

**THE MITOCHONDRIA-RICH CELLS IN THE GILLS OF AIR-  
BREATHING FISHES**

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**EXTENDED ABSTRACT ONLY – DO NOT CITE**

**Introduction**

Mitochondria-rich cells are mainly found in the gill filaments of teleosts. Pavement cells accounts for more than 90% of the gill surface (mostly in the lamellae) and are considered to be the site of gas exchange. In general, mitochondria-rich cells are believed to be the site of ion extrusion by fish in seawater and ion uptake by fish in fresh water. Nevertheless, several studies reported the presence of lamellar mitochondria-rich cells (MRC<sub>L</sub>s) in various teleost species, and MRC<sub>L</sub>s are inducible in a certain species acclimated in fresh water or deionized water. Most of the conclusions on the role of MRC<sub>L</sub>s have been drawn on a species-specific basis and thus far there has been no systematic investigation of their functions. Because of their rather large and oval shape, MRC<sub>L</sub>s will increase diffusion distance and therefore impede gas exchange. If a fish can exchange gases from other parts of the body, the gills may play a more important role in alternative physiological functions such as osmoregulation and acid-base balance. This led our attention to air-breathing fishes which have the ability to exchange gases directly with the aerial environment. In this study, we have included two independent experiments to elucidate the presence of MRC<sub>L</sub>s is correlated with the life history and air-breathing ability of the fish. First, we compared the relative changes in the amounts of filament and lamellar MRCs in air-breathing and non-air-breathing fishes. Second, the distribution of both MRC<sub>F</sub>s and MRC<sub>L</sub>s in 12 orders, 28 families, 56 genera and 67 species (including 30 species from the literature) was examined by scanning electron microscopy, zinc-iodide osmium staining (ZIO) or immunofluorescent staining.

**Experimental Designs**

*The Relative Amounts of MR Cells in Filaments and Lamellae*

Six fish species were included in this experiment. They were *Colisa lalia*, *Trichogaster trichopterus*, *Periophthalmus cantonensis*, *Oreochromis mossambicus*, *Monodactylus argenteus* and *Toxotes jaculator*. The first three species were air-breathers, while the latter three were not. For every fish species, at least four individuals from each water condition were collected for MR cell determination.

#### *The association between lamellar MR cells and air-breathing ability in 67 fish species*

For analysis purpose, fish were classified into (1) air-breathing fish, (2) fish species that have never been described as air-breathers but belong to the families with known air-breathing species, and (3) non-air-breathing fish according to Graham (1997). The association between air-breathing ability and the distribution of  $MRC_{LS}$  was tested using a 2×2 Fisher exact statistical analysis. The experiments and handling on the animals comply with the current laws of Taiwan, ROC.

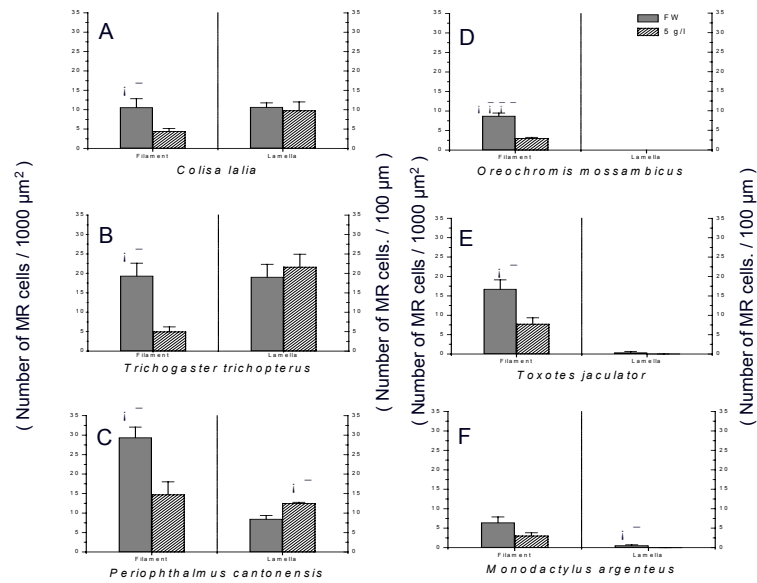
### **Results**

For both *C. lalia* and *T. trichopterus*, a significantly higher amount of  $MRC_F$  was found in those from freshwater than from 5 g L<sup>-1</sup> (Fig. 1). However, this difference was not found in the numbers of lamellar MR cells between the two media in either species. *Periophthalmus cantonensis* is an amphibious, peripheral freshwater fish. The amount of MR cells in filaments was significantly higher in freshwater condition. On the contrary, lamellar MR cells were significantly increased in 5 g L<sup>-1</sup> condition than in freshwater condition (Fig. 1).

The secondary freshwater fish, *O. mossambicus*, had no MR cells in the lamellae, and the amount of  $MRC_F$  was significantly higher in the freshwater condition than in the 5 g L<sup>-1</sup> condition. In the two peripheral freshwater fish, *M. argenteus* and *T. jaculator*, very few lamellar MR cells can be found at the base of the lamellae (Fig. 1). A significant higher amount of  $MRC_F$  was found in freshwater condition for *T. jaculator*, while there was no significant difference in the number of  $MRC_F$  between two water conditions for *M. argenteus* (Fig.1).

There were 27 air-breathing fish, 37 non-air-breathing fish and 3 species that have never been described as air-breathers but belong to the families with known air-breathing species according to Graham (1997). There was a significant association between the presence of  $MRC_{LS}$  and the mode of breathing on all three levels of systematic categories (species, genus and family) (Table 1).

Figure 1. The numbers of filament and lamellar MRCs in air-breathing and



non-air-breathing fish..

Table 1. The association between the presence of MRC<sub>L</sub>s and air-breathing ability at (a) species-, (b) genus- and (c) family-levels. \* Significance calculated using 2×2 Fisher exact probability.

	Presence of MRC <sub>L</sub> s		P*
	Yes	No/Few	
<b>a. SPECIES</b>			
Air-breathing species	18	7	3.88×10 <sup>-7</sup>
Non-air-breathing species	2	30	
Species in the families with known air-breathing species	1	2	
<b>b. GENERA</b>			
Air-breathing genus	16	4	4.81×10 <sup>-8</sup>
Non-air-breathing genus	1	26	
Genus in the families with known air-breathing species	1	2	
<b>c. FAMILIES</b>			
Air-breathing family	5	3	0.011
Non-air-breathing family	1	13	

## Discussion

There has been little discussion as to which kind of fish species to be more likely to possess  $MRC_{LS}$ . Whether there is a functional differentiation between gill filaments and lamellae is constantly under debate. In the present study, it appeared that unequal changes in the amounts of  $MRC_{FS}$  and  $MRC_{LS}$

were found in the three species of air-breathing fish acclimated to fresh water and  $5 \text{ g L}^{-1}$ . However, when the multicellular complex of  $MRC_{FS}$  found in saltwater was taken into account, we may have underestimated the amount of  $MRC_{FS}$  in  $5 \text{ g L}^{-1}$  condition and the difference between the relative changes of MRCs in both filaments and lamellae became insignificant. Therefore, our results supported the discussion by Laurent and Perry (1990) that filament and lamellar MRCs have similar functions due to their concurrent change in number.

According to Graham (1997), air breathing is an ancient trait evolved independently in many fishes and there is a great diversity in this bimodal respiratory specialization. Of course, the degree of air-breathing should not be taken as a dichotomous trait but rather a continuous spectrum. But, in this study, for the convenience of statistical analysis and the availability of sample size, we only classified the species into air-breathers and non-air-breathers. Gills, as a multifunction organ, should constantly have their functional differentiation. That is, extra-branchial gas exchange may lead to an increase in the role of ion regulation in gills. Therefore,  $MRC_{LS}$  which would greatly impede respiration were found.

In the present study, only those species that have been documented as air-breathers were included in the analysis. For the species that have never been reported to be air-breathers but belong to the air-breathing families, a better description on their life histories is needed. More studies on the physiology of the air-breathing fishes from an integrative approach would allow a comparison of the relative increase in both  $MRC_{FS}$  and  $MRC_{LS}$  upon salinity change in air-breathing and non-air-breathing fishes.

## Acknowledgements

This study was supported by National Science Council (NSC 89-2311-B-029 -006) and Academia Sinica (Major Group-Research Project), Taiwan, R.O.C. to HCL.

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