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Reactive oxygen species, antioxidants and fish mitochondria

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1. ABSTRACT

In fishes, irrespective of their thermoregulatory capacity or metabolic rate, the main physiological source of reactive oxygen species (ROS) is mitochondria. During active swimming, ROS is by an large provided by red muscle mitochondria. Other tissues such as lens, liver, heart, swimbladder, roe and blood also afford important ROS production and antioxidant levels in resting fish. A close relationship between structure and function is evident in fish mitochondrion with a surface-to-volume optimization by the size of cristae to maximize electron transfer. The mechanism of fish mitochondrial superoxide anion ($O_2^{\bullet -}$) and ROS production as well as the mechanism of mitochondrial coupling and proton leak seems similar to that of mammals. Contrary to mammalian red cells, fish erythrocytes possess nuclei and mitochondria. The presence of cardiolipin and the absence of cholesterol in fish mitochondrial membranes confer a high structural flexibility. The difference in phospholipid unsaturation may explain the greater proton leak in endotherms compared to thermoconformers. The present review summarizes our current understanding in respect to comparative aspects of fish mitochondrial function, with an emphasis on the adaptations to changes in temperature, O_2 availability and O_2 consumption, which are generally coupled to changes in antioxidant *status* and ROS production. Nevertheless, most work on this fascinating area has yet to be done. The literature on the effect of xenobiotics, aquatic contamination, and aquaculture issues are not reviewed. Data on the production of NO and reactive nitrogen species (RNS), on O_2 sensing and on the role of ROS and RNS in cell signalling involving fish mitochondria are almost completely lacking in the literature.

2. INTRODUCTION

Fish comprise roughly two thirds of vertebrates and constitute a biological group that has unique morphological, physiological and biochemical adaptations, which along evolution enabled them to successfully inhabit the marine, brackish- and fresh-water environments and, although temporarily, also the terrestrial environment (1). The majority of fish species is not able to live without a continuous O_2 supply to tissues but carp and goldfish, among few other fishes, are able to survive long periods (days or months) under anoxic conditions (1). Most fish are water-breathing; beside there are a few species of lungfishes and obligatory or facultative air-breathing fish, most from freshwater and brackish-water environments that have developed different types of epithelia for gas exchange (1,2).

Several estimations in different vertebrate species mostly in laboratory animals, indicate that at least 90% of the reactive oxygen species (ROS) generated in the cell is originated in mitochondria (3,4). Therefore studies performed on antioxidants systems in different vertebrate tissues indirectly reflect such inherent ROS production in mitochondria (5). Most fish are thermoconformers and oxyconformers and therefore their metabolic rate depends on environmental temperature and on environmental O_2 tensions (1). As a consequence, the generation rate of ROS and the corresponding level of antioxidant defenses (AD) are fairly interdependent in these fish species (5-8).

Fish mitochondria as well as mitochondria from other vertebrates display a conspicuous and close structure-function relationship. Examples of such relationship are the mitochondria-rich chloride cells found in freshwater fishes (9), in the unusually large and string-like mitochondria found in the ionocytes of teleosts (10), in mitochondria-rich red cells related to the high locomotory performances of tuna-like fish (11), and also in the increased mitochondrial cristae density (without changing the mitochondrial volume density) found in Antarctic and cold-acclimated fish (12).

Until few years ago the attention of comparative respiratory biochemistry and physiology focused mainly on the availability and use of O_2 without taking in consideration the deleterious consequences of ROS generation (5). After the discovery of the enzyme superoxide dismutase (SOD) by McCord and Fridovich in 1969 (13) the studies on antioxidant defenses against the inherent toxicity of ROS began to appear in late seventies, including those on fish (5). Since then, fascinating comparative studies on this topic experienced a continuous and increased interest. We learned that some adaptations of aerobic organisms might not be necessarily and primarily driven to guarantee O_2 supply to the tissues and to "normal" metabolic functions such as growth, weight gain and reproduction, but sometimes the adaptation of the antioxidant system to avoid an harmful oxidative stress related to O_2 reutilization might be more important for fish (an other vertebrates) survival (5,14).

More than 90% of the ROS are generated in mitochondria and *ca*. 2% of the whole O_2 uptake is accounted by ROS generated as obligatory byproducts of oxidative metabolism, as mainly verified in experimental mammals (15,16), a generation of actual and potential harmful products that are counteracted by the antioxidant defense system with a panoply of enzymes and metabolites that are free radical scavengers (4,17). Almost three decades ago, it was shown, mainly using liver and heart of laboratory mammals, that mitochondria are the most important sub-cellular sites for the primary superoxide anion ($O_2^{\bullet}$) production, with subsequent generation of hydrogen peroxide (H_2O_2) by the catalytic action of the mitochondrial Mn-SOD, and the eventual production of significant amounts of the highly toxic hydroxyl radical ($HO^{\bullet}$) (17,18). The denomination of ROS was collectively applied to the three species of the partial reduction of O_2 ($O_2^{\bullet}$, H_2O_2 and $HO^{\bullet}$), with a sort of general acceptance based in the about similar biological effects but overlooking the chemical identity and the molecular mechanisms. The ROS concept has been sometimes extended from the three species of the partial reduction of O_2 to include other chemical toxic chemical species, such as the alkyl ($R^{\bullet}$) and peroxyl ($ROO^{\bullet}$) radicals and singlet oxygen ($^1O_2$).

The antioxidant enzymes exhibited by the different vertebrate groups are very similar and most inter-species differences rely on the quantitative distribution of antioxidant enzymes in different tissues and at different sub-cellular levels (5). However, endotherms such as birds and mammals display higher level of antioxidant enzymes compared to fish (5). Moreover, in marine fish antioxidant enzymes seem to be higher in active fish compared to sluggish species (5). Interestingly, a higher rate of ROS generation, accounting for 3-7% of the O_2 consumption was reported in aquatic invertebrates (19) and this high rates might also occur in fish (5). On the other hand, mitochondria of true endotherms such as birds and mammals apparently produce lower rates of ROS generation compared to fish (5,20-22).

3. FISH MITOCHONDRIA: STRUCTURE-FUNCTION RELATIONSHIPS

Several examples illustrate the tightly interdependence between structure and function in fish mitochondria, including morphological and biochemical adjustments to environmental changes in temperature and in O_2 tensions (9), in salinity (10) and in hydrostatic pressure (23,24).

3.1. General morphological and biochemical adaptations

The mitochondria-rich chloride cells represent the main site of a H^+ pump present in gills of freshwater fishes, which are responsible for the acid-base and ionic regulation (25). The increased mitochondrial volume density found in oxidative red muscle of fish living at or acclimated to low temperatures (26) is another example of mitochondrial adaptation to environmental constraints.

In the temperate Antarctic eelpout *Pachycara brachycephalum* the activity of mitochondrial cytochrome

oxidase correlates with mRNA levels, which is interpreted as a continuous enzyme synthesis despite the decelerating effect of the low temperature, whilst in the cold-acclimated eelpout *Zoarces viviparus* from the North Sea, a lower cytochrome oxidase was determined (27). In this regard, the temperature-compensatory effect showed by the Antarctic species seems to be similar to that described for mammals (9). On the other hand, in contrast to mammalian striated muscle, fish lack distinct subcellular populations, despite some differences between mitochondria occurring beneath the cell membrane and those interlaced throughout the myofibrils, as observed in rainbow trout red muscle (28). As a big difference, mammalian red cells lack mitochondria but all other vertebrates, such as fishes, amphibians, reptiles and birds, have mitochondria, nuclei and other organelles as well (1,2).

3.2. Fuel supply for fish mitochondria

The function of fish mitochondria as well as the mitochondria of other vertebrates depends on the adequate supply of O_2 and of substrates, *i.e.* metabolic fuels, that correspond to the specific requirements of each tissue. The main fuel for vertebrate mitochondria is carbohydrate (1). Teleosts (bony fishes, the main diversified fish group, having more than 30,000 species) use glutamine as the main oxidative substrate for mitochondrial energy production in the red muscle, but ketone bodies are also used in other fish tissues (29). In tuna red muscle the levels of several glycolytic enzymes are among the highest reported and mitochondrial density in this tissue is also very high, similar to the highest values reported for vertebrates (30). In terms of energy mobilization in mitochondria, high-performance fish elevate their relative reliance on intramuscular fuel reserves and decrease their relative use of circulatory fuels, similarly to the strategy described for endurance-adapted mammals (31).

Similar to the mammalian heart, the teleost heart generates ATP through oxidation of glucose and fatty acids (32). However, contrary to the mammalian heart, non-pathogenic ventricular hypertrophy in rainbow trout *Oncorhynchus mykiss* do not decrease mitochondrial fatty acid oxidation and ATP regeneration, thereby maintaining the organ capacity for O_2 metabolism after adaptation to intense chronic exercise (33). Fatty acid beta-oxidation occurs in both peroxisomes and mitochondria, however mitochondrial beta-oxidation seems to be quantitatively more important compared to other processes as energy source in fish tissues (34).

A very interesting fish adaptation associated with feeding is the sparing of antioxidant enzymes when submitted to severe starvation, despite that the liver experienced a 10-fold decrease in size (35). In that study, the Atlantic cod *Gadus morhua* no changes or even increases in the activities of antioxidant enzymes (catalase and glutathione S-transferase) were observed in liver and white muscles, whilst the activities of important mitochondrial enzymes such as cytochrome oxidase and citrate synthase were significantly downregulated (35). A similar response was found in the spawning salmon, and is likely to occur in other fish facing the same environmental constraints (9).

Interestingly, elasmobranch fishes (sharks, rays, skates and chimaeras, with less than thousand species already described), use urea as the main osmolyte for osmoregulatory purposes, implying a mitochondrial adaptation to urea for this and other physiological functions (2,7).

3.3. Composition of fish mitochondrial membrane

Some important differences in the composition of liver mitochondrial membranes were reported for three phylogenetic distinct marine fish species (36). The fatty acid content of the phospholipids in the elasmobranch *Raja erinacea* showed a higher percentage of saturated and a lower percentage in polyunsaturated fatty acids, as compared to other non-elasmobranch species.

The peculiar presence of the phospholipid cardiolipin and the absence of cholesterol is likely to maintain a high membrane flexibility in fish mitochondria as well as in mitochondria of other vertebrates (29). Modifications in membrane phospholipid composition involving polyunsaturated fatty acids (PUFA) have been proposed to play a role in the H^+ leak across mitochondrial membranes (25). Rats consuming fish as a primary lipid source show lower mitochondrial ROS levels, an effect attributable the diet (25). However, increased unsaturation of fatty acids leads to a potential enhancement of the rate of endogenous lipid peroxidation induced by ROS (4), thus increasing the oxidative challenge to fish tissues (5-7).

3.4. Antioxidant protection in fish mitochondrial membrane

The rates of H^+ leak in endotherms have been reported higher as compared to thermoconformers (37), and the adaptative value of this mechanism might rely upon the decrease of ROS generation (38). Fishes, in special those from the Antarctic seas, possess relatively high proportion of PUFA in mitochondria as well as in other membranes (39). Thus fish tissues are prone to lipoperoxidation processes and indeed, the malonaldehyde and TBARS levels usually found in fish liver are much higher than the values reported for mammals (5). Vitamin E is considered the most abundant and important nutritional (exogenous) chain-breaker antioxidant in the cell and organelle membranes of vertebrates (4) and it is understood that the vitamin E physiological function is prevent membranes from the augmented oxidative damage associated with ROS propagation reactions (4). It seems that Antarctic fish compensate this threat of increased oxidative damage by maintaining high levels of vitamin E in plasma and incorporated into the membranes. Plasma from two Antarctic species, *Pagothenia borchgrevinki* and *Trematomus bernarchii* showed much higher (five to six fold) vitamin E content than those of two temperate water fish species. Accordingly, an evaluation of the vitamin E contents of different organs and tissues of several freshwater and marine fish species from Brazil showed relatively low levels, in the 1-40 microM range (6,7), compared to Antarctic fish but in the same range of values reported for mammals (5). Beside alpha-tocoferol, which is considered the more important constituent of vitamin E in plasma and tissues from fish and other vertebrates, the

contents of gamma-tocoferol, that is considered a better antioxidant compared than alpha-tocoferol (4), is higher in fish than in mammals (5-8). An unusual constituent of the vitamin E group, alpha-tocomonoenol, which is occurs in Antartic fishes, apparently provides a higher antioxidant protection to fish mitochondria than alpha-tocoferol (40).

Aged red cells from trout show a lower mitochondrial membrane potential compared to younger ones, and the effect can be reversed by antioxidant supplementation (41). Fish erythrocyte has been proven to provide an excellent model for studies on ROS and antioxidant (5), especially for age-related studies, because these cells possess mitochondria and nucleus and show changes that are associated to aging (1,2).

4. TEMPERATURE EFFECT

A beautiful example of co-evolution adaptation is seen in the temperature effect exhibited by endothermic fish belonging to the families Tunnidae, Scombridae and Xyphiidae (teleosts) and Lamnidae (sharks) that enable them to sustain a remarkable fast swimming capacity (1,2). These fish take advantage of endothermy to reach unusually high locomotory performances for foraging in the food-abundant surface of the open ocean. Another temperature specialization is found in fish inhabiting Antarctic waters in which the mitochondrial volume density is increased in oxidative muscle. Irrespective of all these adaptations, they have in common remarkable high mitochondrial densities in their red muscles.

4.1. Mitochondrial adaptations in fast-swimming endothermic fish

The high temperatures (~30-35°C) reached by the axial red muscles of these endothermic fish not only accelerate the O_2 diffusion mediated by myoglobin to mitochondria, but also improve synaptic communication and probably fasten the clearance of blood lactate (11). In the swordfish *Xyphias gladius* brain and eye temperatures are above ambient (up to 13°C). The heat-generating organ is located at the base of the brain and their cells possess one of the highest mitochondrial density (55-70%) reported in the animal kingdom (11), as high as those found in hearts of hummingbirds and of Antarctic icefish (2). Furthermore, the billfish is able to enhance the temperature of a specialized structure called the heater organ (11). Unlike the mammalian brown adipose tissue in which uncoupling of ATP synthesis optimizes heat production, the mitochondria of the heater organ are coupled and do not express the corresponding uncoupling protein (42).

4.2. Temperature adaptations in non-endothermic fish

The stripped bass is a non-endothermic fish species that almost double the mitochondrial volume in red muscles after cold acclimation (11). This adaptation is believed not only to increase the oxidative capacity of the locomotory tissue but also to enhance O_2 diffusion by reducing diffusion distances (11). In the low-temperature adapted Arctic fishes, myoglobin O_2 dissociation rates are faster than those measured in other vertebrates, thus intracellular O_2 diffusion is not compromised (43).

4.3. Adaptation to the cold: specialization of Antarctic fish species

The main biochemical mechanism to generate more energy in vertebrate cells consists in the switch of mitochondria from the resting from state 4 to the active state 3 to increase the rate of ATP synthesis associated with an increased O_2 utilization (18). Nevertheless, other adaptations for increased energy demand occurs as seen in the increased mitochondrial mass without the concomitant increase in cell division, which is an adaptation found in cold-acclimated and Antarctic fish (44). The augmented mitochondrial density in polar fish seems to be the main determinant evolutionary adaptation instead of the metabolic cold adaptation earlier proposed (44).

It is well known that fish species living in the thermally stable Antarctic environment possess a very high packing density of mitochondria, as high as those described for tuna red muscle (30). The mitochondrial density of the Antarctic fish species *Pleuragramma antarcticum* is around 60% of their muscle fibers (44). In addition, mitochondrial volume density is increased in the oxidative muscle of fishes living or acclimated to low temperatures, contrary to what happens in moderately active fish species, in which decreased mitochondrial density were found (26).

The cytochrome oxidase activities of Antarctic fish species are higher than those of temperate species. In the Antarctic fish *Trematomus bernachii* from the Notothenoidae family the substrate binding properties of mitochondria occurs at very low temperatures. Apparently, this fish has no need to acclimate mitochondrial function to higher temperature than 3°C, and the so-called Arrhenius break temperature showed the lowest value recorded for any organism (46). Other nototheniid species *Notothenia coriiceps* from Antartida fails to exhibit a temperature compensation regarding its respiration rate, and again the higher activities found for mitochondrial enzymes seems to rely on an increased mitochondrial volume (44).

Interestingly, this surface-to-volume adaptation exhibited by Antarctic and by cold-acclimated fish, in which the mitochondrial cristae density is increased without changing volume density likely fasten the intracellular delivery of O_2 and related oxidative metabolites (12), is the opposite of the strategy of erythrocyte swelling found in stressed fish (47), likely to diminish the binding of organic phosphate to hemoglobin and thereby favoring O_2 uptake under environmental hypoxia (48).

4.4. Antioxidant adaptation in Antarctic fish

Regarding antioxidant adaptation, red-blooded Antarctic fish species exhibit nearly five-fold higher heart catalase activities than Antarctic icefish species which are devoid of hemoglobin. Red-blooded Antarctic fishes also show a higher erythrocytic Cu,Zn-SOD activity than other fish species (49). Also, the blood Cu,Zn-SOD activity of mammalian and bird species from Antartida are in the same level as red-blooded fish species, but this activity is one order of magnitude higher compared to that of white-blooded Antarctic fish species (50). Again, these studies

revealed a straight correlation between antioxidant levels and O_2 consumption, reinforcing the concept that this relationship is widespread and tightly coupled in thermoconforming fish (5).

Modifications in membrane phospholipid content might be responsible for some mitochondrial responses to thermal acclimation (26). The structural differences in mitochondrial membrane composition such as the degree of phospholipid unsaturation, which characterize organisms inhabiting different thermal environments, might account for mitochondrial properties that are dependent on temperature (26). Interestingly, fish living or acclimated at low temperatures are able to maintain high locomotory performances during most part of their ontogenies. However, this ability seems to be jeopardized only during spawning and gametogenesis (26).

Mammalian mitochondria respond to physiological stimulation inducing a coordinate upregulation of both nuclear and mitochondrial-encoded cytochrome oxidase transcripts, and the basic mechanism is supposed to occur also in fish (9). Nevertheless, in Antarctic fishes net cytochrome oxidase synthesis is upregulated despite the decelerating effect of low temperatures, probably reflecting a relative increase of enzymatic synthesis over the degradation rates (27).

5. OXYGEN TENSION, ROS GENERATION AND MITOCHONDRIAL FUNCTION

Many fish species face drastic daily or seasonal changes in O_2 availability and O_2 consumption (1). Some species have developed different and sometimes bizarre strategies to survive in freshwater environments, as in the case of the Amazon basin and the Paraguayan Chaco, where O_2 tensions in water may diurnally shift from anoxia or severe hypoxia to hyperoxia (1). Adaptations from surfacing, in which obligatory water-breathing fish are able to draw the better oxygenated waters near to the air-water interface, to different epithelia for gas exchange with the air (lungfish, electric eel, *Synbranchus*, among others) are relatively common in those environments (1,2). Other fish species possess molecular adaptations: the Root effect hemoglobins and *retia mirabile* associated with local acidification act in concert and generates hyperoxic O_2 to fill the swim bladder or to ensure high O_2 supply to the retina (51). The tolerance to the deleterious consequences of those drastic O_2 changes or to hyperoxic O_2 tensions that tissues and mitochondria must cope with such unusual O_2 levels is still demanding more studies and challenging interpretation regarding the corresponding antioxidant protection.

5.1. Adaptations to different oxygen tension

Active thermoconforming fish species sustain higher O_2 consumption rates for longer time intervals than less active species (52). Accordingly, comparative studies of their antioxidants defenses and ROS production rates revealed linear relationship with O_2 consumption rates (5-8,19). Endotherms exhibit generally a 10-100 fold higher ROS production in different tissues compared to invertebrates and fish (5,19), and this difference corresponds roughly to differences in specific metabolic rates (2). Interestingly, fish hepatocytes exhibit metabolic depression at O_2 tensions which are at least 2–3 orders of magnitude higher than the O_2 tension required for 50% activity of mitochondrial respiratory chain (53). As a consequence, it can be expected that the antioxidant enzyme activities were primarily determined by tissue O_2 consumption and/or tissue O_2 availability. Increases in the number of mitochondria are usually associated with increases in tissue-specific activity of cytochrome oxidase, thereby corresponding to the augmented tissue O_2 consumption. Accordingly, the antioxidant enzyme activities found in different tissues of more active fish are higher compared to sedentary or bottom-dwelling species (6,7). A similar direct relationship was found between antioxidant enzyme activities and hemoglobin content with O_2 consumption of different fish species (5).

5.2. Adaptations to hypoxia

The role of the hypoxia-inducible factor 1alpha (HIF-1alpha) seems to be well established in mammals (19) but not in fish (53). However, other aspects related to fish metabolism under hypoxia, such as the regulation of erythrocyte ATP and GTP concentrations remain to be clarified (53). ATP and GTP are the two more important energy molecules and also the main organic O_2 affinity regulators in the nucleated fish erythrocytes (48).

Juveniles of *Leporinus elongatus* exposed to moderate and severe hypoxia showed similar responses in liver and blood (14) the main mitochondrial site of ROS generation after hypoxia and during reoxygenation. The antioxidant enzymes glutathione peroxidase and glutathione S-transferase were enhanced whilst lipid peroxidation and oxidized glutathione showed decreased levels especially in the liver of fish exposed to severe hypoxia. This study strongly support the idea that fish and probably other vertebrates have metabolic priorities maintaining or even elevating their antioxidant protection in detriment of other important metabolic functions such as growth and weight gain (14).

5.3. Adaptations to hyperoxia

As mentioned above, some fish species having Root effect hemoglobins and *retia mirabile* are able to generate internal hyperobaric O_2 to ensure a high O_2 supply to the swimbladder or to the retina (51). Other fish species can tolerate hyperoxia or drastic changes in O_2 availability, especially in tropical freshwater environments (1). However, chronic exposure of fish to high O_2 levels provoke mitochondrial swelling and functional disruption in a way similar to that described for mammalian mitochondria (5).

5.4. The oxygen cascade and mitochondrial oxygen utilization

Irrespective of the tissue specific metabolic rate the O_2 cascade ultimately allows a PO_2 below 2 Torr at the mitochondrial cristae level (54). Accordingly, the minimum intracellular PO_2 for maximum cytochrome oxidase turnover about 0.5 Torr, and 0.2 Torr of O_2 is sufficient to

saturate its enzymatic capacity (55). In this regard, it is very tempting to speculate that along evolution vertebrates reached an upper limit in the efficiency of O_2 delivery to tissues, considering the morphological limitations of O_2 diffusion barriers together with the functional limitations of the circulatory and respiratory system. The circulatory and diffusion system for O_2 delivery to the tissues is based in an O_2 gradient from the environment to mitochondria, sometimes called the O_2 cascade, with mitochondrial O_2 tensions approximately one hundred times lower at the mitochondrial cristae compared to atmospheric tensions (54). Inside the cells there are O_2 gradients that seem to be restricted to the vicinity of mitochondria, thus the mitochondrial location is a major factor determining the O_2 tensions experienced by intracellular structures (53).

In addition, the low-yield but fast energy supply obtained from the glycolitic pathway present mainly in special situations, *i.e.* during the flight or fight syndrome (2), is a widespread strategy present in all vertebrates, which might also be a way to avoid an excess of ROS production in animal tissues under such conditions (5). This is also in accordance to the O_2 restriction found in the tracheolar respiratory system of insects. Insects are able to deliver almost atmospheric O_2 tensions to their tissues because O_2 diffuses ~10,000 times faster in gas phase of the tracheolar branches to reach directly the mitochondrial membranes compared to the slow O_2 transport in body fluids (2). These organisms seem to maintain O_2 levels sufficient to their requirements but as low as possible to minimize oxidative damage, keeping the spiracles closed most of the time, thereby limiting O_2 utilization and ROS overgeneration (56). This strategy showed by insect mitochondria contrasts with that of temperate and polar aquatic organisms, in which the priority is to counteract the deceleration effect associated with very low temperatures, maximizing O_2 delivery to the tissues via reducing O_2 diffusion distances (19).

The fish gill is a multifunctional organ responsible for gas exchange, ionic and acid-base regulations, excretion of nitrogenous wastes (9), and also elimination of H_2O_2 (57). Under hypoxia or under osmoregulatory stress fish experience a chloride cell proliferation in the lamellae, which may jeopardizes gas exchange through the augmented blood-to-water diffusion distance (9). In this regard, the impact of cell proliferation on gas transfer, blood acid-base status and on HbO_2 binding properties are well established, but the impact on the antioxidant capacity has been overlooked.

The antioxidant depletion and increased ROS generation following hypoxia and reoxygenation is well known in vertebrates (5). At a first moment hypoxia elicits a drastic decrease in antioxidants, characterizing the O_2 paradox, but later it may induce the recovery of antioxidant levels in detriment of other functions, such as growth and weight gain (14), a strategy similar to that obtained in fish after severe starvation (34). It is tempting to interpret the increased lamellar thickness observed in fish for ion and pH regulation (9) as a temporarily strategy to impede an excess of O_2 uptake and consequently to avoid the inherent oxidative challenge, preparing adequate antioxidant defenses for the organism when O_2 levels resume (5,14). This sort of adaptation contrasts with that (mentioned in section 4) showed by invertebrates and fish inhabiting cold aquatic environments that have high mitochondrial volume density, thus being able to maximize mitochondrial O_2 uptake through reducing O_2 diffusion distances (19,43).

The mechanism of mitochondrial ROS production through the initiation reactions involving the generation of $O_2^{\bullet}$ described for mammals (3,41) seems to be similar to that of fish, despite the fact that fish generally show a higher rate (3-7%) of ROS production (5,19). Nevertheless, there are only few numbers available in the literature concerning ROS production in invertebrates and non-mammalian vertebrates (5,19). This needs to be better established and further studies are necessary for a proper understanding of the homeostasis of fish mitochondrial ROS production if compared with mammalian mitochondria (4,58,59).

6. PERSPECTIVES

The knowledge on many aspects of fish mitochondrial function, such as the mitochondrial regulation of O_2 sensing, the role of hypoxia inducible transcription factor (HIF-alpha), the ROS/RNS generation and the modulation of antioxidant enzymes and defenses, is incipient, However, there is relatively abundant information on other areas of fish mitochondrial properties, as the morphological, physiological and biochemical adaptations and responses to environmental constraints, especially to temperature and to O_2 tensions. Moreover, almost nothing is known in relation to NO and RNS metabolism in fish mitochondria. The same gap applies to the specific sites of ROS production in fish mitochondria, which are probably the same as those described for mammals. Also, the turnover of ROS/RNS, their impact in fish pathologies, and the nature of oxidative stress promoters in fish mitochondria need further studies. Comparative studies have improved the understanding in different biological areas, and the same is expected in relation to ROS/RNS and antioxidants, in fish mitochondria. Further studies are required to obtain adequate information on these topics.

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8. REFERENCES

1. Lagler KL, J.E. Bardach, R.R. Miller & D.R. MayPassino (Eds): Ichthyology. John Wiley & Sons, New York (1977)

2. Schmidt-Nielsen, K: Animal Physiology – Adaptation and Environment. Cambridge University Press, Cambridge, 699 (1977)

3. Boveris A & B. Chance: The mitochondrial generation of hydrogen peroxide. General properties and effect of hyperbaric oxygen. *BiochemJ* 34, 707-717 (1973)

4. Halliwell B & JMC Gutteridge: Free Radicals in Biology and Medicine. 4th ed, Oxford Univ Press, London (2002)

5. Wilhelm Filho D. J.L. Marcon, C.G. Fraga & A. Boveris: Antioxidant defenses in vertebrates: emphasis on fish and mammals. *Trends Comp Biochem Physiol* 7, 33-45 (2000)

6. Wilhelm Filho D, C. Giuvili & A. Boveris: Antioxidant defenses in marine fish. I. Teleosts. *Comp Biochem Physiol* 106C, 409-414 (1993)

7. Wilhelm Filho D & A. Boveris: Antioxidant defenses in marine fish. II. Elasmobranchs. *Comp Biochem Physiol* 106C, 415-418 (1993)

8. Janssen BL, J.J. Childress, F. Baguet & J.F. Rees: Reduced enzymatic antioxidative defense in deep-sea fish. *J Exp Biol* 203, 3717-3725 (2000)

9. Perry SF: The chloride cell: structure and function in the gills of freshwater fishes. *Annu Rev Physiol* 59, 325-347 (1997)

10. Muders K, R. Anken, M. Ibsch & H. Rahmannet: Extremely elongated mitochondria in ionocytes of the saccular epithelium of a teleost, *Oreochromis mossambicus* (Cichlidae). *Adv Space Res* 33, 1406-1410 (2004)

11. Hazel JR: Thermal biology. In: The physiology of fishes. Ed: Evans, DH, CRC Press, Boca Raton (1993)

12. St-Pierre J, J.A. Buckingham, S.J. Roebuck & M.D. Brand: Topology of superoxide production from different sites in the mitochondrial electron transport chain. *J Biol Chem* 277, 44784-44790 (1998)

13. McCord JM & I. Fridovich: Superoxide dismutase: an enzymic function for erythrocuprein (hemocuprein). *J Biol Chem* 244, 6049-6055 (1969)

14. Wilhelm Filho D, M.A. Torres, E. Zaniboni-Filho, R.C. Pedrosa: Effect of different oxygen tensions on weight gain, feed conversion, and antioxidant *status* in piapara, *Leporinus elongatus* (Valenciennes, 1847). *Aquaculture* 213, 120-127 (2005)

15. Chance B, H. Sies & A. Boveris: Hydroperoxide metabolism in mammalian organs. *Physiol Rev* 59: 527-605 (1979)

16. Turrens J: Mitochondrial formation of reactive oxygen species. *J Physiol* 552, 335-344 (2003)

17. Boveris A: Mitochondrial production of superoxide radical and hydrogen peroxide. *Adv Exp Med Biol* 78, 67-82 (1977)

18. A Boveris & H Cadenas: Cellular sources and steady-state levels of reactive oxygen species. In: Oxygen, gene expression and cellular function. Eds: Mazzaro D & Clerch L, Marcel Dekker, NY, 1-25 (1997)

19. Abele DK & S. Puntarulo: Formation of reactive species and induction of antioxidant defence systems in polar and temperate marine invertebrates and fish. *Comp Biochem Physiol* A138, 405-415 (2004)

20. Sohal RS, H.-H. Ku & S. Agarwal: Biochemical correlates of longevity in two closely related rodent species. *Biochem Biophys Res Comm* 196, 7-11 (1993)

21. Barja G, S. Cadenas, C. Rojas, R. Pérez-Campo & M. López-Torres: Low mitochondrial free radical production per unit O_2 consumption can explain the simultaneous presence of high longevity and high metabolic rate in birds. *Free Radic Res* 21, 317-328 (1994)

22. Brunet-Rossini AK: Reduced free-radical production and extreme longevity in the little brown bat (*Myotis lucifugus*) versus two non-flying mammals. *Mech Ageing Develop* 125, 11-20 (2004)

23. Sebert P,M. Theron & A. Vettier: Pressure and temperature interactions on cellular respiration: a review. *Cell Mol Biol* 50, 491-500 (2004)

24. Amerand A, A. Vettier, P. Sebert & C. Cann-Moisan: In vitro effect of hydrostatic pressure exposure on hydroxyl radical production in fish red muscle. *Redox Report* 10, 25-28 (2005)

25. Ramsey, JJ, M.E. Harper, S.J. Humble, E.K. Koomson, J.J. Ram, L. Bevilaqua & K. Hagopian: Influence of mitochondrial fatty acid composition on protin leak and H_2O_2 production in liver. *Comp Biochem Physiol* 140B, 99-108 (2005)

26. Guderley, H: Locomotor performance and muscle metabolic capacities: impact of temperature and energetic status. *Comp Biochem Physiol* B139, 371-382 (2004)

7. Hardewig, R, P. L.M. van Dijk, C. D. Moyes & H. O. Pörtner: Temperature-dependent expression of cytochrome-*c* oxidase in Antarctic and temperate fish. *Am J Physiol* 277, R508-R516 (1999)

28. Battersby, BJ & C.D. Moyes: Are there distinct subcellular populations of midtochondria in rainbow trout red muscle? *J Exp Biol* 201, 2455-2460 (1998)

29. Ballantyne JS: Mitochondria: aerobic and anaerobic design, lessons from molluscs and fishes. *Comp Biochem Physiol B: Comp Biochem* 139, 461-467 (2004)

30. Suarez RK: Upper limits to mass specific metabolic rates. *Annu Rev Physiol* 58, 583-605 (1996)

31. Weber, JM & F. Haman: Pathways for metabolic fuels and oxygen in high performance fish. *Comp Biochem Physiol* A113, 33-38 (1996)

32. WR Driedzic: Cardiac energy metabolism. In: Fish Physiology, vol. 12. Eds: Hoar WS, Randall DJ & Farrell AP, Academic Press, NY (1992)

33. Clark, JJ, R.J. Clark, J.T. McMinn & K.J. Rodnick: Microvascular and biochemical compensation during ventricular hypertrophy in male rainbow trout. *Comp Biochem Physiol* B139, 695-703 (2004)

34. Henderson, RJ: Fatty acid metabolism in freshwater fish with particular reference to polyunsaturated fatty acids. *Arch Anim Nutr* 49, 5-22 (1996)

35. Guderley, H, D. Lapointe, M. Bédard & J.-M. Dutil: Metabolic priorities during starvation: enzyme sparing in liver and white muscle of Atlantic cod, *Gadus morhua* L. C*omp Biochem Physiol* A135, 347-356 (2003)

36. Glemet, HC & J.S. Ballantyne: Comparison of liver mitochondrial membranes from an agnathan (*Myxine glutinosa*), an elasmobranch (*Raja erinacea*) and a teleost (*Pleuronectes americanus*). *Mar Biol* 124, 509-518 (1996)

37. Brand MD, P. Couture, P.L. Else, K.W. Withers & A.J. Hulbert: Evolution of energy metabolism: proton permeability of the inner membrane of liver mitochondria is greater in a mammal than in a reptile. *Biochem J* 275, 81-86 (1991)

38. Skulachev, VP: Uncoupling: new approaches to an old problem of bioenergetics. *Biochem Biophys Acta* 1363, 100-124 (1998)

39. Gieseg SP, S. Cuddihy, J.V. Hill & W. Davison: A comparison of plasma vitamin C and E levels in two Antarctic and two temperate water fish species. *Comp Biochem Physiol* 125, 371-378 (2000)

40. Yamamoto Y, A. Fujisawa, A. Hara & W.C. Dunlap: An unusual vitamin E constituent (alpha-tocomonoenol) provides enhanced antioxidant protection in marine organisms adapted to cold-water environments. Proc Natl Acad Sci USA 98, 13144-13148 (2001)

41. Tiano L, P. Ballarini, G. Sanoni, M. Wozniak & G. Falcioni: Morphological and functional changes of mitochondria from density separated trout erythrocytes. *Biochim Biophys Acta* 157, 118-128 (2001)

42. Brand MD, C. Affourtit, T.C. Esteves, K. Green, A.J. Lambert, S. Miwa, J.L. Pakay & N. Parker: Mitochondrial superoxide: production, biological effects, and activation of uncoupling proteins. *Free Radic Biol Med* 37, 755-767 (2004)

43. Sidell BD: Intracellular oxygen diffusion: the roles of myoglobin and lipid at cold body temperature. *J Exp Biol* 201, 1119-1128 (1998)

44. Johnston IA, H.E. Guderley, C.E. Franklin, T. Crockford & C. Kamunde: Are mitochondria subject to evolutionary temperature adaptation? *J Exp Biol* 195, 293-306 (1994)

45. Johnston IA, J.-P. Camm & M. White: Specialisations of swimming muscles in the pelagic Antarctic fish Pleuragramma antarcticum. *Mar Biol* 100, 3-12 (1988)

46. Weinstein RB & G.N. Somero: Effects of temperature on mitochondrial function in the Antarctic fish *Trematomus bernachii*. *J Comp Physiol* B168, 190-196 (1998)

47. Wilhelm Filho D, J.L. Marcon, F.X. Caprario & A. Nollis: Intraerythrocytic nucleoside triphosphates in marine fish. *Comp Biochem Physiol* 102A, 323-331 (1992)

48. Weber RE & F.B. Jensen: Functional adaptations in hemoglobins from ectothermic vertebrates. *Annu Rev Physiol* 50, 161-179 (1988)

49. Ansaldo M, C.M. Luquet, P.A. Evelson, M.J. Polo & S. Llesuy: Antioxidant levels from different Antarctic fish caught aroound South Georgia and Shag Rocks. *Polar Biol* 23, 160-165 (1999)

50. Witas H, T. Gabryelak & B. Matkovics: Comparative studies on superoxide dismutase and catalase activities in livers of fish and other Antarctic vertebrates. *Comp Biochem Physiol* 77C, 409-411 (1984)

51. Pelster B: The generation of hyperbaric oxygen tensions in fish. *News Physiol Sci* 16, 287-291 (2001)

52. Bennett AF: Activity metabolism of the lower vertebrates. *Annu Rev Physiol* 400, 447-469 (1978)

53. Nikimmaa M: Oxygen-dependent cellular functions – why fishes and their aquatic environment are a prime choice of study. *Comp Biochem Physiol* 133, 1-16 (2002)

54. Pörtner HO & M.K. Grieshaber: Critical PO_2 (s) in oxyconforming and oxyregulation animals: gas exchange, metabolic rate and the mode of energy production. In: The vertebrate gas transport cascade. Adaptations to environment and mode of life. Ed: J.E.P.W. Bicudo. CRC Press, Boca Raton (1993)

55. Chance B: Pyridine nucleotide as an indicator of the oxygen requirements for energy-linked functions of mitochondria. *Circ Res* 38: 31-39 (1976)

56. Hetz S & T.J. Bradley: Isects breathe discontinuosly to avoid oxygen toxicity. *Nature* 433, 516-519 (2005)

57. Wilhelm Filho D, B. González-Flecha & A. Boveris: Gill diffusion as a physiological mechanism for hydrogen peroxide elimination by fish. *Braz J Med Biol Res* 27, 2879-2882 (1994)

58. Bell EL, B.M. Emerling & N.S. Chandel: Mitochondrial regulation of oxygen sensing. *Mitochondria* 5, 322-332 (2005)

59. Jezek, P & L. Hlavatá: Mitochondria in homeostasis of reactive oxygen species in cell, tissues, and organism. *Int J Biochem Cell Biol* 37, 2478-2503 (2005)

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