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Morphological Analysis on Air-Breathing Fishes: An Overview

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Abstract*: This article reports about the air-breathing fishes which deals with morphology, histochemical analysis of respiratory members and muscles, morphometrics and development of respiratory organts, hematology and other parameters. The scientific significance and application of the studies of functional morphology and physiology in understanding the alteration caused by pollutants have also been elucidated*

Keywords: air-breathing

I. INTRODUCTION

The air-breathing structures appeared in freshwater fish during the late Silurian or early Devonian period and are thought to have evolved as adaptation to hypoxic water conditions due to severe periodic droughts (Smith 1931;Johansen 1970).Recent studies on air-breathing fish have demonstrated that various types of morphological and physiological adaptations have made it possible for fish to utilize aquatic and aerial respiration. The morphological and physiological adaptations in these fishes are designed in a manner so that they derive maximum advantage of their surrounding environment. There is an intimate relationship among the physico-chemical characters of habitat, nature of biota, food chain and finally the morpho-physiological adaptation of animals.

II. MORPHOLOGY

The structures of the different types of respiratory organs have been studied for many years by several scientists. Munshi (1961, 1962, 1962a, 1968), Hughes and Munshi (1973), Dutta (1968), Jordan (1976), Peters (1978), and Burggren (1979) found/ that certain air-breathing fishes such as *Clarias batrachus, Heteropneustes fossilis*, Blue gourami, *Trichogaster trichopterus*, *Anabas, Macropodus* and *Betta* possess labyrinthine air-breathing organs. These organs are derived from the epibranchial regions of the first and second branchial arches, and extend dorsally as plate-like organs to fill the suprabranchial chamber and the air-sacs which are the extensions of the opercular cavity or branchial chamber. The earlier hypothesis of Munshi (1962b) that the "respiratory islets" of *H.fossilis* and *C. batrachus* are modified lamellar structures has been confirmed later by electron microscopic studies of Hughes and Munshi (1973a). According to them, the cells forming the vascular spaces in the dendritic organs and respiratory islets are typical pillar cells of the gills.

Although the suprabranchial chambers in the Anabantidae group are extensions of branchial chambers and the labyrinthine organs which have developed on the epibranchials, the "respiratory islets" of the accessory respiratory organs have evolved in different fashion. The hypothesis that the labyrinthine organs and the respiratory islets of the suprabranchial chambers represent modified gill structure (Munshi 1968) is now no longer tenable as the electron microscopic studies of Hughes and Munshi (1973a) revealed that in *Anabas testudineus* the pillar cells of the air-breathing organs do not have the same relationship as in gills, but are modified epithelial cells. Obviously we are dealing with analogous rather than homologous structures that are used to serve the same function, supporting the contiguous vascular units which make up these respiratory organs. Such a supporting function is clearly of importance. During evolution, different structural arrange-ments have been selected to provide surfaces with minimum diffusion barrier between blood and the respiratory medium (Hughes and Munshi 1968). Further, the blood capillaries of the respiratory islets of Anabas are unique structures, having a series of most characteristic type of unicellular valves ever discovered in any animal system so far (Hughes and Munshi 1973a). These valves control the movement of blood through the respiratory islets. In *Amphipnous cuchia* and *Channa* sp. the suprabranchial chambers are the extensions of

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the pharynx. The vascular mucosa of the air-sacs have evolved independently (Hughes and Munshi 1973a) and not from the gill lamellae as it appears in the histological preparations under light microscope (Munshi 1962a; Munshi and Singh 1968).

In Amphipnous (Monopterus) cuchia, the structure of the valve is very unique. It projects freely into the papilla lumen and appears to be involved in the regulation of blood flow through individual papilla (Hughes and Munshi 1973a). These structures were discovered for the first time and are not only new contributions to knowledge, but open up new lines of further research.

In Periophthalmus vulgaris and Boleopht-halmus boddaereti, opercular chambers are modified for air-breathing purposes. The accessory respiratory organs of P. vulgaris provide an excellent example of adaptation by modification of opercular chambers (Singh and Munshi 1969). In this fish, the opercular chambers have enlarged and become vascularized for respiratory purposes. Intricate mechanisms for opening and closing of the inhalant and exhalent apertures have evolved and the branchiostegal apparatus has developed a special type of safety valve which is workable by stripes of muscles. Further, the studies on ultra structure of gills of these air-breathing fishes have made new contributions to our knowledge. The earlier hypothesis of Munshi and Singh (1968a) that the pillar cells are modified smooth muscle cells have been confirmed (Munshi 1976).

Some other forms of accessory gas exchange organs have evolved in numerous groups offish in order to obtain oxygen from air. These air-breathing organs may be in the form of modified swimbladders, pharyngeal cavities, stomach and intestine (Johansen 1966; Munshi 1976; Singh 1976). Kramer and McClure (1980) found that similar to other callichthyids, the posterior end of the intestine of Corydoras aeneus works as an accessory respiratory organ and its anterior end is provided with a muscular bulb. Generally, these organs are utilized for oxygen uptake and gills are used for the elimination of CO_2, and these processes seem to be similar to aquatic respiration (Randall et.al 1978; Burggren 1979).

It has been observed by Jordan (1976) that some air-breathing fishes are facultative air-breathing and can survive indefinitely on dissolved on dissolved oxygen, while some such as *Protopterus, Lepidosiren* and *Electrophorus* are obligate air-breathers that will drown when access to air is denied. She also found that Clarias batrachus is a bimodally breathing teleost. C. Batrachus possesses an air-breathing organ with highly branched dentritic organs or respiratory 'trees' that develop as outgrowths of the second and fourth gill arches, and are located in the suprabranchial chamber.

III. HISTOCHEMISTRY

3.1 Histochemical Studies Of The Respi-ratory Membrance

A complete knowledge of the cellular structure of the respiratory membrane of the air-breathing organ is essential to understand its physiology. The histochemical study of the respiratory membrane reveals five kinds of specialized cells: (a) mucous cells, (b) adicophil granular cells, (c) basophil mast cells, (d) large bi-or trinucleate glandular cells, and (e) mitochondria rich cloride cells in the gills and accessory respiratory organs of many air-breathing fishes (Munshi 1960). The typical goblet type of mucous glands are present in large numbers in freshwater fishes such as, Catla catla, Labeo rohita, Channa punctatus, Mastracembellus armatus, Clarias batrachus, Heteropneustes fossilis. In C. catla these respond to the chloride test (Munshi 1964). This means that besides mucous secretion, they also play an important role in chloride regulation. In air-breathing fishes *viz* C. *punctatus,* C. *batrachus* and *H. fossilis* only few mucous cells give positive reaction with Ag N03/HN03 test for chloride. The medullary hormones of adrenal have profound effect on the mucous cells of air-sacs and the gills of *H. fossilis* and M. *aculeatum* respectively (Guha *et al* 1967).The sulphated acid mucopolysaccharide component of the mucous keeps the air-sac moist and lubricated during gaseous exchange.

The acidophil granular cells of the gill epithelia are diastase resistant, PAs-positive. Those belonging to the connective tissue system are PAs-negative, however, almost all the granular cells are PAs-positive after extraction of lipids. The granules of these cells are composed of tyrosine-rich protein. The cells also appear to contain a large amount of RNA. The eosinophilic granular cells appear to contain carbohydrate, protein and lipid firmly bound with each other. Some of the cells give positive reactions for alkaline phosphatase. These cells do not respond to $AgN0_3/HN0_3$ test for chloride (Singh and Munshi 1968).

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The basophilic mast cells are present in large numbers in the sub-epithelial connective tissues of the gill lamellae of *Hilsa ilisha.* They are closely associated with blood capillaries (Munshi 1960). It is quite meaningful that mast cells (which are reservoirs of heparin and histamine) are found in the gills of fishes.

The bi- and trinucleate glandular cells are found in the gills of siluroid fishes. They have been derived from the glands of skin of these fishes (Munshi 1960). The cytomorphosis of these glands has been studied for the first time and throw light on their origin (Mittal and Munshi 1970).

Chloride cells are found in good numbers in many of the air-breathing fishes *viz Anabas* and *Clarias* which live in brackish waters also. The endoplasmic reticulum is very well developed and a large number ofmitochondria is found in these cells (Hughes and Munshi 1973a)

Large amounts of reserve fats have been discovered in true air-breathing organs of amphibious fishes (Singh *et a11973).* The stored lipids lie in the well-developed fat cells of the connective tissue layer between the respiratory islets and the muscles of the airsacs. A direct correlation exists between the vascularity of the organs and the concentration of the fat globules, and they contain both acidic and neutral fats. The pharmacological action of adrenalin and atropine was effective in bringing about complete mobilization of the fat deposits of the air-sacs of *Saccobranchusfossilis in vivo* condition.

3.2 Enzyme Histochemistry Of The Respiratory Muscles

The gill ventilation is under the influence of buccal pressure and opercular suction pumps. These pumps are operated by means of a series of respiratory muscles. The enzyme histochemistry of these respiratory muscles has opened up a new field of investigation and it also reveals that the muscles operating these respiratory pumps are composite in nature. Red, white and intermediate muscle fibers have been distinguished in the respiratory muscles depending on their intensity of reaction for succinic dehydrogenase (Munshi *et a11975).* It has been noted that the muscles innervated by the facialis nerve are dominated by red fibers, whereas those innervated by trigeminal are dominated by white muscle fibers (Ojha and Munshi 1975). The cytochemical differentiation of the muscle fibers will reflect their metabolic activities during gill ventilation. The combined study of ultra structure and enzyme activities of muscle fibers provides an in depth understanding of the muscle physiology which can be correlated with behavioral patterns of air-breathing fishes.

Both electron microscopy and enzyme determination techniques were used by Hochachka *et al* (1978) and Johnston (1979) to determine the ultra structures and enzyme activities of white and red muscle fibers of *Aruana* and *Arapaima,* both obligate air breathers. Their study reveals that the white muscle fibers in both species possess a rather similar ultra structure, characterized by large diameter, very few mitochondria, and few capillaries. They also found that white muscle fibersof *Aruana* displayed higher levels of enzyme activity, while enzymes in aerobic metabolism occurred at about one half the levels in *Arapaima.* No red muscle was found in *Aruana, but* it was present in *Arapaima* and was fueled by glycogenand lipid droplets. Their studies led to a revealing conclusion that the surface skimmer sustained a higher oxidative capacity in its myotomal muscles than that of the facultative air-breather.

IV. MORPHOMETRICS AND DEVELOPMENT OF RESPIRATORY ORGANS

An earlier study (Das 1927) detailed only the morphometric aspect of the air-breathing organ of *Channa striatus* and C. *punctatus* during their ontogenic development. Whereas the more recent morphometric studies (Hughes *et a11973;* Hakim *et al1978)* explain the role of the gas exchange machinery of the amphibious fishes during their development and growth. Recently, Dube and Munshi (1974) have observed that *Anabas testudineus* of the lower weight group survived for a longer period than that of higher weight group, when prevented from surfacing. They reasoned that as the fish grow, the rate of increase in gill surface becomes less than that in the surface of the accessory respiratory (labyrinthine) organ.

Dube and Munshi (1974)also found out that the O2gas-diffusing capacity of the gill of Anabas testudineus decreases at faster rate with increasing body weight than that of Clarias and Heteropneustes. These findings explain why Anabas ofhigher weight group dies when not allowed to breathe atmospheric air whereas the gills and skin of Clarias and Heteropneustes are efficient enough to take care of the total metabolic demands of the fishes as they grow in size.The estimation of the diffusion capacity of the accessory respiratory organs of the air-sacs of Amphipnous are less suited for oxygen uptake than that of Anabas.

The morphometry of respiratory surface area can be used for gas transfer by using the following equation (Hughes and Morgan 1973)

$$VO_2 = K (A\ PO_2)/T.$$

where V02 = 02 uptake in ml 02/min; A is the area in cm2 for gaseous exchange; P02 is the mean difference between the oxygen tensions of water and blood; K is the permeation coefficients (ml 02/m/cm2 mm Hg/min).

On the basis of morphometric deduction of the surface area, its physiological aspects can be inferred. These studies further have practical importance in rearing and transporting of these air-breathing fishes (Munshi and Ojha 1974; Munshi and Dube 1974; Munshi et aI1974). Other researchers (Lenfant and Johansen 1968; Farber and Rahn 1970; Hughes and Singh 1970a, b, 1971; Singh and Hughes 1971; Magid and Babiker 1975;Stevens and Holeton 1978;Magnuson et al1982; Ischimatsu and Itazawa 1983a, b) have also shown that there are considerable variations among air-breathing fishes in the degree of dependence on aquatic or aerial respiration depending on degree of development and efficiency of the respiratory and related structures, environmental limitations and metabolic needs of the fish.

V. ECOLOGY, POLLUTANTS AND AIR-BREATHING FISHES

The extensive use of insecticides is continuously polluting fresh water. There are manifold effects of insecticides on living organisms including economically important fishes. They are also responsible for a number of physiological and biochemical disturbances. Metasystox is known to be the most widely used insecticide against paddy sucking aphids, spiders, mites, saw flies etc. It has been proven by Natarajan (1981) that in the pesticide contaminated water, the air-breathing organs of Channa striatus playa very important role by extracting O2 from air during their stay in such contaminated water. He also observed the existence of DDT and Dieldrin-induced anemia as shown by the low erythrocyte count, low Hb content, light MCH and colour index in Channa punctatus. The progressive decrease in erythrocyte count, Hbconcentration and total leucocyte count were found in C. punctatus exposed to malathion and methyl parathion. It has also been observed by Natarajan (1978) that when a climbing perch, Anabas scandens is exposed to a lethal dose of sumithion, it used its air-breathing organs extensively to overcome the pollution stress for survival.

VI. HEMATOLOGY

Hematology And Other Blood Para-meters

A series of hematological studies on Amphipnous, Anabas, Channa and Heteropneustes indicate that the oxygen-carrying capacity of blood is related to body size of these amphibious fishes (Dube and Munshi 1973; Mishra *et al* 1977; Pandey *et al 1977).* Recently, a comparative study of the bloods of 45 species ofAmazonian fishes (Powers *et al* 1979) pointed out that thereis a significant difference between water and airbreathers CO_2 tension in the blood. Air-breathing fishes have much higher CO_2 tension in the blood and the arterial CO_2 tension of water breathers is generally below 5 torr (Rahn 1966).Whereas, in the air breathersCO_2 tension ranges from 15to 43 torr (Rahn and Garey 1973).

Thus, it is apparent that with the evolution ofair-breathing mechanism, adjustments occur at the molecular level of CO_2 which is the causative factor for efficient hemoglobin function to counteract the increased CO_2 load. Therefore, SOIPe of the differences in the hemoglobin of air and water breathers are related to the effect of carbon dioxide on the hemoglobins of the air-breathers (Farmer 1979). Further, while studying the effects of CO_2 on hemoglobin function of air-breathing fish, Farmer investigated three major points. First, to what extent are the oxygen binding properties of non-mammalian hemoglobins get influenced by CO_2 and whether these properties are independent of pH or not. Second, whether or not the hemoglobins of water breathers differ from the hemoglobins ofair-breathers with respect to the magnitude of the effect of carbamino CO_2 affinity. Third, whether or not there is a correlation between Bohr effectand effect of carbamino CO_2 on the O_2 affinity of hemoglobin. He also found that the blood CO_2 content of air-breathing fish and amphibians is much higher than that of water breathers, but hemoglobin showed no adaptation to an increased CO_2 load. But the drop in oxygen affinity of hemoglobin caused by CO_2 is increased by increasing pH for each hemoglobin examined.

VII. CONCLUSION

Being a warm water tropical fish the air-breathers are not only widespread in India, but the Indian biologists have made significant contributions to the understanding of their functional morphology. Gross morphology and the behavioural aspects of the air-breathing fishes have been researched more intensively than the applied aspects of physiology and effects of pollutants on morphology.

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