RESPIRATORY ASPECTS OF FISH OF THE AMAZON

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Introduction

The main environmental driving force in the Amazon is the regular flood pulse. It affects essentially all living organisms of the Amazon. At the port of Manaus a regular crest of 10 meters, occasionally more, is observed (see Val and Almeida-Val, 1995). As the water rises the contiguous river area is flooded and new habitats become available for aquatic animals. These floodplain areas, known as varzea, present a set of very peculiar conditions which include drastic changes in oxygen availability, large changes in pH, high levels of hydrogen sulfide and methane, significant changes in water temperature, among others.

The amount of dissolved oxygen is the result of complex interactions of physical, chemical, and biological factors. These factors are strongly influenced by the annual flood pulse and so is the amount of dissolved oxygen. However, much more significant are the daily changes in oxygen. For example, oxygen levels can drop to zero at night in varzea lakes and reach high saturation levels at noon the very next day. In addition, the amount of dissolved oxygen of a particular water body is strongly influenced by other specific characteristics which include floating macrophyte cover, diurnal thermal stratification, winds, organic decomposition, among others (Kramer et al., 1978; Junk, 1984). Simultaneously to oxygen shortage, many fish species of the Amazon have to face high levels of hydrogen sulfide, low water pH, significant changes in water temperature, and extremes of ion levels.

A peculiar characteristic of Amazonian water bodies is the horizontal microstratification of oxygen concentration near the water surface. The oxygen dissolved in the first centimeter of the water layer is the sole source of oxygen for many aquatic organisms during deep hypoxic or even anoxic conditions. The amount of oxygen dissolved in this first water layer may represent as much as 90% of the total oxygen dissolved in the water column. Many aquatic organisms developed important strategies to uptake oxygen from this part of the water column (reviewed by Almeida-Val et al., 1993; and Val and Almeida-Val, 1995).

The fish of the Amazon have developed multiple adaptive solutions to improve oxygen transfer under the extreme environmental conditions they live. These adaptive solutions include behavioural, morphological, physiological, and biochemical adjustments. These adjustments occur in different degrees in different groups of fish. Many fish species improve several of these adjustments simultaneously. Several papers and reviews have addressed these adaptive solutions.
in fish of the Amazon (Kramer et al., 1978; Val, 1993; 1996; Brauner et al., 1995; Val and Almeida-Val, 1995; Val et al., 1996). The present paper will focus on the adjustments of hemoglobin and intraerythrocytic phosphate levels.

**Adjusting the levels of circulating hemoglobin**

To cope with low dissolved oxygen many fish species regulate their hematocrit and hemoglobin levels. Catecholamines that are released during stressful conditions (hypoxia and acidosis, for example) induce spleen contraction (Nilsson and Grove, 1975; Randall and Perry, 1992; Moura, 1994) resulting in a release of new red blood cells into the circulation. These new red blood cells include a significant number of immature red blood cells.

A significant increase in circulating hemoglobin and hematocrit has been observed in *Colossoma macropomum* exposed to stressful conditions (Table 1). Out of three situations analysed, hypoxia exposure resulted in the higher increase in hemoglobin concentration (43%) followed by temperature (39%) and exercise (23%). Exercised *Colossoma* had simultaneous decrease in spleen weight and hemoglobin content (Moura, 1994). Adrenaline administration resulted in similar results, suggesting that at least for *Colossoma*, the increase in blood hemoglobin concentration is the result of adrenergic stimulation of spleen. As expected a significant portion of the released red blood cells were immature, including proerythrocytes.

**Table 1.** Hematocrit and hemoglobin concentration in *Colossoma macropomum* exposed to different stressful condition.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Ht (%)</th>
<th>Hemoglobin (g/dL)</th>
<th>Increase in circulating Hb (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normoxia</td>
<td>22.7±1.6</td>
<td>6.9±0.5</td>
<td>43</td>
</tr>
<tr>
<td>Hyoxia</td>
<td>32.0±0.7</td>
<td>9.9±0.6</td>
<td></td>
</tr>
<tr>
<td>20 °C</td>
<td>20.6±0.2</td>
<td>6.1±0.6</td>
<td>39</td>
</tr>
<tr>
<td>35 °C</td>
<td>24.0±1.5</td>
<td>8.5±0.5</td>
<td></td>
</tr>
<tr>
<td>Resting</td>
<td>29.2±0.6</td>
<td>5.8±0.1</td>
<td>23</td>
</tr>
<tr>
<td>Exercise</td>
<td>32.8±0.7</td>
<td>7.1±0.2</td>
<td></td>
</tr>
</tbody>
</table>

Similar results have been reported for *Hoplosternum littorale*, *Pterygoplichthys multiradiatus*, *Semaprochilodus insignis*, *S. taeniurus*, *Mylossoma duriventris*, *Brycon cephalus*, among others (Monteiro et al., 1987; Val 1993; Val and Almeida-Val, 1995). Interestingly, the adjustments of circulating levels of hemoglobin occur simultaneously with other morphological, physiological, and biochemical adjustments, supporting the hypothesis that many of these adjustments are under a single control factor. It has been suggested that this factor would be the catecholamines.

**Hemoglobins**

The hemoglobin exists at the limit of its solubility inside the red blood cell. Considering that the deoxyhemoglobin is less soluble than its counterpart the oxyhemoglobin form (Riggs, 1979), and that the oxygen transport capacity decreases as the hematocrit values diverge from a optimum (Wells and Weber, 1991), fish have developed many ways to adjust gas transfer.

Hemoglobin-oxygen affinity is strongly influenced by pH changes. As the pH decreases the Hb-O₂ affinity decreases. So, the first challenge for a fish facing stressful condition is to maintain or
even to increase the intraerythrocytic pH (pHi). According to Primett et al. (1986) the catecholamines play an important role regarding this aspect by acting on β-adrenergic receptors of red blood cells causing the cell to swell and raising pHi, at least in rainbow trout. Studying *Colossoma macropomum* exposed to hypoxia and to severe exercise we also have detected no significant change in pHi despite the significant reduction of plasma pH in both conditions. The hemoglobins of *Colossoma* are strongly influenced by pH changes. So, any change in pHi that affect Hb-O₂ affinity would be lethal.

Decreased oxygen solubility in water and increased metabolic demand are the major problems faced by fishes experiencing increased water temperature. Therefore, fish must increase oxygen uptake, even though there is less oxygen available, and/or decrease the metabolic demand. However, in fish of the Amazon we have observed a decreased Hb-O₂ affinity at high temperatures (Powers et al., 1979; Val and Almeida-Val, 1995). To overcome this problem the fish may adjust the intraerythrocytic levels of organic phosphates as in juveniles of *Colossoma* and *Symphysodon*.

**Intraerythrocytic phosphates**

ATP and GTP are the major organic phosphates detected in the erythrocytes of fish of the Amazon. The levels of these phosphates in fish show a wide intraspecific variation as a consequence of many different factors such as exercise, temperature, nutrition, and oxygen availability. Wide interspecific variations have also been reported for animals exposed to similar physiological and environmental conditions. In addition to ATP and GTP, other organic phosphates have been detected in the erythrocytes of fish of the Amazon such as 2,3 DPG in *Hoplosternum littorale*, IPP (inositol pentaphosphate) in *Arapaima gigas*, and IP₃ (inositol diphosphate) in *Lepidoziren paradoxo* (Val, 1993; Isaacks et al., 1977; Bartlett, 1978). In all fish species so far studied these intraerythrocytic phosphates act as a negative modulator of hemoglobin-oxygen affinity (see Nikinmaa, 1990; and Val and Almeida-Val, 1995 for references).

In general the effect of GTP on Hb-O₂ affinity is more pronounced than that of ATP. When only two hydrogen bonds are allowed in the binding site the effect of GTP is similar to that of ATP. This is true for many fish species of the Amazon. In juveniles of *C. macropomum*, however, ATP is stronger allosteric modulator of Hb-O₂ affinity than GTP (Figure 1). We have suggested that this may be due to differences in the structure of the hemoglobins of this fish species. *Colossoma macropomum* have at least five different hemoglobin fractions. The proportions of these fractions show significant seasonal variation. Since each hemoglobin fraction may have different binding site configuration, this figure may change seasonally too. In addition, Hb-O₂ affinity is substantially affected by Hb:NTP (GTP and ATP) which changes seasonally in this fish species (Val, unpublished data). The ratio Hb:NTP also changes according to specific environmental conditions including exercise, temperature, pH, and oxygen availability.
As presented elsewhere, *C. macropomum* expands the lower lips in about two hours when exposed to low oxygen. The lips are used to funnel the oxygen-rich water surface through the gills (Braum and Junk, 1982; Almeida-Val et al., 1993; Val and Almeida-Val, 1995). Val (1995) estimated that about 30% of the blood oxygen content is supplied using the lips in specimens of *Colossoma* exposed to hypoxia. Interestingly, the appearance of lips results in a decrease of plasma lactate (Almeida-Val et al., 1993) and in an increase in Hb:NTP ratio. In addition, Hb:NTP ratio is lower in animals exposed to hypoxia but denied access to water surface compared to those allowed to skim.

The fish of the Amazon are exposed to extremes of dissolved oxygen in a single day, as mentioned above. They may face hypoxia or even anoxia at night and hyperoxia in very next day. The levels of intraerythrocytic phosphate are substantially affected during these changes in oxygen. Short term exposure (6h) of specimens of *Serrasalmus rhombeus*, known locally as piranha preta, to hypoxia and hyperoxia resulted in a significant decrease of Hb:NTP (ATP and GTP) and in an increase in Hb:GTP ratio, compared to normoxia exposed animals, respectively. No significant changes in Hb:ATP were observed in animals exposed to hyperoxia compared to normoxia. An accompanying paper (Marcon and Val, this volume) shows that both Hb:ATP and Hb:GTP in *C. macropomum* are affected by long term exposure to hypoxia and hyperoxia. The decrease in Hb:NTP during hypoxia have been largely documented and have been related to an increase in oxygen transfer by increasing Hb-O₂ affinity. Taking into account that excess of oxygen is toxic, the increase in Hb:NTP (particularly GTP) in animals exposed to hyperoxia may be equally adaptive. Increased Hb:NTP result in a reduced Hb-O₂ affinity which reduces the chance of overloading the tissue with oxygen.

Acknowledgements

The present paper is based on the research supported by CNPq and INPA. The author is the recipient of a research fellowship from CNPq.

References


