

Gas Exchange and Control of Respiration in Air-Breathing Teleost Fish

M.L. Glass and F.T. Rantin

Abstract Atmospheric air at sea level contains 30 times more O₂, when compared to fully O₂-saturated water and, in addition, the O₂ content of water is ever-changing. The gill systems of fish are highly efficient for O₂ extraction, but this cannot save the animal if its O₂ supply is insufficient. This explains why air-breathing in fish has evolved in at least 60 independent lines. Lungfish and bichirs (Polypteriidae) possess true lungs, whereas other air-breathing organs (ABOs) can be derived from the swimbladder as in the gar pike (*Lepidosteus*) and the bowfin (*Amia*). In *Hypostomus sp.* (Loricariidae) the ABO is a modified part of the digestive system. The functions of gills, ABOs and lungs all depend on surfactants. Aerial breathing increases with activity and/or reduced O₂ availability in the water. In addition, increases of temperature result in larger air-breathing efforts. These responses are adjusted by O₂ receptors, located in the gills, whereas the role CO₂/H⁺-receptors is minor in actinopterygian fish.

1 Introduction

1.1 Air and Water as Respiratory Media

Compared to air, water is an oxygen poor environment, because the O₂ solubility in water is low. At 20°C, the O₂ concentration in the fully saturated water is 0.007 (l⁻¹l⁻¹) against 0.2095 (l⁻¹l⁻¹) in air, which implies that air-breathers have 30 times more O₂ available when compared to aquatic breathers in fully saturated water. The relative density and the viscosity of water and air should also be taken

M.L. Glass (✉)

Department of Physiology, Faculty of Medicine of Ribeirão Preto, University of São Paulo, Av. Bandeirantes 3900, 14.049-900 Ribeirão Preto, SP, Brazil,
E-mail: mlglass@rfi.fmrp.usp.br

into account. The water density is no less than 800 times higher compared to the density of air (water: 1.00 kg l^{-1} ; air: 0.0013 kg l^{-1}) and the water viscosity is 50-fold higher than atmospheric air at sea level. The joint analysis of these variables gives an impression of the difficulties faced by water-breathers to maintain an aerobic metabolism. The low O_2 availability in the aquatic environment forces water-breathers to ventilate the respiratory surface area a much higher volume of inert mass with high density and viscosity, which leads to a high metabolic cost of gill ventilation. Gills are absent in most aquatic invertebrates, but they may ventilate some specific part of the body. Fish possess a highly sophisticated countercurrent system, in which the blood passes the gills in the opposite direction to the inspired and expired water flow. It should also be pointed out that the O_2 content in water depends on photosynthesis, which changes on a daily and seasonal basis. Further determinants of O_2 levels are water movement, currents, changes in temperature, and salinity (Dejours 1981).

The metabolic cost of gill ventilation in water-breathing fish is species-specific. It ranges between 3.7 and 5.7% of the total oxygen uptake ($\dot{V}\text{O}_2$) in the sharksucker, *Echeneis naucrates*, which is an unusually low value (Steffensen and Lomholt 1983), while the highest value (43%) was measured in the tench, *Tinca tinca* (Schumann and Piiper 1966). Gill ventilation consumed about 13–22% of the total $\dot{V}\text{O}_2$ in carp, *Cyprinus carpio*, 10–25% in trout, *Onchorhynchus mykiss* (Hughes and Shelton 1962; Hughes and Saunders 1970), and 22–28% in plaice, *Pleuronectes platessa* (Edwards 1971). The cost of ventilation may increase in response to hypoxia, since hyperventilation is an attempt to compensate for the reduced O_2 level. The metabolic cost of gill ventilation in normoxic Nile tilapia, *Oreochromis niloticus*, is about 3% in normoxia, increasing to 20% when the inspired PO_2 falls to 35 mmHg (Fernandes and Rantin 1994). Likewise, the hypoxia-tolerant and sedentary erythrinid fish traíra, *Hoplias malabaricus*, spends 3% of its total $\dot{V}\text{O}_2$ to ventilate its gills when in normoxia, but hypoxia ($\text{PwO}_2 = 25 \text{ mmHg}$) increases the cost of ventilation to no less than 13% (Rantin et al. 1992).

Air-breathing organs (ABOs) have evolved in at least 60 independent lines of teleost and holeost fish (Graham 1997). Fish of bimodal respiration have large advantages when exposed to hypoxic water. Transition to air-breathing will alleviate the hypoxic conditions, which obviously increases the chances for survival. In addition, air-breathing assures access to a steady O_2 supply at a much higher concentration.

1.2 Evolution of the Atmosphere

The early atmosphere contained hydrogen and helium, which the solar wind gradually removed, after which CO_2 became the prevalent gas. This prevalence of CO_2 is still predominant in the atmospheres of Venus and Mars (Stearns and Hoekstra 2005). Geochemical research indicates that evolution of photosynthesis occurred in precursors to the cyanobacteria, which suggests that the catalyzing

reaction of the photosystem II oxidizing complex emerged some 3 billion years ago (Dismukes et al. 2001), and this event would gradually lead to an ozone protection resulting from the photosynthesis. In addition, Dismukes et al. (2001) point out that oxidation of water is one of the most challenging multielectron reactions in biology.

Estimates for the atmospheric O₂ levels are available from the beginning of the Silurian period to the end of the Permian period, which ranges from 444 to 250 million years ago. There is evidence that atmospheric O₂ levels in the early Devonian could have reached up to 25%, exceeding our actual level of 20.95%. The middle and late Devonian periods were characterized by reduced O₂ levels (13% O₂), but then between 340 and 250 million years ago, the O₂ levels rose and might have reach up to 35% (Bernier and Canfield 1989; Scott and Glasspool 2006). As pointed out by Dudley (1998), it is unlikely that 35% O₂ was exceeded, because this value is an approximate threshold for spontaneous combustion of the atmosphere. Recent studies agree with an O₂ peak of up to 25% in the early Devonian, followed by low O₂ levels of about 15% during the beginning of the Late Devonian (Frasnian) period (Clack 2007). Based on Dehadrai and Tripathi (1976), Daniels et al. (2004) proposed that Devonian bony fish may have developed aerial respiration due to low oxygen levels. The message of this short section is that ambient O₂ availability is a principal determinant of the levels of metabolism and of new strategies for respiratory function; one of these is bimodal respiration.

1.3 Hypoxia and Hypercarbia

Based on Junk (1984), Nelson et al. (2007) listed some adverse conditions for O₂ uptake, which are particularly common in tropical and subtropical regions, and these are: (1) respiratory rates may become larger than the production of O₂ by photosynthesis, (2) a stagnant air/water interface impedes an adequate distribution of O₂, and (3) a poor light penetration decreases photosynthesis.

By comparison, the atmosphere above us seems a stable source of O₂. At sea level and 20°C, the PO₂ would be about 155 mmHg with 20.95% O₂. The atmospheric source of O₂ will obviously be advantageous for fish exposed to frequent reductions of ambient O₂ levels. Hypercarbia is the condition of elevated CO₂ levels in the environment, whereas hypercapnia is elevated CO₂ levels within the body. Temperature is also an important factor, since higher temperatures increase the metabolic demands, and, the higher the temperature, the larger are the increases of gill ventilation in response to hypoxia (Glass et al. 1990). An ever-changing O₂ availability is a challenge to the O₂-oriented respiratory control of teleost and holoost fish (cf. Dejours 1981), and each species has a critical PO₂ threshold (PcO₂) below which the normal $\dot{V}O_2$ can no longer be maintained. Holoosts and teleosts compensate for an excess of CO₂ (hypercarbia) by active increases of plasma [HCO₃⁻] levels, which returns pHa towards the normal (normocarbic) value. About 90% of acid–base

relevant ions are mediated by specialized cells within the gill epithelia, and the kidney contributes the rest (Heisler and Claiborne 1986).

The P_{CO_2} of an exclusively water-breathing fish often reflects the O_2^- availability of the habitat. As a typical example, carp (*Cyprinus carpio*) is very resistant to hypoxic conditions, and its Hb– O_2 dissociation curve (ODC) has a very high Hb– O_2 affinity, which implies that a high O_2 saturation is preserved until P_{aO_2} falls to extremely low pressures ($P_{50} = 9.4 \text{ mmHg}$ at 20°C) (Albers et al. 1983), which explains its survival under severely hypoxic conditions (Soncini and Glass 2007). Conversely, dourado (*Salminus maxillosos*) is a river fish capable of a high swimming speed, and it is distributed in regions of Southern Brazil. At $\text{pHa } 7.7$, its P_{50} is high (21 mmHg at 25°C .) This leaves dourado vulnerable to reductions of O_2 , because moderately hypoxic conditions will reduce its Hb– O_2 saturation and, thereby, decrease its swimming performance (Salvo-Souza et al. 2001).

1.4 Gill Function and O_2 Extraction

The O_2 extraction of a gas exchanger can be defined as:

$$\text{EO}_2 = \dot{V}\text{O}_2 \cdot (\dot{V}_G \cdot C_{\text{I}\text{O}_2})^{-1},$$

An alternative expression is:

$$\text{EO}_2 = (C_{\text{I}\text{O}_2} - C_{\text{E}\text{O}_2}) \cdot C_{\text{I}\text{O}_2}^{-1},$$

where $\dot{V}\text{O}_2 = \text{O}_2$ uptake, $\dot{V}_G =$ the water flow irrigating the gill, $C_{\text{I}\text{O}_2}$ and $C_{\text{E}\text{O}_2} = \text{O}_2$ contents of the inspired and expired water (Dejours 1981). The difference $(C_{\text{I}\text{O}_2} - C_{\text{E}\text{O}_2}) \cdot C_{\text{I}\text{O}_2}^{-1}$ is the amount of O_2 extracted by the blood flow and $C_{\text{I}\text{O}_2}$ is the total inspired amount of O_2 . The degree of O_2 extraction by fish gills is high and often about 85% (Lomholt and Johansen 1979). This is very high compared to the extraction of a human lung, which is only about 30%. Evidently, the gas exchange is very effective, because the blood flows in the opposite direction to the water movement, and the gas exchange takes place within the blood lacunae of the secondary lamellae (cf. Piiper 1992).

A cardio-vascular shunt is defined as a quantity of incompletely saturated blood that bypasses the gas exchanges surfaces. As the second step, the shunted blood fraction mixes into the saturated blood from the gas exchanger, and the larger this shunt, the lower Hb– O_2 saturation. Such shunts are apparently small or absent in fish gills, although there is some evidence for shunted blood within the basal channels of the secondary lamellae of rainbow trout (*Oncorhynchus mykiss*) (Tuurala et al. 1984). On the other hand, convincing evidence against major shunts was provided by Eddy (1974), who constructed an in vivo Hb– O_2 dissociation curve for tench (*T. tinca*). The saturation of the oxygen dissociation curve (ODC) ranged from 75 to 95%, but the in vivo arterial point was in some cases located on the upper portion of the oxygen dissociation curve (ODC), which indicates that shunts

are minimal. Most teleost fish have almost hyperbolic ODCs ($n_{\text{Hill}} \sim 1.0$ to 1.3), which implies that PO_2 , even when high, cannot saturate the blood. Therefore, the incomplete saturation can be due to the shape of the ODC and not to cardiovascular shunt (Glass and Soncini 1997). Application of hyperoxia permits calculation of the shunt fraction based on the mixed venous and arterial points. Such measurements are available for pacu (*Piaractus mesopotamicus*), in which the shunt fraction was less than 10%. The equation cannot distinguish between diffusion limitation and ventilation–perfusion mismatching, which leaves 10% shunt as an overestimated value (Soncini and Glass 1997). Consistent data were obtained by Gilmour and Perry (1994), when they exposed trout (*O. mykiss*) to an aquatic PO_2 of 548 mmHg, which increased PaO_2 to a range within 352–502 mmHg. The characteristics of teleost gills are impressive, but in spite of this the fish may not survive severely hypoxic conditions. This explains why air-breathing in fish has evolved within many different and, mainly, tropical taxonomic groups.

Some fish avoid hypoxia by skimming the air/water interface, which has the highest O_2 level. This respiratory behavior is known as aquatic surface respiration (ASR) and is common to a number of tropical fish that inhabit lakes with frequent hypoxic conditions (Kramer and McClure 1982). An example is pacu (*P. mesopotamicus*). To escape from hypoxia, this species performs ASR, which is facilitated by a gradual swelling and extension of the lower lip (Saint-Paul and Bernardino 1988; Val and Almeida-Val 1995). Pacu immediately initiates ASR even in moderate hypoxia (50–70 mmHg), while the full development of swelling may take no less than 3 h or more (Rantin et al. 1998).

1.5 Air-Breathing Organs and Their Function

Respiration by a lung or an ABO requires surfactant to reduce surface tension. Daniels et al. (2004) addressed the role of surfactant in fish in two air breathers: the pirarucu (*Arapaima gigas*) and the tarpon (*Megalops cyprionoides*), along with the exclusively water-breathing snapper (*Pagrus auratus*). They concluded that the fish surfactants share basic features with the alveolar Type II cells of tetrapod lungs, and proposed that surfactant in lungs or swimbladders are homologous. The swimbladder and the lungs are of separate ontogenetic origin (Perry 2007), and Daniels et al. (2003) proposed that surfactant systems already existed before lungs and swimbladders evolved. It also turns out that the surfactant of Osteichthyes is uniform, and may represent the vertebrate prototype.

Adequate O_2 uptake and CO_2 elimination can be obtained via modifications of structure and function, which often leads to surprising solutions. In this section, we present a selection of ABOs and their function, beginning from the most ancient forms. The text takes us from real lungs to respiration using modified stomach tissue.

1.6 *Polypterus senegalus* (*Polypteridae*)

The genera *Polypterus* sp. and *Erpetoichthys* [earlier *Calamoichthys*] form a prominent group of ancient fish. It has been argued that they are a phylogenetically isolated group and that they are the most original extant representatives of actinoterygians (Carroll 1987), and their habitats are tropical waters in Africa. Land vertebrates, lungfish and Polypteriformes possess lungs of a ventral, posterior pharyngeal origin and these are pleisiomorphic paired structures (Perry 2007), i.e. they are ancestral trait and are not apomorphic structures.

There is little information available on the respiratory physiology of Polypteriformes. Studying a specimen of *P. senegalus*, Babiker (1984) reported that the fish in aerated water would surface only once or twice between 11p.m. and 3a.m., which would classify the specimen as a marginal obligatory air-breather. Juvenile specimens (10–30 g) were exclusively gill breathers, which proved fatal during aquatic hypoxia. Larger specimens (290–460 g) survived due to air-breathing, and Magid et al. (1970) measured their lung gas PO_2 , which increased from about 30 to 100 mmHg during inhalation, with a concomitant reduction of $PaCO_2$ from about 14 to 9 mmHg. In this context, it should be mentioned that aerial respiration in fish increases $PaCO_2$ and lowers pH_a and, according to Dejours (1981): “the more an animal depends on pulmonary breathing, the higher its PCO_2 ”. The reason for this is the 30-fold difference of O_2 content in water and in atmospheric air. To compensate for this difference, an exclusively gill-breathing fish must ventilate a 30-fold higher volume, when compared to an air breather. A high degree of gill ventilation leaves a carp with a $PaCO_2$ of 2–3 mmHg (Glass et al. 1990), while the $PaCO_2$ *P. senegalus* would be about 3-fold higher due to additional aerial ventilation. Reductions of PwO_2 markedly increased pulmonary ventilation of *P. senegalus*. As a second defense strategy, the O_2 loss to near anoxic water was minimal, due to a shutdown of gas exchange by the gills (Lomholt and Glass 1987). This is consistent with Babiker (1984), who reported that small specimens (10–30 g) would not develop air-breathing, whereas larger individuals (>100 g) could survive in near-anoxic water by lung ventilation.

1.7 *The gars (Lepisosteus sp.) and the bowfins (Amia calva)* *Amiidae, Amiiformes*

The gar pikes and the bowfins form a sister group which is ramified with the teleosts (Filleul and Lavoué 2001). The gar and the bowfins are restricted to North America, and their ABOs are gas bladders (Hedrick and Jones 1999). The ventilation of an ABO may seem irregular, but this is not the case for *Amia*. Hedrick et al. (1994) applied a spectral analysis to evaluate air-breathing behavior, and two distinct types of breaths were recorded. The first type began with an expiration followed by inhalation. A second type of breath consisted of an inhalation and the authors very

reasonably suggested that this behavior represented an adjustment related to buoyancy. Upon removal of this component, a very regular pattern emerged, with an interval of 30 min between breaths at the surface (temperature 20–24°C), which indicated the presence of a central rhythm generator and/or peripheral O₂-receptor input.

The effects of activity on air-breathing in *Amia* and *L. oculatus* were evaluated by Farmer and Jackson (1998), who first studied the animal at rest and then at stepwise increases of swimming speed (temp. 19–23°C). It turned out that *L. oculatus* at rest obtained less than 2% of total O₂ uptake from aerial respiration, but it turned out that swimming activity increased the contribution of O₂ from air-breathing to no less than 53% of total $\dot{V}O_2$. The corresponding numbers for aerial respiration in *Amia* were 10% at rest and no less than 66% of total $\dot{V}O_2$. As the authors pointed out, the capacity to increase ventilation of the ABO is important for activity, and not only for survival in hypoxic waters.

As expected, the long-nosed gar increased ventilation of the ABO, when exposed to hypoxic water. In addition, it reduced gill ventilation, which minimized O₂ losses to the water (Smatresk et al. 1986). Similar responses have been reported for *Polypterus* in near-anoxic water (Lomholt and Glass 1987).

There is evidence for membrane-bound carbonic anhydrase located in the ABO of *Amia*, and it resembles mammalian carbonic anhydrase IV as regards inhibition characteristics and membrane attachment (Gervais and Tufts 1998). Curiously, glucose is a major source of fuel in the Florida gar (*L. plathyrrinchus*), and the enzyme activities of its ABO resembles those of a mammalian lung, and are different from the enzymes of fish swimbladders (Frick et al. 2007). Evidently, the function of the ABO can be sophisticated, and it turned out that mechanoreceptors are present in *L. oculatus* (Smatresk and Azizi 1987). Two types of receptors were identified. A rapidly adapting type was identified, while a slowly adapting type turned out to be CO₂-sensitive. Application of hypercarbia (6–10% CO₂) decreased firing rate of the slowly adapting receptors to the slowly adapting receptors. Very similar responses have been reported for lungfish (DeLaney et al. 1983) and land vertebrates (Milsom 2002), which would suggest a very ancient origin, if the receptors turn out to be identical (Smatresk et al. 1987).

1.8 *Channa argus* (Channidae)

The snakehead fish *Channa argus* is carnivorous and widely distributed in temperate zone of East Asia. Ventilation of the ABO is obligatory during the summer season, whereas survival by gill ventilation alone is possible during the winter. The ABO of *Channa* is placed within the gill system. The first and second gill arches are perfused by the anterior ventral aorta, after which the blood traverses the bilaterally and dorsally positioned ABO. Differently, the posterior ventral aorta supplies blood to the third and fourth gill arches, bypassing the ABO (Andresen et al. 1987). Animals weighing 1–2 kg were studied at 15–25°C to evaluate the combined effects of

hypoxia and temperature on aerial ventilation. Glass et al. (1986) exposed *Channa* to normoxia (~ 155 mmHg) and hypoxia (75 and 35 mmHg). Aquatic hypoxia was tested and the responses were weak but significant at 25°C, while the response at 15°C failed to reach statistical significance. On the other hand, aerial hypoxia had a large effect on ventilation of the ABO. At 15°C, the ventilation of the ABO rose 2.8-fold with reduction of gas phase PO₂ from 155 to 35 mmHg, and at 25°C the same reduction increased aerial ventilation 3.4-fold. Curiously, this response to hypoxia is also amplified by high temperature in the South American lungfish (*Lepidosiren paradoxa*) and in ectothermic land vertebrates, including the toad *Chaunus scheideri* (earlier *Bufo paracnemis*) (Kruhøffer et al. 1987) and the turtle *Chrysemys picta bellii* (Glass et al. 1983); see Fig. 1.

In addition, *Channa* had a weak increase of aerial ventilation in response to aquatic hypoxia, while the South American lungfish (*L. paradoxa*) had no increase of pulmonary ventilation in response to aquatic O₂ levels. This raises questions about the positions and roles of the involved O₂ chemoreceptors.

Figure 1 clearly shows that ventilatory responses to hypoxia in air breathers become strongly reduced when temperature decreases. The dependence of exclusive water breathers may be different. Carp (*Cyprinus carpio*) increased gill ventilation in response to light hypoxia at 10, 20 and 25°C. These responses were significant both at 10, 20 and 25°C. Moreover, the gain of the responses could be expressed as:

$$(\dot{V}_G \text{ hypoxia} / \dot{V}_G \text{ normoxia}) \cdot 100\%.$$

Expressed in this way, it became clear that the percentage increase was independent of temperature. In other words, the control value and ventilation at a level of hypoxia were scaled up or down by the same factor, which differs from responses to gas-phase hypoxia, which fade away in response to low temperature (Glass et al. 1990; Soncini and Glass 2000).

Channa seemed to respond to hypercarbia (range 0–8%), but this effect did not reach significant levels. Likewise, Graham and Baird (1982) reported that *Hypostomus* would elicit ABO breathing more easily, if hypercarbia (~ 10 mmHg) was added. Moreover, juvenile specimens of the bichir (*P. senegalus*) increased gill ventilation in response to 0.8%CO₂ in the water. Therefore, it seems that a weak CO₂-related component is present, and this topic will be taken up later.

1.9 *Hypostomus* sp. (Loricariidae)

Facultative air-breathers ventilate exclusively use gills when in normoxic water, whereas the ABO is ventilated, when the water turns hypoxic. This applies to the armored catfish *Ancistrus chagresi*, *Hypostomus plecostomus* and *Hypostomus regain*, which have species-specific thresholds for the onset of aerial ventilation. Thus, *A. chagresi* initiated ventilation of the ABO when PwO₂ fell to 33 mmHg, whereas *H. plecostomus* already ventilated the ABO, when PwO₂ dropped to

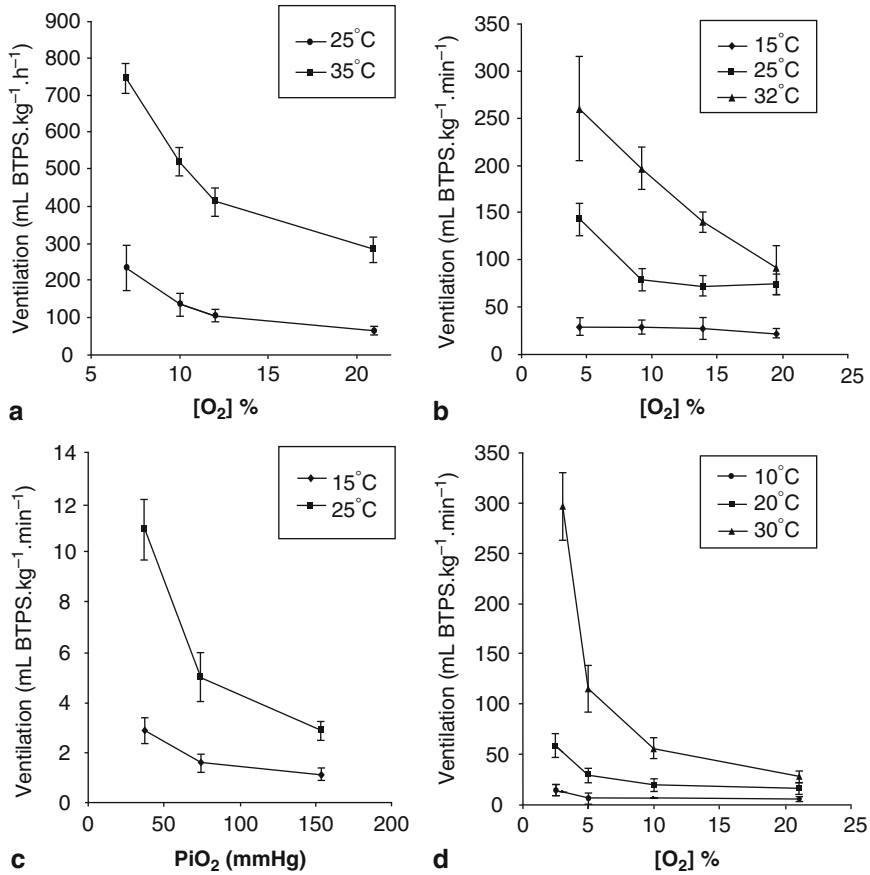


Fig. 1 This figure compares the effects of hypoxia combined with increases of temperature. **a** The South American lungfish *Lepidosiren paradoxa* (da Silva et al., in preparation). **b** The toad *Chaunus schneideri* (Kruhøffer et al. 1986). **c** The teleost fish *Channa argus*, a facultative air-breather equipped with an air-breathing organ. (Glass et al.). **d** The turtle *Chrysemys picta bellii* modified from Glass et al. (1983). These responses are similar, although they are obtained from very distant groups

the higher threshold of 60 mmHg. Application of light hypercarbia (~10 mmHg) changed both thresholds to the higher values 64 mmHg and 79 mmHg, which was in agreement with the relative sensibility of the two species (Graham 1982). Their ABO is modified tissue located within the stomach. The stomach tissue of the catfish *H. plecostomus* is quite different from that of typical fish, since the wall is transparent, and the mucosal layer is smooth, and capillaries are abundant in the arterial part of the stomach. Part of the epithelium contains respiratory epithelial cells, where the air-blood is thin (0.25–2.02 μm) (Podkowa and Goniakowska-Witalinska 2003).

Rhinelepis strigosa (cascudo preto) has been studied in some detail. It will not surface when in well-oxygenated water at 25°C, but air-breathing was initiated

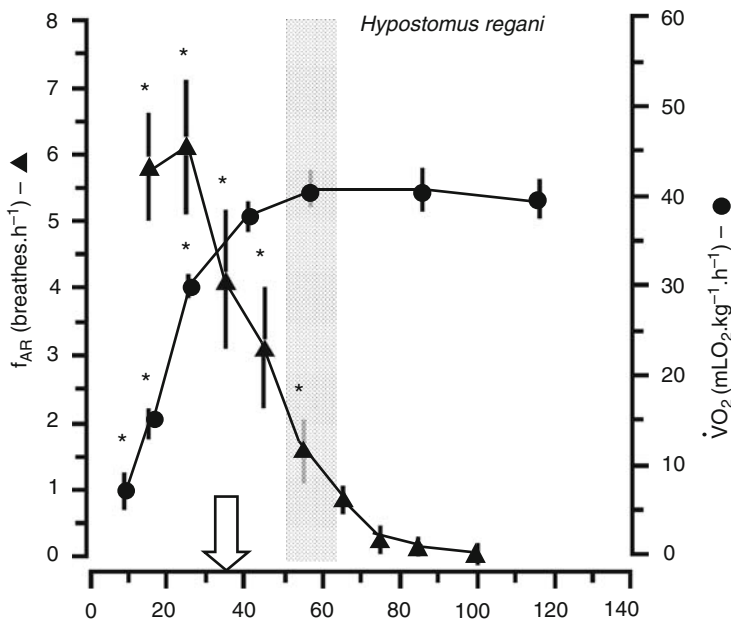


Fig. 2 The threshold O₂ tension for air breathing (*shaded area*) and air-breathing frequency (*filled triangle*) of *H. regani* with free access to the water surface and aquatic $\dot{V}O_2$ (*filled circle*) when the access to the water surface was denied during progressive hypoxia. *Arrow* indicates the P_cO₂. *Asterisks* indicate significant difference compared to the normoxic values. Values are mean \pm SEM. (Modified from Mattias et al. 1998)

below a P_wO₂ of 30 mmHg and it peaked at 7 breaths · h⁻¹ at 10 mmHg (Takasusuki et al. 1998), which certainly proves the efficiency of respiration by an ABO. In this context, it should be remembered that temperature and O₂ levels interact, and behavioral responses may include dislocation to a lower temperature and/or a higher O₂ level (Schurmann and Steffensen 1994).

Mattias et al. (1998) measured $\dot{V}O_2$ and gill respiration during progressive hypoxia in the facultative air-breather *H. regani*. Air-breathing was absent in aerated water, but *H. regani* maintained aquatic $\dot{V}O_2$ at 31 mL O₂ kg⁻¹ h⁻¹ down to the critical oxygen tension (P_cO₂) of 34 mmHg (temperature 25°C). Gill ventilation and ventilation of the ABO reached their peak values within the P₁O₂ range from 56 to 25 mmHg (Fig. 2).

1.10 *Clarias* sp. (Clariidae)

Members of the genus *Clarias* ventilate the ABO in a regular manner, and some species maintain a low level of air-breathing, when in normoxia. In spite of this, some studies question which species are obligatory or facultative air breathers.

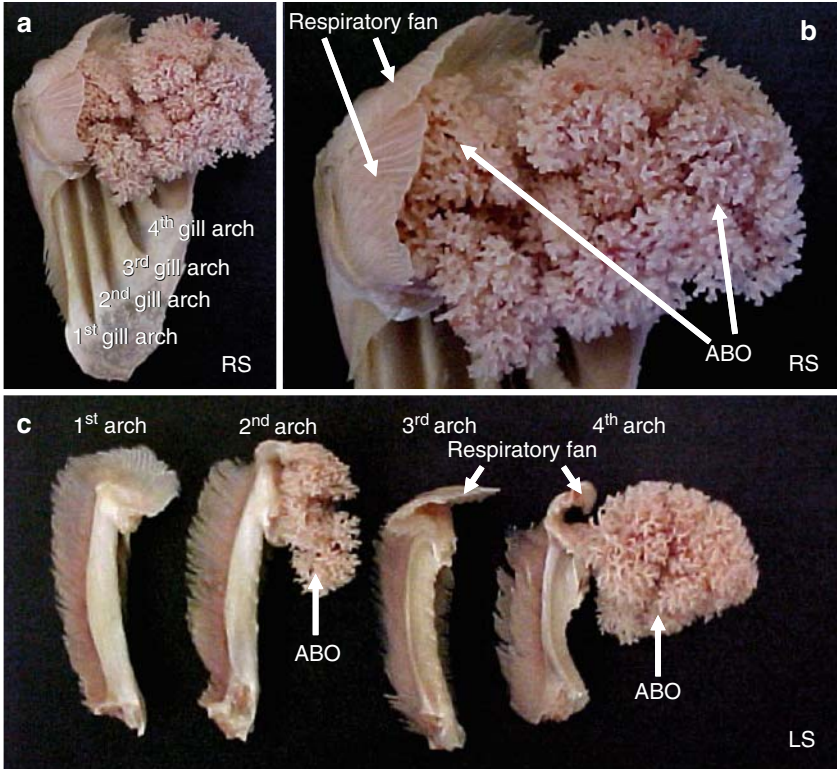


Fig. 3 Gill structures of the African catfish, *Clarias gariepinus*, showing: (a) general view of the four gill arches with the ABO on top (RS – right side), (b) closer look at the ABO showing the respiratory fan and the arborescent organ, which is the gas-exchange unit (RS – right side), and (c) Details of the four gill arches showing the branchial system with lamellae, ventilatory fan in the 3rd and 4th gill arches and the arborescent organ (ABO) in the 2nd and 4th gill arches (LS – left side)

Most of the studies on *C. batractus*, *C. lazera*, *C. gariepinus* and *C. macrocephalus* concluded that they are facultative air breathers (Hora 1935; Magid 1971; Donnelly 1973; Jordan 1976; Bevan and Kramer 1987), but some authors consider the first two species as obligatory air-breathers (Greenwood 1961; Singh and Hughes 1971).

The ABO in this genus (Fig. 3) consists of two chambers in superbranchial position and located within the posterior–dorsal part of the opercular cavity. This space is nearly filled up with arborescent gill organs, which are extensions of the second and fourth gill arches. In addition, the openings to each chamber are equipped with valves to prevent water to enter. These valves are fan shaped extensions and are located on the last gill lamellae of the interior gill arch. Aerial respiration involves a modified gill epithelium, including the inner surface of these openings, its base, the

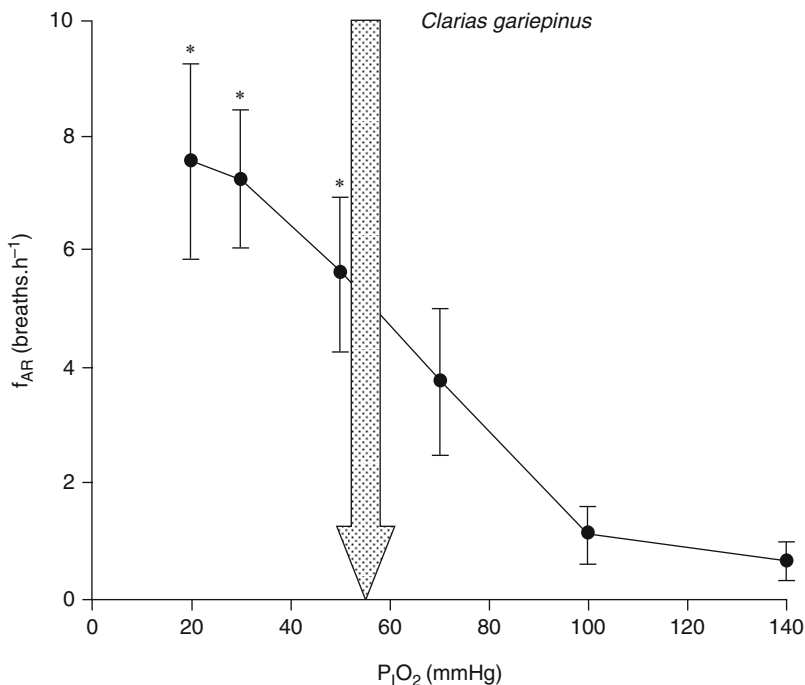


Fig. 4 Air-breathing frequency of the African catfish, *Clarias gariepinus*, as a function of the inspired PO_2 . The arrow indicates the critical oxygen tension ($PcO_2 = 55$ mmHg) for this species. Notice that the f_{AR} increased significantly near the PcO_2 to reach maximum values (10-fold in relation to the initial values) at a P_iO_2 of 20 mmHg. Asterisks represent statistical significance in relation to the normoxic condition (unpublished data)

walls of the interior chamber, and the end of the arborescent surfaces (Munshi 1976; Singh et al. 1982).

The African catfish *Clarias gariepinus* is a facultative air-breather, which can maintain an adequate $\dot{V}O_2$ down to a critical PO_2 of about 55 mmHg. Below this tension, the fish increases the \dot{V}_G considerably, due to a larger V_T . At this point the species develops hypoxic bradycardia before the air-breaths, followed by tachycardia after the surface episode (unpublished data). Figure 4 shows the frequency of air breathing (f_{AR}) of *C. gariepinus* in response to graded hypoxia.

1.11 Oxygen and CO_2/H^+ Receptors

The ever-changing conditions in water are matched by a highly O_2 -oriented respiratory control in holoost and teleost fish. Oxygen receptors are located within the gill system to monitor blood gases, or alternatively, the inspired water (Burlinson and Milsom 1995a, b, 1990). These external receptors are important for homeostasis

of the blood. As an example, carp (*Cyprinus carpio*) was exposed to light hypoxia ($P_{wO_2} = 97$ mmHg), but P_{aO_2} remained at ~ 30 mmHg during both normoxia and hypoxia. Simultaneously, gill ventilation increased from 296 to 470 ml $kg^{-1} min^{-1}$, due to stimulation by the external O_2 receptors. In addition, there is evidence for $[O_2]$ receptors in carp, since gill ventilation increased 50%, after reduction of $[O_2]_a$ from 6.8 to 4.4 vol% by application of CO. Carp were also exposed to hypercarbia (normoxia, 7 mmHg and 14 mmHg, which increased gill ventilation twofold. Unfortunately, the increases of gill ventilation correlated with significant reductions of $[O_2]_a$ due to hypercarbia-induced Root and Bohr-shifts (Soncini and Glass 2000). Therefore, it was not possible to pin down the exact modality of the underlying receptors.

Gilmour et al. (2005) studied cardiac and respiratory responses to hypercarbia in tambaqui (*Colossoma macroporum*). Stepwise increase of P_{aCO_2} augmented the amplitude and frequency of ventilatory movements, which suggested the presence of CO_2 receptors. The tested range was $PCO_2 = 7-25$ mmHg, but information on conditions in the habitat would have been useful. The responses to CO_2 predominated whereas the effects of pH changes were weaker. As a problem, it is difficult to distinguish between specific CO_2/H^+ receptors and $[O_2]$ receptors. The latter type can be stimulated by reduction of $[O_2]$ due to Bohr shifts and Root effects (cf. Soncini and Glass 2000).

Perry et al. (2004) addressed the exposure of tropical fish to large fluctuations of ambient $[O_2]$ levels. One choice was the air-breathing erythrinid jeju (*Hoplerythrinus unitaeniatus*), an active freshwater predator, widely distributed in tropical and subtropical regions of South America (Kramer 1978; Rantin and Johansen 1984). The air-breathing organ (ABO) of jeju is the swimbladder, which is subdivided into an anterior and a posterior chamber (Fig. 5), which is subdivided into an anterior richly vascularized respiratory portion, and a nonrespiratory caudally oriented sac. As a facultative air-breather, jeju relies primarily on its gills for gas exchange as long as the water remains normoxic or moderately hypoxic, but it ventilates its ABO as a facultative option, when exposed to severe aquatic hypoxia (Kramer 1978; Stevens and Holeton 1978; Graham 1997). It turned out that this species had no release of plasma catacholamines, regardless of levels of aquatic hypoxia. With reductions of P_{wO_2} the ventilation of the ABO increased in a hyperbolic manner down to $P_{wO_2} = 10$ mmHg. On the other hand, its hypoxia-tolerant relative traira (*Hoplias malabaricus*) depends completely on gill ventilation, and its catacholamine levels increased no less than 18-fold, which clearly documents the advantages of an ABO. Catacholamines have been considered as a stimulus to gill ventilation (Randall and Taylor 1991), but their release failed to increase ventilation of the ABO in jeju. Oliveiro et al. (2004) evaluated the effects of aquatic hypoxia on jeju, and it turned out that the critical PO_2 was $P_{wO_2} = 40$ mmHg, and 50% of its time was spent at the surface, when P_{iO_2} was reduced to 20 mmHg (Fig. 6). In this species, air-breathing was totally abolished after complete branchial denervation (cranial nerve IX to first gill arch and all branches of cranial nerve X innervating the four gill arches). This indicates that the control of air-breathing in jeju involves O_2 chemoreceptors distributed on all gill arches (Lopes 2003).

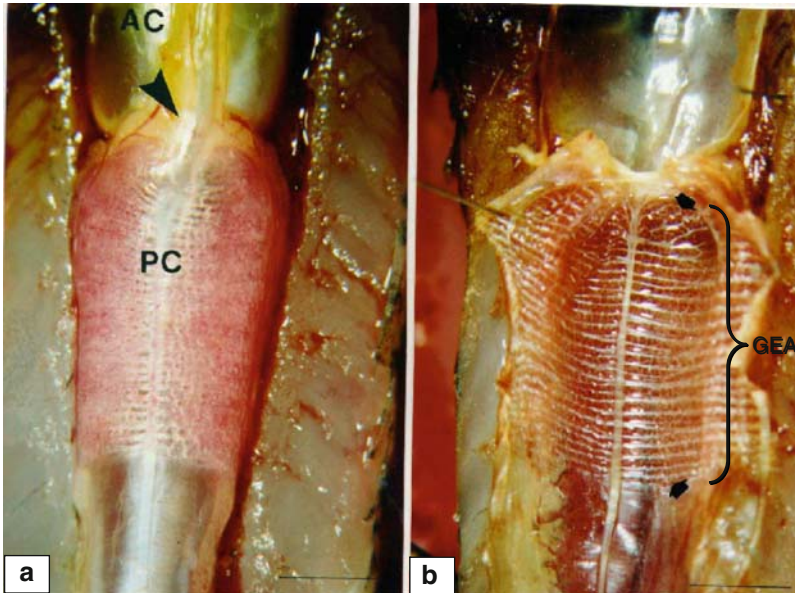


Fig. 5 Swimbladder of jeju, *Hoplerhynchus unitaeniatus*. **a** Intact organ showing the anterior membranous and nonvascularized portion (AC – anterior chamber) and the gas exchange unit (PC – posterior chamber with the vascularized anterior segment). **b** Opened bladder showing the gas exchange area (GEA). Courtesy from Dr. Marisa Narciso Fernandes (UFSCar, São Carlos, SP, Brazil)

Several studies report that hypercarbia stimulates ventilation of the gills and/or an ABO, but these effects are usually weak and, therefore, hard to pin down. By contrast, the responses to hypoxia are large and predominant. The existence of central acid–base receptors in holoost and teleost fish is still a disputed topic. Hedrick et al. (1991) applied central superfusion of mock CSF to the ventricular system of *Amia calva*, but this failed to stimulate ventilation of the ABO.

The long-nosed gar (*L. osseus*) gave a different and partly controversial result. Wilson et al. (2000) reported that hypercarbia increased the frequency of the air-breathing motor output from the *in vitro* brain stem. Smatresk and Cameron (1982) had earlier reported a modest increase of aerial ventilation in the gar pike (*Amia calva*) exposed to hypercarbia. It is not a surprise that an isolated preparation can change firing characteristics, if it is isolated from a possible frequency modulator, and the discovery of central respiratory neurons is highly important.

1.12 The Air-Breathing Descendants of the Sarcopterygians

Sarcopterygians (lobe-finned fish) are in a key position in vertebrate evolution, because their descendants were the land vertebrates (Tetrapoda), the lungfish

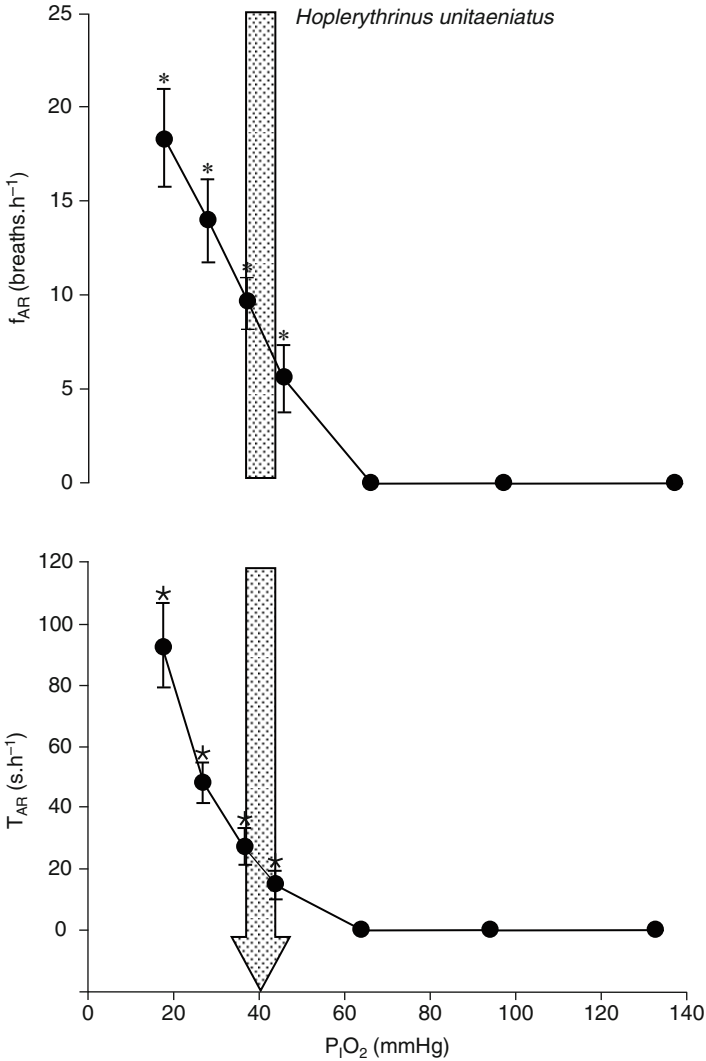


Fig. 6 Air breathing frequency (f_{AR}) and time spent at the surface (T_{AR}) of jeju, *Hoplerythrinus unitaeniatus* as a function of the inspired PO_2 . The arrow indicates the critical oxygen tension ($PcO_2 = 40$ mmHg) for this species. Asterisks represent statistical significance in relation to the normoxic condition (modified from Oliveiro et al. 2004)

(Dipnoi) and the coelacanth (Actinistia). Currently, the most likely sister group constellation is lungfish with the tetrapods (Toyama et al. 2000; Brinkmann et al. 2004), and a fossil (*Styloichthys*) estimated to be no less than 417 million years old fossil, which may be their last common ancestor (Zhu and Yu 2002). The South American lungfish *Lepidosiren paradoxa* inhabits the Amazon and Paran-Paraguai regions, while the African lungfish *Protopterus* inhabits West and South Africa with four

species. The Australian lungfish (*Neoceratodus forsteri*) inhabits rivers within the Queensland region, and it has well-developed gills combined with a simple lung (Kind et al. 2002). The gills of *Protopterus* and *L. paradoxa* are highly reduced, while the lungs are well developed, and the O₂ uptake from the water is only 5% at 25°C and nil at 35°C, whereas the CO₂ outputs were 50 (25°C) and 25% (35°C) (Amin-Naves et al. 2004). Lungfish have true lungs with a diffusing capacity (D_LO₂) is on level with that of a bullfrog (Bassi et al. 2005; Moraes et al. 2005), but the capacity for a similar-sized mammal is 16-fold higher (Takezawa et al. 1980). Just like land vertebrates, *L. paradoxa* and *P. annectens* have central chemoreceptors involved in acid–base regulation (Sanchez et al. 2001; Gilmour et al. 2007) and can be stimulated by CO₂ and H⁺ (Amin-Naves et al. 2007a). The central chemoreceptors of *L. paradoxa* provide 80% of acid–base-related drive to ventilation, while 20% of the drive is provided by peripheral receptors. These relative contributions of peripheral and central CO₂/H⁺ drives are largely identical to those of tetrapods (Amin-Naves et al. 2007b). Intrapulmonary receptors that are stimulated by stretch and inhibited by high CO₂ levels are found in many land vertebrates (Milsom 2002), and are also present in *L. paradoxa* and *Protopterus* (Delaney et al. 1983). Many highly specific physiological mechanisms are evidently common to tetrapods and lungfish, which certainly back up the idea of a sister group.

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