

Review

Going with the flow or life in the fast lane: contrasting mitochondrial responses to thermal change

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Summary

Temperature is one of the most important environmental factors affecting the physiology of animals. Seasonal fluctuations in temperature are of particular importance in aquatic ectotherms since their body temperature is in equilibrium with their environment. When an organism faces adverse environmental conditions, it can either remain active or enter into metabolic depression, adopting the strategy that maximises its fitness. Physiological responses to environmental stress occur at many different levels of organisation in an animal. Here, we focus on mitochondria, given their central importance in cellular energy metabolism. We contrast the thermal biology of skeletal muscle mitochondria from cold-active species with

that of species that spend their winters in a metabolically depressed state. Specifically, we examine the modifications of mitochondrial properties during thermal/seasonal acclimation and examine mechanisms by which these modifications can arise. While compensatory responses to cold acclimation include increases in mitochondrial abundance, in the oxidative capacities of individual mitochondria and adjustments of ADP affinities, metabolic depression can reduce tissue levels of mitochondrial enzymes and mitochondrial proton leak rates.

Key words: mitochondria, seasonal temperature change, temperature compensation, metabolic depression.

Introduction

Prokaryotic life exists over an impressively broad thermal range, but multicellular animals are limited to a much narrower range of temperatures. Although most animals have specialised for life within an even smaller portion of this range, they may still experience marked daily or seasonal temperature fluctuations. These thermal fluctuations have functional consequences at many levels of biological organisation. Endotherms achieve considerable thermal independence from their habitat by conserving much of their metabolically generated heat, thus maintaining a thermal differential with their habitat. In contrast, the body temperature of ectotherms is determined primarily by external heat sources. Whereas the body temperature of terrestrial ectotherms can differ from that of their immediate habitat, the majority of fish and many aquatic ectotherms have body temperatures identical to those of their habitat. Some clues as to why active animal life is limited to a fairly narrow thermal range are provided by the marked functional impact of thermal fluctuations upon ectotherms.

Temperature affects virtually all levels of biological organisation, from the rates of molecular diffusion and of biochemical reactions, to membrane permeability, to cellular, tissue and organ function and to their integration in the whole

organism. On an evolutionary time scale, biochemical compensation for thermal effects is shown by the maintenance of substrate affinities by metabolic enzymes, such as lactate dehydrogenase (LDH) and malate dehydrogenase (MDH), isolated from organisms living at different temperatures, as elegantly illustrated by the extensive studies of Somero (1997). Marked thermal fluctuations would presumably reduce the regulatory effectiveness of these biochemical properties, as illustrated by the far-reaching consequences of the difference in thermal and kinetic sensitivities of the LDH allozymes of the mummichog *Fundulus heteroclitus* (Powers and Schulte, 1998). Temperature shifts may alter the equilibrium between synthesis and degradation of biological structures, change metabolic requirements, favour certain functions over others and alter trophic interactions. Given the extent of these thermal effects, it is not surprising that animals show a variety of strategies, from biochemical to behavioural, to cope with thermal change. The increasingly obvious problem of global warming has given greater urgency to the understanding of biological responses to temperature, particularly in the ectothermal organisms that have limited independence from changes in environmental temperature.

When faced with changes in temperature, ectothermal animals must 'choose' between trying to maintain activity and submitting to the constraints of the law of Arrhenius. This fundamental law reflects the impact of temperature upon the frequency of molecular collisions and formally describes the thermal dependence of rate processes. When organisms are faced with reduced environmental temperatures, they can (i) slow their physiological processes by submitting to the Q_{10} effects, (ii) enhance the effects of Q_{10} on rate processes (hibernation, torpor) or (iii) offset the Q_{10} effects by maintaining functions/capacities using compensatory modifications. When environmental warming is accompanied by hostile or difficult conditions (hypoxia, decreased food availability), some organisms opt out of these demanding conditions by entering into aestivation. When thermal changes are part of recurring cycles (i.e. seasons) in a given habitat, endemic organisms may have 'adapted' to these cycles so as to maximise their overall fitness (reproductive success). The challenge for the scientist is to unravel both the mechanisms by which these functional modifications are achieved and their importance in the species' life cycle.

Mitochondria are of particular interest in questions of thermal biology, primarily because of their pivotal role in energy metabolism but also because of their origin as symbiotic organelles. Vestiges of this symbiotic origin are apparent in the limited permeability of the mitochondrial inner membrane, the functional importance of the electrochemical potential across this membrane and their DNA content. To examine the thermal biology of mitochondria, one must consider the thermal sensitivity of both the phospholipid bilayers and the membrane and soluble proteins of which they are composed. In oxidative muscle, the primary role of mitochondria is the provision of ATP for muscle contraction. Much as endurance training increases the mitochondrial content of mammalian muscle (Holloszy and Coyle, 1984), cold acclimation of many fish species increases the mitochondrial oxidative capacity of skeletal muscle (Sänger, 1993). This change can be due both to greater mitochondrial volume densities and to higher oxidative capacities of isolated mitochondria. This review examines how the properties of muscle mitochondria from ectotherms are modified during thermal acclimation and seasonal acclimatisation and compares mitochondrial physiology in organisms that remain active in the cold and those that undergo metabolic depression at cold temperatures. The mechanisms underlying these changes will be examined.

Mitochondrial responses to cold acclimation and mitochondrial thermal sensitivity

Amongst ectotherms, fish are particularly interesting for the study of muscle mitochondria, given the anatomical separation of fibre types. The standardised methods for measuring sustained swimming (Beamish, 1978; Kolok, 1999) allow the thermal sensitivity of swimming and of mitochondrial performance to be compared. For any given species of fish,

sustained swimming performance increases with temperature to an optimum and then decreases (Fry and Hart, 1948; Brett, 1967). Temperate species, such as goldfish (*Carassius auratus*), salmon (*Oncorhynchus nerka*), carp (*Cyprinus carpio*) and striped bass (*Morone saxatilis*) (Fry and Hart, 1948; Brett, 1967; Rome et al., 1984; Sisson and Sidell, 1987), can shift their thermal optimum of sustained swimming during thermal acclimation. Sustained swimming requires oxidative ATP production, so adjustments in the thermal sensitivity of the catalytic and regulatory properties of muscle mitochondria may accompany changes in the thermal sensitivity of sustained swimming during thermal acclimation.

Cold acclimation leads many temperate-zone fish to increase the mitochondrial volume densities in red and white muscle or the activities of mitochondrial enzymes (for reviews, see Sängler, 1993; Guderley and St-Pierre, 1996). The proportion of aerobic fibres in the swimming musculature rises during acclimation of goldfish to temperatures below 5 °C (Johnston and Lucking, 1978; Sidell, 1980). Both the capacity for lipid oxidation and the volume density of lipid droplets in muscle fibres rise during cold acclimation of striped bass (5 versus 25 °C) (Jones and Sidell, 1982; Egginton and Sidell, 1989). Such shifts are seen during laboratory acclimation (5 versus 18 °C) and seasonal cold acclimatisation of rainbow trout (*Oncorhynchus mykiss*) (Dean, 1969; Guderley and Gawlicka, 1992; Thibault et al., 1997; St-Pierre et al., 1998; Egginton et al., 2000). In striped bass, acclimation to 5 °C increases the concentration of fatty acid binding protein sufficiently to counteract the expected decrease in its diffusion coefficient (Londraville and Sidell, 1996). Both the eurythermal carp (*Cyprinus carpio*) and the cold-water short-horn sculpin (*Myoxocephalus scorpius*) modify the metabolic and contractile properties of their muscle during cold acclimation (Johnston et al., 1985; Guderley and Johnston 1995; Temple and Johnston, 1998; Temple et al., 2000). Cold acclimation also enhances muscle oxidative capacity for other temperate-zone fish, including chain pickerel *Esox niger* (Kleckner and Sidell, 1985), crucian carp *Carassius carassius* (Johnston and Maitland, 1980) and the sticklebacks *Pungitius pungitius* and *Gasterosteus aculeatus* (Guderley and Foley, 1990; Guderley et al., 1994, 2001). Overall, both in oxidative and glycolytic fish muscle, mitochondria seem to be a primary target of thermal compensation. This observation is interesting given that mitochondria in oxidative and glycolytic fibres experience different kinetic environments and patterns of recruitment.

Little is known about the genetics of the capacity for thermal acclimation. When the responses of different stickleback families to thermal acclimation (8 versus 23 °C) were examined, thermal compensation of citrate synthase activity in oxidative and glycolytic muscles was apparent in all families even though the thermal sensitivity of growth differed considerably among families (Guderley and Houle-Leroy, 2001).

Low temperatures could limit mitochondrial performance (i) by reducing mitochondrial oxidative capacity, (ii) by limiting diffusive exchange between the cytoplasm and mitochondria

Table 1. Changes in muscle ultrastructure with thermal habitat and thermal acclimation in fish

Habitat or acclimation temperature	Species	Fibre type	Mitochondria		Myofibril volume density (%)	Lipid droplet volume density (%)	Mitochondrial cristae density ($\mu\text{m}^2 \mu\text{m}^{-3}$)
			Volume density (%)	% sub-sarcolemmal			
Antarctic	<i>Trematomus newnesi</i> ¹	O	31±1.9	67.8±2.7	36±1.9	3±0.7	35.8±8.2
	<i>Gobionotothen gibberifrons</i> ²	C	15.9±0.7	NA	40.1±0.9	NA	29.63±1.6
	<i>Chionodraco rastrospinosus</i> ²	C	20.1±0.7	NA	24.5±1.26	NA	21.52±0.7
	<i>Chaenocephalus aceratus</i> ²	C	36.5±2.1	NA	25.1±1.6	NA	20.0±0.8
Sub-Antarctic							
	<i>Champocephalus esox</i> ¹	O	51±2.6	37.9±5.5	38±2.6	0	43.9±2.1
Summer	<i>Eliginops maclovinus</i> ¹	O	33±7.3	60.1±3.3	45±1.9	0.3±1.0	39.2±3.9
Winter	<i>Eliginops maclovinus</i> ¹	O	31±1.4	69.9±3.1	45±2.1	0.4±0.2	NA
Summer	<i>Paranotothenia magellanica</i> ¹	O	27±1.5	65.5±2.3	46±1.7	0.4±0.2	45.1±4.7
Temperate							
5 °C	<i>Morone saxatilis</i> ³	O	44.8±2.4	NA	42.8±1.7	7.9±1.4	51.8±1.8
25 °C	<i>Morone saxatilis</i> ³	O	28.6±1.8	NA	65.8±2.1	0.6±0.3	46.9±1.4
5 °C	<i>Morone saxatilis</i> ³	G	4.0±0.4	NA	85.2±0.5	NA	49.3±1.6
25 °C	<i>Morone saxatilis</i> ³	G	2.7±0.3	NA	88.8±0.2	NA	52.1±1.5
Winter (1 °C)	<i>Oncorhynchus mykiss</i> ⁴	O	26.9±0.9	42	56.3±1.5	10.0±1.0	40.2±0.6
Summer (16 °C)	<i>Oncorhynchus mykiss</i> ⁴	O	27.0±1.0	41.5	60.3±0.7	7.5±0.6	36.4±1.2
Summer acclimation							
5 °C	<i>Carassius carassius</i> ⁵	O	27±2.2	NA	NA	NA	25.8±1.0
25 °C	<i>Carassius carassius</i> ⁵	O	11±1	NA	NA	NA	15.5±0.8
Winter acclimation							
5 °C	<i>Carassius carassius</i> ⁵	O	21±0.8	NA	NA	NA	13.9±1.0
25 °C	<i>Carassius carassius</i> ⁵	O	10±0.7	NA	NA	NA	10.7±0.1
Autumn acclimation							
2 °C	<i>Carassius carassius</i> ⁶	O	24±1	34.6±2.8	42±1	NA	NA
28 °C	<i>Carassius carassius</i> ⁶	O	12±1	22.2±1.2	58±0.8	NA	NA

Muscle fibres are oxidative (O), glycolytic (G) or cardiac (C).

Volume densities are % fibre volume. ¹Johnston et al. (1998); ²O'Brien and Sidell, 2000; ³Egginton and Sidell (1989); ⁴St-Pierre et al. (1998); ⁵Kilarski et al. (1996); ⁶Johnston and Maitland (1980).

Values are means±S.E.M. (N values are given in original papers); NA, not available.

or (iii) by modifying the sensitivity of mitochondria to regulatory molecules. Mitochondrial oxidative capacities are typically measured, *in vitro*, at saturating ADP and carbon substrate concentrations. How these empirically determined capacities compare with the rates attained during intracellular delivery of adenylates, substrates, oxygen and effectors, is little understood, particularly for non-mammalian systems. Given our lack of knowledge of the conditions under which mitochondria function *in vivo*, it is difficult to assess the exact nature of the thermal limitations. Mitochondrial proliferation could offset the first two problems, increases in the oxidative capacity of isolated mitochondria the first and adjustment of regulatory properties the third.

The numerous studies cited above indicate quantitative changes in mitochondrial abundance during thermal acclimation. Most have interpreted these changes in terms of the catalytic capacity, few have examined other possibilities. Analysis of the 'raison d'être' of these changes must take into account that increases in muscle oxidative capacity with cold

acclimation occur in both cold-active (striped bass, rainbow trout) and cold-inactive (crucian carp) species as well as occurring in both oxidative and glycolytic muscles. In other words, it is important to emphasise that cold-induced increases in aerobic capacity also occur in systems that do not require high intensities of mitochondrial metabolism (e.g. cold-inactive species and glycolytic muscle).

Diffusional limitations

Diffusive processes are commonly considered to have low thermal sensitivities, with Q_{10} values approaching unity. However, within complex biological fluids such as the cytosol, diffusion of small molecules has a thermal sensitivity (within the physiological range) similar to that of enzyme-catalysed processes (Sidell and Hazel, 1987). Such thermal limitations could be overcome by modifying any of the physical or chemical parameters that establish rates of diffusion. Proliferation of mitochondrial membrane would increase the

surface area over which diffusion could occur, facilitating net diffusive transfer between the mitochondrial and cytosolic compartments. While this would facilitate the delivery of oxygen, carbon substrates and adenylates to the mitochondrial compartment, it could be energetically costly, because the proton leak (futile cycling of protons across the inner membrane of the mitochondria) increases as a function of inner membrane surface area (Porter et al., 1996).

Despite its central importance in muscle metabolism, the delivery of oxygen to mitochondria *in situ* is not well understood, partly because of the technical problems of measuring mitochondrial oxygen affinities and because intracellular oxygen gradients may change with shifts in cellular metabolic rate. According to Gnaiger et al. (1998), the higher oxygen affinities of mitochondria from heart compared with those from liver are due to the greater excess of cytochrome *c* oxidase (CCO) in heart mitochondria. High mitochondrial oxygen affinities might facilitate oxygen uptake during intense aerobic work. Gnaiger's inter-tissue comparison suggests that facilitation of oxygen uptake is of central importance in mitochondrial design. That a high proportion of muscle mitochondria are subsarcolemmal, particularly in Antarctic and sub-Antarctic fish (Table 1), is compatible with the suggestion that access to oxygen is a limiting factor for mitochondrial physiology.

Sidell and coworkers suggest that the increased lipid contents of muscle after cold acclimation facilitate oxygen movements (Egginton and Sidell, 1989; Desaulniers et al., 1996). Hence, the contact between lipid droplets and mitochondrial membranes visible in electron micrographs of skeletal muscle and the reticular nature of mitochondria *in vivo* could facilitate intracellular movements of oxygen (Longmuir, 1980; Desaulniers et al., 1996). Such a role is suggested by the mitochondrial architecture in the heart of the icefish *Chionocephalus aceratus*, a species that lacks both haemoglobin and myoglobin. Although the heart mitochondria are present at high volume densities, their cristae densities are fairly low (Table 1), suggesting that maximisation of aerobic capacity is not the prime reason for the mitochondrial proliferation. Rather, the high mitochondrial volume densities would serve to establish an intracellular reticulum within which oxygen could diffuse according to its gradients (O'Brien and Sidell, 2000). The problem of effective oxygen delivery at low temperatures is underscored by the capacity of fish myoglobins to bind and release oxygen more rapidly at low temperatures than those from mammals (Sidell, 1998). Overall, these data suggest that, during cold acclimation, both intracellular lipids and mitochondria proliferate to facilitate oxygen movements.

Nature rarely uses structures for only one purpose, especially ones with a high energetic value, so it is unlikely that intracellular lipids in cold-acclimated fish serve only to store and deliver oxygen to mitochondria. Accordingly, oxidative muscle from 5 °C-acclimated rainbow trout and striped bass shows an enhanced capacity for lipid oxidation in comparison with that from warm-acclimated conspecifics

(Dean, 1969; Jones and Sidell, 1982). The higher levels of fatty acid binding protein in cold-acclimated striped bass should facilitate movement of fatty acids between the lipid droplets and the mitochondria (Londrville and Sidell, 1996).

Given the role of mitochondria in ATP generation, as well as the potential for rapid generation of phosphocreatine (PCr) in the vicinity of mitochondria, it is apparent that increasing the mitochondrial volume density in muscle fibres could decrease intracellular gradients for these important molecules. In a novel approach combining nuclear magnetic resonance studies of ATP and PCr diffusion and reaction-diffusion analysis, Hubley et al. (1997) showed that the intracellular diffusion coefficients of ATP and PCr are not altered by thermal acclimation. Further, the cold-acclimation-induced increases in mitochondrial volume density in oxidative muscle have no impact upon the intracellular gradients of ATP and PCr. Only at the mitochondrial volume densities typical of glycolytic muscle do the increases resulting from cold acclimation reduce intracellular gradients of PCr. No effect was apparent on the concentration gradients of ATP. Thus, mitochondrial contents in oxidative muscle, even of warm-acclimated fish, seem to exceed greatly the levels required to minimise gradients of ATP and PCr within the fibres. These results illustrate that mitochondrial proliferation might serve different purposes in red and white muscle.

Changes in mitochondrial properties with thermal acclimation

The oxidative capacities of isolated mitochondria can change during thermal acclimation. Mitochondria from the red muscle of 5 °C-acclimated sculpin (*Myoxocephalus scorpius*) have higher rates of pyruvate and palmitoyl carnitine oxidation (oxygen uptake per milligram mitochondrial protein) at a given assay temperature than those isolated from 15 °C-acclimated fish (Guderley and Johnston, 1996). Seasonal acclimatisation to cold (or decreasing) temperatures also enhances the oxidative capacity (oxygen uptake per milligram protein) of mitochondria isolated from trout oxidative muscle (Guderley et al., 1997; St-Pierre et al., 1998; Guderley and St-Pierre, 1999). Not surprisingly, these changes are accompanied by modifications in the fatty acid composition of mitochondrial phospholipids. Both the overall level of unsaturation and the proportion of specific unsaturated fatty acids (22:6) in mitochondrial phospholipids increase with cold acclimatisation (Guderley et al., 1997). Although the rate of mitochondrial respiration per gram of muscle rises with cold acclimation in goldfish, the activity of CCO (international enzyme units per gram muscle) increases considerably more than the rate of respiration (Van Den Thillart and Modderkolk, 1978). The rise in respiration rate per gram muscle is at least partly due to an increased mitochondrial abundance (Tyler and Sidell, 1984) but may also reflect increased molecular activities of the electron transport chain enzymes such as CCO (Wodtke, 1981b). In contrast, long-term (>12 weeks) cold (7 °C) acclimation of the sea bass *Dicentrarchus labrax* reduces rates

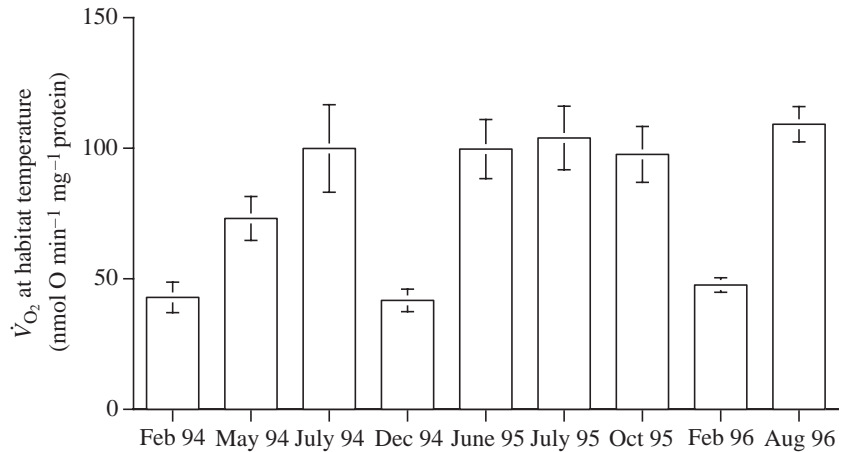


Fig. 1. Maximum rates of oxygen uptake by isolated mitochondria from trout oxidative muscle respiring at habitat temperatures. Values from Feb 94, Dec 94 and Feb 96 did not differ but were lower than those for the other times (ANOVA, *a posteriori* comparisons, $P < 0.05$). Data from Guderley and St-Pierre (1999). Values are means \pm S.E.M., $N=9$.

of glutamate oxidation at 20 °C by mitochondria isolated from liver and heart compared with those from 22 °C-acclimated bass (Trigari et al., 1992). No changes in membrane lipid saturation accompany cold acclimation in sea bass.

Increases in the number of mitochondria and in the oxidative capacity per milligram mitochondrial protein are complementary strategies that both enhance muscle oxidative capacity. Whereas greater volume densities of mitochondria could compromise contractile capacity by reducing myofibrillar volume density, increasing the cristae surface density or enhancing the oxidative capacity per milligram of mitochondrial protein would not have this negative effect. In oxidative fibres from fish living in a variety of thermal habitats, mitochondria occupy between 10 and 50% of fibre volume (Table 1), an impressive variation, particularly considering that mitochondrial volume densities in mammalian skeletal muscle are typically less than 10% (Schwerzmann et al., 1989; Vock et al., 1996). Only cardiac muscle attains similar volume densities in mammals (Conley et al., 1995), with the highly aerobic hummingbird muscles reaching 35% and bee and blowfly muscles at 40–43% (Suarez, 1996).

The high mitochondrial volume densities in fish oxidative muscle may reflect the fairly continuous contractile activity of these fibres, particularly in active pelagic fish. It has frequently been suggested that, during evolutionary specialisation for low habitat temperatures, mitochondrial volume densities increase, but when closely related fish species with similar activity patterns are compared, this relationship only holds for benthic species (Johnston et al., 1998). Cristae densities vary, both among and within species, with most values lying between 20 and 40 $\mu\text{m}^2 \mu\text{m}^{-3}$ (Table 1), although values as high as 72 $\mu\text{m}^2 \mu\text{m}^{-3}$ have been reported in mitochondria from tuna oxidative muscle (Moyes et al., 1992). Thus, at the ultrastructural level, both the volume fraction and the cristae surface density of muscle mitochondria can vary during thermal acclimation and during evolutionary adaptation to temperature. Further, the oxidative capacity per milligram of mitochondrial protein could be enhanced by changes in the properties of membrane phospholipids.

To ascertain the relative importance of ultrastructural and

qualitative strategies in the response of muscle mitochondria to thermal acclimation, we examined the mitochondrial volume densities, the cristae surface densities, the oxidative capacities of isolated mitochondria and the aerobic capacities of oxidative fibres from seasonally acclimated rainbow trout, *Oncorhynchus mykiss*. Cold acclimation increases mitochondrial cristae density without changing mitochondrial volume density (Table 1) (St-Pierre et al., 1998). The simultaneous increase in substrate oxidation rates per milligram of mitochondrial protein and in CCO and citrate synthase activities per milligram of mitochondrial protein markedly enhanced muscle aerobic capacity in cold-acclimated trout. Thus, winter-acclimated trout achieved a considerable increase in muscle aerobic capacity by packing more cristae into their mitochondria and by increasing the oxidative capacity per milligram of mitochondrial protein. The decrease in myofibrillar volume density with cold acclimation was due to the increase in the proportion of fibre volume occupied by lipids (Table 1) (St-Pierre et al., 1998).

Whereas laboratory acclimation seeks to vary only temperature, in natural habitats, temperature changes in a cyclic fashion and in conjunction with other environmental variables. Animals may use decreasing temperatures as a cue to 'foresee' upcoming cold conditions. A given temperature change may provide a different signal and lead to a distinct response according to the time of year at which it is experienced (Kilarski et al., 1996; Seddon and Prosser, 1997; Guderley et al., 2001). Further, the simultaneous changes in temperature and photoperiod may intensify seasonal changes (Taylor et al., 1996). We examined the onset, duration and effectiveness of compensatory modifications in maximal oxidative capacities of mitochondria isolated from oxidative muscle of rainbow trout acclimated to seasonal changes in temperature and photoperiod in an outdoor holding pond. Given the preference of trout for cool temperatures, we predicted essentially complete compensation of maximal oxidative capacities at lower temperatures. The mitochondria show seasonal cycles of their maximal rates of protein-specific substrate oxidation at any given temperature (Guderley and St-

Pierre, 1999). In general, increases in the maximal capacity of pyruvate oxidation were sufficient to compensate for seasonal changes in temperature except during the winter months, when rates at habitat temperature were depressed relative to other periods (Fig. 1).

Overall, there is considerable evidence that thermal acclimation and seasonal acclimatisation of fish lead to compensatory modifications in mitochondrial properties that minimise thermal variation of mitochondrial oxidative capacity. However, given the mismatch between aerobic capacity and metabolic rate in many systems (Suarez, 1996; Suarez et al., 1997), it is unlikely that muscle mitochondria are functioning at these maximal capacities even under extreme work loads. The high mitochondrial volume densities argue for a role in minimising diffusive limitations or increasing regulatory sensitivity.

Thermal sensitivity of mitochondrial regulatory properties

For several substrate affinities of ectothermal mitochondria, the impact of temperature is either small or is minimised by physiological shifts in pH with temperature. The oxygen affinity of isolated mitochondria from goldfish (*Carassius auratus*) oxidative muscle is independent of temperature (7–30 °C) (Bouwer and Van Den Thillart, 1984). The co-variation of pH with temperature (8–22 °C) maintains constant pyruvate affinities for mitochondria from rainbow trout muscle (Blier and Guderley, 1993a). Under similar experimental conditions, the pH gradient in mitochondria isolated from carp red muscle remains at approximately 0.4 units at 10, 20 and 30 °C (Moyes et al., 1988). Physiological shifts in pH and P_{CO_2} with temperature minimise the thermal sensitivity of the succinate affinity of mitochondria isolated from iguana (*Dipsosaurus dorsalis*) liver (Yacoe, 1986). For 15 °C-acclimated short-horn sculpin, the ADP affinity of mitochondria from oxidative muscle is independent of temperature, but in 5 °C-acclimated fish it decreases markedly when temperature rises, even when pH co-varies with temperature (Guderley and Johnston, 1996). However, these studies did not examine whether these 'physiological shifts in pH with temperature' actually occurred *in vivo* in the tissues under study.

In contrast to the above responses, the ADP affinity of rainbow trout muscle mitochondria is quite temperature-sensitive, with markedly higher apparent K_m values ($K_{m,app}$) at 8 than at 15 or 22 °C (Blier and Guderley, 1993b). ADP is both a substrate for and a regulator of oxidative phosphorylation (Brand and Murphy, 1987). Free ADP levels in fish muscle range from 20 to 100 $\mu\text{mol l}^{-1}$ (Van Waarde et al., 1990), near the values of the mitochondrial $K_{m,app}$ for ADP (Blier and Guderley, 1993b; Guderley and Johnston, 1996). Thermal acclimation (4 and 24 °C) does not change adenylate levels in oxidative muscle of continuously swimming brook trout *Salvelinus fontinalis* (Walesby and Johnston, 1980), suggesting that seasonal shifts in adenylate concentrations would not alleviate the thermal sensitivity of the ADP affinity of trout mitochondria.

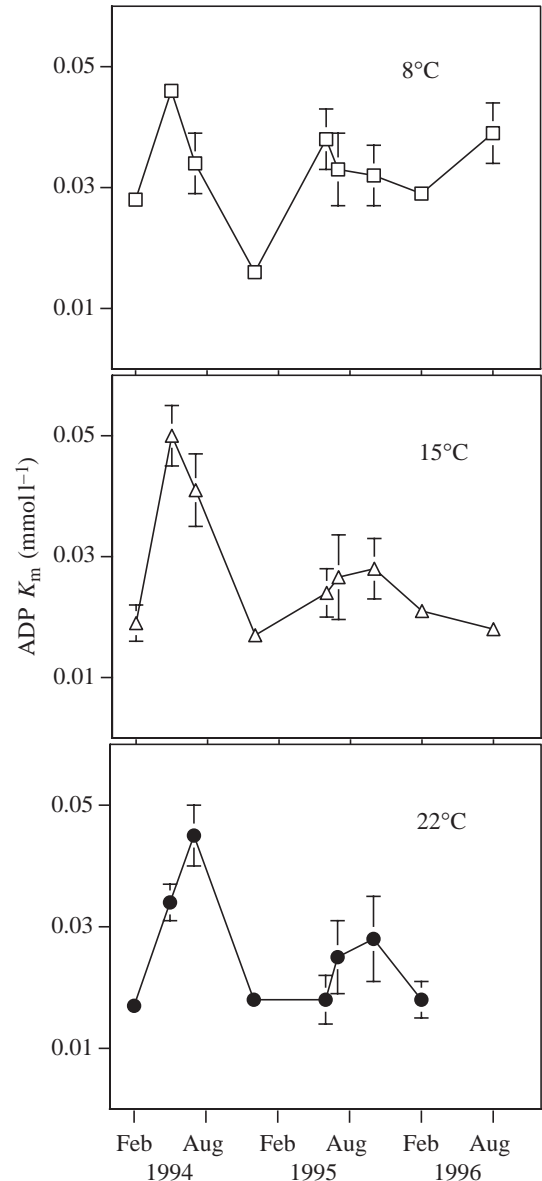


Fig. 2. Cycles in ADP affinity (K_m) of mitochondria isolated from trout oxidative muscle after seasonal acclimatisation. Data from Guderley and St-Pierre (1999). Values are means \pm S.E.M., $N=5-9$.

Thermal independence of mitochondrial sensitivity to ADP would seem the simplest means of maintaining regulatory mechanisms throughout the trout's thermal range. We therefore examined whether the thermal sensitivity of the ADP affinity of trout muscle mitochondria changed throughout the annual cycle. We hypothesised that, during cold periods, the mitochondria would have a greater ADP affinity at low temperatures. At any given temperature, the ADP affinity of isolated mitochondria was highest during cold months, showing a clear annual cycle (Guderley and St-Pierre, 1999) (Fig. 2). Thus, the estimated $K_{m,app}$ for ADP at habitat temperatures showed less seasonal variation than the ADP $K_{m,app}$ at a given experimental temperature. A reduction in ADP affinity with decreasing experimental temperature

occurred through much of the year. Only in December and July was there no change in $K_{m,app}$ with assay temperature. Thus, mitochondria from trout oxidative muscle underwent a seasonal modulation that decreased the seasonal variation of mitochondrial sensitivity to ADP.

The ADP affinity of mitochondria is set by a variety of processes including, as a minimum, the adenine nucleotide translocase (ANT) and the F_1 -ATPase. Further, when mitochondrial ADP affinities are assessed using the ADP sensitivity of oxygen uptake, changes in the capacity of other components, such as the electron transport chain, may influence the observed affinities. Changes in membrane characteristics (see below), particularly in the levels of cardiolipin and in the degree of polyunsaturation of phospholipid acyl chains, influence the activity of membrane-bound proteins such as ANT and the F_1 -ATPase (Hoch, 1992), providing a potential mechanism whereby such seasonal modulation could occur. The above demonstration of the seasonal modulation of mitochondrial ADP affinity suggests that it is a regulated parameter. However, cells rarely allow their ATP levels to become as low as those used during these measurements. The sensitivity of ANT to the [ADP]/[ATP] ratio suggests that the ADP sensitivity in the presence of ATP (and its intracellular fluctuations) differs from the values we determined. However, in the absence of acclimatory changes in adenylate levels (Walesby and Johnston, 1980), this should not modify the seasonal patterns.

Overall, cold acclimation leads many fish species to modify both the abundance and the properties of their muscle mitochondria. Overcoming diffusional limitations, maintaining oxidative capacity despite decreases in temperature and conservation of sensitivity to ADP may underlie these changes. That such changes occur in glycolytic and oxidative muscles suggests an 'aim' of reducing the use of anaerobic glycolysis. That cold-inactive species also show these 'compensatory' increases may reflect issues of oxygen delivery (see below) or the requirements of activity at the cold temperatures experienced after dormancy.

Mitochondrial responses in cold-inactive species

In the face of acute environmental stress, the first lines of physiological defence are activated within seconds to compensate for the adverse effects on physiological functions. If the environmental insult persists, more profound changes will occur, leading to a reorganisation of cellular metabolism. In the face of decreasing temperatures, some animals increase their aerobic capacity to stay active, as seen in the previous section. However, other organisms accentuate the effects of temperature by reducing their standard metabolic rate to a greater extent than would be predicted by Q_{10} effects alone, a phenomenon called metabolic depression. Other harsh environmental conditions, including reductions in oxygen, food and water availability, can trigger metabolic depression in organisms. However, the importance of temperature is emphasised by the fact that changes in temperature are often

accompanied by alterations in these other environmental factors. Metabolic depression is reflected at the cellular level by a reduced ATP turnover (Boutilier and St-Pierre, 2000; Brand et al., 2000, 2001). When ATP supply becomes limiting, vital cell functions gain priority over accessory ones, thereby leading to a hierarchy of ATP-consuming processes (Buttgereit and Brand, 1995). In other words, organisms entering metabolic depression are analogous to computers working on 'power-saving mode'.

The common frog *Rana temporaria* spends several months each year in ice-covered ponds (Pinder et al., 1992). During this time, the animal does not feed and it can become exposed to severe shortages of oxygen because of reduced rates of photosynthesis by aquatic plants. When frogs are submerged in cold hypoxic water, to mimic the over-wintering conditions under ice cover, they gradually decrease their standard metabolic rate to 25 % of that of control animals (with access to air) after 2 months (Donohoe and Boutilier, 1998). The hypometabolic state sustained by the frog during over-wintering submergence is mostly aerobic (Donohoe and Boutilier, 1998). Therefore, the over-wintering frog has been used as a model organism to examine mitochondrial metabolism during long-term aerobic metabolic depression. The only other ectotherm that has been used to investigate these responses is the snail. The entry into metabolic depression for the snail is called aestivation because it is triggered by reduced food and water availability associated with hot temperatures. Most of this section will focus on the over-wintering frog, although we will refer to the snail model when appropriate.

For overwintering frogs, skeletal muscle is thought to be primarily responsible for the overall metabolic depression for two reasons: (i) it makes up the largest proportion of the frog's body mass (Boutilier et al., 1997) and (ii) its metabolic rate conforms to oxygen availability (West and Boutilier, 1998) such that a reduced, or even transiently interrupted, blood supply leads to a marked skeletal muscle hypometabolism. In fact, cold-submerged frogs drastically reduce the blood supply to their skeletal muscle to shunt more blood to the skin for the extraction of oxygen (Boutilier et al., 1986, 1997). For this reason, the extent of metabolic depression in frog skeletal muscle is thought to be higher than in any other tissue. Cardiomyocytes isolated from metabolically depressed frogs did not display a reduction in respiration rate compared with those isolated from controls (Currie and Boutilier, 2001).

The aerobic capacity of frog skeletal muscle and of snail hepatopancreas is decreased during metabolic depression, as indicated by a reduction in the activity of citrate synthase (CS) and CCO (St-Pierre and Boutilier, 2001; Stuart et al., 1998a,c). A reduction in aerobic capacity could be due (i) to a decrease in mitochondrial volume density, (ii) to an alteration in the intrinsic properties of the mitochondria or (iii) to a combination of both. Metabolic depression in frogs led to a greater decrease in the activity of CS in the isolated mitochondria than in tissue homogenates (St-Pierre and Boutilier, 2001). Therefore, the reduction in aerobic capacity of frog skeletal muscle during

metabolic depression can be explained by changes in the intrinsic properties of the mitochondria. No studies have examined the effect of metabolic depression in ectotherms on tissue ultrastructure, notably mitochondrial volume density, so it is difficult to exclude such changes.

Since overwintering submergence is associated with hypoxic environmental conditions, it is interesting to examine what happens to the affinity of mitochondria for oxygen. This question is particularly important since it was shown that there is no change in the O_2 -affinity of mitochondria when mammals are exposed to hypoxia (Jones et al., 1991). However, mammalian cells exposed to hypoxia display a reorganisation of their mitochondrial network (Jones et al., 1991). Interestingly, the mitochondria isolated from the skeletal muscle of over-wintering frogs showed an increase in affinity for oxygen (a decrease in P_{50}) in their active (state 3) state compared with controls (Table 2). At present, it is not known whether these changes in mitochondrial O_2 -affinity are accompanied by a reorganisation of the mitochondrial network in frog muscle cells. Importantly, the increase in mitochondrial O_2 -affinity of metabolically depressed frogs is likely to have functional implications since the intracellular P_{O_2} of their skeletal muscle is probably similar to the mitochondrial P_{50} values during over-wintering submergence.

In fact, frogs recruit anaerobic metabolism during the initial period of submergence in hypoxic water, as indicated by an elevated plasma lactate concentration (Donohoe and Boutilier, 1998). The plasma lactate concentration decreased steadily and returned to control values after 2 months of submergence when the ATP demand was met by aerobic metabolism (Donohoe and Boutilier, 1998). The vast majority of the lactate entering the circulation is thought to originate from the hypo-perfused skeletal muscle (Donohoe and Boutilier, 1999). Taken together, these data indicate that the skeletal muscle of over-wintering frogs might be a good example of a compromise between reducing aerobic capacity and facilitating oxygen uptake.

A coordinated reduction in ATP supply and demand is required to enter a viable hypometabolic state. It is often assumed that all the resting oxygen consumption of cells is used to drive ATP turnover. This is not true. In fact, 20% of the standard metabolic rate (SMR) of a mammal is used to drive the proton leak (Rolfe and Brown, 1997). The proton leak is also thought to be an important contributor to the SMR in ectotherms. Upper estimates of the contribution of the proton leak rate to the resting respiration rate of hepatocytes from lizard, lamprey, frog and snail are around 25% (Brand et al., 2001). Knowing that many organisms decrease their metabolic rate to 20% of control levels during metabolic depression and that the proton leak accounts for approximately 20% of SMR, it is imperative that the proton leak is reduced in hypometabolic states, otherwise the remaining metabolism would be extremely inefficient. The proton leak rate of mitochondria isolated from the skeletal muscle of frogs submerged in hypoxic water for 4 months is half that of controls (Table 2).

Table 2. *Properties of skeletal muscle mitochondria from control and overwintering frogs*

Mitochondrial properties	Change during overwintering submergence (%)
CS activity ¹	-52
State 3 rate ²	-58
State 4 rate ²	-52
Proton leak rate ³	-51
State 3 P_{50} value ²	-55
State 4 P_{50} value ²	0

The change during overwintering submergence represents the difference between values for control and 4-month-hypoxic submerged frogs as a percentage of the control value.

¹St-Pierre and Boutilier (2001); ²St-Pierre et al. (2000b), data taken from experiments performed at 3 °C; ³St-Pierre et al. (2000a).

Citrate synthase (CS) P_{50} values express the K_m values for oxygen for the isolated mitochondria.

The mechanism responsible for the reduction in proton leak rate is a decrease in the activity of the electron transport chain (St-Pierre et al., 2000a). A reduced activity of the electron transport chain will generate a smaller membrane potential, thereby considerably reducing the proton leak rate, because of the steep dependence of the proton leak on membrane potential. The reduction in proton leak rate in over-wintering frogs is not due to a decrease in proton conductance. For cells to preserve their metabolic efficiency (P/O ratio), proton leak rate has to be reduced by the same extent as total respiration rate. Indeed, it was found that the proportion of total respiration rate devoted to the proton leak was similar in hepatopancreatic cells from control and aestivating snails (Bishop and Brand, 2000). The mechanisms behind the reduction in proton leak rate in hepatopancreatic cells from aestivating snails are not yet known. Together, these results indicate that the proton leak is, indeed, reduced during metabolic depression to preserve, or possibly even to increase, metabolic efficiency.

Overall, the data presented above show that a decrease in oxygen consumption rate at the animal level can be equalled at the tissue, cellular and mitochondrial levels, supporting the idea that metabolic depression is reflected at all levels of biological organisation.

How are mitochondrial contents/properties changed during thermal acclimation?

Tissue oxidative capacities can be increased by enhancing the oxidative capacities of existing mitochondria, by increasing the number of mitochondria or by a combination of the two. Given the influence of the membrane environment on the functioning of membrane proteins, changes in membrane composition are a prime mechanism by which the oxidative capacities of existing mitochondria can be modified. Considerable remodelling of the phospholipid and fatty acid

composition of mitochondrial membranes occurs during thermal acclimation (Wodtke, 1981b; Van Den Thillart and De Bruin, 1981; Hazel and Williams, 1990; Hazel, 1995). In fact, homeoviscous adaptation (i.e. the maintenance of a constant membrane fluidity) of mitochondrial membranes during thermal acclimation is more complete than that in other subcellular fractions in green sunfish (*Lepomis cyanellus*) liver and goldfish brain (Hazel and Williams, 1990).

If changes in membrane composition with cold acclimation have a compensatory impact upon catalytic rates of membrane proteins, molecular activities of membrane proteins should increase during cold acclimation. Wodtke (1981a,b) addressed this question in oxidative muscle of carp with concurrent measurements of CCO activity and cytochrome *a/a₃* contents in tissue fractions and isolated mitochondria. Cold- and warm-acclimated carp do not differ in the concentration of mitochondrial cytochromes per milligram mitochondrial protein, but differ markedly in the molecular activity of CCO, measured at a common experimental temperature. While the turnover numbers for the carp enzyme are considerably lower than those of honeybees, they are within the range of values obtained for rat heart (Suarez et al., 1999). The increases in succinic dehydrogenase and CCO activity per milligram of protein in the mitochondrial fraction of skeletal muscle during cold (10 *versus* 32 °C) acclimation of goldfish and carp can be explained by changes in the lipid composition of the mitochondrial membrane (Hazel, 1972a,b; Wodtke, 1981a). Given the differences in the acclimatory responses of a variety of ectotherms, it would be interesting to ascertain whether membrane-based modifications in the molecular activity of mitochondrial cytochromes form a common response during thermal acclimation.

Cold exposure produces an initial activation of the Δ^9 -desaturase in carp liver endoplasmic reticulum that follows the same time course as the changes in membrane order (Wodtke and Cossins, 1991). Only the secondary increase in desaturase activity appears to be due to synthesis of additional enzyme, as reflected by the increases in the levels of its mRNAs (Gracey et al., 1996). The activation of the desaturase is central to the modifications of phospholipid acyl chain composition during cold acclimation. These observations suggest that the activity of membrane proteins can be changed without changing their number simply by modifying the physical properties of the phospholipid bilayer.

Thermal acclimation leads to specific changes in the acyl composition of the membrane phospholipids as well as to shifts in the proportions of the different phospholipid head-groups. Typically, cold acclimation increases the proportion of phosphatidyl ethanolamine (PE) to the detriment of phosphatidyl choline (PC) and increases the proportion of unsaturated fatty acids (Hazel and Williams, 1990; Hazel, 1995). The membrane-destabilising effects of PE would facilitate function at lower temperatures. Changes in the proportions of the phospholipid head-groups and of saturated and monoenoic species in trout kidney plasma membrane occur within hours of the onset of thermal change (Hazel and

Landrey, 1988a,b; Carey and Hazel, 1989). In contrast, changing the proportions of long-chain, polyunsaturated fatty acids requires weeks of thermal acclimation. Specific phospholipids may demonstrate distinct changes in their acyl chain saturation with thermal acclimation. Thermal acclimation shifts the relative abundance of phospholipid classes in the inner and outer hemilayers of the inner mitochondrial membranes.

To identify the phospholipid categories in these hemilayers, mitoplasts are prepared from the isolated mitochondria and reacted with a non-penetrating marker to identify PE. Cold-acclimated rainbow trout have a higher proportion of PE in the inner hemilayer of the inner mitochondrial membrane than warm-acclimated trout (Miranda and Hazel, 1996). Given that, on average, only a small number of lipid molecules is thought to separate membrane proteins or protein aggregates, localised changes in membrane properties may be critical in establishing the activities of membrane proteins (Somero, 1997). However, the considerable mobility of membrane lipids suggests that specific lipid/protein interactions may be exceptional and that general membrane properties are central in determining the molecular activities of membrane proteins.

Despite the considerable information indicating marked changes in membrane properties with thermal acclimation, it is important to recognise that other abiotic parameters, such as salinity and pressure, and intracellular conditions specific to the species under study may influence membrane lipid composition. A case in point is provided by the differences in membrane acyl composition that reflect the presence of urea as an intracellular solute in elasmobranchs. The membranes of elasmobranchs have much lower polyunsaturated fatty acid contents and shorter fatty acid chains than those of non-urea-retaining fish living at the same temperature and salinity (Glemet and Ballantyne, 1996). Such specific effects must be considered when interpreting the functional significance of membrane properties. Nonetheless, the maintenance of membrane function during thermal acclimation seems to reflect changes in membrane composition more than physicochemical changes in the surrounding fluids (Hazel et al., 1992).

Although the changes in mitochondrial phospholipids support the concept of homeoviscous adaptation, it is important to recognise that many membrane systems respond to thermal change in ways that do not. Overall, Hazel (1995) argues that the maintenance of the dynamic properties of membranes through changes in phospholipid structure seems better able to explain the range of membrane responses to temperature. Instead of emphasising the fluidity at a given body temperature, this interpretation underscores the importance of adjusting the temperatures for membrane phase transitions [the fluid–gel transition and the transition to inverted vesicles (H_{II} transition)] so that the organismal temperature is centred between them (Hazel, 1995). Such adjustments preserve the capacity for membrane traffic yet prevent these activities from occurring in an unregulated fashion. Adjustment of the balance between bilayer-stabilising and bilayer-destabilising lipids becomes the principal means of regulating dynamic phase

behaviour. Virtually all the changes in mitochondrial phospholipids during cold acclimation maintain the dynamic phase behaviour of the membrane. Thermal acclimation can require the production of new mitochondria, so the maintenance of a capacity for membrane fusion and access to the H_{II} transition will be required.

A fundamental relationship between membrane properties and the activities of membrane enzymes forms the basis for an intriguing hypothesis suggesting that membrane acyl chain composition acts as the pacemaker for basal metabolic rate (Hulbert and Else, 1999). These authors point out that most of the processes that determine metabolic rate are carried out by membrane-bound systems (ion pumps, the proton leak, protein synthesis, oxidative phosphorylation, etc.). They propose that membrane polyunsaturation increases the activity of membrane-bound proteins, accelerates leak-pump cycles and thereby increases the cost of maintenance of cellular homeostasis. By formalising the central role of protein/lipid interactions in setting metabolic rate, this hypothesis unifies many observations concerning the determination of basal metabolic rate and membrane composition in mammals. It is valuable to evaluate thermo-acclimatory adjustments of membrane composition in this context, particularly since many of the adjustments of membrane composition occur both when isolated cells from ectotherms and when the intact organisms are exposed to thermal change (Hazel, 1995). Although increases in membrane unsaturation will enhance the activity of membrane proteins, they will also accelerate the leak-pump cycles. Differential impacts on the leak or pump function could modify tissue energetic requirements and be important during adjustments of metabolic rate.

A fascinating insight into the importance of rearrangements in membrane lipids comes from the aestivation-induced changes in mitochondrial membranes of snail hepatopancreas (Stuart et al., 1998a,b). As temperature change is not required to induce aestivation, these membrane changes can be interpreted solely in the context of the determination of metabolic requirements. Perhaps the most dramatic result is the similarity in the decreases in mitochondrial cardiolipin content and tissue activity of CCO. The phospholipid content of the mitochondrial fraction was drastically reduced by aestivation, whereas the protein content of the mitochondrial fraction was unchanged. The CCO activity in the mitochondrial fraction was high and unchanged by aestivation (Stuart et al., 1998a). Aestivation also causes profound changes in the fatty acid composition of all phospholipid classes and in the proportions of the phospholipids in mitochondria from snail hepatopancreas. That hepatopancreas phospholipid content did not decrease suggests that the cardiolipin lost from the mitochondria may be sequestered elsewhere in the cells. Cardiolipin molecules had a markedly greater content of saturated fatty acids in aestivating than in active snails (Stuart et al., 1998b). These authors suggest that these changes reduce the metabolic requirements of the hepatopancreas by decreasing the surface area over which the proton leak, a major determinant of basal metabolic rate, occurs as well as reducing

the activity of the proton pumps. They suggest that the sequestration of cardiolipin within the hepatopancreas cell facilitates a rapid return to normal activity once aestivation is terminated.

Coordination of the production of mitochondria

Whenever mitochondrial proliferation occurs, it requires coordinated control of the nuclear and mitochondrial genomes, adjustments for differences in message half-lives to maintain the required stoichiometries and a measured production of phospholipid bilayers into which the membrane proteins are incorporated. Further, the rate of production of mitochondria must exceed their rate of breakdown during the normal turnover of cellular constituents. While most mitochondrial proteins are encoded in the nuclear genome, a significant number of critical membrane-bound respiratory proteins are coded in the mitochondrial genome. No single master control gene has been found that controls mitochondrial proliferation. Nonetheless, several proteins are involved in the control of both the nuclear- and mitochondrial-encoded respiratory genes. Although at least eight factors are known to modulate the transcription rates of genes for mitochondrial proteins, a shared sensitivity to these regulatory factors could allow coordinated expression of the mitochondrial components (Moyes et al., 1998). As described below, these considerations have recently been brought to bear on mitochondrial dynamics during thermal acclimation. The marked proliferation induced by cold acclimation in some species (i.e. cyprinids and striped bass), the anatomical separation of muscle fibre types and their use in distinct swimming behaviours suggest that piscine models may help elucidate fundamental properties of the control of mitochondrial biogenesis in muscle.

To elucidate the control patterns operative during mitochondrial proliferation, the time scale and stoichiometries of protein and message production and the changes in candidate regulatory factors need to be determined. Despite 100-fold differences in the activities of CS in a range of trout tissues, the contents of CS mRNA per milligram of DNA and the activity of CS per CS mRNA varied less. The ratio of mitochondrial RNA (mtRNA) to mitochondrial DNA (mtDNA) was higher in skeletal muscle than in other tissues, despite lower levels of mtDNA per gram (Leary et al., 1998). Although cold acclimation significantly increased the activity of mitochondrial enzymes (CS and CCO), particularly in glycolytic muscle, no increase in mitochondrial mRNAs (for CCO and F_1 -ATPase), ribosomal RNA (rRNA) or mtDNA copy number were observed, perhaps because of the considerable variability among individuals (Battersby and Moyes, 1998). The increases in activity occurred for both matrix and membrane-bound enzymes, so changes in the phospholipid bilayer cannot be the cause. Since the number of copies of mtDNA did not change with thermal acclimation, a simple increase in copy number is not the basis of the enhanced expression at low temperature.

The temperature-dependence of the expression of CCO

differs between permanently cold-adapted and temporally cold-acclimated zoarchid fishes. For permanently cold-adapted Antarctic eelpout (*Pachycara brachycephalum*) and warm-acclimated North Sea eelpout (*Zoarces viviparus*), low levels of enzyme activity correspond with low levels of mitochondrial message. Cold-acclimation of the North Sea eelpout only slightly increases the amount of mRNA for two mitochondrially encoded CCO subunits (CCOI and CCO II RNA per milligram of total RNA), but markedly enhances the mRNA levels per gram of white muscle (Hardewig et al., 1999). Similar trends occurred for nuclear message, resulting in higher levels of message for a given enzyme activity in the cold-acclimated North Sea fish. Cold acclimation seems to perturb a general relationship between specific respiratory gene message content and translation or post-transcriptional processes. Effectively, long-term cold adaptation seems to have led to a compensation of translational capacity that does not occur during short-term cold acclimation.

Warm acclimation leads to decreases in muscle aerobic capacity that may largely reflect the impact of protein degradation rather than specific modification of rates of transcription. When white sucker (*Catostomus commersoni*) were exposed to an increase in water temperature from 9 to 28 °C, significant changes in muscle enzyme levels occurred, and levels of mitochondrially encoded mRNAs did not change but levels of nuclear-encoded mRNAs did. Changes in enzyme activities did not simply follow mRNA levels (Hardewig et al., 2000). A mismatch in the production of proteins coded in the nucleus and the mitochondria could have led to the formation of non-functional proteins. The lack of parallel changes in the levels of mRNAs and the activities of the corresponding enzymes coupled with the sustained, but modest, increase in heat-shock protein 70 (HSP70) suggest that protein degradation is the major factor changing enzyme activities during warm acclimation in the white sucker. Effectively, the enzyme most affected by this process, CS, is not easily protected by HSP70, leaving it more vulnerable to degradation.

A major question underlying the dynamics of mitochondrial proliferation is which of the many potential signals may be responsible. Catalytic limitations are frequently invoked to explain such increases (see above). Such catalytic limitations should logically manifest themselves by imbalances between the rates of ATP use and its aerobic regeneration (hypermetabolic stress). This could lead to the production of anaerobic end-products that could signal the need for metabolic changes. At the high critical temperature, lactate and succinate accumulate in white muscle of zoarcids, revealing such a mismatch (Van Dijk et al., 1999). However, in response to gradual warming such as that typically used to initiate warm acclimation, no accumulation of anaerobic end-products is apparent in glycolytic fibres of white sucker (*Catostomus commersoni*) (Hardewig et al., 2000). Bioenergetic signals, including increases in lactate levels, decreases in ATP levels, inhibition of oxidative phosphorylation and hypoxia, did not induce transcription of HSP70 or HSP30 in trout red blood cells (Currie et al., 1999). As reviewed by Somero (1997), the

thermal sensitivity of the regulatory elements that set the rates of HSP synthesis may reside in the molecules themselves. Clearly, the role of these chaperones is critical in maintaining cellular populations of healthy proteins, so their influence will be considerable during the adjustment of cellular contents of mitochondria. It will be fascinating to compare the regulatory mechanisms controlling mitochondrial properties in systems undergoing metabolic depression at low temperatures with those operative in systems showing thermal compensation of oxidative capacities.

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References

- Battersby, B. J. and Moyes C. D.** (1998). Influence of acclimation temperature on mitochondrial DNA, RNA and enzymes in skeletal muscle. *Am. J. Physiol.* **275**, R905–R912.
- Beamish, F. W. H.** (1978). Swimming capacity. In *Fish Physiology*, vol. 7 (ed. W. S. Hoar and D. J. Randall), pp. 101–187. New York: Academic Press.
- Bishop, T. and Brand, M. D.** (2000). Processes contributing to metabolic depression in hepatopancreas cells from the snail *Helix aspersa*. *J. Exp. Biol.* **203**, 3603–3612.
- Blier, P. U. and Guderley, H. E.** (1993a). Effects of pH and temperature on the kinetics of pyruvate oxidation by muscle mitochondria from rainbow trout (*Oncorhynchus mykiss*). *Physiol. Zool.* **66**, 474–489.
- Blier, P. U. and Guderley, H. E.** (1993b). Mitochondrial activity in rainbow trout red muscle: the effect of temperature on the ADP-dependence of ATP synthesis. *J. Exp. Biol.* **176**, 145–157.
- Boutillier, R. G., Donohoe, P. H., Tattersall, G. J. and West, T. W.** (1997). Hypometabolic homeostasis in overwintering aquatic amphibians. *J. Exp. Biol.* **200**, 387–400.
- Boutillier, R. G., Glass, M. L. and Heisler, N.** (1986). The relative distribution of pulmocutaneous blood flow in *Rana catesbeiana*: effects of pulmonary or cutaneous hypoxia. *J. Exp. Biol.* **126**, 33–39.
- Boutillier, R. G. and St-Pierre, J.** (2000). Surviving hypoxia without really dying. *Comp. Biochem. Physiol. A* **126**, 481–490.
- Bouwer, S. and Van Den Thillart, G.** (1984). Oxygen affinity of mitochondrial state III respiration of goldfish red muscle: the influence of temperature and O₂ diffusion on K_m values. *Mol. Physiol.* **6**, 291–306.
- Brand, M. D., Bishop, T., Boutillier, R. G. and St-Pierre, J.** (2000). Mitochondrial proton conductance, standard metabolic rate and metabolic depression. In *Life in the Cold* (ed. G. Heldmaier, S. Klaus and M. Klingenspor), pp. 413–430. Berlin: Springer-Verlag.
- Brand, M. D., Boutillier, R. G., Wass, M., St-Pierre, J. and Bishop, T.** (2001). Mitochondrial proton leak in metabolic depression. In *Molecular Mechanisms of Metabolic Arrest: Life in Limbo* (ed. K. B. Storey), pp. 59–76. Oxford: BIOS Scientific Publishers.
- Brand, M. D. and Murphy, M. P.** (1987). Control of electron flux through the respiratory chain in mitochondria and cells. *Biol. Rev.* **62**, 141–193.
- Brett, J. R.** (1967). Swimming performance of sockeye salmon in relation to fatigue time and temperature. *J. Fish. Res. Bd. Can.* **24**, 1731–1741.
- Buttgereit, F. and Brand, M. D.** (1995). A hierarchy of ATP-consuming processes in mammalian cells. *Biochem. J.* **312**, 163–167.
- Carey, C. and Hazel, J. R.** (1989). Diurnal variation in membrane lipid composition of Sonoran desert teleosts. *J. Exp. Biol.* **147**, 375–391.
- Conley, K. E., Christian, K. A., Hoppeler, H. and Weibel, E.** (1995). Heart mitochondrial properties and aerobic capacity are similarly related in a mammal and a reptile. *J. Exp. Biol.* **198**, 739–746.
- Currie, S. and Boutillier, R. G.** (2001). Strategies of hypoxia and anoxia tolerance in cardiomyocytes from the overwintering common frog, *Rana temporaria*. *Physiol. Biochem. Zool.* **74**, 420–428.
- Currie, S., Tufts, B. L. and Moyes, C. D.** (1999). Influence of bioenergetic stress on heat shock protein gene expression in nucleated red blood cells of fish. *Am. J. Physiol.* **276**, R990–R996.

- Dean, J. M. (1969). The metabolism of tissues of thermally acclimated trout (*Salmo gairdneri*). *Comp. Biochem. Physiol.* **29**, 185–196.
- Desaulniers, N., Moerland, T. S. and Sidell, B. D. (1996). High lipid content enhances the rate of oxygen diffusion through fish skeletal muscle. *Am. J. Physiol.* **271**, R42–R47.
- Donohoe, P. H. and Boutilier, R. G. (1998). The protective effects of metabolic rate depression in hypoxic cold submerged frogs. *Respir. Physiol.* **111**, 325–336.
- Donohoe, P. H. and Boutilier, R. G. (1999). The use of extracellular lactate as an oxidative substrate in the oxygen-limited frog. *Respir. Physiol.* **116**, 171–179.
- Egginton, S., Cordiner, S. and Skilbeck, C. (2000). Thermal compensation of peripheral oxygen transport in skeletal muscle of seasonally acclimated trout. *Am. J. Physiol.* **279**, R375–R388.
- Egginton, S. and Sidell, B. D. (1989). Thermal acclimation induces adaptive changes in subcellular structure of fish skeletal muscle. *Am. J. Physiol.* **256**, R1–R9.
- Fry, F. E. J. and Hart, J. S. (1948). Cruising speed of goldfish in relation to water temperature. *J. Fish. Res. Bd. Can.* **7**, 169–175.
- Glemet, H. C. and Ballantyne, J. S. (1996). Comparison of liver mitochondrial membranes from an agathan (*Myxine glutinosa*), an elasmobranch (*Raja erinacea*) and a teleost fish (*Pleuronectes americanus*). *Mar. Biol.* **124**, 509–518.
- Gnaiger, E., Lassnig, B., Kuznetsov, A., Rieger, G. and Margreiter, R. (1998). Mitochondrial oxygen affinity, respiratory flux control and excess capacity of cytochrome *c* oxidase. *J. Exp. Biol.* **201**, 1129–1139.
- Gracey, A. Y., Logue, J., Tiku, P. E. and Cossins, A. R. (1996). Adaptation of biological membranes to temperature: biophysical perspectives and molecular mechanisms. In *Animals and Temperature* (ed. I. A. Johnston and A. Bennett). *Soc. Exp. Biol. Semin. Ser.* **59**, 1–23.
- Guderley, H. and Foley, L. (1990). Anatomic and metabolic responses to thermal acclimation in the ninespine stickleback, *Pungitius pungitius*. *Fish Physiol. Biochem.* **8**, 465–474.
- Guderley, H. and Gawlicka, A. (1992). Qualitative modification of muscle metabolic organization with thermal acclimation of rainbow trout, *Oncorhynchus mykiss*. *Fish Physiol. Biochem.* **10**, 123–132.
- Guderley, H. and Houle-Leroy, P. (2001). Family origin and the response of threespine stickleback, *Gasterosteus aculeatus*, to thermal acclimation. *J. Comp. Physiol. B* **171**, 91–101.
- Guderley, H., Houle-Leroy, P. and Gagné, A. (2001). Thermal acclimation, growth and burst swimming of threespine stickleback: enzymatic correlates and influence of photoperiod. *Physiol. Biochem. Zool.* **74**, 66–74.
- Guderley, H. E. and Johnston, I. A. (1996). Plasticity of fish muscle mitochondria with thermal acclimation. *J. Exp. Biol.* **199**, 1311–1317.
- Guderley, H., Lavoie, B. A. and Dubois, N. (1994). The interaction among age, thermal acclimation and growth rate in determining muscle metabolic capacities and tissue masses in the threespine stickleback, *Gasterosteus aculeatus*. *Fish Physiol. Biochem.* **13**, 419–431.
- Guderley, H. and St-Pierre, J. (1996). Phenotypic plasticity and evolutionary adaptations of mitochondria to temperature. In *Animals and Temperature* (ed. I. A. Johnston and A. Bennett). *Soc. Exp. Biol. Semin. Series* **59**, 127–152.
- Guderley, H. and St-Pierre, J. (1999). Seasonal cycles of mitochondrial ADP sensitivity and oxidative capacities. *J. Comp. Physiol. B* **169**, 474–480.
- Guderley, H., St-Pierre, J., Couture, P. and Hulbert, A. J. (1997). Plasticity of the properties of mitochondria from rainbow trout red muscle with seasonal acclimatization. *Fish Physiol. Biochem.* **16**, 531–541.
- Hardewig, I., Van Dijk, P. L. M., Leary, S. C. and Moyes, C. D. (2000). Temporal changes in enzyme activity and mRNA levels during thermal challenge in white sucker (*Catostomus commersoni*). *J. Fish Biol.* **56**, 196–207.
- Hardewig, I., Van Dijk, P. L. M., Moyes, C. D. and Pörtner, H. O. (1999). Temperature dependent expression of cytochrome-C oxidase in Antarctic and temperate fish. *Am. J. Physiol.* **277**, R508–R516.
- Hazel, J. R. (1972a). The effect of temperature acclimation upon succinic dehydrogenase activity from the epaxial muscle of the common goldfish (*Carassius auratus* L.). I. Properties of the enzyme and the effect of lipid extraction. *Comp. Biochem. Physiol.* **43B**, 837–861.
- Hazel, J. R. (1972b). The effect of temperature acclimation upon succinic dehydrogenase activity from the epaxial muscle of the common goldfish (*Carassius auratus* L.). II. Lipid reactivation of the soluble enzyme. *Comp. Biochem. Physiol.* **43B**, 863–882.
- Hazel, J. R. (1995). Thermal adaptation in biological membranes: is homeoviscous adaptation the explanation? *Annu. Rev. Physiol.* **57**, 19–42.
- Hazel, J. R. and Landrey, S. R. (1988a). Time course of thermal adaptation in plasma membranes of trout kidney. I. Headgroup composition. *Am. J. Physiol.* **255**, R622–R627.
- Hazel, J. R. and Landrey, S. R. (1988b). Time course of thermal adaptation in plasma membranes of trout kidney. II. Molecular species composition. *Am. J. Physiol.* **255**, R628–R634.
- Hazel, J. R., McKinley, S. J. and Williams, E. E. (1992). Thermal adaptation in biological membranes: interacting effects of temperature and pH. *J. Comp. Physiol. B* **162**, 593–601.
- Hazel, J. R. and Williams, E. E. (1990). The role of alterations in membrane lipid composition in enabling physiological adaptation of organisms to their physical environment. *Prog. Lipid Res.* **29**, 167–227.
- Hoch, F. L. (1992). Cardiolipins and biomembrane function. *Biochim. Biophys. Acta* **1113**, 71–133.
- Holloszy, J. O. and Coyle, E. F. (1984). Adaptations of skeletal muscle to endurance exercise and their metabolic consequences. *J. Appl. Physiol.* **56**, 831–838.
- Hubley, M. J., Locke, B. R. and Moerland, T. S. (1997). Reaction-diffusion analysis of the effects of temperature on high-energy phosphate dynamics in physical skeletal muscle. *J. Exp. Biol.* **200**, 975–988.
- Hulbert, A. J. and Else, P. L. (1999). Membranes as possible pacemakers of metabolism. *J. Theor. Biol.* **199**, 257–274.
- Johnston, I. A., Calvo, J., Guderley, H., Fernandez, D. and Palmer, L. (1998). Latitudinal variation in the abundance and oxidative capacities of muscle mitochondria in perciform fishes. *J. Exp. Biol.* **201**, 1–12.
- Johnston, I. A. and Lucking, M. (1978). Temperature induced variation in the distribution of different types of muscle fibre in the goldfish (*Carassius auratus*). *J. Comp. Physiol.* **124**, 111–116.
- Johnston, I. A. and Maitland, B. (1980). Temperature acclimation in crucian carp (*Carassius carassius* L.): morphometric analysis of muscle fibre ultrastructure. *J. Fish Biol.* **17**, 113–125.
- Johnston, I. A., Sidell, B. D. and Driedzic, W. R. (1985). Force-velocity characteristics and metabolism of carp muscle fibres following temperature acclimation. *J. Exp. Biol.* **119**, 239–249.
- Jones, D. P., Aw, T. Y., Bai, C. and Sillau, A. H. (1991). Regulation of mitochondrial distribution: an adaptive response to changes in oxygen supply. In *Responses and Adaptation to Hypoxia: Organ to Organelle* (ed. S. Lahiri, N. S. Cherniak and R. S. Fitzgerald), pp. 25–35. New York: Oxford University Press.
- Jones, P. L. and Sidell, B. D. (1982). Metabolic responses of striped bass (*Morone saxatilis*) to temperature acclimation. II. Alterations in metabolic carbon sources and distributions of fiber types in locomotory muscle. *J. Exp. Zool.* **219**, 163–171.
- Kilariski, M. W., Romek, M., Kozłowska, M. and Görlich, A. (1996). Short-term thermal acclimation induces adaptive changes in the inner mitochondrial membranes of fish skeletal muscle. *J. Fish Biol.* **49**, 1280–1290.
- Kleckner, N. and Sidell, B. D. (1985). Comparisons of maximal activities of enzymes from tissues of thermally-acclimated and naturally-acclimatized chain pickerel (*Esox niger*). *Physiol. Zool.* **58**, 18–28.
- Kolok, A. S. (1999). Inter-individual variation in the prolonged locomotor performance of ectothermic vertebrates: a comparison of fish and herpetofaunal methodologies and a brief review of the recent fish literature. *Can. J. Fish. Aquat. Sci.* **56**, 700–710.
- Leary, S. C., Battersby, B. J. and Moyes, C. D. (1998). Inter-tissue differences in mitochondrial enzyme activity, RNA and DNA in rainbow trout (*Oncorhynchus mykiss*). *J. Exp. Zool.* **201**, 3377–3384.
- Londrville, R. L. and Sidell, B. D. (1996). Cold acclimation increases fatty acid binding protein concentration in aerobic muscle of striped bass, *Morone saxatilis*. *J. Exp. Zool.* **275**, 36–44.
- Longmuir, I. S. (1980). Channels of oxygen transport from blood to mitochondria. *Adv. Physiol. Sci.* **25**, 19–22.
- Miranda, E. J. and Hazel, J. R. (1996). Temperature-induced changes in the transbilayer distribution of phosphatidylethanolamine in mitoplasts of rainbow trout (*Oncorhynchus mykiss*) liver. *J. Exp. Zool.* **274**, 23–32.
- Moyes, C. D., Battersby, B. J. and Leary, S. C. (1998). Regulation of muscle mitochondrial design. *J. Exp. Biol.* **201**, 299–307.
- Moyes, C. D., Buck, L. T. and Hochachka, P. W. (1988). Temperature effects on pH of mitochondria isolated from carp red muscle. *Am. J. Physiol.* **254**, R611–R615.
- Moyes, C. D., Mathieu-Costello, O. A., Brill, R. W. and Hochachka, P. W. (1992). Mitochondrial metabolism of cardiac and skeletal muscles from a fast (*Katsuwonus pelamis*) and a slow (*Cyprinus carpio*) fish. *Can. J. Zool.* **70**, 1246–1253.

- O'Brien, K. M. and Sidell, B. D. (2000). The interplay among cardiac ultrastructure, metabolism and the expression of oxygen-binding proteins in Antarctic fishes. *J. Exp. Biol.* **203**, 1287–1297.
- Pinder, A. W., Storey, K. B. and Ultsch, G. R. (1992). Estivation and hibernation. In *Environmental Physiology of the Amphibians* (ed. M. E. Feder and W. W. Burggren), pp. 250–274. Chicago: The University of Chicago Press.
- Porter, R. K., Hulbert, A. J. and Brand, M. D. (1996). Allometry of mitochondrial proton leak: influence of membrane surface area and fatty acid composition. *Am. J. Physiol.* **271**, R1550–R1556.
- Powers, D. A. and Schulte, P. M. (1998). Evolutionary adaptations of gene structure and expression in natural populations in relation to a changing environment: a multidisciplinary approach to address the million-year saga of a small fish. *J. Exp. Zool.* **282**, 71–94.
- Rolfe, D. F. S. and Brown, G. C. (1997). Cellular energy utilization and molecular origin of standard metabolic rate in mammals. *Physiol. Rev.* **77**, 731–758.
- Rome, L. C., Loughna, P. T. and Goldspink, G. (1984). Temperature acclimation: improved sustained swimming performance in carp at low temperatures. *Science* **228**, 194–196.
- Sänger, A. M. (1993). Limits to the acclimation of fish muscle. *Rev. Fish Biol. Fish.* **3**, 1–15.
- Schwerzmann, K., Hoppeler, H., Kayar, S. R. and Weibel, E. R. (1989). Oxidative capacity of muscle and mitochondria: correlation of physiological, biochemical and morphometric characteristics. *Proc. Natl Acad. Sci. USA* **86**, 1583–1587.
- Seddon, W. L. and Prosser, C. L. (1997). Seasonal variations in the temperature acclimation response of the channel catfish, *Ictalurus punctatus*. *Physiol. Zool.* **70**, 33–44.
- Sidell, B. D. (1980). Response of goldfish (*Carassius auratus* L.) muscle to acclimation temperature: alterations in biochemistry and proportions of different fibre types. *Physiol. Zool.* **53**, 98–107.
- Sidell, B. D. (1998). Intracellular oxygen diffusion: the roles of myoglobin and lipid at cold body temperature. *J. Exp. Biol.* **201**, 1119–1128.
- Sidell, B. D. and Hazel, J. R. (1987). Temperature affects the diffusion of small molecules through cytosol of fish muscle. *J. Exp. Biol.* **129**, 191–203.
- Sisson, J. E. and Sidell, B. D. (1987). Effect of thermal acclimation on muscle fibre recruitment of swimming striped bass (*Morone saxatilis*). *Physiol. Zool.* **60**, 310–320.
- Somero, G. N. (1997). Temperature relationships: from molecules to biogeography. *Handbook of Physiology*, section 13, vol. 2 (ed. W. H. Dantzer), pp. 1391–1444. Oxford: Oxford University Press.
- St-Pierre, J. and Boutilier, R. G. (2001). Aerobic capacity of frog skeletal muscle during hibernation. *Physiol. Biochem. Zool.* **74**, 390–397.
- St-Pierre, J., Brand, M. D. and Boutilier, R. G. (2000a). The effect of metabolic depression on proton leak rate in mitochondria from hibernating frogs. *J. Exp. Biol.* **203**, 1469–1476.
- St-Pierre, J., Charest, P. M. and Guderley, H. (1998). Relative contribution of quantitative and qualitative changes in mitochondria to metabolic compensation during seasonal acclimatisation of rainbow trout, *Oncorhynchus mykiss*. *J. Exp. Biol.* **201**, 2961–2970.
- St-Pierre, J., Tattersall, G. J. and Boutilier, R. G. (2000b). Metabolic depression and enhanced O₂ affinity of mitochondria in hypoxic hypometabolism. *Am. J. Physiol.* **279**, R1205–R1214.
- Stuart, J. A., Gillis, T. E. and Ballantyne, J. S. (1998a). Compositional correlates of metabolic depression in the mitochondrial membranes of estivating snails. *Am. J. Physiol.* **275**, R1977–R1982.
- Stuart, J. A., Gillis, T. E. and Ballantyne, J. S. (1998b). Remodeling of phospholipid fatty acids in mitochondrial membranes of estivating snails. *Lipids* **33**, 787–793.
- Stuart, J. A., Ooi, E.-L. and Ballantyne, J. S. (1998c). Maximal activities of enzymes of intermediary metabolism in the estivating terrestrial snail *Cepaea nemoralis*. *Comp. Biochem. Physiol.* **120B**, 417–423.
- Suarez, R. K. (1996). Upper limits to metabolic rates. *Annu. Rev. Physiol.* **58**, 583–605.
- Suarez, R. K., Staples, J. F. and Lighton, J. R. B. (1999). Turnover rates of mitochondrial respiratory chain enzymes in flying honeybees (*Apis mellifera*). *J. Exp. Zool.* **284**, 1–6.
- Suarez, R. K., Staples, J. F., Lighton, J. R. B. and West, T. G. (1997). Relationships between enzymatic flux capacities and metabolic flux rates: Non-equilibrium reactions in muscle glycolysis. *Proc. Natl. Acad. Sci. USA* **94**, 7065–7069.
- Taylor, S. E., Egginton, S. and Taylor, E. W. (1996). Seasonal temperature acclimatisation of rainbow trout: cardiovascular and morphometric influences on maximal sustainable exercise level. *J. Exp. Biol.* **199**, 835–845.
- Temple, G. K. and Johnston, I. A. (1998). Testing hypotheses concerning the phenotypic plasticity of escape performance in fish of the family Cottidae. *J. Exp. Biol.* **201**, 317–331.
- Temple, G. K., Wakeling, J. M. and Johnston, I. A. (2000). Seasonal changes in fast-starts in the short-horn sculpin: integration of swimming behaviour and muscle performance. *J. Fish Biol.* **56**, 1435–1449.
- Thibault, M., Blier, P. B. and Guderley, H. (1997). Seasonal variation of muscle metabolic organisation in rainbow trout (*Oncorhynchus mykiss*). *Fish Physiol. Biochem.* **16**, 139–155.
- Trigari, G., Pirini, M., Ventralla, V., Pagliarani, A., Trombetti, F. and Borgatti, A. R. (1992). Lipid composition and mitochondrial respiration in warm- and cold-adapted sea bass. *Lipids* **27**, 371–377.
- Tyler, S. and Sidell, B. D. (1984). Changes in mitochondrial distribution and diffusion distances in muscle of goldfish upon acclimation to warm and cold temperatures. *J. Exp. Zool.* **232**, 1–9.
- Van Den Thillart, G. and De Bruin, G. (1981). Influence of environmental temperature on mitochondrial membranes. *Biochim. Biophys. Acta* **640**, 439–447.
- Van Den Thillart, G. and Modderkolk, J. (1978). The effect of acclimation temperature on the activation energy of state III respiration and on the unsaturation of membrane lipids of goldfish mitochondria. *Biochim. Biophys. Acta* **510**, 38–51.
- Van Dijk, P. L. M., Tesch, C., Hardewig, I. and Pörtner, H. O. (1999). Physiological disturbances at critically high temperatures: a comparison between stenothermal Antarctic and eurythermal temperate eelpouts (Zoarcidae). *J. Exp. Biol.* **202**, 3611–3621.
- Van Waarde, A., Van Den Thillart, G., Erkelens, C., Addink, A. and Lugtenburg, J. (1990). Functional coupling of glycolysis and phosphocreatine utilization in anoxic fish muscle. *J. Biol. Chem.* **265**, 914–923.
- Vock, R., Weibel, E. R., Hoppeler, H., Ordway, G., Weber, J.-M. and Taylor, C. R. (1996). Design of the oxygen and substrate pathways. V. Structural basis of vascular substrate supply to muscle cells. *J. Exp. Biol.* **199**, 1675–1688.
- Walesby, N. J. and Johnston, I. A. (1980). Temperature acclimation in brook trout muscle: adenine nucleotide concentrations, phosphorylation state and adenylate energy charge. *J. Comp. Physiol.* **139**, 127–133.
- West, T. G. and Boutilier, R. G. (1998). Metabolic suppression in anoxic frog muscle. *J. Comp. Physiol.* **168B**, 273–280.
- Wodtke, E. (1981a). Temperature adaptation of biological membranes. The effects of acclimation temperature on the unsaturation of the main neutral and charged phospholipids in mitochondrial membranes of the carp (*Cyprinus carpio*). *Biochim. Biophys. Acta* **640**, 698–709.
- Wodtke, E. (1981b). Temperature adaptation of biological membranes. Compensation of the molar activity of cytochrome C oxidase in the mitochondrial energy-transducing membrane during thermal acclimation of the carp (*Cyprinus carpio* L.). *Biochim. Biophys. Acta* **640**, 710–720.
- Wodtke, E. and Cossins, A. R. (1991). Rapid cold-induced changes of membrane order and 9 desaturase activity in endoplasmic reticulum of carp liver: a time-course study of thermal acclimation. *Biochim. Biophys. Acta* **1064**, 343–350.
- Yacoe, M. E. (1986). Effects of temperature, pH and CO₂ tension on the metabolism of isolated hepatic mitochondria of the desert iguana, *Dipsosaurus dorsalis*. *Physiol. Zool.* **59**, 263–272.