

Hypoxia adaptation in fish of the Amazon: a never-ending task

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In addition to seasonal long-term changes in dissolved oxygen and carbon dioxide, water bodies of the Amazon present periodic short-term episodes of hypoxia and even anoxia. To preserve gas exchange and acid base balance, fish of the Amazon have developed multiple adaptive solutions which occur at all biological levels. These solutions are thought to represent adaptive convergence rather than phylogenetic relatedness. Fish of the Amazon exposed to different experimental conditions adjust, for example, several parameters to improve oxygen transfer from the gas-exchange site to the tissues. These parameters include morphological changes such as the development of the lower lip in *Colossoma*, changes in ventilation rates, changes in circulatory parameters, increased circulating red blood cells, decreased levels of intraerythrocytic phosphates, and adjustments of intraerythrocytic pH (pHi). These adjustments that allow fish to survive both short- and long-term hypoxia occur in different degrees in different fish species and may or may not occur simultaneously. In addition, these adjustments in oxygen transfer affect many other parameters, particularly acid-base status. We suggest that these adjustments are initiated as soon as the animal detects the environmental change in oxygen availability and are mediated by a single factor, possibly one of the catecholamines. In this paper we aim to show that adaptation to hypoxia is a never-ending task for the fish of the Amazon.

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Introduction

The story of hypoxia adaptation in fish of the Amazon is also a story of changing environments. To understand why hypoxia adaptation in fish of the Amazon is a never-ending task, a general view of the Amazon basin is fundamental. During the Cretaceous, Gondwanaland broke up in the southern hemisphere and two continents appeared: South America and Africa. The Andes mountains then appeared in the western part of the Amazon at the end of the Miocene and induced stupendous changes in the Amazon basin. The Pacific drainage of upper tributaries of the Amazon river was cut and the whole Amazon basin became Atlantic oriented. As a consequence a complete new set of habitats was made available and seasonal river water level oscillations became the main driving force of the Amazon basin (reviewed by Val & Almeida-Val 1995).

Junk, Bayley & Sparks (1989) refer to the river water level oscillation as flood pulses. In fact, a crest of 10 m, occasionally more, occurs every year on the main river channel in front of Manaus. Since the Amazon basin is a flat land, 80% of the whole region is under the 30 m contour line, which means that a significant part of the basin is flooded every year making new habitats in the flooded forest (*igapó*) and in the floodplain areas (*várzea*) available to fish. In addition, the river water level oscillation causes the appearance and disappearance of many other aquatic formations of the Amazon such as *paraná*s (channels), *igarapés* (small streams), beaches, etc. During high water levels the water bodies (lakes, *paraná*s, *igarapés*, etc) are all interconnected. In the *várzea* and *igapós* only the upper part of the trees remains above the water. During low water levels, the receding water leaves behind small water bodies. Chemical, physical, and biological parameters of the water change accordingly. Thus, virtually all living organisms of the Amazon are affected by such a predictable annual flood pulse.

The amount of dissolved oxygen in waters of the Amazon basin is determined by complex interactions of physical, chemical and biological processes. Such processes include photosynthesis, respiration, light penetration, organic decomposition, molecular oxygen diffusion, wind, water body depth and shape, temperature, etc. Since all these parameters are affected by the regular river water level oscillation, a seasonal change in dissolved oxygen in a given place and time, related to the flood pulse, is observed. These changes are called long-term changes in dissolved oxygen. Indeed, mixed patterns of dissolved oxygen occur simultaneously. For example, during high water levels extremes of oxygen may occur in different parts of the same lake as a consequence of water plant cover. During the low water season anoxic conditions are observed in such places owing to the decomposition of the water plants. Such anoxic conditions occur together with high levels of dissolved hydrogen sulphide and methane (reviewed by Val & Almeida-Val 1995).

Because water of varying characteristics occurs in the many aquatic formations of the Amazon, not surprisingly extremes of dissolved oxygen are observed at a given moment. So, while low levels of dissolved oxygen are observed in *várzea* lakes and *igapós* for example, normal or even over-saturated levels are observed in open areas such as in the flowing river. Since these systems are interconnected during high water levels, fish have the choice of staying in the *várzea* lakes where food is available or leaving such places in search of better oxygen conditions.

In parallel to these long-term changes in oxygen, extreme variations in dissolved oxygen tend to occur in a very short period of time. In the lakes of the Marchantaria Island, for example, oxygen levels may drop to zero at night and reach over-saturated levels at noon the very next day (Junk, Soares & Carvalho 1983; Val 1986; Val & Almeida-Val 1995). Short-term changes in dissolved oxygen force rapid respira-

tory adjustments in aquatic animals, particularly fishes (Val 1996).

Another remarkable characteristic of the Amazon water is the microstratification of dissolved oxygen. It has been speculated that the top millimetres of the water column contain as much as 90% of all water-dissolved oxygen. The oxygen dissolved in the first 10 cm of the water column of many aquatic habitats of the Amazon represents the sole source of oxygen for several aquatic animals during the regular episodes of hypoxia/anoxia (reviewed by Val 1996). So, aquatic habitats of the Amazon can be seen as an ever-changing environment regarding oxygen availability.

Adaptations to chronic hypoxia in fish of the Amazon

The Amazon basin encompasses the richest fish fauna in the world. The current number of fish species is estimated to range from 1300 to 2500, including representatives of almost all freshwater groups of fishes (Roberts 1972; Böhlke, Weitzman & Menezes 1978; Rapp-Py-Daniel & Leão 1991). Representatives of primitive orders such as Lepidosireniformes and Laminiformes coexist with advanced teleosts, the Pleuronectiformes and Tetraodontiformes. The dominant group is the Ostariophysi which is by far the largest teleost group of fish currently living in the Amazon. Fink & Fink (1979) refer to it as an example of explosive radiation from a small number of ancestors. Characiformes is the richest order of teleosts in the Amazon, including more than 1200 fish species, followed by the Siluriformes (catfishes) with 1000 fish species (Table 1). Such richness has been explained by the dynamics of the Amazon basin, the size and diversity of habitats, the open character of the basin, the species paucity in some parts of the basin, and the genetic and physiological plasticity of the animals which has been related to the amplification of the genome in early vertebrates and has provided them with the capacity to face the new environmental condi-

tions after the uplift of the Andes mountains. Complex organism–environment interactions set the distribution of fishes across the myriad of habitats of the Amazon (Almeida-Val, Val & Hochachka 1993; Almeida-Val & Farias 1996).

This adaptive radiation of fish of the Amazon has occurred and is still occurring in an ever-changing environment. Ethological, morphological, anatomical, physiological and biochemical adjustments are improved to maintain the essential homeostasis during the regular natural environmental changes. These adjustments occur in different degrees in different groups of fishes, enabling them to survive extreme environmental conditions such as acidic and ion-poor waters (Gonzalez, Wood, Wilson, Patrick, Bergman, Narahara & Val 1998; Wood, Wilson, Gonzalez, Patrick, Bergman, Narahara & Val 1998), high hydrogen sulphide and methane levels (Brauner, Ballantyne, Randall & Val 1995; Val, Marcon, Costa, Barcellos, Maco-Garcia & Almeida-Val 1998) and deep hypoxia or even anoxia (Val 1993; Val & Almeida-Val 1995; Val *et al.* 1998). They are thought to represent adaptive convergence rather than phylogenetic relatedness (Almeida-Val *et al.* 1993).

According to Randall, Burggren, Farrell & Haswell (1981), hypoxia and anoxia have been common events in the evolutionary history of waters since the Cambrian period and fish, consequently, have evolved many different strategies to face such environmental constraints. Air-breathing is one solution and it was tried independently in many unrelated groups of fish of the Amazon. Simultaneously, those fish species unable to utilise air as a source of oxygen have developed many modifications directed towards enhancing oxygen transfer to tissues, including behavioural, morphological, physiological, and biochemical adjustments. It is likely that such adaptive adjustments and the mechanisms of detection of environmental changes in oxygen availability and/or physiological needs have evolved simultaneously as a way to increase survival capacity (Arthur, Hogan, Bebout, Wagner & Hochachka 1992; Almeida-Val & Farias 1996; Hochachka 1996; Val 1996; Walker & Henderson 1996).

Behavioural adjustments

Behavioural change is the most common adaptive response of fish of the Amazon to low oxygen. When fish face oxygen depletion in their environment the simplest way to avoid the problem is to leave the environment, i.e. to escape. Junk *et al.* (1983) reported a significant decrease in species richness and abundance when oxygen levels were low in Camaleão Lake, a typical *várzea* lake of Marchantaria island. The authors showed that only those fish species specialised in some way to tolerate low levels of oxygen remain in the lake during periods of oxygen shortage (Table 2). Most of the water-breathing fish species are able to detect a current oxygen depletion or even to anticipate it, leaving the place before large drops occur. Lateral migrations, i.e. migrations that involve small distances normally between the river and floodplain areas, have been described for several fish species of the Amazon and they are related to oxygen availability in the lakes among other environmental constraints (Cox-Fernandes 1989). In addition to these migrations, other fish species change their natural habitat to explore alternative sources of oxygen. Sometimes, for example, several of these animals are

Table 1 Main orders of fish of the Amazon with an indication of estimated number of species in the Amazon and in the World. Data originally reviewed by Val & Almeida-Val (1995). Note that Gymnotiformes are endemic to the Amazon. ^a not all freshwater

Order	Species in the world	Species in the Amazon
Lepidosireniformes	5	1
Laminiformes	1	1
Rajiformes	70 ^a	12
Osteoglossiformes	206	4
Clupeiformes	331	17
Characiformes	1335	1200
Siluriformes	2211	1000
Gymnotiformes	54	54
Batrachoidiformes	64	1
Cyprinodontiformes	845	30
Synbranchiiformes	15	1
Perciformes	7791	350
Pleuronectiformes	538	5
Tetraodontiformes	329	5

Table 2 Families of fish occurring at Camaleão Lake during low dissolved oxygen with an indication of their main adaptation to hypoxia. After Junk *et al.* (1983), Val & Almeida-Val (1995)

Families/Subfamilies	Species	Main adaptation	Type
Anostomidae	<i>Schizodon fasciatus</i> <i>Leporinus fasciatus</i> <i>Rhythiodus microlepis</i>	Unknown	Unknown
Bryconinae	<i>Brycon melanopterus</i>	Aquatic surface respiration	Lip expansion associated with physiological adjustments
Callichthyidae	<i>Hoplosternum littorale</i> <i>Callichthys callichthys</i> <i>Hoplosternum thoracatum</i>	Air breathing	Stomach and intestine
Cichlidae	<i>Cichlasoma</i> spp <i>Astronotus ocellatus</i> <i>Pterophyllum scalare</i>	Metabolic depression	Metabolic depression and gene regulation
Curimatidae	<i>Curimata latior</i> <i>Curimata laticeps</i> <i>Curimata kneri</i> <i>Curimatella meyeri</i>	Unknown	Unknown
Electrophoridae	<i>Electrophorus electricus</i>	Air breathing	Pharyngeal, branchial, and mouth diverticula
Erythrinidae	<i>Hoplerythrinus unitaeniatus</i> <i>Erythrinus erythrinus</i>	Air breathing	Modified swimbladder
Lepidosirenidae	<i>Lepidosiren paradoxa</i>	Air breathing	Lung
Loricariidae	<i>Liposarcus pardalis</i> <i>Plecostomus</i> sp	Air breathing	Stomach and intestine
Myleinae	<i>Colossoma macropomum</i> <i>Piaractus brachypomum</i> <i>Mylossoma duriventre</i> <i>Mylossoma aureum</i>	Aquatic surface respiration	Lip expansion associated with physiologic and metabolic adjustments
Osteoglossidae	<i>Arapaima gigas</i> <i>Osteoglossum bicirrhosum</i>	Air breathing	Aquatic surface respiration
Prochilodontidae	<i>Prochilodus nigricans</i> <i>Semaprochilodus taeniurus</i> <i>Semaprochilodus insignis</i>	Physiological	Adjustments of Hb:NTP ratios
Synbranchidae	<i>Synbranchus marmoratus</i>	Air breathing	Pharyngeal, branchial, and mouth diverticula

seen exploring the water-air interface or gulping air when dissolved oxygen is low. Species of *Pterygoplichthys* and *Hoplosternum* surface rhythmically to gulp air when exposed to hypoxia, using their vascularized stomach and intestine as an accessory air-breathing organ (Kramer & McClure 1981; Val 1995). According to Jensen, Nikinmaa & Weber (1993) this is a 'sophisticated version of the escape response'. Val (1995) has shown that the air-breathing organ contributes as much as 70% of the total oxygen transferred to the tissues in specimens of Loricariidae exposed to deep hypoxia. Interestingly, as we shall see later, these behavioural adjustments may be improved simultaneously with physiological and biochemical adjustments, possibly mediated by a single factor. Indeed, as the animal reaches a place where oxygen is available again, the oxygen transfer system and the metabolic machinery are reorganised accordingly.

Morphological adjustments

Several fish species of the Amazon are able to expand the lower lip to skim the oxygen-rich water surface. The

expanded lip is not vascularized and has no gas exchange function. It serves to funnel the surface layer of water, which is richer in oxygen as mentioned above, across the gills. Interestingly, the lip is expanded to different extents and at different oxygen thresholds in many non-related fish species such as species of the genera *Colossoma*, *Brycon*, *Triportheus*, and *Mylossoma* (see Val & Almeida-Val 1995). In *Colossoma* exposed to deep hypoxia ($Pw_{O_2} = 30$ mmHg) the lower lip expands in 2 h and retracts in about the same time when oxygen returns to normal levels. Because of the contribution of the expanded lip to oxygen uptake, no significant difference in the blood oxygen content is observed between specimens of *Colossoma* exposed to normoxia and those exposed to hypoxia with access to the water surface. However, in animals denied access to the water surface a significant decrease of 35% in blood oxygenation is estimated during hypoxia (Almeida-Val *et al.* 1993; Val 1996).

The lip expansion in *Colossoma* results in a stabilisation of plasma lactate levels as described by Almeida-Val *et al.* (1993). In a recent series of experiments we have in addition

shown that the level of organic phosphates in the erythrocytes of this fish species is also restored to levels similar to those observed in animals kept under normoxia and well above those in animals denied access to the water surface (unpublished data). This is a clear indication that lip expansion in *Colossoma* helps the animal to extract almost normoxic water, and thus the haemoglobin oxygen affinity can be reduced towards normoxic values. Consequently, the partial pressure gradient between capillary blood and tissue can be increased.

In the facultative air-breathers *Pterygoplichthys* and *Hoplosternum* exposed to deep hypoxia, the use of the accessory air-breathing organ is followed by a significant reduction of gill perfusion. This is needed to prevent oxygen leaking to the hypoxic environment. Again, as these adjustments are made the levels of organic phosphates are restored to normal levels to maintain oxygen transfer to tissues. In addition to these adjustments, morpho-functional adaptations of gills including changes in the number and length of gill filaments, and size and frequency of the secondary lamellae, have been described for several tropical fish species (Fernandes 1996).

Physiological and biochemical adjustments

In addition to behavioural and morphological adjustments, fish may respond to low oxygen by adjusting several physiological and biochemical parameters, such as: (i) increasing ventilatory frequency, ventilatory volume and heart rate; (ii) increasing the number of circulating erythrocytes, haemoglobin concentration and hematocrit value; (iii) increasing haemoglobin-oxygen affinity by changing intracellular levels of allosteric modifiers, specially decreasing Hb:NTP ratios; (iv) using hemoglobins with different functional properties; (v) changing gene expression to adjust metabolic pathways; and (vi) depressing metabolism.

Increasing gill ventilation by increasing tidal volume and/or respiratory frequency is a strategy used by many fish species to maintain arterial PO₂ during hypoxia exposure (reviewed by Milson 1993). Rantin & Kalinin (1996) have shown that *Colossoma macropomum* hyperventilated (2.2-fold higher), increased intrabuccal pressure four times, but presented no significant change in cardiac frequency when exposed to deep hypoxia (20 mmHg). Kalinin *et al.* (1996) have also shown several adjustments in the ventilatory flow, intrabuccal and intraopercular volume of *Hoplias malabaricus* and *H. lacerdae*. In general the responses are instantaneous and are related to stimulation of oxygen chemoreceptors (Milson 1996).

Hematocrit, circulating red blood cells and haemoglobin concentration are significantly increased in animals exposed to both natural and experimentally induced hypoxia (see Rowley, Hunt Page & Maiwaring 1988; Val 1993; Val & Almeida-Val 1995). However, there is a limitation in increasing circulating red blood cells which is the extra heart work needed to pump a more viscous blood. In fact, oxygen transport capacity decreases as the hematocrit diverges from the optimum (see Val 1995). Air-breathers, however, do present higher values for haemoglobin and hematocrit than water-breathers. Interestingly, the water-air breathing transition is followed by a significant increase in circulating haemoglobin and hematocrit values. Clearly, when oxygen is available

again these parameters return to normal levels. It has been suggested that these mechanisms are modulated by a single factor, possibly one of the catecholamines.

Moura (1994) has shown that the increase in circulating red blood cells in tambaqui occurs simultaneously with a decrease in spleen weight and haemoglobin content. She showed also that this mechanism in tambaqui is adrenergically stimulated as has been described for several other fish species (see Randall & Perry 1992). In addition to alpha adrenergic stimulation, beta adrenergic stimulation has been described for fish of the Amazon. Val, Menezes & Wood (1998), for example, have shown that a beta adrenergic response was clearly present in two (*Colossoma* and *Semaprochilodus*) out of six species analysed when surveying the erythrocytes of Amazonian teleosts of the Rio Negro for the presence of adrenergically mediated Na⁺/H⁺ exchangers.

The major organic phosphates modulating haemoglobin oxygen affinity in fish are ATP and GTP (see Val 1996; Weber 1996). In addition to these two compounds, inositol pentaphosphate (IPP) and 2,3-diphosphoglycerate (2,3 DPG) are encountered in the erythrocytes of *Arapaima* and *Hoplosternum*, two fish species endemic to the Amazon basin, respectively (Bartlett 1978; Val, Affonso, Souza, Almeida-Val & Moura 1992; Val 1993). In all fish species so far analysed, exposure to hypoxia results in a significant decrease in the levels of ATP and GTP within the erythrocytes. This decrease correlates with an increase in haemoglobin oxygen affinity which improves oxygen uptake at the gills during environmental hypoxia. In general, the qualitative and quantitative differences between species generally correlate with differences in the structural properties of the haemoglobins that are species-specific (Weber 1996). However, whereas NTP predominates in the erythrocytes of juveniles of *Arapaima* that are water breathers, IPP predominates in adults without any apparent change in haemoglobin structure (Val 1986; Weber 1996).

The mechanism related to the adjustment of intraerythrocytic levels of ATP and GTP is unknown. Greaney & Powers (1978) suggested that the decrease in NTP levels would be a direct consequence of low oxygen supply to oxidative phosphorylation, an explanation that has not been accepted (Nikinmaa 1990). Dalessio, DiMichele & Powers (1991) have reported a significant decrease (44%) in ATP and GTP levels in *Fundulus heteroclitus* erythrocytes incubated with adrenaline. This explanation can not be generalised either (Nikinmaa 1992; Randall & Perry 1992; Val *et al.* 1998). In fish of the Amazon, a significant change in NTP levels is observed before catecholamines are released (Val 1993). In addition, the erythrocytes of several species are not sensitive to adrenaline (Val *et al.* 1998).

Adjustments of NTP levels are improved as soon as the animal is exposed to a new condition either physiologically or environmentally induced. *Piaractus brachypomum*, for example, exhibits a cyclic change in Hb:NTP ratio that correlates with diurnal changes in oxygen availability in their natural environment (Val 1996). In *Colossoma*, the intraerythrocytic levels of ATP and GTP are partially recovered as soon as the lip is extended and the animal has access to the water surface. In *Pterygoplichthys*, a facultative air-breather, a similar situation is observed. These examples suggest that these fish spe-

cies continuously adjust their intraerythrocytic microenvironment.

Multiple haemoglobins appear to be present in most fish species. Haemoglobin multiplicity has been considered an adaptation to unstable environments because there is a clear association between structural and functional heterogeneity for several species such as: the catfish *Hoplosternum littorale* (Pérez 1980); the catfish *Pterygoplichthys pardalis* (Brunori, Bonaventura, Focesi, Galdames-Portus & Wilson 1979), the prochilodontid *Semaprochilodus taeniurus* (Val 1983); the catfish *Callichthys callichthys* (Wilhelm & Weber 1983); the anostomid *Leporinus friderici* (Peterson, Schwantes, De Luca & Schwantes 1989), among others. This observation has been strengthened since some fish species present cyclic changes in the concentrations of specific haemoglobin components during the thermoacclimatory process (reviewed by Pérez, Rylander & Nirchio 1995). Cyclic changes in the concentration of specific haemoglobin fractions related to other environmental factors, presumably oxygen availability, have also been described for *Colossoma* in the Amazon (Val 1986). However, it should be stated that no clear relationship exists between haemoglobin heterogeneity and environmental characteristics when data from marine, freshwater, and estuarine fishes are pooled (Pérez *et al.* 1995; Val & Almeida-Val 1995).

One of the most common mechanisms for surviving hypoxia in nature is the depression of oxidative metabolism and the subsequent activation of the anaerobic metabolism (Nilsson 1995). Suppressing energy demands is common during hypoxia exposure when the temperature is low (Van Waversheld, Addink & van den Thillart 1989). Some temperate fish present modified metabolic pathways to cope with oxygen depletion; the goldfish and the crucian carp, for example, produce ethanol and carbon dioxide as end products to avoid accumulation of acidic products in their body during long term hypoxia exposure (Shoubridge & Hochachka 1983). Tropical fish can not rely on temperature drops to decrease their metabolic rates and avoid high energy demands. However, to deal with such situations, some fishes of the Amazon do depress their metabolism when exposed to hypoxia to decrease energy demands and to avoid end product accumulation. *Cichlasoma amazonarum* (Almeida-Val & Farias 1996) and *Astronotus ocellatus* (Muusze, Marcon, van den Thillart & Almeida-Val 1998) show metabolic depression after 24 h of hypoxia exposure. Catecholamines increased in *Astronotus ocellatus* and *Serrasalmus nattereri* exposed to hypoxia, resulting in the activation of anaerobic glycolysis and in metabolite level changes. Additionally, many fish of the Amazon present phenotype adjustments which consist in down-regulating enzyme levels and up-regulating glycolytic capacity (Almeida-Val *et al.* 1993; Almeida-Val & Hochachka 1995; Val & Almeida-Val 1995). Moreover, some adjustments in isozyme tissue distribution, mainly in LDH (lactate dehydrogenase, E.C. 1.1.1.27), occur in many fish species of the Amazon (Almeida-Val, Schwantes & Val 1990; Almeida-Val, Farias, Silva, Duncan & Val 1995). LDH is the key enzyme of the anaerobic end of glycolysis and its isozyme system is tissue regulated according to metabolic requirements.

Phenotypic adjustments

The occurrence of phenotypic adjustments in fish of the Amazon was reported in water-breathing fish 10 years ago (Almeida-Val 1986). Adjustments in gene expression are known to occur according to environmental changes. These adjustments are referred to as phenotype plasticity by several authors (reviewed by Almeida-Val 1998).

During the last fifteen years the concept of phenotype plasticity has emerged in the literature including a new idea about diversity (West-Eberhard 1989). According to Walker (1998) the interest in phenotypic plasticity as a relevant evolutionary factor was resuscitated by the idea that gene regulation orchestrates the time order of ontogenesis and metabolism, and establishes the direct biomechanical connection between environmental stimuli and specific genetic loci via perception, hormones and regulatory proteins. Fish have sophisticated systems to detect environmental changes in order to modulate their physiology and biochemistry accordingly. Catecholamines, cortisol, oxygen itself, HIF (hypoxia induced factor), among others, play an important role in such modulation (Randall & Perry 1992; Hochachka 1996). These aspects are poorly described in fish of the Amazon.

In fact, in addition to the above described cyclic adjustments of haemoglobin phenotypes, we have shown for the enzyme lactate dehydrogenase (LDH; E.C. 1.1.1.27) that the adjustments of metabolic profiles are possible through the regulation of enzyme levels (coarse adjustments) and through adjustments of isozyme levels and their distribution among tissues (fine adjustments). Many Amazon fish species present LDH adjustments according to environmental conditions. We have described heart LDH isozyme changes in *Colossoma macropomum* that could be related to oxygen availability in its environment (Almeida-Val *et al.* 1990). In this case, LDH kinetics were adjusted accordingly (Almeida-Val & Val 1990). Another interesting example comes from cichlids, a highly 'plastic' group of animals. Species of cichlids so far analysed present two patterns of LDH tissue distribution which are related to the oxygen availability in their natural habitats (Almeida-Val *et al.* 1995). Among these animals, some species are even considered anoxia tolerant like the Oscar, *Astronotus ocellatus* (Muusze *et al.* 1998), and may present two patterns for LDH, as described in *Cichlasoma amazonarum*, *Astronotus ocellatus*, and *Cichla monoculus* (Almeida-Val *et al.* 1995). The species *Cichlasoma amazonarum* is one of the best examples of phenotype plasticity regarding LDH tissue distribution. When exposed to long-term (50 days) severe hypoxia (36 mmHg), this fish species exhibits a modification of LDH distribution, particularly in heart, liver, and brain. Activation of isozyme A₄ was observed in heart and brain while isozyme B₄ was deactivated in skeletal muscle and activated in liver. These changes were followed by changes in LDH absolute activities and pyruvate inhibition (Figure 1). Such plasticity allows the animals to regulate their anaerobic metabolism in order to avoid accumulation of end products. While these data relate to oxygen depletion, increase in oxygen content may also induce LDH gene regulation. In fact, exposure of *Serrasalmus rhombeus* (the black piranha) to hypoxia and hyperoxia (6 h) results in tissue isozyme adjustments, i.e., LDH-A₄ (muscle type) is activated in heart and liver (Figure 2).

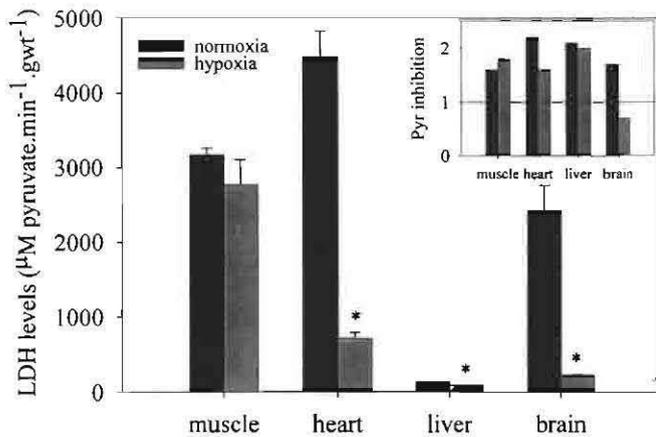
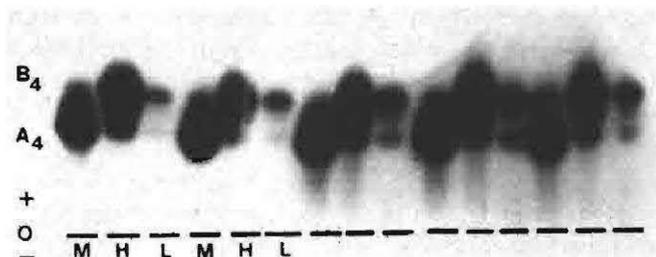
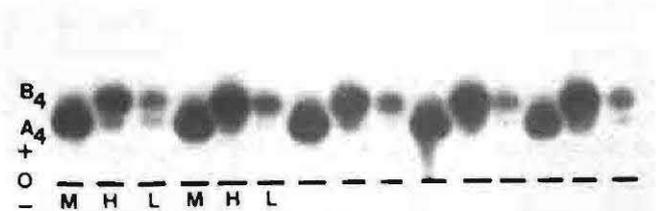


Figure 1 Changes in tissue LDH levels from *Cichlasoma amazonarum* exposed to deep hypoxia for 50 days (after Almeida-Val *et al.* 1995).



Hyperoxia



Normoxia



Hypoxia

Figure 2 Changes in tissue distribution of LDH isozyme in *Serrasalmus rhombus* exposed to hyperoxia, normoxia and hypoxia (6 h). M – muscle; H – heart; L – liver.

Indeed, there is the 'other side of the coin' regarding oxygen availability in an ever-changing environment that we should address at this point. Each time the animal copes with hypoxia it has to cope with recovery from the hypoxic state when the oxygen becomes available again. The re-perfusion of some organs such as heart, brain and liver with an oxygen rich blood may cause irreversible damage as the animal can

not avoid the presence of oxyradicals (Marcon 1997). Antioxidant defences against hyperoxia include an up-regulation of antioxidant enzymes among others. Hypoxia exposure can also induce antioxidant defences in some Amazon fish, and this is so because the animal is preventing the risk of cell damage when oxygen is available again (Marcon 1997).

Concluding remarks

In fish exposed to hypoxia (natural or experimental, short or long term), behavioural, physiological and biochemical adjustments are made to preserve the oxygen supply to metabolising tissues. Many of these adjustments are prompt whereas others are on a long-term basis. Behavioural (lateral migrations and aquatic surface respiration, for example) and morphological (lip extension, stomach/intestine vascularization) adjustments result in changes in oxygen availability and oxygen transfer mechanisms are readjusted accordingly. Long-term adjustments in the concentrations of specific haemoglobin components or LDH profiles are equally important regarding this aspect. So, adjustments of oxygen transfer in fish of the Amazon which experience a diversity of habitats and long and short-term changes in their environmental conditions are a never-ending task.

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