Histopathological analysis of the respiratory organs of *Channa striata* subjected to air exposure

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**ABSTRACT**

Effects of air exposure on the respiratory organs of *Channa striata* possessing bimodal respiration for exploitation of water (via gills and skin) as well as air (through air-breathing organs - suprabranchial chamber, ABOs) have been investigated. On air exposure the fish survived for 8 h. Following air exposure the fine, thin-walled blood capillaries (BLCs) at the surface of the ABO swelled and bulged out due to congestion when the blood came very close to air in the lumen. In the initial periods, mucous cells (MCs) of all three respiratory organs showed periodic fluctuations in their density and staining properties and stain for sulphated moieties known to hold an additional quantity of water. The sub-epithelial connective tissues of the ABO and skin also contained a large quantity of sulphated mucopolysaccharides. Subsequently, severe wear and tear and sloughing leading to haemorrhage took place from the skin. The outer cellular layers of the epidermis sloughed off. The density of sacciform-granulated cells (SGCs) increased and stained strongly with PAS technique (almost negative in controls). Air exposure also caused extensive damage in the gills. In the initial periods the BLCs showed severe congestion, causing extensive bulging and protrusion onto the surface. Later, the epithelial linings of gill filaments (PL) as well as respiratory lamellae (SL) were detached and lifted up. Subsequently, the neighbouring SL fused, causing decreased surface area, thereby reducing the efficiency of gills. The ladder-like arrangements of the pillar cells - blood capillaries (PLCs-BLCs) also collapsed. PAS-positive materials appeared within these PLCs. Subsequently, the BLCs dilated and showed congestion. The RBCs of gills also showed PAS staining. A thin layer of sulphated slime often covered the respiratory epithelia. Prior to death of the fish, the cells of the gills degenerated extensively. Thus, air exposure also prevented normal branchial respiration and the fish died due to anoxia and other physiological disorders.

**Key words:** air-exposure, *Channa striata*, desiccation, histopathology, respiratory organs

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Introduction

*Channa striata* inhabits O$_2$-deficient muddy and marshy waters (GUENTHER, 1880). All members of the family Channidae have acquired the capacity for gas exchange with water in their gills and skin, as well as with air, through their suprabranchial chamber (air-breathing organs, ABO) (MUNSHI, 1962; ISHIMATSU and ITAZAWA, 1981; ISHIMATSU and ITAZAWA, 1993). This enables these species to survive during extended periods of being buried in moist soil (HORA and PILLAY, 1962). These obligate air-breathing fish are also known to survive in a state of torpor in semi-fluid mud or below hard-baked mud crusts (GUENTHER, 1880). Also, it has been a regular practice on the Indian sub-continent to transport these fish to markets in bamboo baskets, and the fish remain alive even when kept out of the water for prolonged periods.

Therefore, in this paper efforts have been made to understand how the structural adjustments in all the three respiratory organs (ABOs, skin, gills) helped the fish extend their survival period when they face conditions of extreme drought, desiccation and air exposure. While the gills are the main organs for respiration, ABOs and the highly vascularized skin constitute the accessory respiratory organs (AROs) (Fig. 1). The ABOs are located dorsally to the gill arches (Fig. 1). Anteroventrally, the ABO opens into the buccal cavity and posterolaterally into the opercular chamber.

![Channa striata and its accessory respiratory organs](image)

Fig. 1. *Channa striata* and its accessory respiratory organs
Materials and methods

Healthy specimens of *C. striata* (18-20 cm in length) belonging to a single population were acclimated in the laboratory for 25 days prior to experimentation. Ten groups of ten fish each were removed from water and placed on separate dry plastic aquaria in the laboratory for exposure to the air (atmospheric humidity 72%, room temperature 29 to 32 °C) for 8 h, beyond which they could rarely survive. The fish were assumed to be dead when they did not respond to shaking by glass rods and failed to revive when returned to water. Control groups were retained in tap water.

At regular intervals of air exposure (0, 2, 4, 6, 8 h) 5 living fish from each experimental group, as well as control aquaria, were sacrificed by decapitation. The second gill holobranch, along with the entire suprabranchial chamber from both sides of the fish, were fixed in absolute ethanol, aqueous Bouin’s fluid and 10% neutral formalin. Skin fragments, 6 mm x 6 mm, from the dorsal surface of the body between the anterior part of the dorsal fin and lateral line canal were also fixed. Six µm paraffin sections were stained with Ehrlich’s haematoxylin and eosin (H/E) for histopathological analyses. Certain carbohydrate moieties were stained by periodic acid-Schiff (PAS), alcian blue pH 2.5 (AB 2.5), AB 2.5/PAS, alcian blue pH 1.0 (AB 1.0) techniques (PEARSE, 1985). The entire experiment was repeated three times.

Results

The ABO of control fish was lined by a stratified epithelium (Figs 2, 4). The underlying connective tissues were also richly vascularized with a large number of fat cells (Fig. 2). Fine sub-epithelial blood capillaries penetrated into the epithelial lining (without breaking open the basement membrane) where they anastomosed extensively and reached to the surface of the ABOs (Figs 2, 3). These minute blood channels (BLCs) terminated in the form of vascular papillae (VP) (Fig. 3). Supporting epithelial cells (ECs) separated the neighbouring VP. The aerial surface (the projecting part) of the papilla was surrounded by a thin (one or two EC thick) respiratory epithelium (RE). The epithelial lining was provided with a large number of strongly PAS, moderately to strongly AB 2.5 and faintly AB 1.0 positive mucous cells (MCs) (Fig. 4) which took a dark greenish-
S. Chandra and T. K. Banerjee: Histopathological analysis of the respiratory organs of *Channa striata* subjected to air exposure

violet colour with AB 2.5/PAS techniques. The MCs were present mostly at the outer surface of the epithelium, where their contents very often formed patches in the form of a slimy layer.

Between 2-4 h of exposure, the BLCs forming the VPs swelled and bulged out at the surface of the ABO due to intensive engorgement with seven or more RBCs. The blood capillaries coursing through the epithelium towards the surface (Fig. 5) also showed congestion. Subsequently,
due to a bulged finger-like projection, the BLCs sometimes gave the false appearance of SL of a gill (Figs 6, 8).

The density and staining properties of the MCs fluctuated at many stages of exposure. After 2 h and 6h onwards, large numbers of voluminous MCs stained strongly with AB 1.0 and dark greenish-blue with AB 2.5/PAS were located throughout the epithelium (Figs 5, 6). However, after 4 h they were aggregated mostly in the ML (Fig. 5) with decreased staining intensities. Subsequently, after 8h the number of the MCs decreased substantially (Fig. 9).

The chemical morphology of the sub-epithelial connective tissues also became altered following air exposure (Figs 7, 9). After 2 h they stained strongly greenish blue with AB 2.5/PAS (Fig. 7). With AB 1.0, strongly stained connective tissues remained restricted to certain patches only. The intensity of AB 2.5/PAS reaction decreased marginally after 4h. The AB 1.0 positive areas, however, increased after 4h onwards after exposure.

**Skin.** The skin of *C. striata* is made up of three layers: the epidermis, dermis and subcutis (MITTAL and BANERJEE, 1975a, MITTAL and BANERJEE, 1975b) (Figs 10, 11). The epidermis is a stratified epithelium whose cellular constituents include epithelial cells (ECs), sacciform granular cells (SGCs), MCs and a few ionocytes (Chloride cells) (Figs 10, 11). The epidermis is sub-divided into three layers: an outermost layer (OL), a middle layer (ML) and a basal layer (BL) (Fig. 10). The OL is made up of several layers of
ECs. At the surface chloride cells, OL, MCs, and SGCs open (Fig. 10). The ML is composed of unicellular glands, MCs and SGCs. ECs fill the spaces between the gland cells (Fig. 10). A single row of low columnar ECs constitutes the BL (Fig. 10). The MCs stain moderately with PAS and
strongly with AB 2.5. The contents of the SGCs are mainly proteinaceous in nature. The scaly dermis (Fig. 11) is made up of an outer loosely arranged \textit{stratum laxum} and a compactly arranged \textit{stratum compactum}. The sub-epidermal connective tissues are richly vascularized. A small amount of acidic (AB 1.0 negative) mucopolysaccharides is also present in this layer.

Within 2 h, the MCs exposed skin discharged their contents profusely (Fig. 12) that formed a thick AB 1.0 and 2.5 positive coating. While the
MCs remained unstained with PAS during the entire period of exposure, most of them stained moderately to strongly with AB 1.0 (Fig. 14) and weakly to moderately (peripheries darker) with AB 2.5 after 2 h. With AB 2.5/PAS, the same MCs took on a moderate greenish-blue colour. Air exposure also caused hyperplasia of ECs in the lower layers (ML and BL). Due to decreased density of the AB 2.5 positive MCs, the quantity of slime (AB 1.0 negative and AB 2.5 positive) on the body surface decreased. However, a large number of voluminous sac-like moderately to strongly AB 1.0 positive MCs reappeared (Figs 13, 14). The SGCs at this stage showed increased density and reached the surface layer, with their bulky body extending deep into the ML (Fig. 13). Their secretory contents became basophilic and stained weakly to moderately magenta with PAS and AB 2.5/PAS preparations. After 6 h, the epidermis showed wear and tear, enormous shrinkage and the ECs, especially in the ML, became flat spindle-shaped (Fig. 15). The MCs stained faintly with AB 1.0 and AB 2.5 and in many places became irregularly formed. Very often, they poured their contents onto the surface which formed a thick, weakly AB 2.5 positive coating (Fig. 16). The density of the SGCs decreased greatly (Fig. 15). The flat, compressed ECs at the surface started sloughing (Fig. 15) which was further aggravated after 8 h when extensive peeling-off of the outer layer (Fig. 16), along with a thick coating of strongly AB 1.0 positive slime (Fig. 17), took place. With AB 2.5, the MCs stained faintly. A large number of AB 1.0 positive MCs regenerated in the lower layer.
Following exposure, a large number of fine blood capillaries appeared in the loose connective tissues that were noticeably prominent after 4 h. The moderately basophilic sub-epidermal connective tissues stained weakly to moderately greenish-blue with AB 1.0, AB 2.5, and light magenta with PAS and bluish-pink with AB 2.5/PAS techniques after 2 h. However, the fine connective tissue fibrils, along with the ground substance just above the scale surface, stained moderately with PAS, very strongly (greenish-blue) with AB 1.0 and AB 2.5 and AB 2.5/PAS. After 4 h, the staining intensity of the entire sub-epithelial connective tissues decreased. However, after 8 h the staining became strongly positive with all the above-mentioned techniques.

**Gills.** The vascular components of the secondary lamellae (SL) in control gill (or after 0 h of exposure) are made up of alternately arranged pillar cells (PLCs) - blood channels (BLCs) that remained covered by a thin respiratory epithelium (RE). The MCs are mostly observed in the epithelium of the gill filaments or primary lamellae (PL) (Fig. 18). A few saucer-shaped MCs are also present in the SL. The MCs take on a dark greenish-black colour with AB 2.5/PAS (Fig. 19). The MCs on the PL stain light greenish-blue, with their periphery taking on a dark blackish-green colour with AB 2.5/PAS. They stain moderately to strongly with AB 2.5 and weakly to moderately with AB 1.0. No PAS positive MC was noticed in PL or SL.
Extensive inter-cellular vacuolisation with widespread hyperplasia of cells of the epithelial linings of PL and SL resulted in their increased thickness after 2 h of exposure (Fig. 20). Lifting of the epithelial lining both from the PL as well as SL was very commonly observed (Fig. 20). BLCs of the SL became considerably engorged with RBCs which stained positively with PAS method. The density of the MCs (both in SL and PL) increased greatly (Fig. 20). These cells stained negatively with PAS (Fig. 20), moderately to strongly with AB 2.5 and AB 1.0, and greenish-blue with AB 2.5/PAS techniques.

Vacuolisation aggravated further after 4 h (Fig. 21). At this stage, a good number of round, large vacuoles of uniform size began appearing in the PL, as well as SL. A small amount of basophilic slimy substance stained positively with AB 2.5 and AB 1.0 was frequently observed, especially in the inner lining of these vacuoles. The size and density of these vacuoles in the hyperplastic PL in general and SL in particular increased in the subsequent stages (Figs 22, 23). The BLCs of the SL, however, became greatly dilated and engorged with RBCs after 6h (Fig. 23). Often, more than one of the BLCs, especially those at the base of the SL, became fused together at certain stages (Fig. 21). Even though mild wear and tear of the epithelial linings of the PL and SL was noticed at this stage, no haemorrhage or rupture of the BLCs was detected.
The density of the MCs decreased slightly after 4h. A slightly basophilic fuzzy substance regularly sloughed from the surface of the SL. However, a thin layer of AB 2.5 positive (AB 1.0 and PAS negative) mucus coated the surface of the SL between 4 to 6 h of exposure. After 6h, neighbouring SL merged. At several sites the typical ladder-like arrangement of the PLC-BLC collapsed (Fig. 23). However, the gills regenerated partially and regained some of their lost staining properties at several other sites. The blood capillaries running through the gill filament showed extensive congestion and engorgement with a large number of RBCs. After 8 h, the adhesion and merging of the neighbouring SL was extensive, with regular lifting of the RE from the entire surface of the SL. However, the density of the RBCs within the BLCs decreased greatly. The density of the MCs decreased further at this stage, with loss of the slimy coating over the SL.

**Discussion**

The present study indicates the supra-branchial chamber of *C. striata* to be a less efficient ABO for aerial respiration than those of *C. batrachus* (CHANDRA, 2001) and *H. fossilis* (PARASHAR and BANERJEE, 1999a; PARASHAR and BANERJEE, 1999b; PARASHAR and BANERJEE, 1999c); when taken out of water, *C. striata* survives for 8 h only, while *H. fossilis* subsists 17 h; *C. batrachus* remain alive for 27-31 h. This is because air ventilation in
Channa needs water as an essential compliment; hence, the efficiency of ABO is reduced considerably when the fish is taken out of water (ISHIMATSU and ITAZAWA, 1993). In Channa, expiration precedes inspiration (ISHIMATSU and ITAZAWA, 1981). During the expiratory phase, water is drawn into the ABO through the gill cavity, translocating the gas into the anterior buccal cavity. Subsequently, for expiration the fish opens its mouth at the water surface, leaving no residual gas in the organ. Inspiration starts with depression of buccal floor and adduction of the opercular cavity, while the branchioostegal membrane closes the gill opening. The air thus introduced into the buccopharyngeal cavity is subsequently translocated into the ABO. Hence, presence of water is always needed for continuation of the process of aerial respiration in C. striata. Also, this slows down significantly as soon as the fish is removed from water. The early but extensive damage of the skin of C. striata (earlier than that of C. batrachus, CHANDRA, 2001) may be another reason for early death of the species.

The BLCs of the gills of the exposed C. striata show enormous congestion. This perhaps brings additional numbers of RBCs to aerial O₂ for gaseous exchange to compensate the loss of water breathing, at least temporarily.

The sub-epithelial network of the blood capillaries of the ABO simultaneously becomes engorged with RBCs following the stress of air exposure. All these structural adaptations narrow the barrier distance between the atmospheric air and the blood in the ABO to supplement the failure of branchial respiration. JOHANSEN (1970) observed that that gill breathing in the primitive air-breather Amia calva varied reciprocally with O₂ tension of the ABO, because blood bypasses the gas exchange blood vessels of the gills when the gills are not the primary site for the uptake of O₂. Engorgement of the BLCs (VPs) of the ABO and gills in air exposed fish support this view. However, the gills later showed extensive damage (e.g. merger of neighbouring BLCs (Fig. 21), fusion of neighbouring SL and lifting of the RE (Fig. 20) which resulted in failure of branchial respiration in the air. Formation of such non-tissue spaces (Fig. 21) following lifting of RE often slows down, at least temporarily, the evaporation of water across the gill surface. However, this also decreases the diffusion distance for gaseous exchange. Thus, the ultimate death of
fish may be due to failure of respiration, haemorrhage and desiccation, along with the collapsing of several other important physiological processes (e.g. CO$_2$ and N$_2$ elimination). Many of the above mentioned histopathological alterations observed under the stress of air exposure greatly resemble those also observed in the gills of several air-breathing fish exposed to different ambient xenobiotics (ROY and MUNSHI, 1992; DUTTA, 1997; DUTTA et al., 1996; HEMALATHA and BANERJEE 1997a; HEMALATHA and BANERJEE 1997b; PARASHAR and BANERJEE; 1999a; PARASHAR and BANERJEE, 1999b; PARASHAR and BANERJEE, 2002). While O$_2$ absorption is the dominant feature of air-breathing (TODD and EBELING, 1966), aquatic breathing remains very important in CO$_2$ (JOHANSEN, 1970) and N$_2$ elimination.

Continuous elaboration of the slime also keeps the respiratory surfaces clean as the mucus facilitates removal of a part of trapped intoxicating agents, pathogens and foreign matters from the surface of the gills (POWELL et al., 1992), skin (BANERJEE, 1993) and ABO (present study), especially when they are outside water. However, this protection does not last long as the MCs become exhausted following continuing exposure. This causes exfoliation of necrotic ECs (Fig. 16). Like amphibians, the skin of C. striata also acts as an accessory respiratory organ (MITTAL and BANERJEE, 1975a; MITTAL and BANERJEE, 1975b) because the quantity of sulphated mucopolysaccharides in the sub-epithelial connective tissues of the dermis (and ABOs also) of this fish increases during air exposure. According to PARASHAR and BANERJEE (1999a) the problem of CO$_2$ retention in fishes could also be partially solved by their moist skin, which contains a large amount of sulphated mucopolysaccharides in the subepithelial connective tissues (MITTAL and MUNSHI, 1971; MITTAL and BANERJEE, 1974; MITTAL and BANERJEE, 1975a; MITTAL and BANERJEE, 1975b).

The SL of gills of C. striata give the appearance of a large number of prominent, voluminous round, empty space-like vacuoles after 4h and onward of exposure (Figs 22, 23). Ammonia is the major excretory product in a large number of air breathing fishes, including Channa (SAHA and RATHA, 1989). It appears that these vacuoles in the gills of this fish may lodge this metabolic waste gas (ammonia) because elimination of ammonia from the surface of the gills during air-exposure is not possible in absence of water. SAHA and RATHA (1989) also noticed that due to the absence of the
enzyme argininosuccinate synthetase of the ornithine-urea cycle in the liver and kidney, active conversion of ammonia into urea was not possible in *Channa*. Thus, as a compensatory measure, these vacuole-like spaces may have developed to accumulate the toxic ammonia or amino acids in which the free ammonia very often becomes converted in living systems. As already mentioned, the survival period of air exposed *C. striata* is much less than that of *C. batrachus* and *H. fossilis*. Another reason for the early death of the air exposed *C. striata* may perhaps be due to continuation of toxicity of ammonia which in the other two fishes (*C. batrachus* and *H. fossilis*) become de-toxified by being converted into urea (SAHA and RATHA, 1989).

**Conclusion**

The fish survived out of water for several hours due to prolongation of aerial respiration through their accessory respiratory organs, which also failed to function subsequently. However, the fish died not only due to failure of respiratory mechanisms but also due to retention of toxic metabolites, including nitrogenous waste (ammonium) products.

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S. Chandra and T. K. Banerjee: Histopathological analysis of the respiratory organs of *Channa striata* subjected to air exposure


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Izloženi su učinci izlaganja zraku dišnih organa ribe dvodihalice *Channa striata* koja za disanje koristi vodu (putem škrga i kože) ili zrak (putem organa zračnog disanja odnosno suprabranhijalne komore). Izložene zraku ribe su preživjele 8 sati. Nakon izloženosti zraku, nježne krvne kapilare tankih stijenki na površini organa zračnog disanja, odebljale su i izbočile se zbog kongestije kad je krv došla vrlo blizu zraku u lumenu organa. Sluzniène stanice svih triju dišnih organska pokazivala su u početnim razdobljima povremeno kolebanje u gustoæi i sposobnosti bojanja sulfatnih dijelova poznatih po zadržavanju dodatne kolièine vode. Subepitelno vezivno tkivo organa za zračno disanje i kože također je sadržavalo veliku kolièinu sulfatnih mukopolisaharida. Kao posljedica velike istrošenosti i ljuštenja javilo se krvenje na koži. Vanjski slojevi stanica epidermisa su se ljuštili. Gustoæa vreæastih zrnatih stanica se poveæala i stanice su se snaæno obojale PAS metodom (kontrola gotovo negativna).

Izloženost zraku uzrokovala je i opseæna ošteæenja škrga. Krvne kapilare su u početnom razdoblju bile zadebljane, nabubrene s izboèinama na površini. Kasnije su se bazalni epitel škrganih listiæa i diæni nabori odvojili i otkinuli. Potom su se susjedni diæni listiæi spojili dovodeæi do smanjenje površine, a time i do smanjene uèinkovitosti škrga. Palisadni poredak stupèastih stanica krvnih kapilara takoðer je propao. PAS pozitivni materijal pojavio se u stanicama škrganih listiæa. Nakon toga su se tanke stijenke krvnih kapilara proširile i zadebljale. Diæne stanice su se takoðer obojale PAS metodom. Tanak sloj sulfatne sluzi æesto je prekrivao diæni epitel. Neposredno prije uginuæa škrgane stanice su degenerirale. Tako je izloženost zraku takoðer sprijeæila normalno branhijalno disanje i ribe su uginule zbog nedostatka kisika i drugih fizioloških poremeæaja.

**Kljuène rijeèi:** izloženost zraku, *Channa striata*, isušenje, histopatologija, diæni organi

52

*Vet. arhiv* 74 (1), 37-52, 2004