The Journal of Experimental Biology 214, 1888-1895

doi:10.1242/jeb.053330

RESEARCH ARTICLE

Origins of variation in muscle cytochrome *c* oxidase activity within and between fish species

Katharina Bremer and Christopher D. Moyes*
Department of Biology, Queen's University, Kingston, ON, Canada, K7L 3N6
*Author for correspondence (chris.moyes@queensu.ca)

Accepted 8 February 2011

SUMMARY

Mitochondrial content, central to aerobic metabolism, is thought to be controlled by a few transcriptional master regulators, including nuclear respiratory factor 1 (NRF-1), NRF-2 and peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α). Though well studied in mammals, the mechanisms by which these factors control mitochondrial content have been less studied in lower vertebrates. We evaluated the role of these transcriptional regulators in seasonal changes in white muscle cytochrome *c* oxidase (COX) activity in eight local fish species representing five families: Centrarchidae, Umbridae, Esocidae, Gasterosteidae and Cyprinidae. Amongst centrarchids, COX activity was significantly higher in winter for pumpkinseed (2-fold) and black crappie (1.3-fold) but not bluegill or largemouth bass. In esociforms, winter COX activity was significantly higher in central mudminnow (3.5-fold) but not northern pike. COX activity was significantly higher in winter-acclimatized brook stickleback (2-fold) and northern redbelly dace (3-fold). Though mudminnow COX activity increased in winter, lab acclimation to winter temperatures did not alter COX activity, suggesting a role for non-thermal cues. When mRNA was measured for putative master regulators of mitochondria, there was little evidence for a uniform relationship between COX activity and any of *NRF-1*, *NRF-2α* or *PGC-1α* mRNA levels Collectively, these studies argue against a simple temperature-dependent mitochondrial response ubiquitous in fish, and suggest that pathways which control mitochondrial content in fish may differ in important ways from those of the better studied mammals.

Key words: fish, mitochondria, white muscle, acclimation, acclimatization, cytochrome *c* oxidase, NRF-1, NRF-2α, PGC-1α.

INTRODUCTION

The ability to adjust mitochondrial oxidative capacity is critical to controlling energy production under changing physiological and environmental conditions. In mammals, mitochondrial content changes in response to factors such as exercise (Holloszy, 1967), electrical stimulation (Baar et al., 2002) and cold exposure (Puigserver et al., 1998; Wu et al., 1999). Fish respond in a similar manner to activity (e.g. Farrell et al., 1991), but they also induce mitochondrial proliferation in response to cold acclimation and winter acclimatization (Egginton and Sidell, 1989; Egginton et al., 2000; Guderley, 1990). Unlike the situation in mammals, where cold exposure induces hypermetabolism, cold treatment of fish depresses metabolism, leading to a suite of morphological, physiological, biochemical and genetic modifications (Egginton and Johnston, 1984; Johnston and Maitland, 1980; Johnston and Wokoma, 1986; Orczewska et al., 2010; Shaklee et al., 1977). The underlying cause of cold-induced mitochondrial proliferation remains elusive but it is thought to reflect a degree of thermal compensation to overcome the negative thermodynamic effects on metabolism and metabolic enzymes (Egginton and Sidell, 1989; Hazel and Prosser, 1974).

Regardless of the reason why fish remodel mitochondrial energy metabolism in the cold, the question of how such a change is achieved remains. Mitochondrial biogenesis is a complex process requiring exquisite coordination of nuclear and mitochondrial genes. Each oxidative phosphorylation (OXPHOS) complex is composed of multiple subunits, many of which have paralogues. All mitochondrial OXPHOS complexes, except complex II, have subunits encoded by both nuclear genes and mitochondrial DNA (mtDNA). Transcriptional regulation plays a very important role in the control of mitochondrial biogenesis (Hock and Kralli, 2009). As described in recent reviews (Hock and Kralli, 2009; Scarpulla, 2010), central to this pathway are three transcription factors that have been identified as master regulators of mitochondrial biogenesis: nuclear respiratory factor 1 (NRF-1), NRF-2 and peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α). The two NRFs are DNA-binding proteins that associate with elements in the promoters of many genes that encode mitochondrial proteins. Once localized at the promoter, they directly (NRF-1) or indirectly (NRF-2) bind PGC-1α, forming a complex that recruits the general transcription machinery, activating transcription. The coactivator PGC-1α is the most important member of the gene family including two homologues, PGC-1β and PGC-1-related coactivator (PRC), which have overlapping roles with functional specialization (Hock and Kralli, 2009; Scarpulla, 2010).

Each of these transcription factors that play a role in mammalian mitochondrial biogenesis is also present in fish, but there is some uncertainty about their respective roles in lower vertebrates. In a study on thermal acclimation of goldfish (*Carassius auratus*), cold induced increases in COX activity, *COX4* mRNA and *NRF-1* mRNA, but *PGC-1α* mRNA was decreased (LeMoine et al., 2008). Likewise, in three-spined stickleback (*Gasterosteus aculeatus*), cold acclimation induced increases in COX, *COX4* mRNA and *NRF-1* mRNA but not *PGC-1α* mRNA (Orczewska et al., 2010). The lack of a uniform pattern of correlation between PGC-1α and

mitochondrial changes prompted the suggestion that the pathway of regulation in fish may differ not just from that of mammals but also between species. The diminished importance of PGC-1α may be due to mutations in the NRF-1 binding domain (LeMoine et al., 2010) and it has been suggested that PGC-1β may be more important in the control of mitochondrial biogenesis in some fish (LeMoine et al., 2008).

In this study, our first goal was to investigate the patterns seen in the seasonal response of a broad range of ecologically diverse freshwater fish species. Secondly, we wanted to better understand the regulatory mechanisms that underlie the compensatory effects in mitochondrial enzyme content in lower vertebrates compared with those in higher vertebrates.

MATERIALS AND METHODS

Fish

Eight local fish species were caught in Lake Opinicon and its nearby marshlands, about 50 km north of Kingston, ON, Canada. Lake Opinicon is a large (787 ha, or 7.87 km^2), but relatively shallow (~50% is <5 m; maximum depth <11 m) and wind-exposed lake (Keast and Fox, 1992). Because of these physical conditions, it is classified as a polymictic lake (Agbeti and Smol, 1995), with water mixing in summer as well as spring and autumn, thereby reducing thermal variation regionally (Crowder et al., 1977; Keast and Fox, 1992). The four sunfish (Centrarchidae) representatives, pumpkinseed (*Lepomis gibbosus*, Linnaeus), bluegill (*Lepomis macrochirus*, Rafinesque), black crappie (*Pomoxis nigromaculatus*, Lesueur) and largemouth bass (*Micropterus salmoides*, Lacépède), as well as northern pike (*Esox lucius*, Linnaeus) were caught by hook and line. Brook stickleback (*Culaea inconstans*, Kirtland), northern redbelly dace (*Chrosomus eos*, Cope) and central mudminnow (*Umbra limi*, Kirtland) were caught in minnow traps in the marsh around the lake. No longer than 1 h after being caught, animals were anaesthetized in buffered (0.8 g l^{-1} $NaHCO_3$) 0.4 g l^{-1} tricaine methanesulphonate and killed by cutting the spinal cord. White muscle samples were taken from the epaxial muscle below the dorsal fin but above the lateral line and immediately frozen in liquid nitrogen. Fish were caught in summer 2008 and 2009 and in winter/spring 2009/2010 (Fig. 1). Morphometric data including mass, length and condition factor of the fish are summarized in Table 1. As these fish were sampled from their natural environment, experiencing the whole range of seasonally changing stimuli, it is not surprising that some of them showed significant differences in their condition factors between summer and winter.

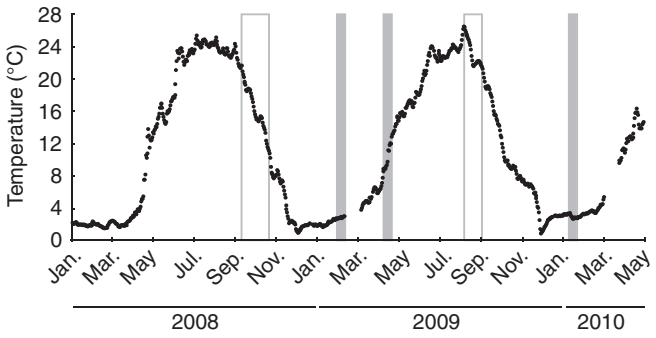

Fig. 1. Daily mean water temperature (°C) at a depth of 3.3 m in Lake Opinicon. Boxed areas indicate sampling periods (open, summer; filled, winter).

Acclimation experiment

Mudminnows, caught in the marsh area around Lake Opinicon during summer 2008, were held in a 723 l tank attached to a flow-through system in the Animal Care facilities of Queen's University, Kingston. Water was supplied by the city system taken from Lake Ontario and filtered through a carbon filter prior to serving the fish tank. Fish were acclimated to the ambient water temperature of Lake Ontario for 4 weeks before the first sampling event. During the beginning of the experiment, water temperatures of Lake Ontario were the same as those in the marshlands around Lake Opinicon, but decreased at a slightly lower rate than field water temperatures throughout the experiment. Samples were taken every 27–42 days (Fig. 2A). Acclimation temperatures were defined as the mean temperature over the 3 weeks prior to each sampling event (Fig. 2A).

Cytochrome *c* oxidase activity

For enzyme extraction, white muscle samples were powdered under liquid nitrogen. About 50 mg was homogenized in 20 volumes of cold extraction buffer (25 mmol l^{-1} K_2HPO_4, 1 mmol l^{-1} EDTA, 0.6 mmol l^{-1} lauryl maltoside, pH 7.4) using a 7 ml Tenbroeck tissue grinder (Wheaton Industries, Millville, NJ, USA). Homogenates were not centrifuged and were mixed prior to enzyme measurements. Enzyme activity was determined spectrophotometrically (Molecular Devices, Sunnyvale, CA, USA) at 550 nm and 25°C in 96-well plates (Corning, Corning, NY, USA) using assay buffer (25 mmol l^{-1}

Table 1. Morphometric data for all seasonally acclimatized fish species and temperature-acclimated central mudminnow

	Mass (g)	Fork length (cm)	Fulton's condition factor*
Seasonal acclimatization			
Brook stickleback	0.58±0.1 / 0.85±0.1†	3.91±0.1 / 4.33±0.1†	0.95±0.03 / 1.03±0.07
Northern redbelly dace	1.88±0.2 / 7.49±0.5†	5.73±0.2 / 8.61±0.2†	0.98±0.04 / 1.17±0.05†
Pumpkinseed	171.34±5.2 / 111.21±8.4†	19.98±0.2 / 17.27±0.4†	2.15±0.02 / 2.14±0.05
Bluegill	123.07±3.8 / 87.77±6.5†	17.64±0.2 / 16.24±0.4†	2.24±0.05 / 2.03±0.04†
Black crappie	193.52±34.2 / 180.46±20.1	22.48±1.2 / 21.83±0.7	1.57±0.06 / 1.66±0.06
Largemouth bass	392.08±95.4 / 629.60±80.0	30.29±3.7 / 33.85±1.5	1.39±0.11 / 1.53±0.06
Northern pike	594.84±83.4 / 690.88±91.3	47.38±2.3 / 47.69±2.8	0.54±0.01 / 0.62±0.02†
Central mudminnow	5.15±0.8 / 3.69±0.8	7.68±0.4 / 6.72±0.6	1.07±0.03 / 1.02±0.04
Temperature acclimation (central mudminnow)			
Time point a (see Fig. 2)	5.26±0.8[a]	8.10±0.3[a]	0.95±0.03[a]
Time point b	3.40±0.4[a]	7.15±0.2[a]	0.92±0.03[a]
Time point c	2.14±0.2[b]	6.47±0.2[b]	0.78±0.02[a]
Time point d	2.11±0.2[b]	6.66±0.2[b]	0.71±0.02[b]
Time point e	2.02±0.2[b]	6.41±0.3[b]	0.74±0.02[b]

*After Ricker (1975). †Significant differences between seasons. Labels a–e represent sampling events during the experiment. For each parameter, data points sharing a superscript (a or b) are not significantly different from each other.

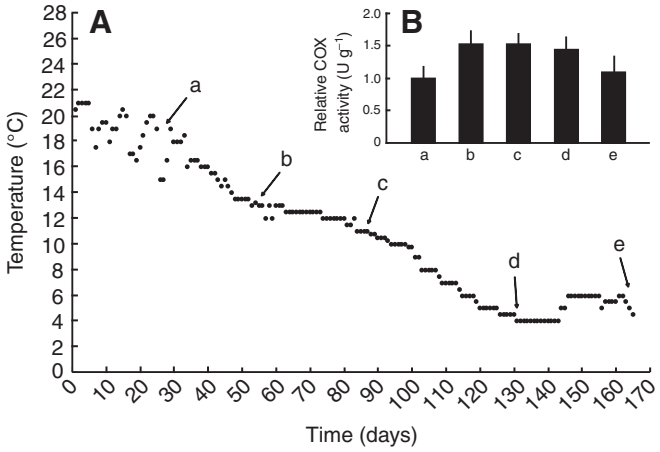

Fig. 2. Time course of water temperature (°C) and white muscle sampling events (a–e) during the lab acclimation experiment with central mudminnow (A), and corresponding relative cytochrome *c* oxidase activity (U g^{-1}) in white muscle (B) (a–c: *N*=6; d and e: *N*=8). Results in B are plotted as means + s.e.m.

K_2HPO_4, 0.6 mmol l^{-1} lauryl maltoside, pH 7.4) and reduced cytochrome *c* (0.05 mmol l^{-1}) as the substrate. Cytochrome *c* was reduced by ascorbic acid, then dialysed exhaustively in 25 mmol l^{-1} K_2HPO_4 (pH 7.4) and frozen in aliquots. All samples were measured in triplicate.

RNA extraction and reverse transcription

RNA was isolated from powdered white muscle samples using a slight modification of the single-step method by guanidinium thiocyanate–phenol–chloroform extraction (Chomczynski and Sacchi, 2006). The purified RNA pellet was dissolved in diethylpyrocarbonate-treated water and quantified at 260 nm prior to storage at –80°C. Additionally, absorption at 230, 270 and 280 nm was measured to test salt, phenol and protein contamination, respectively. Samples showed a 260 nm/230 nm ratio of 1.86±0.2, a 260 nm/270 nm ratio of 1.43±0.0 and a 260 nm/280 nm ratio of 2.14±0.1. Reverse transcription of RNA and the removal of genomic DNA were carried out using the QuantiTect Reverse Transcription Kit (Qiagen, Mississauga, ON, Canada) according to the manufacturer's instructions.

Real-time PCR

All analyses were performed on an ABI 7500 Real Time PCR System (Foster City, CA, USA) using the following protocol: 10 min at 95°C followed by 40 cycles of 15 s at 95°C, 15 s at the annealing temperature (Table 2), 34 s at 72°C. The efficiency of each primer set for every species was determined by real-time PCR with an appropriate dilution series of cDNA prior to the sample analyses. Based upon these results, an appropriate cDNA concentration for each reaction was chosen. Samples were then assayed in duplicate in a 25 µl total reaction volume containing 5 µl cDNA [100 ng of *NRF-1*, *NRF-2*α, *PGC-1*α, TATA-binding protein (*TBP*) and *18S*; 50 ng β-*actin*], 12.5 µl FastStart Universal SYBR Green Master mix (Roche Applied Science, Penzberg, Bavaria, Germany), 3.5 µl

Table 2. Forward and reverse real-time PCR primer sequences for target genes

	Forward primer (5′–3′)	Reverse primer (5′–3′)	Annealing temperature (°C)
NRF-1			
PS, BG, LM, BC	gcaccatctgactgcacagaata	acctggatgagcgagactgtt	65
RD	aaccgtagtgcagacgatca	tggcaattctgacgcatctg	61
BS	gggaaggagagctgcaagcc	gacaccctctgtttctgctcctc	61
PI	cgttgcggaccatcgttaagaact	agtttgtgagggcacaaggtga	61
MM	cgttgcggaccatcgttaagaact	agcgatattctgtgcggtcagatg	61
*NRF-2*α			
PS, BG, LM, BC	actctggagccatctggaactt	aggctgatcaatggtgacggta	61, 59 (BC)
RD	ttgaaggctaccgcaaagagca	cataacccaaactgcccagtggat	61
BS	acagattcagctctggcagttc	tccggctggttgagtttgaa	61
PI	ctctggagtcatctggagttgctt	acctgtaggtcaatggtgactgtg	61
MM	gaccagaagccacagtcacaat	gctctgcttcagcaccttgatt	61
*PGC-1*α			
PS, BG, LM, BC	ggacgtgaccaatgccagtga	atagctgagttgggagtttgcgg	61, 59 (LM)
RD	gacgtgaccaatgccagtgat	atagctgagctgggagttagca	61
BS	agtctccaaatgaccacaaggg	gggttccagcaatctccaca	59
PI	caatgccagtgaccagagctgt	ttatagctgagctgggagtttgcg	61
MM	cgtgaccaatgcaagtgaccaga	gattgtagctgagctgggagttcg	61
TBP			
PS, BG, LM	cacacatcaacagttcggcagcta	aaccttggcacctgtgagtacaac	61, 59 (LM)
BC	cctgcaaagttcctggacttca	tcctggaaacagctctggttca	61
RD	agtggtgcagaagttgggttttcc	atgtgtaagcaccaggccctctaa	61
BS	tcacggttagctgccaggaaatac	ttcaagccgaatggggaacttcac	61
PI	gagaatacgagagccaaggacaac	tcctggaaacagctctggttca	61
MM	tggtgtgtacaggagccaaaagtga	agctgcccaccatgttctgaat	61
β-*Actin*			
PS, BG, LM, BC, RD, BS	tccaggctgtgctgtccctgta	gtcaggatcttcatgaggtagtc	61, 59 (LM, BC)
PI, MM	gacaggatgcagaaggagat	acatctgctggaaggtggaca	61
*18S**			
All species	ggcggcgttattcccatgacc	ggtggtgcccttccgtcaattc	61

*Data from Braun et al. (2009).

PS, pumpkinseed; BG, bluegill; LM, largemouth bass; BC, black crappie; RD, northern redbelly dace; BS, brook stickleback; PI, northern pike; MM, central mudminnow.

NRF, nuclear respiratory factor; PGC-1α, peroxisome proliferator-activated receptor-γ coactivator-1α; TBP, TATA-binding protein.

double-distilled H_2O and 2 µl each forward and reverse primer (final concentration, 0.58 µmol l^{-1}). Controls were run with water to ensure the absence of contamination. Results were analysed according to the ΔCt (cycle threshold) method using *TBP*, β-*actin* and *18S* ribosomal RNA as housekeeping genes with their calculated geometric mean for each sample as standardized Ct (Pfaffl et al., 2004). Specific primers were designed to amplify single products of 91–175 bp in length (Table 2). Species-specific real-time PCR primers were constructed after larger amplicons were generated from consensus primers, cloned and sequenced.

Statistics

All data are presented as means + s.e.m. for temperature comparison within one species and are plotted relative to summer values. The significance of differences between the two seasons for each species was tested by the non-parametric Mann–Whitney *U*-test for enzyme activity, relative mRNA levels of target genes and the morphometric data. The significance of differences between multiple groups in the acclimation experiment was tested by the Kruskal–Wallis test. Results were tested at a significance level of 95%.

RESULTS

COX activity

White muscle COX activity was not affected by season in three out of eight species: bluegill, bass and pike. For simplicity, we term these species non-compensators. The remaining five species (pumpkinseed, crappie, dace, stickleback and mudminnow) showed significant increases (1.3- to 3.5-fold) in white muscle COX activity in winter (Fig. 3). These species were denoted as compensators.

There was no phylogenetic pattern that distinguished compensators and non-compensators. Within centrarchids, pumpkinseed and crappie compensated whereas bluegill and bass did not. Within Esociformes, mudminnow compensated but pike did not show thermal compensation.

Mudminnows were held at seasonal temperatures from autumn through to winter. Mean temperatures dropped by 14°C, from 19°C at the beginning to 5°C at the end of the experiment (Fig. 2A). In contrast to acclimatized fish, the lab-acclimated fish showed no significant changes in COX activity (Fig. 2B).

Transcription factors and coactivator mRNA levels

The responses of each species in terms of transcription factor mRNA levels are depicted in Fig. 4. A previous study on goldfish acclimated to 5 and 20°C (LeMoine et al., 2008) found that cold acclimation was accompanied by an increase in COX activity and *NRF-1* mRNA but a decrease in *PGC-1*α mRNA (Fig. 4A). In extending this approach to eight other species, there was no clear relationship between COX changes and the patterns seen in *NRF-1*, *NRF-2*α and *PGC-1*α mRNA levels. Five species showed thermal compensation in COX activity: dace, stickleback, pumpkinseed, crappie and mudminnow.

Among the compensators, *NRF-1* mRNA increased in pumpkinseed (3-fold) and crappie (5-fold) by a factor that was much greater than the increase seen in COX activity. In contrast, no change was seen in *NRF-1* mRNA in dace, stickleback or mudminnow (Fig. 4), each of which showed a greater change in COX activity than did pumpkinseed and crappie. The level of mRNA for *NRF-2*α, a subunit of NRF2, did not change in any compensator, except for crappie, which showed a 2-fold increase in the cold (Fig. 4).

As with goldfish, *PGC-1*α mRNA decreased significantly in three compensators: stickleback (3-fold), pumpkinseed (10-fold) and mudminnow (2-fold). There was no significant change in *PGC-1*α mRNA in crappie. In contrast to these species, dace *PGC-1*α mRNA increased significantly (7.5-fold) in winter fish (Fig. 4).

As noted above, there were three species of fish that failed to show a seasonal response in COX activity (Fig. 3). In these species, *NRF1* mRNA did not change in pike, but significantly increased in bluegill (5-fold) and bass (9-fold). *NRF-2*α mRNA increased in bass (3-fold) but did not change in bluegill or pike. *PGC-1*α mRNA did not change in any of these species between seasons (Fig. 4).

DISCUSSION

Low body temperatures lead to pleiotropic effects on metabolism and physiology, and long-term responses to temperature result in many physiological and morphological changes in ectotherms. A cold-induced increase in mitochondrial content, whether expressed in terms of morphometry (e.g. volume density, cristae density) or marker enzyme activity (e.g. COX, citrate synthase), probably corrects shortfalls in ATP production in the cold (Guderley, 2004). In mammals, the regulation of mitochondrial biogenesis is attributed to a set of master regulators of transcription that coordinate the expression of genes encoding mitochondrial proteins (Hock and Kralli, 2009). However, a survey of the literature on fish models suggests that the process in fish may differ from that seen in mammals (LeMoine et al., 2008). In this study, we focused on the expression patterns seen in white muscle mitochondria of a variety of species found regionally, exploring the underlying control of these patterns.

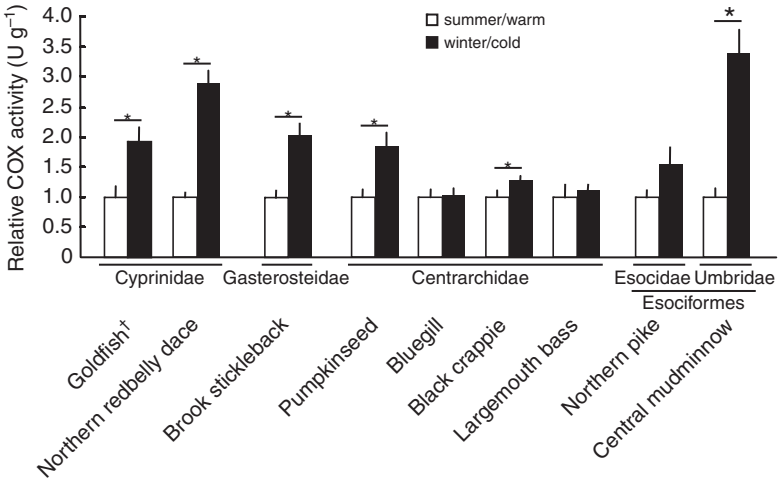

Fig. 3. Cytochrome *c* oxidase (COX) activity in white muscle of fish collected in summer and winter. Sample sizes for summer/winter were as follows: northern redbelly dace, 7/8; brook stickleback, 8/8; pumpkinseed, 8/7; bluegill, 8/7; black crappie, 13/10; largemouth bass, 7/10; northern pike, 8/8; and central mudminnow, 8/10. Results are expressed relative to summer/warm values and are plotted as means + s.e.m. Mean (+s.e.m.) enzyme activity (U g^{-1}) for summer/winter was as follows: northern redbelly dace, 4.6 (0.3)/13.4 (1.0); brook stickleback, 2.6 (0.3)/5.2 (0.5); pumpkinseed, 1.4 (0.2)/2.5 (0.3); bluegill, 3.0 (1.0)/3.1 (0.4); black crappie, 4.5 (0.5)/5.8 (0.3); largemouth bass, 4.1 (0.8)/4.6 (0.4); northern pike, 1.4 (0.1)/2.1 (0.4); and central mudminnow, 4.0 (0.6)/13.5 (1.6). Significant differences (P<0.05) between seasons are indicated by an asterisk. †Goldfish data for 4 and 20°C were obtained from previous studies (LeMoine et al., 2008).

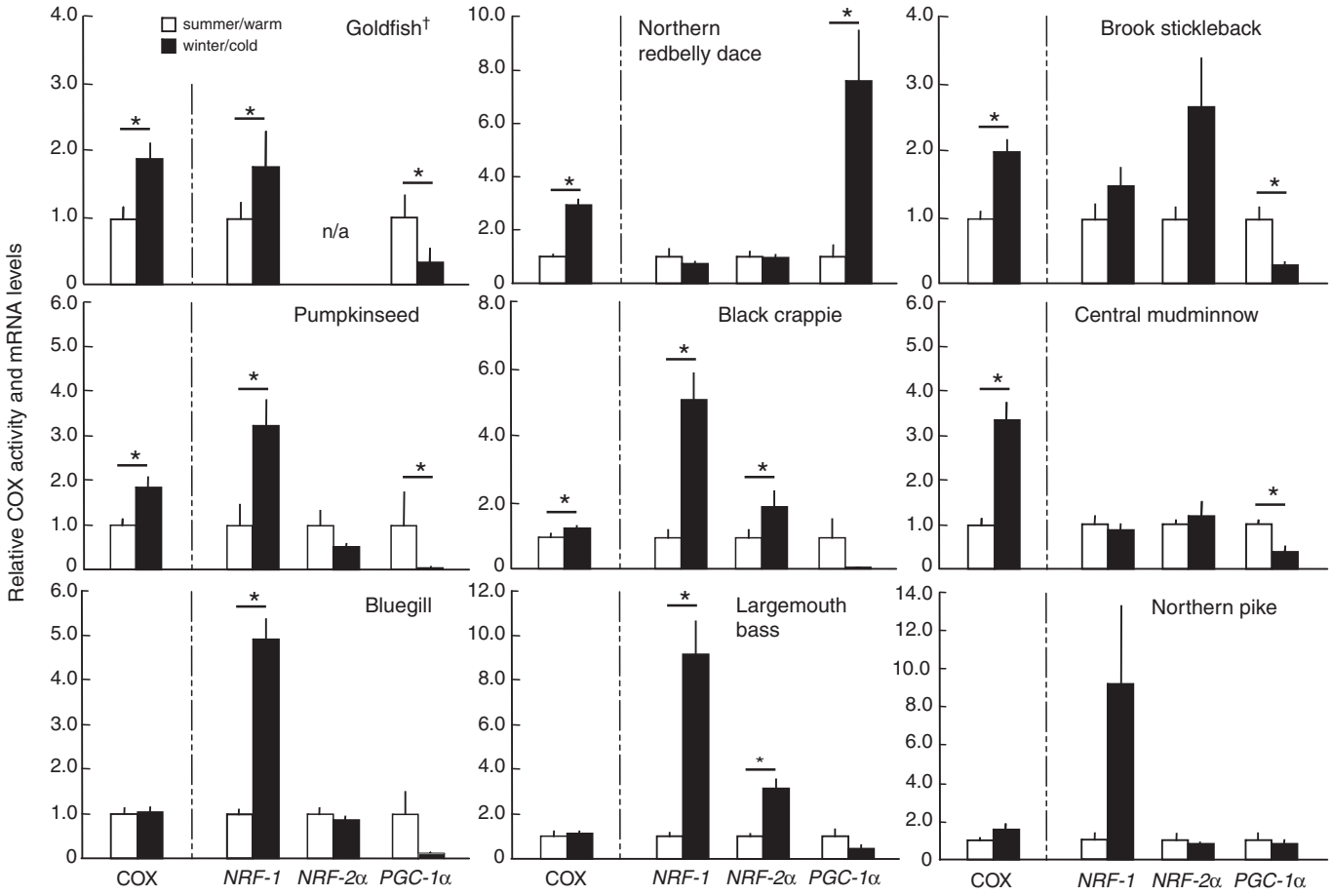

Fig. 4. Relative COX activities mRNA levels of target genes in white muscle of fish collected in summer and winter. Sample sizes for summer/winter were as follows: northern redbelly dace, 7/7; brook stickleback, 5/7; pumpkinseed, 7/6; black crappie, 7/6; central mudminnow, 8/6; bluegill, 7/7; largemouth bass, 6/7; northern pike, 7/7. Results are expressed relative to summer/warm values and are plotted as means + s.e.m. Significant differences (P<0.05) between seasons are indicated by an asterisk. n.a., not available. †Goldfish data for 4 and 20°C were obtained from previous studies (LeMoine et al., 2008).

Species-specific seasonal thermal response in enzyme activity

Cold-induced changes in mitochondrial markers, first shown in goldfish (Freed, 1965), have since been shown in many other ectotherms – crustaceans, amphibians, reptiles and annelids (Bullock, 1955; Heinrich, 1977; Sommer and Pörtner, 2004). In contrast, some temperate species fail to change mitochondrial enzyme content in relation to temperature or season. The reasons for the different responses among studies and species are not clear, nor are the mechanisms by which the changes arise.

The variation in response among species does not appear to have a simple taxonomic basis. As in a previous study on centrarchids (Tschantz et al., 2002), we saw increases in COX activity in some species (pumpkinseed, 2-fold; crappie, 1.3-fold) but not others (bluegill, bass). We found a striking difference in the response of two esociforms: northern pike and mudminnow. The lack of response in pike is similar to the situation in chain pickerel (*Esox niger*), which show a very modest response to cold acclimation (20% increase in COX activity) and no response to winter acclimatization (Kleckner and Sidell, 1985). The closest relatives to Esociformes are the Salmoniformes, which typically experience only a mild response to the cold, generally less than a 75% increase in COX activity (Battersby and Moyes, 1998; Blier and Guderley, 1988; Egginton et al., 2000; Guderley and Gawlicka, 1992; Guderley et al., 1997). Thus, the dramatic response of mudminnows is atypical within this broader group.

Other taxonomic groups appear to have a more uniform response to the cold. Sticklebacks (Gasterosteidae) all appear to show a pronounced increase in mitochondrial enzyme activities in the cold: brook stickleback (Fig. 3), three-spine stickleback (Orczewska et al., 2010; Vézina and Guderley, 1991) and nine-spine stickleback (Guderley and Foley, 1990). Cyprinids (carp and true minnows) also show increases in muscle mitochondrial enzyme activities: dace (Fig. 3), goldfish (Freed, 1965; LeMoine et al., 2008), roach (*Rutilus rutilus*) and tench (*Tinca tinca*) (Heap et al., 1985). The lack of a thermal response seen in tropical zebrafish (Duggan et al., 2011; McClelland et al., 2006) is perhaps not surprising as the lower lethal temperature (7°C) for this species (Lawrence, 2007) lies above the temperature at which thermal compensation is usually seen (<5°C).

Collectively, these studies suggest that there are few phylogenetic patterns that could indicate which species change their muscle mitochondrial enzymes with temperature. Differences are seen in species that are closely related and even show similar locomotor patterns. Both mudminnow and pike are ambush predators whereas both bluegill and pumpkinseed swim in a labriform mode, yet these species pairs differ in their responses to season.

Acclimation *versus* acclimatization

One challenge in reconciling our results with those from previous studies is related to the nature of the thermal challenge and confounding effects of other seasonal factors that accompany the cold. Independent studies on the responses of rainbow trout (*Oncorhynchus mykiss*) to acclimatization and acclimation show a similar compensatory response in muscle oxidative capacity (Battersby and Moyes, 1998; Egginton et al., 2000; Guderley and Gawlika, 1992; Thibault et al., 1997). However, there are studies where different responses in acclimation *versus* acclimatization have been found. For example, we found winter acclimatization to increase muscle mitochondrial enzyme activity in black crappie, whereas thermal acclimation had no effect in another study (Tschantz et al., 2002).

The disparity between acclimation and acclimatization is most compelling when the same researchers compared the two treatments. For example, chain pickerel (Kleckner and Sidell, 1985) and three-spine stickleback (Vézina and Guderley, 1991) have elevated mitochondrial enzyme activity after cold acclimation but the differences diminish when fish are winter acclimatized. We found a marked difference in the response of mudminnow to lab acclimation *versus* field acclimatization. In lab fish, we saw no changes in muscle COX activity even after 35 days at 5°C (Fig. 2B). However, mudminnows captured from the field in early April showed a 3-fold increase in muscle COX activity relative to that of summer fish. Thus, it appears unlikely that the seasonal effects we see in this species are due to simple effects of temperature.

Apart from temperature, the metabolic phenotype could be affected by many of the factors that differ between seasons. Photoperiod can induce modifications in mitochondria in both Atlantic cod (Pelletier et al., 1993) and rainbow trout (Martin et al., 2009). Oxygen is well known to affect the glycolytic phenotype (Chippari-Gomes et al., 2005; Jørgensen and Mustafa, 1980; Lushchak et al., 1998), though its effect on mitochondrial enzymes is still debated (Hoppeler et al., 2003; Klimova and Chandel, 2008). Diet can have complex effects on metabolism, though the effects are most commonly seen in liver (de la Higuera et al., 1999; Hemre et al., 2002; LeMoine et al., 2008; Menoyo et al., 2004). Activity levels change during the season, and these may induce the equivalent of a training response. The significant decrease in the condition factor of the lab-acclimated fish (Table 1) did not have an impact on enzyme activity, nor was the significant increase in enzyme activity in the winter-acclimatized fish mirrored in the condition factors. Reproductive status may also affect muscle mitochondria, through hormone levels or locomotor behaviours, including migrations, mating, spawning activity and territory defence. In our study, stickleback, mudminnow and dace each showed an increase in muscle COX activity. These species begin spawning activity in spring (Abbott, 1870; Peckham and Dineen, 1957; Reisman and Cade, 1967), which includes spawning migrations, nest building and guarding as well as active mating behaviour. A combination of factors triggers spawning in fish, primarily temperature and photoperiod (Lam, 1983). Changes in the latter could play an important role in our observations of mudminnow and dace. Spawning itself induces mitochondrial modifications in rainbow trout (Kiessling et al., 1995).

In summary, this study and others demonstrate a wide range of patterns in the response of muscle aerobic enzymes to thermal history. We have shown evidence that one source of variation is a distinction between thermal acclimation and seasonal acclimatization. Nonetheless, even closely related species with very similar acclimatization histories can have divergent patterns of metabolic remodelling.

Control of mitochondrial oxidative capacity

Whether or not a species changes muscle mitochondrial content, the levels of mitochondria are controlled by genetic mechanisms affecting the transcription of mitochondrial genes, including those encoding proteins of mtDNA replication and transcription, and protein import and assembly (Hock and Kralli, 2009). In mammals, the cascade from transcriptional master regulators to mitochondrial gene expression and mitochondrial biogenesis is well understood (Hock and Kralli, 2009; Scarpulla, 2010). The role of a master regulator of mitochondrial biogenesis was first ascribed to NRF-1 (Evans and Scarpulla, 1990); however, more recent studies support a greater role for coactivator PGC-1α (Scarpulla, 2010). Although a few studies have assessed the potential role of these factors in adaptive remodelling in fish, the extent of the parallel with mammals is not yet clear. In particular, there is little correlation between COX activity and *PGC-1α* mRNA level in different muscles, dietary states, activity levels and temperature treatments (LeMoine et al., 2008; McClelland et al., 2006; Orczewska et al., 2010). This prompted the suggestion that the PGC-1α paralogue PGC-1β may be the major coactivator for mitochondrial gene expression control in fish (LeMoine et al., 2008). Though the role for PGC-1 paralogues may differ in fish *versus* mammals, these studies have found stronger support for a role for NRF-1 as master regulator.

One goal of our study was to broaden the species comparisons to better clarify the potential role of PGC-1α in controlling mitochondrial changes with temperature and/or season. Consistent with previous studies on goldfish (LeMoine et al., 2008) and stickleback (Orczewska et al., 2010), we found in four species a cold-induced increase in COX activity but no increase in *PGC-1α* mRNA level; three of these species (brook stickleback, pumpkinseed and mudminnow) showed a marked decrease in *PGC-1α* mRNA level. Only in dace was an increase in COX activity (3-fold) accompanied by an increase in *PGC-1α* mRNA level (7-fold), which is what is expected from mammalian models (Scarpulla, 2010). The large variation in *PGC-1α* mRNA between species could be related to its role in other cellular processes, including fatty acid oxidation (Vega et al., 2000) and muscle fibre specification (Lin et al., 2002). Alternatively, changes in PGC-1α activity may be related to the coactivation of other DNA-binding proteins, including thyroid hormone receptors, peroxisome proliferator-activated receptors (PPARs), retinoic acid receptors and estrogen-related receptors (Puigserver and Spiegelman, 2003; Schreiber et al., 2003). Thus, even if PGC-1α plays a role in mitochondrial biogenesis in response to temperature, its regulation could be complicated by simultaneous changes in diet, fuel selection, activity level and reproduction (Dean, 1969; Miranda and Pugh, 1997; Suski and Ridgway, 2009). Nonetheless, there is little evidence from fish studies to support a role of PGC-1α in determining changes in COX activity with temperature.

Mammalian studies have shown NRF-1 to be an important regulator of all nuclear-encoded COX genes (Dhar et al., 2008). It acts by binding to NRF-1 promoter elements (Virbasius et al., 1993), where it recruits PGC-1α (Scarpulla, 2010) The interplay between NRF-1 and PGC-1α is an important regulatory axis in mammalian muscle. Furthermore, transcriptional regulation of *NRF-1* appears central to the control of its activity (Hock and Kralli, 2009). Previous studies on fish have shown that increases in *NRF-1* mRNA accompany increases in mitochondrial enzyme activities seen with diet, temperature and exercise (LeMoine et al., 2008; McClelland

Table 3. Trends in COX activities and transcription factor mRNA levels in target species relative to summer/warm values

Species	COX	*NRF-1*	*NRF-2α*	*PGC-1α*
GF	↑	↑	↔	↓
RD	↑	↔	n.a.	↑
PS	↑	↑	↔	↓
BC	↑	↑	↑	↔
BS	↑	↔	↔	↓
MM	↑	↔	↔	↓
BG	↔	↑	↔	↔
LM	↔	↑	↑	↔
PI	↔	↔	↔	↔

Goldfish data obtained from previous studies (LeMoine et al., 2008).
PS, pumpkinseed; BG, bluegill; LM, largemouth bass; BC, black crappie; RD, northern redbelly dace; BS, brook stickleback; PI, northern pike; MM, central mudminnow; GF, goldfish.
NRF, nuclear respiratory factor; PGC-1α, peroxisome proliferator-activated receptor-γ coactivator-1α.

et al., 2006; Orczewska et al., 2010). In this study, pumpkinseed and crappie both showed a significant increase in COX activity and *NRF-1* mRNA level. However, the pattern was not consistent across species. Dace, stickleback and mudminnow each achieved an increase in COX activity without a corresponding increase in *NRF-1* mRNA. Furthermore, two non-compensators (bluegill and bass) showed significant increases in *NRF-1* mRNA level with no change in COX activity. Despite this variation, NRF-1 appears to be the most likely candidate for a role as master regulator of mitochondrial biogenesis in fish. In mammals, post-transcriptional regulation of NRF-1 can play a role in determining its transcriptional activity (Hock and Kralli, 2009), so perhaps this level of regulation is more important in fish.

Like NRF-1, NRF-2 has an important role in the regulation of all nuclear-encoded COX genes in mammals (Ongwijitwat et al., 2006). Its regulation is more complex because the active transcription factor is a heterodimer of NRF-2α and either NRF-2β or NRF-2γ (Gugneja et al., 1995). There is also clear evidence for post-transcriptional regulation of NRF-2 (Vallejo et al., 2000), making measurement of mRNA less informative. Furthermore, apart from its role in the control of mitochondrial genes, it regulates diverse cellular processes (Imaki et al., 2003; Ristevski et al., 2004; Yang et al., 2007). The role of NRF-2 has been less studied in fish but there is little evidence from our study of a role in the thermal remodelling of muscle. Only two species showed seasonal changes in *NRF-2α* mRNA; its level increased in crappie, paralleling changes in COX activity, and bass, which showed no change in COX activity.

The present study has shown that there is no phylogenetic pattern in the response of COX content in muscle to cold acclimation and/or seasonal acclimatization (Table 3). Changes seen seasonally in free-living fish may be confounded by non-thermal cues, such as reproductive status. Furthermore, the role of transcriptional master regulators of mitochondrial biogenesis appears to be more complex (Table 3) than is expected from mammalian studies. The transcriptional factors themselves may act in fundamentally different ways, and there is a potential role for post-transcriptional regulation.

ACKNOWLEDGEMENTS

This study was funded by a Discovery Grant (C.D.M.) of the Natural Sciences and Engineering Research Council (NSERC) Canada. We would like to thank the staff of the Queen's University Biological Station for their support of these studies; in particular, Frank Phelan and Bruce Tufts for their fishing expertise as well as students Sarah Wyness, Katrinka Kocha, Trevor Snider, Christopher Monk, Paul Debes and Scott Taylor for their help in the field.

REFERENCES

Abbott, C. C. (1870). Mud-loving fishes. *Am. Nat.* **4**, 385-391.
Agbeti, M. D. and Smol, J. P. (1995). Winter limnology: a comparison of physical, chemical and biological characteristics in two temperate lakes during ice cover. *Hydrobiologia* **304**, 221-234.
Baar, K., Wende, A. R., Jones, T. E., Marison, M., Nolte, L. A., Chen, M., Kelly, D. P. and Holloszy, J. O. (2002). Adaptations of skeletal muscle to exercise: rapid increase in the transcriptional coactivator PGC-1. *FASEB J.* **16**, 1879-1886.
Battersby, B. J. and Moyes, C. D. (1998). Influence of acclimation temperature on mitochondrial DNA, RNA, and enzymes in skeletal muscle. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **275**, 905-912.
Blier, P. and Guderley, H. (1988). Metabolic responses to cold acclimation in the swimming musculature of lake whitefish, *Coregonus clupeaformis*. *J. Exp. Zool.* **246**, 244-252.
Braun, M. H., Steele, S. L., Ekker, M. and Perry, S. F. (2009). Nitrogen excretion in developing zebrafish (*Danio rerio*): a role for Rh proteins and urea transporters. *Am. J. Physiol. Renal Physiol.* **296**, 994-1005.
Bullock, T. H. (1955). Compensation for temperature in the metabolism and activity of poikilotherms. *Biol. Rev.* **30**, 311-342.
Chippari-Gomes, A. R., Gomes, L. C., Lopes, N. P., Val, A. L. and Almeida-Val, V. M. F. (2005). Metabolic adjustments in two Amazonian cichlids exposed to hypoxia and anoxia. *Comp. Biochem. Physiol.* **141B**, 347-355.
Chomczynski, P. and Sacchi, N. (2006). The single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction: twenty-something years on. *Nat. Protoc.* **1**, 581-585.
Crowder, A. A., Bristow, J. M., King, M. R. and Vanderkloet, S. (1977). Distribution, seasonality, and biomass of aquatic macrophytes in Lake Opinicon (Eastern Ontario). *Nat. Can.* **104**, 441-456.
de la Higuera, M., Akharbach, H., Hidalgo, M. C., Peragón, J., Lupiáñez, J. A. and García-Gallego, M. (1999). Liver and white muscle protein turnover rates in the European eel (*Anguilla anguilla*): effects of dietary protein quality. *Aquaculture* **179**, 203-216.
Dean, J. M. (1969). The metabolism of tissues of thermally acclimated trout (*Salmo gairdneri*). *Comp. Biochem. Physiol.* **29**, 185-196.
Dhar, S. S., Ongwijitwat, S. and Wong-Riley, M. T. (2008). Nuclear respiratory factor 1 regulates all ten nuclear-encoded subunits of cytochrome *c* oxidase in neurons. *J. Biol. Chem.* **283**, 3120-3129.
Duggan, A. T., Kocha, K. M., Monk, C. T., Bremer, K. and Moyes, C. D. (2011). Coordination of cytochrome *c* oxidase gene expression in the remodelling of skeletal muscle. *J. Exp. Biol.* **214**, 1880-1887.
Egginton, S. and Johnston, I. A. (1984). Effects of acclimation temperature on routine metabolism muscle mitrochondrial volume density and capillary supply in the elver (*Anguilla anguilla* L.). *J. Therm. Biol.* **9**, 165-170.
Egginton, S. and Sidell, B. D. (1989). Thermal acclimation induces adaptive changes in subcellular structure of fish skeletal muscle. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **256**, 1-9.
Egginton, S., Cordiner, S. and Skilbeck, C. (2000). Thermal compensation of peripheral oxygen transport in skeletal muscle of seasonally acclimatized trout. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **279**, 375-388.
Evans, M. J. and Scarpulla, R. C. (1990). NRF-1: a trans-activator of nuclear-encoded respiratory genes in animal cells. *Genes Dev.* **4**, 1023-1034.
Farrell, A. P., Johansen, J. A. and Suarez, R. K. (1991). Effects of exercise-training on cardiac performance and muscle enzymes in rainbow trout, *Oncorhynchus mykiss*. *Fish Physiol. Biochem.* **9**, 303-312.
Freed, J. (1965). Changes in activity of cytochrome oxidase during adaptation of goldfish to different temperatures. *Comp. Biochem. Physiol.* **14**, 651-659.
Guderley, H. (1990). Functional significance of metabolic responses to thermal acclimation in fish muscle. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **259**, 245-252.
Guderley, H. (2004). Metabolic responses to low temperature in fish muscle. *Biol. Rev.* **79**, 409-427.
Guderley, H. and Foley, L. (1990). Anatomic and metabolic responses to thermal acclimation in the ninespine stickleback, *Pungitius pungitius*. *Fish Physiol. Biochem.* **8**, 465-473.
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., 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.
Gugneja, S., Virbasius, J. V. and Scarpulla, R. C. (1995). Four structurally distinct, non-DNA-binding subunits of human nuclear respiratory factor 2 share a conserved transcriptional activation domain. *Mol. Cell. Biol.* **15**, 102-111.
Hazel, J. R. and Prosser, C. L. (1974). Molecular mechanisms of temperature compensation in poikilotherms. *Physiol. Rev.* **54**, 620-677.
Heap, S. P., Watt, P. W. and Goldspink, G. (1985). Consequences of thermal change on the myofibrillar ATPase of five freshwater teleosts. *J. Fish Biol.* **26**, 733-738.
Heinrich, B. (1977). Why have some animals evolved to regulate a high body temperature? *Am. Nat.* **111**, 623-640.
Hemre, G.-I., Mommsen, T. P. and Krogdahl, Å. (2002). Carbohydrates in fish nutrition: effects on growth, glucose metabolism and hepatic enzymes. *Aquacult. Nutr.* **8**, 175-194.
Hock, M. B. and Kralli, A. (2009). Transcriptional control of mitochondrial biogenesis and function. *Annu. Rev. Physiol.* **71**, 177-203.

Holloszy, J. O. (1967). Biochemical adaptations in muscle: effects of exercise on mitochondrial oxygen uptake and respiratory enzyme activity in skeletal muscle. *J. Biol. Chem.* **242**, 2278-2282.
Hoppeler, H., Vogt, M., Weibel, E. R. and Flück, M. (2003). Response of skeletal muscle mitochondria to hypoxia. *Exp. Physiol.* **88**, 109-119.
Imaki, H., Nakayama, K., Delehouzee, S., Handa, H., Kitagawa, M., Kamura, T. and Nakayama, K. I. (2003). Cell cycle-dependent regulation of the *Skp2* promoter by GA-binding protein. *Cancer Res.* **63**, 4607-4613.
Johnston, I. A. and Maitland, B. (1980). Temperature acclimation in crucian carp, *Carassius carassius* L., morphometric analyses of muscle fibre ultrastructure. *J. Fish Biol.* **17**, 113-125.
Johnston, I. A. and Wokoma, A. (1986). Effects of temperature and thermal acclimation on contractile properties and metabolism of skeletal muscle in the flounder (*Platichthys flesus* L.). *J. Exp. Biol.* **120**, 119-130.
Jørgensen, J. B. and Mustafa, T. (1980). The effect of hypoxia on carbohydrate metabolism in flounder (*Platichthys flesus* L.). Utilization of glycogen and accumulation of glycolytic end products in various tissues. *Comp. Biochem. Physiol.* **67B**, 243-248.
Keast, A. and Fox, M. G. (1992). Space use and feeding patterns of an offshore fish assemblage in a shallow mesotrophic lake. *Environ. Biol. Fishes* **34**, 159-170.
Kiessling, A., Larsson, L., Kiessling, K.-H., Lutes, P. B., Storebakken, T. and Hung, S. S. S. (1995). Spawning induces a shift in energy metabolism from glucose to lipid in rainbow trout white muscle. *Fish Physiol. Biochem.* **14**, 439-448.
Kleckner, N. W. and Sidell, B. D. (1985). Comparison of maximal activities of enzymes from tissues of thermally acclimated and naturally acclimatized chain pickerel (*Esox niger*). *Physiol. Zool.* **58**, 18-28.
Klimova, T. and Chandel, N. S. (2008). Mitochondrial complex III regulates hypoxic activation of HIF. *Cell Death Differ.* **15**, 660-666.
Lam, T. J. (1983). Environmental influences on gonadal activity in fish. In *Fish Physiology* (ed. W. S. Hoar, D. J. Randall and E. M. Donaldson), pp. 65-116. New York: Academic Press.
Lawrence, C. (2007). The husbandry of zebrafish (*Danio rerio*): a review. *Aquaculture* **269**, 1-20.
LeMoine, C. M. R., Genge, C. E. and Moyes, C. D. (2008). Role of the PGC-1 family in the metabolic adaptation of goldfish to diet and temperature. *J. Exp. Biol.* **211**, 1448-1455.
LeMoine, C. M. R., Lougheed, S. C. and Moyes, C. D. (2010). Modular evolution of PGC-1α in vertebrates. *J. Mol. Evol.* **70**, 492-505.
Lin, J., Wu, H., Tarr, P. T., Zhang, C., Wu, Z., Boss, O., Michael, L. F., Puigserver, P., Isotani, E., Olson, E. N. et al. (2002). Transcriptional co-activator PGC-1α drives the formation of slow-twitch muscle fibres. *Nature* **418**, 797-801.
Lushchak, V. I., Bahnjukova, T. V. and Storey, K. B. (1998). Effect of hypoxia on the activity and binding of glycolytic and associated enzymes in sea scorpion tissues. *Braz. J. Med. Biol. Res.* **31**, 1059-1067.
Martin, N., Kraffe, E. and Guderley, H. (2009). Effect of day length on oxidative capacities of mitochondria from red muscle of rainbow trout (*Oncorhynchus mykiss*). *Comp. Biochem. Physiol.* **152A**, 599-603.
McClelland, G. B., Craig, P. M., Dhekney, K. and Dipardo, S. (2006). Temperature- and exercise-induced gene expression and metabolic enzyme changes in skeletal muscle of adult zebrafish (*Danio rerio*). *J. Physiol.* **577**, 739-751.
Menoyo, D., Izquierdo, M. S., Robaina, L., Ginés, R., Lopez-Bote, C. J. and Bautista, J. M. (2004). Adaptation of lipid metabolism, tissue composition and flesh quality in gilthead sea bream (*Sparus aurata*) to the replacement of dietary fish oil by linseed and soyabean oils. *Br. J. Nutr.* **92**, 41-52.
Miranda, L. E. and Pugh, L. L. (1997). Relationship between vegetation coverage and abundance, size, and diet of juvenile largemouth bass during winter. *N. Am. J. Fish. Manage.* **17**, 601-610.
Ongwijitwat, S., Liang, H. L., Graboyes, E. M. and Wong-Riley, M. T. (2006). Nuclear respiratory factor 2 senses changing cellular energy demands and its silencing down-regulates cytochrome oxidase and other target gene mRNAs. *Gene* **374**, 39-49.
Orczewska, J. I., Hartleben, G. and O'Brien, K. M. (2010). The molecular basis of aerobic metabolic remodeling differs between oxidative muscle and liver of threespine sticklebacks in response to cold acclimation. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **299**, 352-364.
Peckham, R. S. and Dineen, C. F. (1957). Ecology of the central mudminnow, *Umbra limi* (Kirtland). *Am. Midl. Nat.* **58**, 222-231.
Pelletier, D., Guderley, H. and Dutil, J.-D. (1993). Does the aerobic capacity of fish muscle change with growth rates? *Fish Physiol. Biochem.* **12**, 83-93.
Pfaffl, M. W., Tichopad, A., Prgomet, C. and Neuvians, T. P. (2004). Determination of stable housekeeping genes, differentially regulated target genes and sample integrity: BestKeeper-Excel-based tool using pair-wise correlations. *Biotechnol. Lett.* **26**, 509-515.
Puigserver, P. and Spiegelman, B. M. (2003). Peroxisome proliferator-activated receptor-coactivator 1 (PGC-1): transcriptional coactivator and metabolic regulator. *Endocr. Rev.* **24**, 78-90.
Puigserver, P., Wu, Z., Park, C. W., Graves, R., Wright, M. and Spiegelman, B. M. (1998). A cold-inducible coactivator of nuclear receptors linked to adaptive thermogenesis. *Cell* **92**, 829-839.
Reisman, H. M. and Cade, T. J. (1967). Physiological and behavioral aspects of reproduction in the brook stickleback, *Culaea inconstans*. *Am. Midl. Nat.* **77**, 257-295.
Ricker, W. E. (1975). Computation and interpretation of biological statistics of fish populations. *Bull. Fish. Res. Board Can.* **191**, 1-382.
Ristevski, S., O'Leary, D. A., Thornell, A. P., Owen, M. J., Kola, I. and Hertzog, P. J. (2004). The ETS transcription factor GABP is essential for early embryogenesis. *Mol. Cell. Biol.* **24**, 5844-5849.
Scarpulla, R. C. (2010). Metabolic control of mitochondrial biogenesis through the PGC-1 family regulatory network. *Biochim. Biophys. Acta*. doi:10.1016/j.bbamcr.2010.09.019.
Schreiber, S. N., Knutti, D., Brogli, K., Uhlmann, T. and Kralli, A. (2003). The transcriptional coactivator PGC-1 regulates the expression and activity of the orphan nuclear receptor estrogen-related receptor α (ERRα). *J. Biol. Chem.* **278**, 9013-9018.
Shaklee, J. B., Christiansen, J. A., Sidell, B. D., Prosser, C. L. and Whitt, G. S. (1977). Molecular aspects of temperature acclimation in fish: contributions of changes in enzyme activities and isozyme patterns to metabolic reorganization in the green sunfish. *J. Exp. Zool.* **201**, 1-20.
Sommer, A. M. and Pörtner, H. O. (2004). Mitochondrial function in seasonal acclimatization versus latitudinal adaptation to cold in the lugworm *Arenicola marina* (L.). *Physiol. Biochem. Zool.* **77**, 174-186.
Suski, C. D. and Ridgway, M. S. (2009). Winter biology of centrarchid fishes. In *Centrarchid Fishes: Diversity, Biology, and Conservation* (ed. S. J. Cooke and D. P. Philipp), pp. 264-292. Oxford: Wiley-Blackwell.
Thibault, M., Blier, P. U. and Guderley, H. (1997). Seasonal variation of muscle metabolic organization in rainbow trout (*Oncorhynchus mykiss*). *Fish Physiol. Biochem.* **16**, 139-155.
Tschantz, D. R., Crockett, E. L., Niewiarowski, P. H. and Londraville, R. L. (2002). Cold acclimation strategy is highly variable among the sunfishes (*Centrarchidae*). *Physiol. Biochem. Zool.* **75**, 544-556.
Vallejo, C. G., Escrivá, H. and Rodríguez-Peña, A. (2000). Evidence of tissue-specific, post-transcriptional regulation of NRF-2 expression. *Biochimie* **82**, 1129-1133.
Vega, R. B., Huss, J. M. and Kelly, D. P. (2000). The coactivator PGC-1 cooperates with peroxisome proliferator-activated receptor α in transcriptional control of nuclear genes encoding mitochondrial fatty acid oxidation enzymes. *Mol. Cell. Biol.* **20**, 1868-1876.
Vézina, D. and Guderley, H. (1991). Anatomic and enzymatic responses of the three-spined stickleback, *Gasterosteus aculeatus* to thermal acclimation and acclimatization. *J. Exp. Zool.* **258**, 277-287.
Virbasius, C. A., Virbasius, J. V. and Scarpulla, R. C. (1993). NRF-1, an activator involved in nuclear-mitochondrial interactions, utilizes a new DNA-binding domain conserved in a family of developmental regulators. *Genes Dev.* **7**, 2431-2445.
Wu, Z., Puigserver, P., Andersson, U., Zhang, C., Adelmant, G., Mootha, V., Troy, A., Cinti, S., Lowell, B., Scarpulla, R. C. et al. (1999). Mechanisms controlling mitochondrial biogenesis and respiration through the thermogenic coactivator PGC-1. *Cell* **98**, 115-124.
Yang, Z.-F., Mott, S. and Rosmarin, A. G. (2007). The Ets transcription factor GABP is required for cell-cycle progression. *Nat. Cell Biol.* **9**, 339-346.