

**GILL CHLORIDE CELLS AND GAS TRANSFER
IN FISH EXPOSED TO DEIONIZED WATER**

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Abstract

The effect of the proliferation of gill chloride cell on gas transfer was investigated in *Hoplias malabaricus*, exposed to deionized water for 1, 2 and 7 days. Chloride cell proliferation in the lamellar epithelium increased the water-blood diffusion barrier in all except fish kept in deionized water for 1 day. Oxygen consumption ($\dot{V}O_2$) was significantly increased in fish kept in deionized water for 2 days, but all fish groups studied were able to keep $\dot{V}O_2$ constant until the critical oxygen pressure (PcO_2) was reached. No change in PcO_2 was found in any groups. Respiratory frequency showed significant increase in fish kept for 2 days in deionized water while there was an increase in respiratory volume in fish kept 2 days in same water conditions. The arterial O_2 partial pressure showed a significant decrease in normoxia and hypoxia in fish kept in deionized water. Plasma ions and pH were kept almost constant, although osmolarity decreased significantly in fish kept in deionized water for 7 days. These results confirm that the chloride cells in lamellar epithelium reduce the effectiveness of O_2 uptake in *H. malabaricus*. However, other mechanisms should be involved in its capability to maintain the oxygen consumption up to PcO_2 .

Introduction

The fish gill is a multifunctional organ which serves in the passive exchange of oxygen, carbon dioxide, ions and nitrogenous metabolic products, and is also the site of active ion exchange and transport. As the gill is the main surface interface for the transfer of respiratory gases in most teleost fish, the effectiveness of gill structure depends on the lamellar surface, the distance of water-blood diffusion and the magnitudes and matching of gill ventilation and blood flow. However, the gill structure and its large surface area, which facilitates the respiratory gas movement, may give rise to problems in maintaining the ionic and osmotic steady state in fish body fluid.

The fish gill is organized in a sufficiently flexible way to allow fish to inhabit widely diverse and greatly varying environments (Laurent, 1989) and the gill epithelium generally responds to changes in the internal and external medium. Most studies have been addressed to respiratory or ion regulatory function and, over the last two decades, a number of researches have focused on the relationship between the gill morphology and the impairment of ionic homeostasis and its impact on the respiratory gas exchange (Perry, 1998).

Two epithelia are identified in the gill, i.e., the respiratory epithelium covering the lamella, which represents about 96 % of gill surface, and the non-respiratory epithelium covering the gill filament. Three cell types, pavement (PVC), chloride (CC) and mucous (MC) cells, are the main cells found in the outermost cell layer of gill epithelium. PVC is the most common cell type and is located on both filament and lamellar epithelia. MC and CC are dispersed in the leading and the trailing edges of filament or in the interlamellar regions. In some species, CC are found in the lamellae (Laurent, 1984). Morphological transformation of CC of euryhaline fish in response to transfer from freshwater to seawater and vice-versa has been well documented, as well as the CC proliferation on lamellar epithelium under conditions requiring an increase of ion transport capacity (Laurent 1989).

In seawater fish, the CC is the site of Cl^- and Na^+ secretion; however, the role of CC is not well defined in the freshwater fish. Several studies have evidenced that CC in freshwater fish is the site of Na^+ , Cl^- (Avella et al, 1987; Perry et al., 1992) and Ca^{2+} (Flik e Verboost, 1993) uptake. Considering that the ionic composition of freshwater environments is extremely variable, ranging from almost distilled water to water with high ion concentration, CC proliferation has

been accepted as a common response of fish to ion poor water and, sometimes, high proliferation is found in the lamellar epithelium (Perry, 1998).

Contrary to PVC, the CC are round and large in diameter. Thus, increased numbers of CC on the lamella could potentially impair the gas transfer by modifying the diffusive conductance of the gill's surface due to the increased distance of the water-blood barrier diffusion (Bindon et al., 1994a, b; Fernandes et al, 1998). In this context, the purpose of this study was to evaluate the effect of gill CC proliferation on the oxygen transfer with emphasis on the oxygen uptake in normoxic and hypoxic conditions.

Brazilian freshwaters are generally ion poor and soft, with the exception of a few saline lakes on the coastal regions and the south of Brazil's pantanal (swampland) region. A preliminary analysis on the gills of species living in these environments showed great variability in the distribution, number and apical area of CC (unpublished data). Among these species *Hoplias malabaricus* has a large surface area (Fernandes et al., 1994) with an extremely thin respiratory epithelium consisting of PVC with rare chloride cells at the base of lamella (Fernandes e Moron, 1996). However, the exposure of *H. malabaricus* to water devoid of ion (extreme condition) stimulated CC proliferation (Moron, 2000).

Materials and Methods

Adult *Hoplias malabaricus* weighing between 265 and 565 g were collected in the Mogi Guaçu basin, state of São Paulo, Brazil. The fish were kept in 1000 L tanks with continuous aerated flowing water (water composition: $[Na^+] = 2.06 \pm 0.03$, $[K^+] = 2.52 \pm 0.004$, $[Ca^{2+}] = 4.00 \pm 0.013$, $[Cl^-] = 0.5 \pm 0.04$, pH = 7.0) at 25 °C and photoperiod 12L:12D, for at least 1 month before experiments. Fish were fed with live fish (small fish: *Astyanax sp*).

The fish were divided in two groups (n = 54 each); the control and the deionized water group. The control group was transferred to aquaria containing water with the same characteristics as those of the acclimation period, while the group 2 was transferred to aquaria with deionized water ($[Na^+]$, $[Ca^{2+}]$ and $[Cl^-]$ no detectable). Two thirds of the water of each aquarium was renewed daily. One, two and seven days after their transference, fish randomly selected (n = 8) from each group were submitted to respirometry experiments in normoxia (partial oxygen pressure- $PO_2 = 140$ mmHg) and gradual hypoxia to determine the critical oxygen pressure (PcO_2). Oxygen consumption ($VO_2 - mL O_2 kg^{-1} h^{-1}$)

was determined using a flow-through respirometry system (Rantin et al., 1992). Concomitantly, the respiratory parameters (gill ventilation - V_G - $\text{mL}_{\text{H}_2\text{O}} \text{kg}^{-1} \text{min}^{-1}$, ventilatory volume - $V_{S,R}$ - $\text{mL}_{\text{H}_2\text{O}} \text{kg}^{-1} \text{breath}^{-1}$ and respiratory frequency - f_R - breath min^{-1}) and oxygen extraction from water flow (EO_2 - %) were determined by measuring the inspired (PiO_2) and expired (PeO_2) water taken directly from the buccal and opercular cavities of fish via polyethylene tubes inserted in these cavities.

A second set of experiments was carried out after the PcO_2 was determined. The dorsal aortae of the fish was cannulated using a flexible polyethylene tube (PS 50), the experiments consisted of exposing fish to normoxic water following by gradual hypoxia until the PO_2 reached PcO_2 , in which condition the fish were kept for 30 minutes. Samples of blood (500 μL) were taken in normoxia and hypoxia to determine arterial blood PO_2 (PaO_2), make ion analyses ($[\text{Na}^+]$, $[\text{K}^+]$, $[\text{Ca}^{2+}]$ and $[\text{Cl}^-]$) and determine pH.

After these experiments, the gills of each fish were immediately removed and fixed with 2.5% glutaraldehyde in 0,1 M phosphate buffer pH 7.4 at 4 °C and processed for analysis using light microscopy (LM) and scanning electron microscopy (SEM). Sections of 1 μm thick were used to calculate the mean water-blood barrier distance according to the method described by Weibel and Knight (1964). The test-system to estimate distances in layered structures (Gundersen et al., 1988) were superimposed four times in each picture; an average of 100 measurements were made per fish. Cell surface parameters were analyzed using SEM. The apical CC area was determined according to Bindon et al. (1994b) by tracing the cell perimeter in a calibrated computer screen using a special software (Sigmascan, Jandel Scientific). In addition, the CC fractional area (CCFA), which represents the fraction of the epithelial surface of the gill filament occupied by CCs and cell density, was subsequently calculated using the following equations:

$$\begin{aligned} \text{CCFA} &= \Sigma \text{ area of whole and partial CCs} / 10^6 \text{ picture area} \\ \text{Cell density} &= \text{CCFA} / \text{average whole cell area} \end{aligned}$$

The data were expressed as means \pm S.E.M. and the statistical significance of the differences between control fish and the fish kept in deionized water were determined using the GrapPad Instat v. 3.0 to Windows 95. The non-parametric test Kruskal-Wallis ANOVA for multiple comparisons was used to compare the respirometric data followed by the Dunn Multiple Comparison test with 95 % confidence limits whenever a significance difference occurred. The

hematological and CC morphometric data were based on parametric ANOVA tests and the Tukey-Kramer Multiple Comparison with 95 % confidence limits whenever a significance difference appeared.

Results

Figure 1 shows the effect of the reduction of PiO_2 on VO_2 of control fish and on those kept in deionized water for 1, 2 and 7 days. The VO_2 of fish kept for 48 h in deionized water was significantly greater than that of the controls in normoxia. Independently of experimental conditions, VO_2 was kept constant during gradual hypoxia until it reached the PcO_2 .

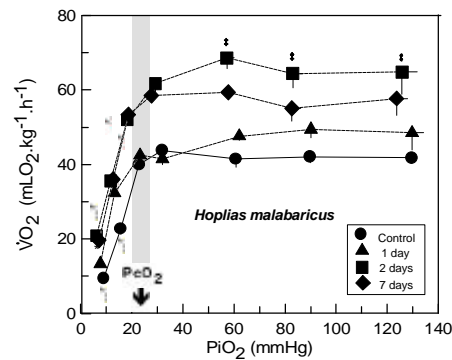


Figure 1. Effect of PiO_2 on the VO_2 in *H. malabaricus* from the control group and group maintained in deionized water 1, 2 and 7 days. The arrow indicates the mean PcO_2 . Points are the means values \pm S.E.M, $n = 8$. (*) indicates significant difference from normoxic data and (+) indicates significant difference from controls.

Below these PiO_2 the VO_2 became dependent upon the environment O_2 availability. No significant difference was found in the PcO_2 of either group and a mean PcO_2 were calculated as $PiO_2 = 21, 6 \pm 0,9$ mmHg. Gill ventilation (V_G) showed no significant increase in normoxia and hypoxia although a significant increase was found in the f_R in fish kept 2 days in deionized water.

The effects of deionized water on PaO₂ is shown in Fig. 2. The PaO₂ of fish kept in deionized water was significantly lower than the control fish. Both the control group and the one kept in deionized water (1, 2 and 7 days) showed a significant decrease in PaO₂ in hypoxia (PiO₂ = PcO₂) compared with normoxic values.

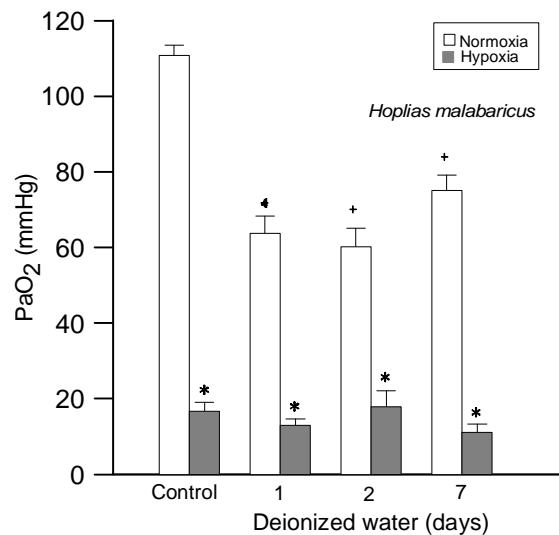


Figure 2. Arterial oxygen partial pressure (PaO₂) in controls and maintained in deionized water *H. malabaricus* in normoxia and hypoxia (PiO₂ = PcO₂). + indicates significant difference (p<0.05) from controls in normoxia; * indicates significant difference (p<0.05) between normoxic and hypoxic conditions in controls and deionized water fish.

Plasma [Na⁺], [K⁺], [Ca²⁺] and [Cl⁻] and blood pH were unaffected in fish kept in deionized water, however osmolarity decreased and was significant lower in fish maintained in deionized water during 2 and 7 days (Table I).

The distance of water-blood barrier on lamellae was smaller in fish kept 1 day in deionized water and greater in fish maintained 2 and 7 days in the same condition (p < 0.05)(Fig. 3). CC proliferated in the fish kept in deionized water and their apical surface area was significantly greater than that of control fish.

Consequently, the fractional surface area of CC on the epithelia also increased ($p < 0.05$).

Table 1. Plasma ion concentration, osmolality and pH in control and fish maintained in deionized water 1, 2 and 3 days. Values are means \pm 1S.E.M.

	Control Group (n=24)	Deionized Water Group (n = 8 each)		
		1 Day	2 Day	7 Day
[Na ⁺] (mEq L ⁻¹)	128.06 \pm 4.6	134.18 \pm 4.2	128.29 \pm 2.9	141.3 \pm 6.6
[K ⁺] (mEq L ⁻¹)	3.7 \pm 0.2	3.4 \pm 0.4	3.2 \pm 0.35	3.7 \pm 0.35
[Ca ²⁺] (mEq L ⁻¹)	1.10 \pm 0.04	1.18 \pm 0.04	1.35 \pm 0.07	1.13 \pm 0.07
[Cl ⁻] (mEq L ⁻¹)	88.11 \pm 6.0	97.8 \pm 10.8	82.5 \pm 9.8	82.9 \pm 9.7
Osmolarity (mmol kg ⁻¹)	278.0 \pm 15.5	255.0 \pm 13.4	238.0 \pm 5.6*	215.6 \pm 11.6*
pH	7.8 \pm 0.04	7.8 \pm 0.07	7.9 \pm 0.04	7.8 \pm 0.02

(*) indicates a significant difference from the control value, $p < 0.05$.

Discussion

The rate of oxygen transfer across the lamellar epithelium is inversely related to the thickness of the water-blood diffusion barrier. The morphometric diffusion capacity given as KA/t where K is the Krogh permeation coefficient, A is the respiratory area and t is the water-blood barrier thickness has been estimated in several fish species (Hughes, 1984). Generally, fish gills with larger surface areas tend to have thinner barriers and consequently much greater diffusing capacities (Hughes, 1984). Thus, the enlargement of the distance of the water-blood diffusion barrier due to CC proliferation in the lamellar epithelium potentially cause an impairment of gas transfer. Early studies have shown a significant reduction of PaO₂ in normoxia and hypoxia (Thomas et al., 1988, Bindon et al. 1994b, Greco et al., 1995).

In the present study, *H. malabaricus* kept in deionized water exhibited two types of responses related to CC proliferation. Fish kept 1day in deionized water did not increase the water-blood diffusion distance although the lamellar thickness increased due to the enlargement of blood spaces and the changes on CC were restricted to those cells in the filament epithelium. Fish kept in deionized water for 2 and 7 days exhibited high CC proliferation in lamellar epithelium and

increased the water-blood diffusion barrier. All fish kept in deionized water showed a significant reduction in P_{aO_2} .

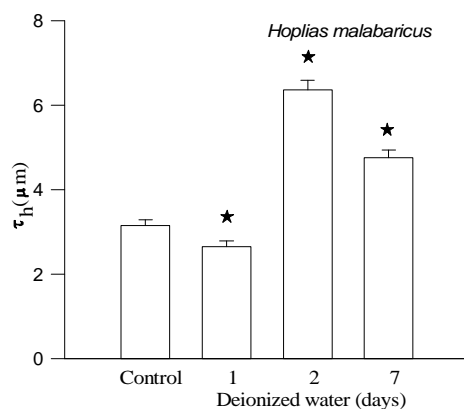


Figure 3. The effect of dionized water on the harmonic mean water-blood barrier distance (τ_h). Values are means \pm S.E.M. (*) indicates significant difference from controls ($p < 0.05$)

However, *H. malabaricus*, whose $\dot{V}O_2$ is low, showed be an oxyregulator species even under this extremely adverse condition. The P_{cO_2} values were similar to those calculated for this species by Rantin et al. (1992), denoting a marked ability to compensate for reduced environmental O_2 pressure. The maintenance of $\dot{V}O_2$ implies the need for ventilatory adjustments in an attempt to keep E_{O_2} from water and, hence, the O_2 cascade from water to tissue. However, E_{O_2} generally decreases with very large ventilation volume which increases the branchial dead space. Increased f_R such as was found in *H. malabaricus* could be a compensatory mechanism to counteract with the potential loss of gas transfer effectiveness associated with changes in the diffusion barrier, as suggested by Greco et al (1995) in their studies with rainbow trout, *Oncorhynchus mykiss* acclimated in softwater. Although, the increase in f_R represents a higher increase in the metabolic cost of ventilation, *H. malabaricus* has lower ventilatory cost than other species already studied (Rantin et al., 1992). Its large respiratory surface area (Fernandes et al., 1994), high O_2 affinity of hemoglobin (Wood and Lenfant, 1979) and high anaerobic capacity (Hochachka et al., 1978) may favor this species to maintain $\dot{V}O_2$.

The plasma ion levels of *H. malabaricus* kept in deionized water remained unchanged despite the significant reduction on plasma osmolarity. These findings are difficult to explain in fish kept in deionized water. Greco et al. (1995) found significant depression in plasma [Cl⁻] in *O. mykiss* acclimated to softwater. Thus, the proliferation of CC and the increase in its apical surface together with the possible activation of other regulatory mechanisms may maintain plasma ion levels in *H. malabaricus* kept in deionized water.

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