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Functional morphology of the respiratory organs of the air-breathing fish with particular emphasis on the African catfishes, *Clarias mossambicus* and *C. gariepinus*

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ABSTRACT

The evolution of air-breathing and transition from water to land were pivotal events that greatly determined the ecological diversification, the advances and the successes of animal life. During their relocation onto land, the so-called bimodal breathers were literally caught at the water-air interface. Among such animals are the diverse air-breathing bony fish. Such taxa, however, strictly do not constitute the so-called ‘bridging animals’, i.e., the inaugural animals that crossed from water to land, nor are they their direct progenitors. The pioneer transitional animals were the Devonian rhipidistian amphibians that possessed a primitive lung which acquired O_2 directly from air and discharged CO_2 back into the same. By having particular morphological and physiological adaptations for terrestrialness, the modern amphibious- and aquatic air-breathers are heuristic analogues of how and why animals relocated from water to land. It has generally been espoused that lack or dearth of O_2 in water, especially in the warm tropical one, was an elemental driver for adoption of air-breathing. There is, however, no direct causal relationship between the evolution of air-breathing and the shift onto land: the move onto land was a direct solution to the existing inimical respiratory conditions in water. This is evinced in the facts that: a) even after attaining capacity of air-breathing, an important preadaptation for life on land, some animals continued living in water while periodically accessing air, b) in the fish species that live in the well-oxygenated waters, e.g., torrential rivers, only few air-breathe and c) air-breathing has still evolved in freshwaters and seawaters, where levels of dissolved O_2 are sufficiently high. Here, the structure and function of the respiratory organs of the air-breathing fish are succinctly outlined. Two African catfishes, *Clarias mossambicus* and *C. gariepinus* are highlighted.

1. Introduction

> “For animal lines moving from water to land, the changes in physical and chemical characteristics of the environment are immense. These changes affect all possible life processes, from respiration and excretion to methods of movement, the functioning of the sense organs and reproduction mechanisms. The transition must therefore have been made very gradually, and some of the transition stages are repeated by present day groups”.
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> Little (1990).

Fish have lived in water for the last ~500 million years (Long, 1995; Long and Gordon, 2004; Pough et al., 2013) and have mainly utilized gills for breathing water. The common English expression `like a fish out of water', which generally expresses experience of or exposure to an unfamiliar challenge or environment, specifically points out the inability of fish to live in and breathe air. Among the contemporary animal life, this is, however, not absolutely correct. About 400 species of fish belonging to 50 families and spanning 70 orders of bony fish (Osteichthyes) to various extents and under different environmental conditions breathe air (Dehadrai and Tripathi, 1976; Liem, 1988; Sayer and Davenport, 1991; Graham, 1997; Graham and Wegner, 2010; Martin, 2014). Some of them, the so-called obligate air-breathers, have adapted to air-breathing to an extent that they die if prevented from accessing air for a particular period of time (Johnston et al., 1983; Graham, 1997). In hitherto water-breathing and water-inhabiting animal species, air-breathing and transition from water to land evolved independently at different times (Dejours, 1988; Randall et al., 1981; Graham, 1997; Joss, 2006; Wright and Turko, 2016). Air-breathing fish possess an assortment of anatomical, physiological, biochemical and

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Abbreviations: ABO, air breathing organ; BGB, blood-gas barrier; DO_2, total morphometric diffusing capacity for oxygen; LOs, labyrinthine organs; SBCMs, suprabranchial chamber (epibranchial) membranes; WBB, water-blood barrier; τht, harmonic mean thickness of the tissue barrier of the BGB or the WBB

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behavioural adaptations by which they acquire O_2 from air and discharge CO_2 back into it (Munshi, 1961, 1968, Munshi, 1976; Hughes and Munshi, 1973; Hughes et al., 1973; Hughes and Weibel, 1976; Graham, 1976, 1997; Kramer et al., 1978; Randall et al., 1981; Maina and Maloiy, 1986). Because of their very different phylogenetic statuses, the diversity of the habitats they occupy, the lifestyles they pursue, the adaptations they possess, the strategies they have adopted and the morphological and functional malleability they display, the air-breathing fish are remarkable (Moore, 1990; Lefevre et al., 2014). Phenotypic plasticity occurs when animals develop a range of relatively fit matching structural features in response to demands compelled by the environment (Pigliucci et al., 2006; Turko et al., 2012). While physiological plasticities can occur quite rapidly in response to environmental change, the morphological ones take longer to happen (Piersma and van Gils, 2011). Air-breathing fish can be differentiated into amphibious- and aquatic taxa (Graham, 1976, 1997; Sayer, 2005; Ashley-Ross et al., 2013): the former live in water while breathing air and the later, the so-called bimodal-breathers, permanently inhabit water and surface periodically to access air. Each time they move back-and-forth between water and air, fluid media of different physicochemical properties (Dejours, 1976, 1988), amphibious- and bimodal breathers have to respond to greatly different physicochemical- and environmental factors such as changes in buoyancy and gravity, hydration and desiccation, low and high O_2 availability, low and high CO_2 accumulation and high and low NH_3 solubility (Wright and Turko, 2016): O_2 and CO_2 diffuse much faster in air than in water; O_2 is more soluble in air than in water and; air contains ~210 ml $O_2\,L^{-1}$ while water (depending on salinity) holds only 5–6 ml $O_2\,L^{-1}$ at 25 °C (Dejours, 1976, 1988; Graham, 2006). Singular traits are needed for the amphibious fish to maintain homeostasis under different environmental conditions. It has been conjectured that organisms that live in heterogeneous or dynamic environments tend to possess greater phenotypic variability compared to those inhabiting relatively stable ones (West-Eberhard, 2003).

Air-breathing evolved in the Silurian, i.e., 438–480 million years ago (mya) (Horra, 1935; Packard, 1974; Schmalhausen, 1968; Randall et al., 1981; Olson and Panchen, 1981; Little, 1990; Graham, 1997; Farmer and Jackson, 1998; Long and Gordon, 2004; Graham and Lee, 2004; Giomi et al., 2014; Icardo et al., 2017), ~100 million years before the vertebrate life shifted from water to land, a process that occurred in the Devonian Period (416 million to 358 mya) (Panchen, 1980; Boucot and Janis, 1983; Little, 1990; Gordon and Olson, 1994; Graham and Lee, 2004; Clement and Long, 2010). While it has been posited that the most important driver for air-breathing was the inimical conditions prevailing in water, especially that of lack of O_2 (hypoxia and anoxia) (Carter and Beadle, 1931; Das, 1940; Saxena, 1963; Eduardo et al., 1979; Randall et al., 1981; Graham and Baird, 1982; Graham, 1983, 1997; Dejours, 1988, 1994; Maina, 1998; Lefevre et al., 2013; Hsia et al., 2013; Giomi et al., 2014), it has also been argued that air-breathing does not correlate directly with deficiency or lack of O_2 in water (Rahn et al., 1971; Rahn and Howell, 1976; Kramer et al., 1978; Farmer and Jackson, 1998; Farmer, 2017) nor does it (air-breathing) correspond with terrestriality (Tatsumi et al., 2016). For example, in the habitat occupied by the Australian lungfish (*Neoceratodus forsteri*), Grigg (1965) observed that activity rather than the O_2 content in water was the main factor which prompted air-breathing. In the striped catfish, *Pangasianodon hypophthalmus*, Lefevre et al. (2013) showed that air-breathing provides significant amounts of O_2, especially with increasing metabolism, e.g., during swimming. While the actual settings under which air-breathing evolved has yet to be fully resolved, regarding the possible mechanisms, processes and strategies that animals adopted to achieve air-breathing, the modern bimodal-breathers are highly instructive. In evolutionary terms, innovatory steps or events which are designated 'evolutionary novelties' (Miller, 1949; Mayr, 1960; Riedl, 1978), 'key innovations' (Lauder and Liem, 1989), 'broad adaptations' (Schaeffer, 1965a, b) or 'derived traits instrumental in acquiring entry into a novel adaptive zone featuring novel selective regimes' (Baum and Larson, 1991) have developed in bimodal breathers. The modern teleosts fish which arose from the holosteans during the late Triassic Period are not the pioneer animals that moved from water to land (Jarman, 1970). Those were the Devonian rhipidistian crossopterygians which had primitive lungs and bony-limbed fins which existed ~350 mya (Jarman, 1970; Kolesová et al., 2007): the primitive lungs acquired O_2 from air and voided CO_2 back into the same (Rahn and Howell, 1976).

2. Diversity, development and function of respiratory organs of air-breathing fish

Environments are naturally dynamic spaces (Davenport, 1985; Fernandez et al., 2007). Animals that occupy them therefore face constant abiotic shifts which include fluctuations in temperature and O_2 availability (Davies, 1975; Davenport and Woolmington, 1981). Certain species of fish have adapted to respiratory challenges by acquiring air-breathing organs (ABOs) which supplement the gills as gas exchangers. By definition, an air-breathing fish is one which can exchange respiratory gases (O_2 and CO_2) with air by utilizing specialized ABOs (Graham, 1997). The structure and the function of the ABOs have been extensively studied (Moussa, 1957; Munshi, 1961, 1968; Saxena, 1962, 1963; Johansen et al., 1968; Hughes and Munshi, 1968, 1973, 1979; Johansen, 1970; Singh and Hughes, 1971; Abdel-Magid, 1971; Singh, 1976; Graham, 1983; Graham and Baird, 1984; Maina and Maloiy, 1986; Bevan and Kramer, 1987; Johnston et al., 1983; Munshi et al., 2001; Lefevre et al., 2011; Huang et al., 2011; Huang and Lin, 2016). Existence of structures such as pillar-like cells in the ABOs (Munshi, 1968; Johansen, 1970; Hughes and Munshi, 1968, 1973, Hughes and Munshi, 1979; Singh, 1976; Maina and Maloiy, 1986), cellular elements which particularly characterize the secondary lamellae of the teleost gills (Maina, 1990, 1991), suggests the ABOs develop from gills or the ABOs are evolutionary modifications or exaptations of the gills (Hughes and Munshi, 1973). For the Indian catfish, *Clarias batrachus*, Olson et al. (1995) observed that: a) ABOs are derived from gill tissue and retain structural features of the arterio-arterial blood vessels which are similar to those of the gill filaments, b) the gill and the blood vessels of the ABOs lie in parallel to each other and both are arranged in series relative to the systemic circulation and; c) the pillar cells that exist in the gills and the pillar cell-like cells of the ABOs are analogous rather than homologous. Regarding the structure of the gills of fish such as *C. batrachus, Heteropneustes (= Saccobranchus) fossilis, Channa punctata, Monopterus (= Amphipnous) cuchia and Boleophthalmus boddaerti,* species studied by among others Hughes et al. (1974a,b), Hughes and Munshi (1979), Munshi et al. (1980), Hughes and Munshi (1986) and Chandra and Banerjee (2004), it was observed that the gill filaments and the secondary lamellae were well-developed in all the species except in *Monopterus* where the lamellae are highly modified, the pillar cells are lacking, and the endothelial cells line the vascular channels, with exception of the marginal ones. According to Hughes and Morgan (1973), Hughes et al. (1974a,b), Hughes and Munshi (1979) and Maina and Maloiy (1986), the thickness of the water-blood barrier (WBB) in the gills of fish that have been investigated ranges from 1.5 to 13 μm, in *Boleophthalmus*, a species that inhabits brackish water, the secondary lamellae have well-developed lymphoid spaces between the two layers of epithelial cells and structural modifications of the gills correlates with the extent of air-breathing. Regarding large respiratory surface area, intense vascularization and thin blood-gas barrier (BGB) and WBB, the morphological similarities between the gills and the ABOs in phylogenetically different fish species shows that similar selective pressures compelled the evolution of the two types of gas exchangers. In *Heteropneustes*, Hughes et al. (1992) determined the harmonic mean thickness of the BGB (τht) of the air-sac was 0.342 μm and the mass-specific total morphometric diffusing capacity for O_2 (DO_2) was 0.001 ml $O_2\,s^{-1}\,mbar^{-1}\,kg^{-1}$. They showed that the air-sac is more efficient