; Hubley, Locke & Moerland 1997).

Conclusions

Fibre size has a major influence on capillary supply to skeletal muscle. Cold acclimation in mammals may depress activity and reduce fibre cross-sectional area; the resulting increase in capillary density and change in fibre type composition may increase the fatigue resistance of the atrophied muscle. In fishes, activity levels are often maintained at low temperatures, leading to fibre hypertrophy and altered composition (e.g. higher mitochrondrial content). Capillary growth and structural remodelling within fibres may ameliorate the adverse effects of low temperature on extra- and intracellular diffusion, respectively, although different strategies to maintain an adequate oxygen tension may be adopted by different species (Figure 11).

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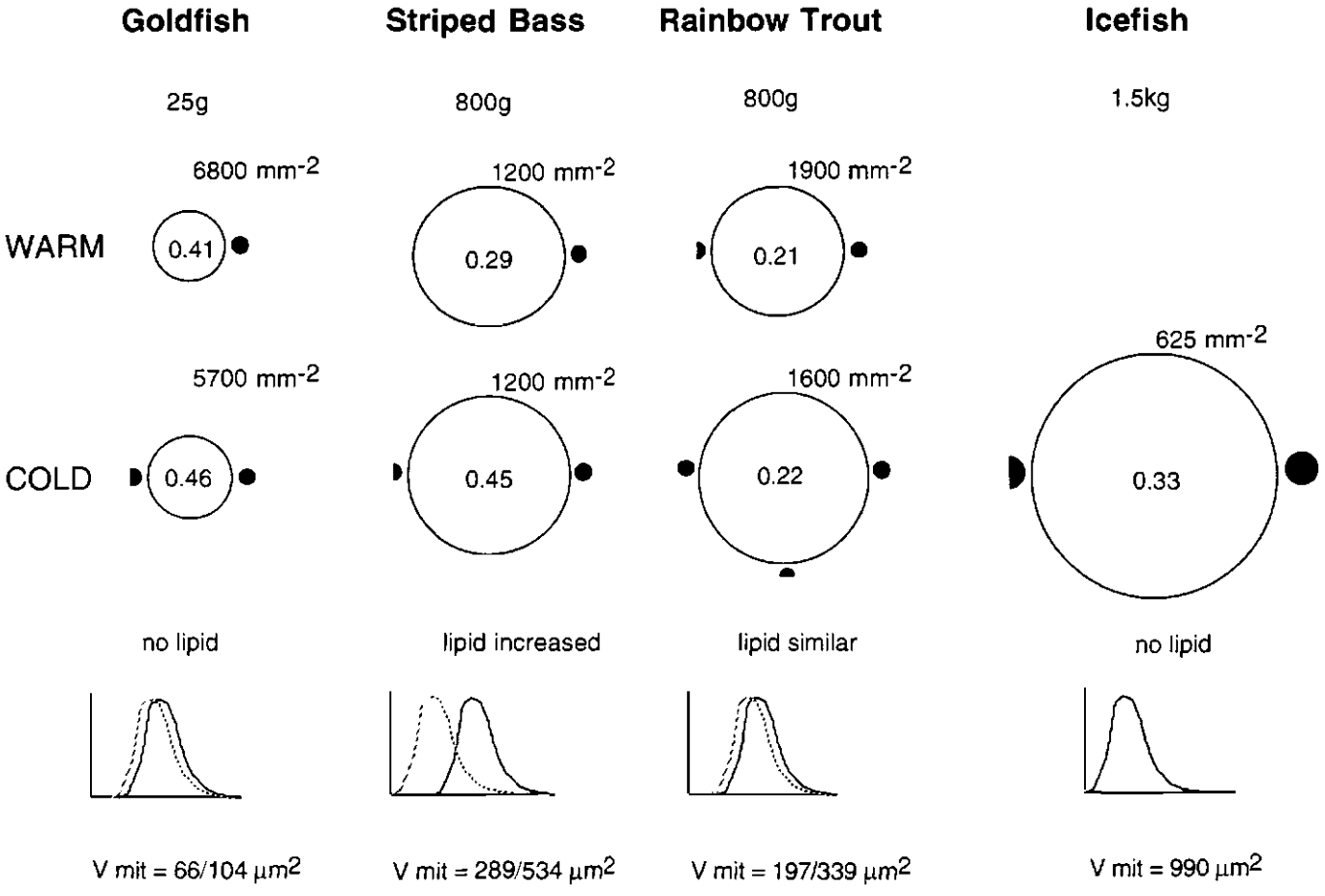

Figure 11 Schematic of the anatomical adaptations for peripheral oxygen transport at high and low temperatures in various fish species mentioned in the text, with preliminary data from Antarctic icefish (*Chaenocephalus aceratus*) added to illustrate the possible limits in tissue geometry. Numbers (top to bottom) refer to body weight, capillary density, mitochondrial volume density and mitochondrial volume (Vmit = % mitochondria × fibre area). Relative fibre size is shown by the open circles, and C:F by the filled circles (note the larger vessels in icefish). Involvement of lipid is indicated, and the distribution of mitochondrial separation is shown.

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