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Ionoregulatory and oxidative stress issues associated with the evolution of air-breathing

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ABSTRACT

Aquatic areas frequently face hypoxic conditions. In order to get sufficient oxygen to support aerobic metabolism, a number of freshwater fish resort to aerial respiration to supplement gill respiration especially in situations with reduced oxygen availability in the water. In many species a concomitant reduction in gill surface area or in gill perfusion reduces possible loss of aerially acquired oxygen to the water at the gills, but it also compromises the ion regulatory capacity of gill tissue. In consequence, the reduced gill contact area with water requires appropriate compensation to maintain ion and acid-base homeostasis, often with important ramifications for other organs. Associated modifications in the structure and function of the gills themselves, the skin, the gut, the kidney, and the physiology of water exchange and ion-linked acid-base regulation are discussed. In air-breathing fish, the gut may gain particular importance for the uptake of ions. In addition, tissues frequently exposed to environmental air encounter much higher oxygen partial pressures than typically observed in fish tissues. Physostomous fish using the swimbladder for aerial respiration, for example, will encounter aerial oxygen partial pressure at the swimbladder epithelium when frequently gulping air in hypoxic water. Hyperoxic conditions or rapid changes in oxygen partial pressures result in an increase in the production of reactive oxygen species (ROS). Accordingly, in air-breathing fish, strategies of ionoregulation may be greatly modified, and the ROS defense capacity of air-exposed tissues is improved.

1. Introduction

In the history of our earth the photosynthesis and the development of photosystem II caused an increase in atmospheric oxygen concentration. This allowed a switch from the previously anaerobic metabolism to aerobic metabolism with a greatly increased the rate of ATP production from carbohydrates. It also opened the door for using fatty acids as a fuel, which cannot be metabolized in the absence of oxygen in eukaryotic organisms. Accordingly, metabolism of heterotrophic organisms became largely dependent on the availability of oxygen. Under conditions of reduced oxygen availability, some organisms or certain tissues may transiently refer to anaerobic metabolism. Hypoxia typically causes a significant reduction in ATP production and results in metabolic depression (St-Pierre et al., 2000; Guppy, 2004; van Ginneken and van den Thillart, 2009; Ali et al., 2012). Coastal areas and certain freshwater systems are prone to transient hypoxic situations (Diaz and Rosenberg, 2008; Diaz and Breitburg, 2009; Jenny et al.,

2016), and in our study we will focus mainly on the freshwater situation.

In aquatic systems, in particular in the Amazon Basin, the oxygen concentration is known to vary greatly, ranging from very low oxygen partial pressures in stagnant water during the night, to clearly hyperoxic conditions at day time when photosynthetic active organisms produce and release oxygen (Val and Almeida-Val, 1995; Muusze et al., 1998; Diaz and Breitburg, 2009; Welker et al., 2013). To supplement oxygen supply during aquatic hypoxia, many fish of the Amazon Basin rely on aquatic surface respiration or even use specific tissues or organs for aerial gas exchange (Val and Almeida-Val, 1995; Graham, 1997). A vascularized stomach or intestine is used for aerial gas exchange by some loriciid and the callichthyid fish. The tambaqui picks up oxygen from the water surface with a largely extended inferior lip under hypoxic conditions, and some species of the erythrinid family of fish use the swimbladder to extract oxygen from the air. Pharyngeal, branchial and mouth diverticula are found among the Electrophoridae and the

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Synbranchidae, while the South-American lungfish *Lepidosiren paradoxa* takes most of the oxygen required for aerobic metabolism using its well-developed lung (Bemis et al., 1987; Val and Almeida-Val, 1995; Glass and Rantin, 2009).

In fish, venous blood is returned to a single atrium and, from the ventricle, the blood is pumped to the gills for reoxygenation. Oxygenated blood passes on to the systemic circulation, and then from the various organs returns to the heart. Accordingly, if under hypoxic conditions the gut or the swimbladder, for example, are used as an air-breathing organ, the oxygenated blood is returned to the heart and during subsequent passage through the gills oxygen may be lost again to the hypoxic water. In hypoxic water a loss of oxygen through the gills has indeed been recorded, for example, in spotted gar (*Lepisosteus osseus*) (Smatresk and Cameron, 1982; Smatresk, 1986), and in bowfin (*Amia calva*) (Johansen et al., 1970b; Randall et al., 1981b). A recent study also documented oxygen loss in some individuals (4 out of 11 animals) of the armoured catfish *Hypostomus aff. pyreneusi* at low PO₂ in the water (Scott et al., 2017).

In consequence, in many air-breathing fish a reduction in the surface area of the gills has been detected compared to purely water breathing species, thus reducing the possibility to lose oxygen obtained by aerial respiration during passage of the blood through the gills (Cameron and Wood, 1978; Graham, 1997; Brauner and Val, 2006; Gonzalez et al., 2010; Scott et al., 2017).

Fish gills, however, are multifunctional organs and serve for respiration, ion uptake and exchange, water homeostasis, acid-base regulation, and nitrogenous waste excretion (Laurent and Perry, 1991; Evans et al., 2005). Therefore, a reduction in gill surface area will compromise their capacity for ion exchange and acid-base regulation.

Another problem encountered with the development of a special air-breathing organ is related to oxygen. Because arterial oxygen partial pressure of water breathing fish typically is much lower than aerial oxygen partial pressure (Gilmour and Perry, 1994; Kristensen et al., 2010), oxygen partial pressure and concentration in the air-breathing structures of fish breathing air certainly are higher than in other tissues. Higher concentrations of oxygen may, however, result in the production of reactive oxygen species (ROS). The accumulation of ROS causes oxidative stress (Lushchak, 2014; Sies, 2015) and results in lipid peroxidation, protein carbonylation, and/or DNA modifications, i.e. formation of oxidized bases, in particular 8-oxoguanine (Lushchak, 2011). In consequence, the inordinate accumulation of ROS causes serious tissue damage and is harmful for the whole organism.

The extensive changes in the anatomy and accompanying physiology of the organs involved in respiration, circulation, and excretion, connected to the evolutionary transition from breathing water to breathing air have been extensively studied in air-breathing fishes (Carter and Beadle, 1931; Johansen et al., 1970a, b; Johansen, 1972; Kramer et al., 1978; Dejours, 1981; Randall et al., 1981a; Ultsch, 1996; Graham, 1997; Martin and Bridges, 1999; Evans et al., 1999; Sayer, 2005; Glass and Rantin, 2009; Ishimatsu, 2012; Milsom, 2012; Lefevre et al., 2014; Wright and Turko, 2016). Far less attention has been given to two other issues: (i) the reduced availability of an aqueous environment as a major source of electrolytes for ionoregulation, counterions for acid-base regulation, and water for diffusive and osmotic exchanges through the gills, with important ramifications for other organs; (ii) the consequences for oxidative stress resulting from the intimate contact of tissues with a medium (air) which has many-fold higher O₂ concentration, and often a higher PO₂ than either the external water or the internal tissues. The evolution of air-breathing may have occurred independently 38–67 times in fish (Graham, 1997; Graham and Lee, 2004). Our goal here is to highlight overall trends that may illuminate common problems and convergent strategies for overcoming them that were employed in the evolution of air-breathing.

2. Ion and water homeostasis

2.1. Reduced contact with the aqueous environment: consequences for gills and other organs

Very simply, as the gills became smaller, less ventilated with water, in some fish more exposed to air, and less perfused with blood due to bypass shunting, critical branchial functions for ionoregulation, coupled acid-base exchange, and osmoregulation had to be modified and/or shifted to other organs. That organ was clearly not the swimbladder or incipient lung (Zheng et al., 2011) as it is not in contact with external water, ions, or acid-base equivalents.

2.2. Changes in gill ionoregulatory function

General trends seen in the gills of air-breathing fish include reductions in overall gill mass and gill surface area, the latter additionally due to stubbier filaments and lamellae, loss or clumping of lamellae, and infilling between lamellae with the interlamellar cell mass (ILCM), all of which contribute to increased blood-to water diffusion distance (Perna and Fernandes, 1996; Graham, 1997; Brauner et al., 2004a). The actual flow of blood through the lamellae is also likely reduced by bypass shunting, increasing physiological dead space. The net effect of these changes is to reduce O₂ uptake across the gills under normal circumstances, and perhaps more importantly, to minimize O₂ losses to external hypoxic water from venous blood entering the gills when the fish is ventilating the air-breathing organ (ABO) (Johansen et al., 1970a; Randall et al., 1981a, b; Smatresk and Cameron, 1982; Smatresk, 1986; Scott et al., 2017). CO₂ and ammonia excretion are less affected, presumably because of their greater lipid solubility, their potential transport by facilitated diffusion carriers such as Rh proteins (Wright and Wood, 2009; Perry et al., 2010), and the greater capacitance of the water for these respiratory gases. Indeed Janis and Farmer (Janis and Farmer, 1999) noted that no living fish has completely lost its gills, and speculated that this is because the gills remain essential for N-waste and CO₂ excretion. These morphofunctional changes accompanying air-breathing that reduce effective gill contact with the water would be expected to (i) compromise active ion uptake and ion versus acid-base exchanges at the gills, and (ii) reduce rates of diffusive ion loss and water exchange.

A useful approach to test these predictions is to compare closely related air-breathing versus water-breathing species such as the erythrinids jeju (*Hopleythrinus unitaeniatus*, a facultative air-breather) versus traira (*Hoplias malabaricus*, an obligate water breather) (e.g. Cameron and Wood, 1978; Hulbert et al., 1978b; Fernandes et al., 1994; Wood et al., 2016) or the osteoglossids pirarucu (*Arapaima gigas*, an obligate air-breather) versus arowana (*Osteoglossum bicirrhosum*, an obligate water-breather) (Hulbert et al., 1978c; Brauner et al., 2004a; Gonzalez et al., 2010). The pirarucu may be particularly instructive as it represents an individual species where increased reliance on air-breathing occurs as development proceeds (Fernandes et al., 2012), such that it transitions from a water-breathing early life stage to an obligate air-breathing adult stage where the lamellae have been completely obliterated externally by formation of an ILCM, and greatly reduced internally by atrophy of the pillar cell channels (Ramos et al., 2013).

With respect to baseline ion fluxes under normoxic conditions, these predictions are only partially supported by the limited physiological data available. For example, branchial unidirectional Na⁺ uptake and efflux rates in normoxic water were actually higher in the jeju (a facultative air-breather) than in the water-breathing traira, despite a 50% lower gill area in the former (Cameron and Wood, 1978). However, after a period of fasting, the traira was able to maintain positive net Na⁺ and Cl⁻ balance with water (Fig. 1B), whereas the jeju could not (Fig. 1A), and plasma ions in the jeju were lower (Wood et al., 2016). The explanation for the higher unidirectional fluxes in the jeju may be

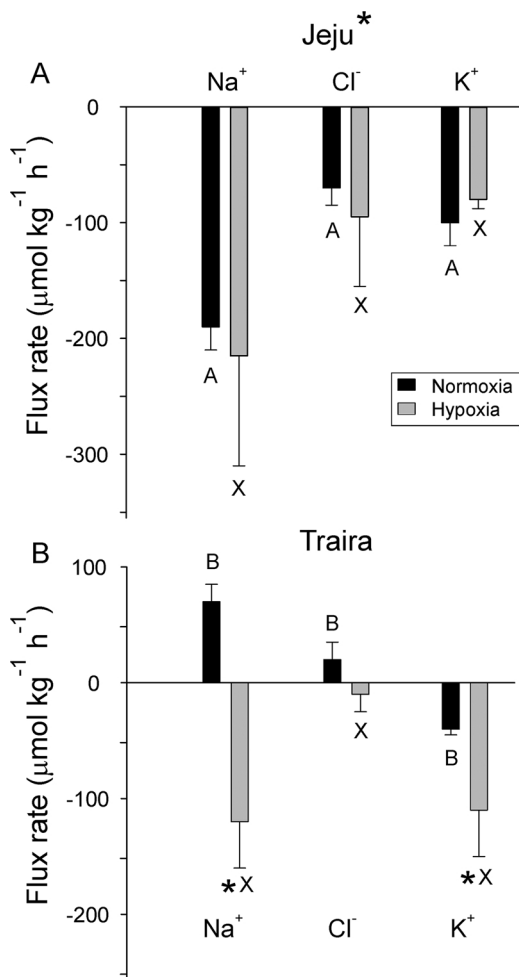


Fig. 1. A comparison between two erythrinid species (A) the facultative air-breather jeju (*Hopleythrinus unitaeniatus*) and (B) the pure water-breather traira (*Hoplias malabaricus*), of net flux rates of Na^+ , Cl^- , and K^+ with the water under normoxia and at 1–2 h of acute severe waterborne hypoxia (15% air saturation). The animals were fasted. Note that under normoxia, the jeju cannot maintain positive ion balance, whereas the traira is in largely positive balance. However, during severe hypoxia, when the jeju resorts to greatly increased air-breathing, there is negligible disturbance of net ion flux rates, whereas the traira, which is obligated to continue water breathing, suffers substantial net ion losses. Capital letters denote significant differences between the two species for a particular ion under either normoxia or hypoxia (means sharing the same letter are not significantly different). * indicates significant differences within a species between hypoxia and normoxia. Large * on jeju indicates a significant overall difference between the two species by two-way ANOVA (adapted from Wood et al., 2016).

the higher specific activities of ion-motive enzymes (Na^+ , K^+ ATPase, v-type H^+ ATPase) recorded in its gill tissue. These may represent a more than sufficient compensation for the reduction in gill surface area and lower number of ionocytes (Hulbert et al., 1978b; Wood et al., 2016). An additional compensation may be the location of the ionocytes. Based on a survey of 66 fish species, both water-breathing and air-breathing, Lin and Sung (Lin and Sung, 2003) concluded that air-breathers were much more likely to have ionocytes on the lamellae where they could be more effective for ion uptake, despite impeding respiratory gas exchange (Greco et al., 1995). Air-breathing is a risky business because it exposes the fish to greater threat of predation (Sloman et al., 2009), so it makes sense that fish will minimize this behavior as much as possible. Having a gill that still functions well for normal ionoregulation in water, despite structural modification for facultative air-breathing when absolutely necessary, facilitates this trade-

off.

In the pirarucu, mass-specific unidirectional Na^+ uptake and efflux rates and the ability to compensate a respiratory acidosis were actually greater in older animals with lower mass-specific gill areas and lower apparent diffusing capacities than in younger animals (Gonzalez et al., 2010). These observations are again counterintuitive, and could not be explained by Na^+ , K^+ ATPase specific activity which was lower in the gills of the older animals. However absolute ionocyte numbers were greater in the gills of older animals, and they were concentrated in the ILCM regions (Ramos et al., 2013), so again, it may be a matter of a more favorable location. The development of long sinuous paracellular channels between the epithelial cells in older individuals (Ramos et al., 2013) may also facilitate ion exchange. Regardless, the overall rates of Na^+ influx and efflux in pirarucu were very low relative to most freshwater fish, and the animals were in net negative Na^+ balance with the water (Gonzalez et al., 2010). Notably, branchial Na^+ K^+ ATPase specific activities were much lower in the pirarucu than in its water-breathing relative, the arowana *Osteoglossum bicirrhosum* (Hulbert et al., 1978c). Similarly, unidirectional Na^+ flux rates were very low (~10% of typical teleost values) in the marbled swamp eel *Synbranchus marmoratus*, a facultative air-breathing synbranchid (Stiffler et al., 1986), two species of African lungfish, the slender lungfish, *Protopterus dolloi* and the Tana (West African) lungfish, *Protopterus annectens* (Sawyer and Pang, 1980; Stiffler et al., 1986; Wilkie et al., 2007; Patel et al., 2009a), and the South American lungfish *Lepidosiren paradoxa* (Stiffler et al., 1986). Like the pirarucu, these dipnoans are obligate air-breathers as adults.

With respect to ion fluxes under conditions where the fish is forced to rely more on air-breathing, the available data are in better support of the predictions. For example, when exposed to severe environmental hypoxia (15% air saturation), jeju escaped the stressor by resorting to air-breathing (Pelster et al., 2016) and exhibited negligible disturbance of net Na^+ , Cl^- , and K^+ balance (Fig. 1A) (Wood et al., 2016). In contrast, the obligate water-breathing traira suffered net losses of all three ions to the external water (Fig. 1B) (Wood et al., 2016), presumably due to the osmorepiratory compromise at the gills, i.e. increased branchial ion losses as a consequence of an increased gill surface area or a decreased diffusion distance to improve oxygen uptake (Randall et al., 1972; Robertson et al., 2015). Similarly, when the facultative air-breathing swamp eel was encouraged to air-breathe by exposure to aquatic hypoxia (20% air saturation), it maintained unidirectional Na^+ fluxes unchanged and net Na^+ flux actually became slightly positive (Stiffler et al., 1986). When facultative air-breathing bowfins (*Amia calva*, holosteans) were exposed to very acidic water pH (3.5), increased air-breathing allowed the animals to better maintain plasma Na^+ and Cl^- levels than in fish which were not allowed to air-breathe (Gonzalez et al., 2001), presumably because of reduced ion losses through the gills. On the other hand, access to air-breathing exacerbated the acid-base disturbance during low pH exposure, as well after exhaustive exercise, presumably because non-respiratory shunting of gill blood flow associated with use of the ABO compromised the capacity of ion uptake-linked acid-base transport processes on the branchial epithelium. When the African Tana lungfish was exposed to “deoxygenated” water (O_2 level not reported), unidirectional Na^+ efflux rate decreased greatly, again probably reflecting decreased ion loss across the gills (Oduleye, 1977). In more extreme circumstances, when branchial access to water was completely denied by placing the animals in air on a wet surface, both the slender African lungfish (Wilkie et al., 2007) and the mangrove killifish (*Kryptopterus marmoratus*, a facultative air-breathing cyprinodont) (LeBlanc et al., 2010) were able to maintain plasma or whole body ions quite stable by low rates of ion and water exchange with the wet surface, presumably through the ventral skin (see Section 2.3.). Interestingly, the epithelial sodium channel (ENaC), which has proven elusive in teleosts, is in fact expressed in the gills, kidney, and rectum of both the Australian lungfish (Uchiyama et al., 2012) and African Tana lungfish (Uchiyama et al., 2015) but not in the

ventral body skin. Expression levels declined during prolonged aestivation.

2.3. Increased use of the skin for ionoregulation

This is a well-studied area (reviewed by (Glover et al., 2013), and there are numerous examples where the skin is used as an auxiliary ionoregulatory organ in air-breathing and amphibious fishes, and indeed even in some exclusively water-breathing fish such as various cichlids (opercular epithelia – e.g. (McCormick et al., 1992; Burgess et al., 1998) and salmonids (cleithral epithelium and body skin – e.g. (Perry and Wood, 1985; Marshall et al., 1992). The skin appears to be the predominant organ of ionoregulation in the early life stages of many fish (reviewed by (Zimmer et al., 2017), so its use in adult fish may be interpreted as the exploitation of a neotenic characteristic. With respect to air-breathing fish, notable examples include the jaw-skin of the goby *Gillichthys mirabilis* (Marshall, 1977), the pectoral fins and general body surface of the shanny *Lipophrys (Blennius) pholis* (Nonnotte et al., 1979), and the general body surfaces of the mangrove killifish *Kryptolebias marmoratus* (LeBlanc et al., 2010), the slender African lungfish (Wilkie et al., 2007), and the marbled swamp eel *Synbranchus marmoratus* (Stiffler et al., 1986). The opercular epithelium of the common killifish, *Fundulus heteroclitus* (Karnaky et al., 1977) is particularly notable, because it has provided what is arguably the most important *in vitro* preparation in fish ionoregulatory physiology, serving as an Ussing chamber-friendly surrogate for the branchial epithelium in literally hundreds of papers dedicated to understanding the cellular mechanisms of ion transport in the gills of euryhaline teleosts (reviewed by (Zadunaisky, 1984; Marshall, 1995; Marshall and Bellamy, 2010). This killifish is usually considered to be an exclusively water-breathing fish, but overlooked is the fact that when faced with adverse water quality, this species can emerge and sustain aerial respiration, albeit at a lower rate, without accumulating an O₂ debt (Halpin and Martin, 1999). Interestingly, we are aware of no studies on whether the opercular epithelium is involved in ion transport when the fish is in air.

Depending on the acclimation condition of the fish, these epithelia appear to be able to actively take up Na⁺ and Cl⁻ from fresh water, and to excrete them into sea water. The skin also appears to be especially important for Ca²⁺ transport. For example, in the freshwater rainbow trout, the general body skin provides about half of the whole body Ca²⁺ influx (Perry and Wood, 1985), and the opercular epithelia in the freshwater killifish may account for the majority of Ca²⁺ uptake (Marshall et al., 1995). African lungfish take up Ca²⁺ from fresh water at typical teleost rates, even though rates of Na⁺ and Cl⁻ uptake are extremely low (Patel et al., 2009a). Effective transport of ions occurs even when there is only a thin film of water contacting the ventral body surface (e.g. (Wilkie et al., 2007; LeBlanc et al., 2010). The skin may also exhibit some capacity for ammonia and urea excretion and acid-base regulation (e.g. (Wood et al., 2005; Hung et al., 2007; Gilmour et al., 2007; Hung et al., 2009; Cooper et al., 2013), though as discussed subsequently (see Section 2.7.), the latter may be curtailed in air-breathing fish, at least for pH regulation in the extracellular compartment. In all cases, there are readily identifiable mitochondria-rich cells or ionocytes in the skin, with the greatest concentrations in the areas where ion transport is greatest – for example, the ventral surface in the mangrove killifish (LeBlanc et al., 2010) and the Tana lungfish (Sturla et al., 2001). During prolonged air exposure, morphological changes occur in the skin which presumably increase ion transport capabilities and/or decrease rates of evaporative water loss (Sturla et al., 2002; LeBlanc et al., 2010), and in terrestrialized lungfish, all but the ventral surface becomes encased in a cocoon (Wilkie et al., 2007). In at least one species, the mangrove killifish, blood flow to the skin can be increased both acutely, through changes in vasomotor tone, and chronically, through angiogenesis, so as to facilitate increased cutaneous O₂ uptake during emersion (Cooper et al., 2012). We are aware of no studies investigating whether a similar phenomenon occurs to facilitate

cutaneous ion exchange in air-breathing fish. However, in an amphibian, the tiger salamander *Ambystoma tigrinum*, experimentally induced reductions in skin blood flow severely compromised cutaneous Na⁺ uptake (Stiffler, 1988). This is a topic worthy of future investigation.

In summary, the skin plays a critical role as a supplementary or alternate ionoregulatory organ to the gills as fish make the transition from water-breathing to air-breathing. However, from a long-term evolutionary perspective, cutaneous ion uptake is just an interim solution because effective cutaneous contact with water, or at least wet surfaces, is eventually lost once organisms transition to life on land. Another mechanism for ion acquisition from the environment must be developed, and that mechanism involves the digestive tract.

2.4. A shift towards ion uptake through the digestive tract

This area has been curiously overlooked until recently, though it must be pointed out that the potential importance of ion uptake from the diet has been overlooked in general by ionoregulatory physiologists, despite evidence that trophic intake can supply > 50% of the normal electrolyte requirements of wild water-breathing fish such as salmonids (Smith et al., 1989; Wood and Bucking, 2011). Indeed a certain amount of Na⁺ uptake through the gut may be absolutely necessary for nutrient absorption because of linked transport mechanisms such as Na⁺-dependent glucose and amino acid uptake. Once air-breathers lose contact with water, all ion acquisition from the environment must occur through the digestive tract.

The only quantitative assessment was performed in African lungfish. Patel et al. (Patel et al., 2009a) measured electrolyte budgets in both the slender lungfish (Fig. 2A) and the Tana lungfish (Fig. 2B) kept under aquatic conditions and fed a 3% ration of bloodworms every 48 h. The diet accounted for 35–47% of Na⁺ intake, 32–33% of Ca²⁺ intake, but only 1–4% of Cl⁻ intake. These figures for Na⁺ and Ca²⁺ acquisition through the gut are comparable to those measured in water-breathing fishes (Wood and Bucking, 2011). The low Cl⁻ intake from the diet is unusual, but may have reflected the low Cl⁻ content of the bloodworm diet. Ion loss rates through the faeces were low, representing 1–20% of total uptake rates, comparable to those in the urine, and far below those through the gills and body surface. These experiments did not test whether dietary uptake accounted for a greater fraction of total uptake when the animals were forced to rely more on air-breathing. However in the Tana lungfish, the “specific dynamic action” effect of feeding itself was accompanied by increased air-breathing, though the relative partitioning of O₂ uptake (~40% from water, ~60% from air) did not change (Iftikar et al., 2008).

Various parts of the gut itself are used as ABOs in many air-breathing fish, raising interesting questions about the tradeoffs between alimentary and respiratory functions. This area has been thoroughly reviewed by Nelson (Nelson, 2014), and there has been one experimental study showing some reduction in nutrient transporter abundance relative to other gut sections in the respiratory portion of the intestine in the Asian weatherloach *Misgurnus anguillicaudatus* (Goncalves et al., 2007). However to date, the focus has been on possible compromises between respiration *versus* digestion and nutrient absorption, not *versus* ion absorption. Given that gut breathing may in fact be the most common method of air-breathing in fish overall (Nelson, 2014), this is an important area for future investigation.

Recently, we examined the Amazonian erythrinid pair, the facultative air-breathing jeju *versus* the obligate water-breathing traira, to see if there was indeed a shift towards ion uptake through the digestive tract with the evolutionary transition to air-breathing (Wood et al., 2016). *In vitro* experiments demonstrated that the capacity for uptake of Na⁺, Cl⁻, and K⁺ through the digestive tract was far greater in the air-breather (Fig. 3A), and this was associated with greater activities of ion-motive enzymes (Na⁺, K⁺ ATPase, v-type H⁺ ATPase) in the intestine (Fig. 3B). *In vivo* experiments demonstrated that the obligate water-breather was able to maintain generally positive ion balance without

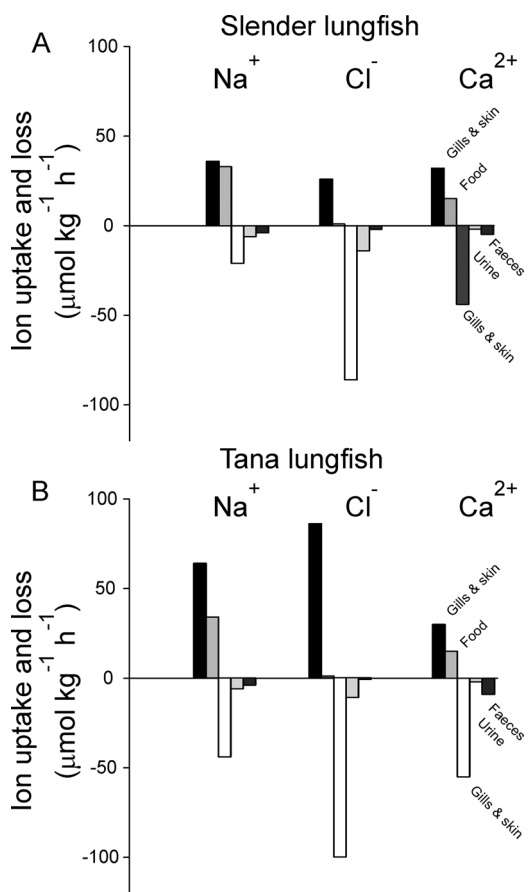


Fig. 2. A quantitative illustration of the routes of uptake and loss of Na⁺, Cl⁻, and Ca²⁺ in two species of African lungfish (A) the slender lungfish (*Protopterus dolloi*) and (B) the Tana lungfish (*Protopterus annectens*) when living in water and feeding on a bloodworm diet. Note the importance of both the gills + skin, and the ingestion of food, in Na⁺ and Ca²⁺ uptake, the importance of only the gills + skin route in Cl⁻ uptake (as the bloodworm diet contains very little Cl⁻), the importance of gills + skin in the efflux of all three ions, and the very small loss rates in faeces and urine. Calculations based on data presented in Patel et al. (2009a) and Patel et al. (2009b).

feeding (Fig. 1B), whereas the facultative air-breather exhibited negative ion balance (Fig. 1A), suggesting that ion uptake from the diet was essential.

These very limited data raise intriguing questions. Does a carnivorous lifestyle favor air-breathing because of the higher Na⁺ and Cl⁻ availability in the diet? If so, how do we explain the fact that most gut-breathing fish are herbivorous (Nelson, 2014)? Is it because the much greater length of the gut in herbivores creates a much greater capacity for ion uptake (Pelster et al., 2015), with sufficient leeway to sacrifice some of this for respiratory gas exchange? Does feeding encourage more or less voluntary air-breathing? If forced to rely more on air-breathing, do bimodal breathers increase ion uptake capacity at the gut and decrease it at the gills? To date, these topics have received minimal attention, and represent a rich area for future investigation by comparative physiologists.

2.5. Improved capacity of the kidney for ionoregulation and osmoregulation

In addition to the skin and the gut, the kidney is another organ that must play a key role in ionoregulation during the water-breathing to air-breathing transition (Smith, 1959; Vize, 2004), but the kidney can only retain and regulate ions, not acquire them from the environment, which is a critical requirement for freshwater fish. Physiological investigations on the fish kidney are technically more difficult than on the

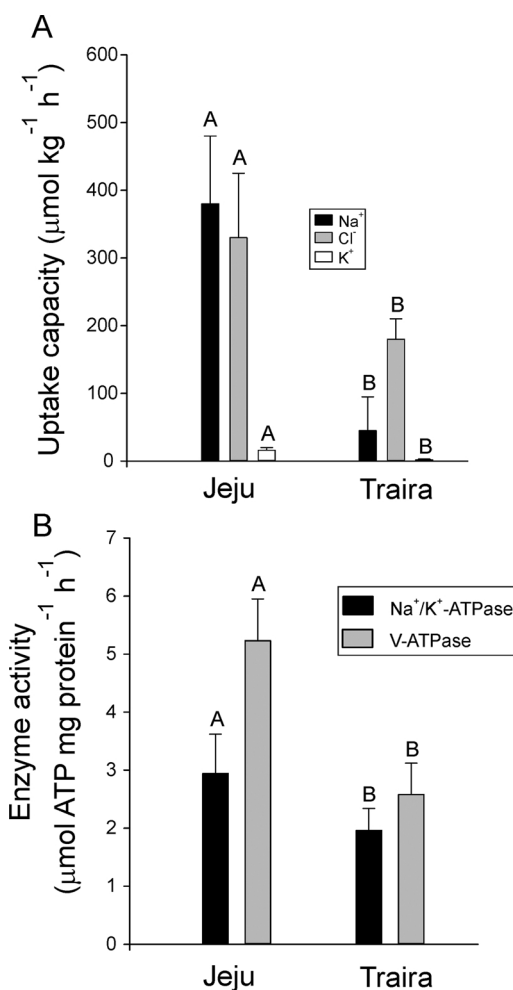


Fig. 3. A comparison between two erythrinid species, the facultative air-breather jeju (*Hopleythrinus unitaeniatus*) and the pure water-breather traira (*Hoplias malabaricus*), of (A) the total absorptive transport capacity of the intestinal tract for Na⁺, Cl⁻, and K⁺, and (B) the average activity levels of the transport enzymes Na⁺, K⁺-ATPase and vH⁺-ATPase over the entire intestinal tract. Note the higher transport capacities and higher enzyme activities in the facultative air-breather. Capital letters denote significant differences between the two species for a particular ion or enzyme (means sharing the same letter are not significantly different) (adapted from Wood et al., 2016).

skin, and as a result there is far less information available. Most studies have focused on structure and metabolic biochemistry.

An early comparison of the Amazonian erythrinid pair, the facultative air-breathing jeju versus the obligate water-breathing traira, found that the kidney was larger in the air-breather but that the overall organization was similar in the two species (Hulbert et al., 1978a). While the basic structure of the nephron was similar to that in other freshwater fish, there was an unusual abundance of mitochondria-rich “chloride cells” in the third (distal) segment of the nephron in both species. The authors considered these cells to be similar to the ionocytes of the gills, and the “active” forms of these cells were more abundant in the jeju. This observation correlated with an approximately 5-fold higher total Na⁺, K⁺ATPase activity in the kidney of the air-breather (Hulbert et al., 1978a). Wood et al. (Wood et al., 2016) also reported a higher Na⁺, K⁺ATPase activity in the kidney of the jeju, but the relative difference was less marked. Nevertheless, the assumed role was a more effective reabsorption of ions from the tubular urine. However, a physiological comparison of the two species through collection of urine via indwelling bladder catheters actually found higher urinary Na⁺ and Cl⁻ losses in the jeju, at least under normoxic conditions (Cameron and

Wood, 1978), so the functional significance remains unclear.

A similar structural and biochemical comparison was made on the Amazonian osteoglossid pair, the obligate air-breathing pirarucu *versus* the obligate water-breathing arowana (Hochachka et al., 1978; Hulbert et al., 1978a). In the pirarucu, the kidney is a remarkable structure, 3–4 times greater in mass than in the arowana, engorged with blood, and lying midventrally along the dorsal surface of the large lung cavity (see Fig. 1 of (Hochachka et al., 1978)): it is hard to believe that there is not a mechanistic, functional relationship between the two organs, though this has never been investigated. In both species, as in the erythrinids, the proximal regions of the nephrons were not unusual, but there was again an unusual abundance of “chloride cells” in the third (distal) segments, and these segments were particularly long in the pirarucu. Curiously, these “chloride cells” presented an inactive morphology in both species.

This inactive appearance was characterized by large cuboidal shape, filled with electron-transparent cytoplasm with numerous mitochondria concentrated in the basal region of the cell. The mitochondria were closely associated with an anastomosing tubular network very similar to that found in inactive chloride cells in the gill. In contrast, the active-type chloride cells were smaller, with extremely electron-dense cytoplasm, packed full of mitochondria and extensive anastomosing membrane networks, as in the active “chloride cells” of the gill, though apical crypts were absent. Nevertheless, total Na^+ , K^+ ATPase activity was 15-fold greater in the pirarucu kidney, while Ca^{2+} -ATPase activity was 3-fold higher, again pointing to a stronger ion reabsorption in the tubules of the air-breather *versus* the water-breather. Glutamate dehydrogenase activity was also about 7-fold higher, suggesting greater ammoniogenic potential and therefore a greater role for the kidney in gluconeogenesis, ammonia excretion, and acid-base balance in the air-breather. Unfortunately, there have been no functional comparisons of the two species, but there were later reports of very high total ammonia ($\sim 10 \text{ mmol L}^{-1}$) (Gonzalez et al., 2010) and CO_2 concentrations (29 mmol L^{-1} - probably mainly HCO_3^-) (Brauner and Val, 1996) and very low Na^+ and Cl^- concentrations ($< 0.5 \text{ mmol L}^{-1}$) (Gonzalez et al., 2010) in pirarucu urine, in accord with these assumptions. There is a clear need for mechanistic studies on kidney function in the pirarucu.

The lungfish, especially members of the obligate air-breathing and aestivating African genus *Protopterus*, are the one group of air-breathing fish in which both the anatomy and physiology of the kidney have been investigated in some detail. Wake (Wake, 1986) presented an excellent summary of the early anatomical literature, and noted that basic nephron morphology was similar among the African, South American, and Australian genera. The renal corpuscles are unusual with groups of 3 or more served by a single afferent arteriole (Guyton, 1935; Ojeda et al., 2008) and at least in the South American lungfish *Lepidosiren paradoxa*, there are vascular shunts between the afferent and efferent glomerular arterioles (Guyton, 1935). The corpuscles undergo rapid morphological changes during aestivation/terrestrialization and return to fresh water (Ojeda et al., 2008). The presence of vascular shunts may facilitate graded glomerular recruitment and the rapid shutdown or reactivation of glomerular filtration when the fish becomes anuric during air exposure or rapidly restores renal excretion upon return to aquatic conditions (Smith, 1931; Godet, 1961; DeLaney et al., 1977; Wilkie et al., 2007). Classically, the neuroepithelial hormone arginine vasotocin (AVT) was thought to be involved in this control (Sawyer, 1966, 1970; Sawyer et al., 1976; Babiker and Rankin, 1979), and more recently nitric oxide (Amelio et al., 2008). Konno et al. (Konno et al., 2010) speculated that enhanced water reabsorption in the distal tubule may also contribute to anuria during aestivation, based on increased co-expression of the vasotocin V-2 receptor and a unique aquaporin paralog (*aqp0*) in the late distal segment. As in the Amazonian teleosts described earlier, the most remarkable features of the lungfish nephrons were large mitochondria-rich cells in the distal segment (Ojeda et al., 2006). The authors characterized these as “flask cells” of two different types; it is unclear whether they are homologous to the “active” and

“inactive chloride cells” of Hochachka et al. (Hochachka et al., 1978) and Hulbert et al. (Hulbert et al., 1978a), although Ojeda et al. (Ojeda et al., 2006) considered them to be important in ion transport.

A number of functional studies have been performed on the kidneys of various lungfish species living in fresh water (Sawyer, 1966, 1970; Sawyer et al., 1976; DeLaney et al., 1977; Babiker and Rankin, 1979; Sawyer et al., 1982; Patel et al., 2009a, b), and are in general agreement that rates of both glomerular filtration (GFR) and urine flow (UFR) are high relative to those of most of water-breathing freshwater teleosts, a difference of 1.5- fold or more. This is in accord with a highly permeable glomerular filtration barrier (Sawyer et al., 1976, 1982; Ojeda et al., 2008) and reflects the curiously high osmotic permeability of these lungfishes (Section 2.6.). Nevertheless, tubular ion reabsorption processes are extremely efficient (Patel et al., 2009b) so electrolyte levels in the urine are very low. Therefore, urinary ion losses are generally comparable to or lower than in freshwater teleosts (Fig. 2), while water excretion rates are higher. There is general agreement that urine production quickly ceases during aestivation/terrestrialization, but to our knowledge, no one has yet examined whether renal function changes when lungfish living in water are forced to rely more on air-breathing because of aquatic hypoxia. However when the marbled swamp eel (*Synbranchus marmoratus*), a synbranchid teleost, was forced to rely almost exclusively on air-breathing by aquatic hypoxia (10% saturation), UFR did not change (Heisler, 1982).

2.6. Changes in water permeability of the body surface

In 1928, Sir James Gray proposed that reduction of epithelial water permeability was probably a necessary exaptation (*i.e.* a “pre-adaptation” that was exploited in support of this new function) for the successful invasion of land (Gray, 1928). However, except for studies on lungfish, this topic has received almost no attention in air-breathing fish, a remarkable oversight. Certainly, the limited available UFR measurements, which are generally considered to be the best estimates of osmotic permeability (Isaia, 1984) do not support this idea in either air-breathing teleosts (*e.g.* (Cameron and Wood, 1978; Heisler, 1982; Brauner and Val, 1996; Gonzalez et al., 2010) or dipnoans (Section 2.5). It appears that the maintenance of high osmotic permeability, despite a reduction in gill surface area, may be necessary for other purposes during the aquatic life of air-breathing fishes. An alternative, though less likely interpretation (one that has never been tested) is that air-breathing fish in water drink the medium to a greater extent than standard water-breathing fish, such that UFR overestimates osmotic permeability.

With respect to diffusive water permeability, as measured by tritiated water turnover (Isaia, 1984), the only study on air-breathing teleosts appears to be that of LeBlanc et al. (LeBlanc et al., 2010) on the mangrove killifish. When these fish were experimentally emerged for 9 days with maintenance of ventral water contact, diffusive water turnover rates increased in freshwater-acclimated animals and decreased in seawater-acclimated individuals. Only the latter response appears adaptive, but in both treatment groups, whole-body water content data was preserved. Additional information is available for several dipnoans of the genus *Protopterus*. Here there is support for Gray’s hypothesis, with general agreement that diffusive water permeabilities of African lungfish living in water are low (20–50%) relative to those of standard water-breathing fish (Oduleye, 1977; Wilkie et al., 2007; Patel et al., 2009b), in parallel to the low rates of ion exchange measured in these same species (Section 2.2.). At least in part, this may be explained by the reduced gill area, as divided chamber experiments showed that about 80% of the diffusive water exchange occurred across the head (presumably the gills), and only about 20% across the rest of the body in slender lungfish and Tana lungfish (Patel et al., 2009b). However, the rate showed little change when the Tana lungfish was subjected to aquatic hypoxia, forcing it to rely more on air-breathing, despite the fact that Na^+ exchange was greatly reduced (Oduleye, 1977). During

experimental terrestrialization of the slender lungfish, diffusive water exchange initially continued at about 70% of aquatic values through the moist surface of the ventral skin, though this declined to about 30–35% after 6–8 months (Wilkie et al., 2007; Patel et al., 2009b). Whole-body water content actually increased substantially over this long period. Upon return to water after this long period in air, diffusive water exchange rates were rapidly restored to control aquatic values (Wilkie et al., 2007). These changes may be correlated with changes in the abundance of aquaporins (water channels). In the Tana lungfish, both *aqp1* and *aqp3* were expressed in gills and skin (Chng et al., 2016), and *aqp0* in the kidney (Konno et al., 2010). During the maintenance phase of aestivation, first mRNA expression and later protein abundance of *aqp1* and *aqp3* declined in the gills, with fairly rapid rebounds (1–3 days) upon return to water after 6 months in air; *aqp1* and *aqp3* changes in the skin and kidney were not analyzed (Chng et al., 2016). However the expression of *aqp0* in the kidney greatly increased during aestivation and declined upon return to fresh water (Konno et al., 2010), reflecting possible changes in tubular water reabsorption (Section 2.5).

Patel et al. (Patel et al., 2009b) noted the paradox that while osmotic permeability is high in lungfish (against Gray's hypothesis), diffusive water permeability is low (in support of Gray's hypothesis), with a ratio of 4–12, in contrast to 2.1–3.3 in standard freshwater teleosts. The two indices measure different properties of the barrier epithelia (Isaia, 1984), and it may be that low diffusive water permeability is the more important characteristic in limiting evaporative water loss in air. Mechanistic investigations, as well as measurements of the two indices in water and evaporative water loss rates in air, both in lungfish and other air-breathing fish, are needed to solve this paradox.

2.7. A shift towards preferential acid-base regulation of the intracellular compartments

Acid-base regulation is intimately linked to ionoregulation. Air-breathing is associated with the retention of CO₂ and potential respiratory acidosis (Dejours, 1981). Heisler's (Heisler, 1982) investigation on the marbled swamp eel (*Synbranchus marmoratus*, a facultative air-breathing synbranchid) provides a classic example. When exposed to severe hypoxia (10% saturation), the animal partially emersed its head and filled its buccal cavity (which serves as an ABO) with air, a behavior that persisted for days. Arterial PaCO₂ increased by about 20 torr, while arterial pH fell by about 0.6 units and remained at this acidotic level for 4–5 days. In contrast, the intracellular pH values of cardiac muscle and white muscle were perfectly regulated over this same time course, and this compensation occurred as a result of transfer of extracellular HCO₃⁻ to the intracellular compartment- *i.e.* tissue acid-base status was regulated at the expense of blood acid-base status. Acid-base exchange with the water was negligible. This was the first example of apparent preferential regulation of intracellular pH over extracellular pH in a fish (though it had also been shown in the bimodally breathing salamander, *Siren lacertian* by (Heisler et al., 1982) and it contrasted with the usual pattern in water-breathing fish where extracellular and intracellular pH are regulated more or less in parallel ("coupled regulation") in response to internal hypercapnia (reviewed by Brauner and Baker, 2009). This "normal" pattern occurs mainly by adjustments in the rates of Na⁺ versus acid (H⁺) and Cl⁻ versus base (HCO₃⁻) exchanges with the water at the gills (Wood, 1991; Evans et al., 2005; Perry and Gilmour, 2006), so one possible explanation is that air-breathing severely compromised the capacities of these exchangers. However, arguing against this are divided chamber experiments on the same species by Stiffler et al. (Stiffler et al., 1986) indicating that 75% of Na⁺ uptake normally occurs across the skin and is unaffected by aquatic hypoxia. Clearly, more work is needed here.

When the armoured catfish (*Liposarcus pardalis* = *Pterygoplichys pardalis*, a catostomid), which is a facultative air-breather, was exposed to severe waterborne hypercarbia (PCO₂ up to 42 torr), an almost

identical phenomenon occurred - excellent regulation of intracellular pH in a variety of tissues with negligible regulation of extracellular pH (Brauner et al., 2004b). Later experiments on this same species with other acid-base challenges (anoxia, exhaustive exercise, HCO₃⁻ loading) provided additional evidence that intracellular pH was preferentially regulated over extracellular pH, even though air-breathing did not occur in any of these exposures (Harter et al., 2014). However there is an earlier report on another air-breathing catostomid (*Hypos-tomus* sp.) that does not appear to follow this pattern (Wood et al., 1979), so the phenomenon may not be universal. Recently, Shartau and Brauner (Shartau and Brauner, 2014) and Shartau et al. (Shartau et al., 2016a) have reported unpublished observations of preferential intracellular pH regulation in three species of facultative air-breathing gar (holosteans).

Brauner et al. (Brauner et al., 2004b) speculated that preferential intracellular pH regulation could in fact be an adaptive characteristic of air-breathers in general, associated with the loss of gill ion versus acid-base exchange function, but noted that it could also be a consequence of the extremely low ion levels of the Amazonian waters in which the studied teleosts live. Repeating these experiments in more ion-rich waters would be instructive, but we are aware of no such studies. However, Baker et al. (Baker et al., 2009) reported a very similar phenomenon of preferential intracellular pH regulation during hypercarbia in a more ancient fish, the sturgeon *Acipenser transmontanus*. This chondrosteian is not an air-breather, but is endemic to similarly ion-poor waters. Brauner et al. (Brauner et al., 2004b) and Harter et al. (Harter et al., 2014) pointed out that Amazonian waters are also often very hypercarbic, far above PCO₂ levels that could be dealt with by HCO₃⁻ accumulation in the blood plasma because the "bicarbonate threshold" (Heisler, 1984) would be limiting, so this could be an additional factor favoring intracellular over extracellular pH regulation. Conditions in Devonian swamps during the invasion of freshwater and subsequent evolution of air-breathing in fish may have been similarly hypercarbic and/or ion-poor (Shartau and Brauner, 2014), and Ultsch (Ultsch, 1987, 1996) has argued that aquatic hypercarbia may have been a selective driver for the evolution of air-breathing. Considered in this light, these various explanations are not mutually inclusive. Indeed, the conclusion of Brauner and Baker (Brauner and Baker, 2009) and Shartau et al. (Shartau et al., 2016a) that the capacity for preferential regulation of intracellular pH is an exaptation for air-breathing seems entirely reasonable. Shartau et al. (Shartau et al., 2016b) have now documented preferential intracellular pH regulation in embryonic turtles, a condition which transitions to coupled extracellular and intracellular pH_i regulation as development proceeds, so it may be a very primitive vertebrate characteristic recapitulated in ontogeny, one that was subsequently exploited for a variety of purposes, including the evolution of air-breathing.

3. ROS defense

3.1. Production of reactive oxygen species

Oxygen is used in respiration as the ultimate electron acceptor and thus completely reduced to water. However, 0.1–2.0% of the oxygen is constantly converted into superoxide radical depending on tissue and substrate concentrations, so that some ROS is continuously produced in cells (Boveris and Chance, 1973; Turrens, 2003; Tahara et al., 2009). The most important site of ROS production is the respiratory chain of the mitochondria. The mitochondrial transmembrane protein, Complex III, appears to be the main site where an electron can escape forming the superoxide anion (Demin et al., 1998; Lushchak, 2011), but complex I and II may also contribute to ROS production (Chandel and Budinger, 2007). Complex IV does not seem to generate ROS. Additional sites of ROS production are plasma membrane NADPH-oxidase, involved in the cytoplasmic NADP⁺/NADPH₂ redox balance, and NO-synthase, which is required for the production of NO. To a minor extent

the endoplasmic reticulum with cytochrome P450 and different cellular oxidases may also contribute to ROS production (Storey, 1996; Chandel and Budinger, 2007; Lushchak, 2011). The catabolism of foreign molecules via cytochrome P450, for example, includes redox steps, which may result in the generation of free radicals. Peroxisomes, containing various oxidases, are additional sites of ROS production, in particular of H_2O_2 production. Peroxisomes are also well known for the presence of catalase, a H_2O_2 degrading enzyme (Schrader and Fahimi, 2008; Smith and Aitchison, 2013).

ROS participate in redox signaling (Finkel, 2011); therefore some level of ROS is essential for the healthy functioning of cells (Monaghan et al., 2009; Metcalfe and Alonso-Alvarez, 2010). Unduly elevated ROS levels, however, cause damage to all sorts of macromolecules. Lipids can be modified by peroxidation, proteins are damaged by prenylation or by oxidation of amino acid residues, and DNA damage is achieved by interaction of ROS with transition metals, causing strand breaks or base modifications. ROS has also been implicated in the accelerated reduction of telomere length (Richter and Zglinicki, 2007; Monaghan et al., 2009). These and other processes may ultimately result in cell death (Lee and Choi, 2003; Hermes-Lima, 2004; Kregel and Zhang, 2006; Welker et al., 2013; Lushchak, 2014).

3.2. Exposure to variable oxygen partial pressures and elevated ROS levels

Changing oxygen availability has frequently been shown to stimulate ROS production. Aquatic organisms experiencing a wide range of oxygen availability therefore must deal with changing and sometimes increasing steady-state levels of reactive oxygen species. ROS production has unequivocally been observed under hyperoxic conditions, and the rate of ROS production increases proportionally as oxygen concentration increases (Turrens, 2003). ROS production is also correlated to the rate of oxygen consumption and the number of mitochondria in the tissue (Storey, 1996), supporting the notion that the mitochondrial electron transport chain is by far the most important site of ROS production. In aquatic systems hyperoxia may result from photosynthetic activity during daytime, and antioxidant responses have for example been observed in estuarine fish (Ross et al., 2001), but also in goldfish (Lushchak et al., 2005a). Johannsson et al. (Johannsson et al., 2014) recorded a daily pattern of H_2O_2 concentration in Lake Magadi water in association with daytime hyperoxia, with highest ROS concentrations in the mid-afternoon, and the lowest concentrations in the early morning. Surplus oxygen used in aquaculture may also result in hyperoxic conditions and an antioxidant reaction (Ritola et al., 1999, 2002; Dong et al., 2011).

As H_2O_2 readily crosses the gills, this waterborne H_2O_2 will certainly affect organisms in the water. In aquatic organisms the accumulation of ROS has been reported under various conditions (see Lushchak, 2011; Welker et al., 2013), and has been shown to induce oxidative stress, for example, in goldfish *Carassius auratus* (Lushchak et al., 2005a), Atlantic salmon *Salmo salar* (Olsvik et al. 2005) and Senegal sole *Solea senegalensis* (Salas-Leiton et al. 2009). Oxidative stress is not solely induced by hyperoxia, but also by much more common hypoxiareoxygenation events. It has been shown repeatedly that reduced oxygen availability (hypoxia) may also cause oxidative stress (Welker et al., 2013), as reported for several fish species like goldfish *Carassius auratus* (Lushchak et al., 2001), common carp *Cyprinus carpio* (Lushchak et al., 2005b), rotan *Percottus glenii* (Lushchak and Bagnyukova, 2007), and the Walking catfish *Clarias batrachus* (Tripathi et al., 2013). The Indian catfish is a facultative air-breather and it has been suggested that not only hypoxic conditions but also high levels of H_2O_2 may stimulate air-breathing behavior (Johannsson et al., 2014). In fact, with changing water levels, high photosynthetic activity, rain, wet and dry seasons, rapid changes in aquatic oxygen partial pressures are frequently expected in tropical freshwater systems like the Amazon.

Many fish use the swimbladder as a buoyancy organ to retain the

status of neutral buoyancy when descending or ascending in the water column. The swimbladder of fish that lost the pneumatic duct (i.e. the connection between the esophagus or pharynx and the swimbladder, = physoclist fishes) typically contains high concentrations of oxygen because oxygen makes up the main fraction of newly secreted gas (Kobayashi et al., 1990). With increasing hyperbaric pressure at depth swimbladder gas therefore becomes extremely hyperoxic (Pelster, 1997, 2011; Pelster, 2015).

Fish using an air-breathing organ like a lung or a swimbladder to supplement oxygen supply in situations of aquatic hypoxia will also face unusually high oxygen partial pressures in the air-breathing organ, although hyperoxic conditions will not be encountered. Arterial oxygen partial pressure of water breathing fish typically is much lower than aerial PO_2 (Gilmour and Perry, 1994; Kristensen et al., 2010) so that the surface tissue of the air-breathing organ when exposed to fresh air will face much higher oxygen partial pressures than other tissues that obtain oxygen via the bloodstream from branchial oxygen uptake. In addition, when switching between aquatic and aerial respiration the air exposed tissue will face rapid changes in oxygen partial pressures. In gas cavities that are not ventilated, less soluble gases will accumulate, while highly soluble gases will be rapidly removed, as nicely shown by Johannes Piiper and coworkers (Piiper et al., 1962; Piiper, 1965). This phenomenon results in the gradual accumulation of nitrogen during periods of non-ventilation. In carp, a physostomous fish with persisting pneumatic duct not using the bladder for aerial respiration, oxygen content may go down to 2% (Hall, 1924). Accordingly, after periods of non-ventilation gulping air at the surface will cause significant increases in bladder PO_2 . Previous studies have indeed shown that swimbladder PO_2 in the jeju may even reach 150 torr in the anterior bladder (Kramer, 1978), and Farrell and Randall (Farrell and Randall, 1978) estimated a value of about 135 torr. Measurement of the oxygen content of the anterior and posterior swimbladder of jeju, a facultative air-breather, revealed values of up to 90% of aerial oxygen content, and under hyperoxic conditions even higher values were recorded. In the swimbladder of the water-breathing traia, however, the oxygen content was close to 20% of aerial oxygen content (Pelster et al., 2018). These results clearly support the notion that epithelia of air-breathing organs may be exposed to high PO_2 values, and that the PO_2 in a swimbladder of a physostomous fish not using the bladder for aerial gas exchange, which therefore is not frequently ventilated, typically is very low.

3.3. Mechanisms of ROS defense

To reduce the danger of ROS related tissue damage organisms have developed sophisticated defense systems to break down and detoxify reactive oxygen molecules (Hermes-Lima, 2004; Lushchak, 2011; Summarwar and Verma, 2012; Welker et al., 2013; Lushchak, 2014). These can include the accumulation of non-enzymatic and small antioxidants like glutathione (GSH/GSSG), thioredoxin (Trx-(SH₂)/Trx-SS), ascorbic acid (vitamin C), retinol (vitamin A) or α -tocopherol (vitamin E). Some of these non-enzymatic antioxidants must be taken up with the diet. Several metals and non-metal elements like selenium (Se) also contribute to the ROS defense system (Hermes-Lima, 2004). Cu, Mn and Zn are constitutive co-factors of antioxidant degrading enzymes such as superoxide dismutase and catalase, for example, and selenium is crucial for glutathione peroxidase and some additional enzymes.

Several enzymes that are highly conserved in evolutionary terms are able to remove ROS, such as catalase and superoxide dismutase (SOD). SOD catalyzes the dismutation of O_2^- into O_2 and H_2O_2 ; catalase decomposes H_2O_2 . Glutathione peroxidase (GPx) also decomposes H_2O_2 using the tripeptide GSH in its reduced form, and glutathione reductase (GR) subsequently reduces GSSG back to GSH at the expense of $NADPH_2$ (Hermes-Lima, 2004). Glucose-6-phosphate dehydrogenase, an enzyme of the pentose phosphate shunt, therefore is an auxiliary enzyme involved in the ROS defense system, because it generates $NADPH_2$, which is required for the GR reaction.

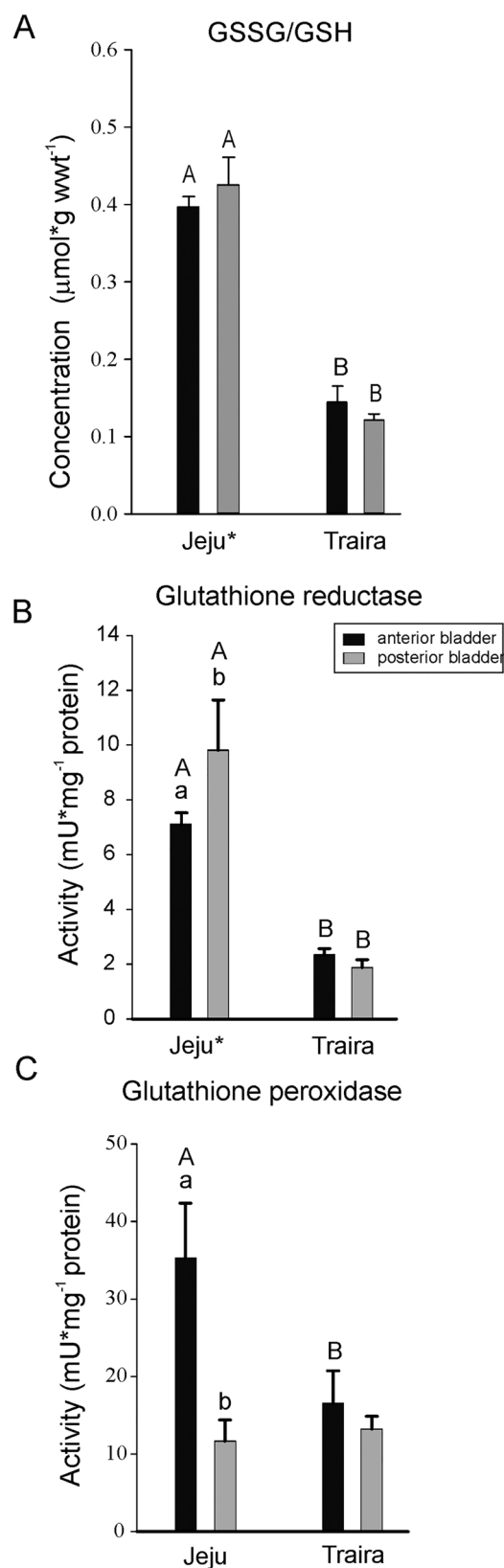
3.4. ROS defense capacity

Morris and Albright (Morris and Albright, 1981) compared catalase, SOD and GPx activities in kidney, heart, gills, and rete mirabile and gas gland of the swimbladder of the oyster toadfish *Opsanus tau* and found especially high activities of SOD in gas gland and rete mirabile tissue, while the other two enzymes were not elevated as compared to the other tissues. Catalase activity was reported to be particularly low in gas gland tissue, and based on histochemical studies it was speculated that most of the activity had to be attributed to catalase present in blood cells (Morris and Albright, 1981).

Measurements of the steady state oxygen content in tissues of seven fish from the Northern Atlantic revealed a positive correlation with SOD activity in combined gas gland and rete mirabile tissue samples, while no correlation between oxygen content and catalase or GPx activity was detected (Morris and Albright, 1984). Experimental deflation of the bladder in *Opsanus tau* caused a subsequent increase in oxygen content of the bladder, but the increase in oxygen content did not change antioxidant enzyme activities (Morris and Albright, 1984). A recent study revealed that the process of silvering, i.e. the preparation of the European eel *Anguilla anguilla* for the spawning migration to the Sargasso Sea, includes an improvement of the oxygen defense capacity in gas gland cells (Schneeberger et al., 2016). As reported by Morris and Albright (Morris and Albright, 1984) for North Atlantic marine fish, SOD activity was elevated in silver eels as compared to yellow eels, but increased concentrations of GSH + GSSG as well as higher activities of GPx and GR suggested that in addition to SOD the glutathione cycle is very important for ROS detoxification in eel gas gland tissue. Catalase activity, in turn, was not affected by the developmental change to the silver eel. This suggests that the mechanisms used to detoxify ROS may vary between species.

In fish using the swimbladder as an air-breathing organ, hyperbaric oxygen partial pressures cannot be expected, but aerial partial pressures will be encountered when frequently ventilating the bladder (see above). A comparison of two closely related erythrinid species, the traira (*Hoplias malabaricus*), and the jeju (*Hoplerythrinus unitaeniatus*), confirmed the assumption that using the swimbladder as an air-breathing organ coincides with an elevated ROS defense capacity of the air-exposed tissue (Pelster et al., 2016). Both fish have a swimbladder consisting of an anterior and a posterior chamber, connected to the esophagus via a ductus pneumaticus. In the facultative air-breathing jeju, the posterior swimbladder is highly vascularized (particularly in its anterior portion), and it is used for aerial gas exchange. In the water-breathing traira, the swimbladder wall mainly consists of connective tissue, and extensive vascularization is completely absent.

Behavioral studies confirmed that traira even under severe aquatic hypoxia does not use the swimbladder for aerial respiration. Jeju, the facultative air-breathing erythrinid fish, referred to breathing air under normoxic conditions, and the frequency of air-breathing significantly increased under hypoxic conditions (Pelster et al., 2016). Total glutathione (GSSG/GSH) concentration in anterior and posterior swimbladder tissue of jeju was significantly higher than in swimbladder tissue of traira, indicating an elevated capacity of swimbladder tissue to defend against ROS (Fig. 4A). Even in muscle tissue of jeju, total glutathione concentration was elevated compared to traira muscle tissue, suggesting that oxygen taken up in the swimbladder is transported to other tissues, which therefore also may be exposed to higher PO₂ values. Comparing swimbladder and muscle tissue in the jeju, swimbladder tissue had much higher total glutathione concentrations, while in traira no significant difference was detected between swimbladder tissue and muscle tissue. This observation again supported the conclusion that the parts of the swimbladder frequently exposed to air are characterized by an increased capacity to defend against ROS molecules (Pelster et al., 2016). Although the posterior part of the jeju swimbladder is used for aerial respiration, due to the breathing cycle both parts of the bladder are exposed to aerial PO₂. Air is initially transferred



(caption on next page)

to the anterior part of the bladder. After exhaling part of the volume in the posterior bladder, air from the anterior part is subsequently transferred to the posterior part, where oxygen is then taken up by the blood (Randall et al., 1981a). Accordingly, both parts of the swimbladder are

Fig. 4. Total glutathione concentration (A), activity of glutathione reductase (B) and of glutathione peroxidase (C) in anterior and posterior swimbladder tissue of two erythrinid species, the facultative air-breather jeju (*Hopleythrinus unitaeniatus*) and the pure water-breather traira (*Hoplias malabaricus*). Using the swimbladder as an air-breathing organ coincides with a significantly elevated capacity of the glutathione based ROS defense system. Capital letters denote significant differences between the two species, small letters denote significant differences between tissues within a species (means sharing the same letter are not significantly different), * indicates significant overall difference between the two species by two-way ANOVA (adapted from Pelster et al., 2016).

exposed to high and variable PO₂ values and therefore may need a higher ROS defense capacity.

Focusing on the activity of ROS degrading enzymes the picture was more complex (Pelster et al., 2016). SOD activity was very similar in the tissues of jeju and traira, and no significant difference between the two species could be detected. Similarly, no significant difference was detected among the different tissues of either jeju or traira. Accordingly, SOD does not appear to play a crucial role for the degradation of ROS in the facultative air-breathing fish jeju. Catalase activity, however, was elevated in jeju swimbladder as compared to traira, and again in the posterior bladder of jeju the activity was significantly elevated as compared to jeju muscle tissue, while in traira no difference between the different tissues could be detected.

The elevated total glutathione concentration detected in jeju swimbladder tissue suggested that the GPx/GR reaction would significantly contribute to ROS breakdown, and indeed the largest difference in enzyme activities between the two erythrinid species was detected for GR activity (Fig. 4B). The highest GR activity was detected in posterior swimbladder tissue of jeju, followed by the anterior bladder, and finally muscle tissue of jeju. All three jeju tissues had higher GR activities than traira tissue, and within traira again no difference between the three tissues was detected. At the species level, in the anterior part of jeju swimbladder GPx activity was higher than in anterior swimbladder tissue of traira (Fig. 4C). These results clearly show that catalase activity and in particular the glutathione system are elevated in order to defend against inordinate ROS production, which may occur due to the varying and high oxygen partial pressures encountered in the swimbladder used for aerial respiration.

Enzyme activities discussed so far have been measured in resting fish with free access to air under normoxic conditions. Incubation of traira under hyperoxic conditions (PO₂ = 400 torr) for 4 h resulted in elevated SOD and GR activities (Pelster et al., 2018). In contrast to the results reported by Morris and Albright (Morris and Albright, 1984) for *Opsanus tau* (see above) this indicates that in erythrinid fish the ROS defense capacities may be rapidly adjusted to changes in ROS production and ROS accumulation in tissues.

4. Conclusion

At first glance, developing an air-breathing organ to improve oxygen supply to tissues in situations of frequent aquatic hypoxia is a simple and elegant solution due to the many-fold higher oxygen capacity of air compared to water and the much higher diffusibility of oxygen in air. Our analysis revealed however, that the situation is much more complex and several additional physiological functions and additional organs are involved for the successful development of a facultative air-breathing fish using a specialized air-breathing organ for aerial gas exchange. Due to the multi-functionality of gills and the unicircular construction of the circulatory system of fish, precautions are required to prevent oxygen loss at the gills in hypoxic water. Modification of the gills, however, affects ion-regulatory capacities. This may result in a shift of ion regulatory capacities to other organs such as the skin or the gut. In addition, transport capacities of the kidney may have to be modified, as well as the transport capacities of remaining gill surfaces. Water permeability of body surfaces must also

be adjusted. Ion transport is intimately connected to movements of acid-base equivalents, so that unavoidably modifications of acid-base regulation are involved, and in air-breathing fish, intracellular pH may be preferentially controlled, at the expense of extracellular pH. Last, but not least, an increased ROS defense capacity is required to prevent oxidative damage to tissues due to rapidly changing and often high oxygen partial pressures at the respiratory surfaces in contact to air.

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References

- Ali, S.S., Hsiao, M., Zhao, H.W., Dugan, L.L., Haddad, G.G., Zhou, D., 2012. Hypoxia-adaptation involves mitochondrial metabolic depression and decreased ROS leakage. *PLoS ONE* 7, e36801.
- Amelio, D., Garofalo, F., Brunelli, E., Loong, A.M., Wong, W.P., Ip, Y.K., Tota, B., Cerra, M.C., 2008. Differential NOS expression in freshwater and aestivating *Protopterus dolloi* (lungfish): heart vs kidney readjustments. *Nitric Oxide* 18, 1–10.
- Babiker, M.M., Rankin, J.C., 1979. Renal and vascular effects of neurohypophysial hormones in the African lungfish *Protopterus annectens* (Owen). *Gen. Comp. Endocrinol.* 37, 26–34.
- Baker, D.W., Matey, V., Huynh, K.T., Wilson, J.M., Morgan, J.D., Brauner, C.J., 2009. Complete intracellular pH protection during extracellular pH depression is associated with hypercarbia tolerance in white sturgeon, *Acipenser transmontanus*. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 296, R1868–R1880.
- Bemis, W.E., Burggren, W.W., Kemp, N.E., 1987. *The Biology and Evolution of Lungfishes*. Alan R. Liss, Inc., New York, pp. 1–383.
- Boveris, A., Chance, B., 1973. The mitochondrial generation of hydrogen peroxide. General properties and effect of hyperbaric oxygen. *Biochem. J.* 134, 707–716.
- Brauner, C.J., Baker, D.W., 2009. Patterns of acid–base regulation during exposure to hypercarbia in fishes. In: Glass, M.L., Wood, S.C. (Eds.), *Cardio-Respiratory Control in Vertebrates*. Springer Verlag, Heidelberg, pp. 43–63.
- Brauner, C.J., Val, A.L., 1996. The interaction between O₂ and CO₂ exchange in the obligate air-breather *Arapaima gigas*, and the facultative air breather, *Liposarcus pardalis*. In: Val, A.L., Almeida-Val, V., Randall, D.J. (Eds.), *Physiology and Biochemistry of the Fishes of the Amazon*. INPA, Manaus, Brazil.
- Brauner, C.J., Val, A.L., 2006. Oxygen transfer. In: Val, A.L., Almeida-Val, V.M.F., Randall, D.J. (Eds.), *The Physiology of Tropical Fishes*. Academic Press, Amsterdam, pp. 277–306.
- Brauner, C.J., Matey, V., Wilson, J.M., Bernier, N.J., Val, A.L., 2004a. Transition in organ function during the evolution of air-breathing; insights from *Arapaima gigas*, an obligate air-breathing teleost from the Amazon. *J. Exp. Biol.* 207, 1433–1438.
- Brauner, C.J., Wang, T., Wang, Y., Richards, J.G., Gonzalez, R.J., Bernier, N.J., Xi, W., Patrick, M., Val, A.L., 2004b. Limited extracellular but complete intracellular acid-base regulation during short-term environmental hypercapnia in the armoured catfish, *Liposarcus pardalis*. *J. Exp. Biol.* 207, 3381–3390.
- Burgess, D.W., Marshall, W.S., Wood, C.M., 1998. Ionic transport by the opercular epithelia of freshwater acclimated tilapia (*Oreochromis niloticus*) and killifish (*Fundulus heteroclitus*). *Comp. Biochem. Physiol. Part A* 121, 155–164.
- Cameron, J.N., Wood, C.M., 1978. Renal function and acid-base regulation in two Amazon erythrinid fishes: *Hoplias malabaricus*, a water breather, and *Hopleythrinus unitaeniatus*, a facultative air breather. *Can. J. Zool.* 56, 917–930.
- Carter, G.S., Beadle, L.C., 1931. The fauna of the swamps of the Paraguayan Chaco in relation to its environment. II. Respiratory adaptations in the fishes. *J. Linnean Soc. Lond. (Zool.)* 37, 327–368.
- Chandel, N.S., Budinger, G.R.S., 2007. The cellular basis for diverse responses to oxygen. *Free Radic. Biol. Med.* 42, 165–174.
- Chng, Y.R., Ong, J.L., Chen, X.L., Hiong, K.C., Wong, W.P., Ip, Y.K., 2016. Molecular characterization of aquaporin 1 and aquaporin 3 from the gills of the African lungfish, *Protopterus annectens*, and changes in their branchial mRNA expression levels and protein abundance during three phases of aestivation. *Front. Physiol.* 7, e532.
- Cooper, C.A., Litwiler, S.L., Murrant, C.L., Wright, P.A., 2012. Cutaneous vasoregulation during short- and long-term aerial acclimation in the amphibious mangrove rivulus, *Kryptolebias marmoratus*. *Comp. Biochem. Physiol. Part B* 161, 268–274.
- Cooper, C.A., Wilson, J.M., Wright, P.A., 2013. Marine, freshwater and aerially acclimated mangrove rivulus (*Kryptolebias marmoratus*) use different strategies for cutaneous ammonia excretion. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 304, R599–R612.
- Dejours, P., 1981. *Principles of Comparative Respiratory Physiology*. Elsevier,

- Amsterdam.
- DeLaney, R.G., Lahiri, S., Hamilton, R., Fishman, P., 1977. Acid-base balance and plasma composition in the aestivating lungfish (*Protopterus*). *Am. J. Physiol. Regul. Integ. Comp. Physiol.* 232, R10–R17.
- Demin, O.V., Kholodenko, B.N., Skulachev, V.P., 1998. A model of O_2^- generation in the complex III of the electron transport chain. *J. Supramol. Struct. Cell. Biochem. Suppl.* 184, 21–33.
- Diaz, R.J., Breitbart, D.L., 2009. The hypoxic environment. In: Richards, J.G., Farrell, A.P., Brauner, C.J. (Eds.), *Hypoxia*. Elsevier, Amsterdam, pp. 1–23.
- Diaz, R.J., Rosenberg, R., 2008. Spreading dead zones and consequences for marine ecosystems. *Science* 321, 926–929.
- Dong, X.Y., Qin, J.G., Zhang, X.M., 2011. Fish adaptation to oxygen variations in aquaculture from hypoxia to hyperoxia. *J. Fish. Aquacult.* 2, 23–28.
- Evans, D.H., Claiborne, J.B., Kormanik, G., 1999. Osmoregulation, acid-base regulation, and nitrogen excretion. In: Horn, M.H., Martin, K.L., Chotkowski, M.A. (Eds.), *Intertidal Fishes: Life in Two Worlds*. Academic Press, New York, pp. 79–96.
- Evans, D.H., Pierrmarini, P.M., Choe, K.P., 2005. The Multifunctional Fish Gill: Dominant Site of Gas Exchange, Osmoregulation, Acid-Base Regulation, and Excretion of Nitrogenous Waste? *Physiol. Rev.* 85, 97–177.
- Farrell, A.P., Randall, D.J., 1978. Air-breathing mechanics in two Amazonian teleosts, *Arapaima gigas* and *Hoplerthrinus unitaeniatus*. *Can. J. Zool.* 56, 939–945.
- Fernandes, M.N., Rantin, F.T., Kalinin, A.L., Moron, S.E., 1994. Comparative study of gill dimensions of three erythrinid species in relation to their respiratory function. *Can. J. Zool.* 72, 160–165.
- Fernandes, M.N., da Cruz, A.L., da Costa, O.T.F., Perry, S.F., 2012. Morphometric partitioning of the respiratory surface area and diffusion capacity of the gills and swim bladder in juvenile Amazonian air-breathing fish, *Arapaima gigas*. *Micron* 43, 961–970.
- Finkel, T., 2011. Signal transduction by reactive oxygen species. *J. Cell Biol.* 194, 7–15.
- Gilmour, K.M., Perry, S.F., 1994. The effects of hypoxia, hyperoxia or hypercapnia on the acid-base disequilibrium in the arterial blood of rainbow trout. *J. Exp. Biol.* 192, 269–284.
- Gilmour, K.M., Euverman, R.M., Esbaugh, A.J., Kenney, L., Chew, S.F., Ip, Y.K., Perry, S.F., 2007. Mechanisms of acid–base regulation in the African lungfish *Protopterus annectens*. *J. Exp. Biol.* 210, 1944–1959.
- Glass, M.L., Rantin, F.T., 2009. Gas exchange and control of respiration in air-breathing teleost fish. In: Glass, M.L., Wood, S.C. (Eds.), *Cardio-Respiratory Control in Vertebrates - Evolutionary and Evolutionary Aspects*. Springer, Heidelberg, pp. 99–120.
- Glover, C., Bucking, C., Wood, C., 2013. The skin of fish as a transport epithelium: a review. *J. Comp. Physiol. B* 183, 877–891.
- Godet, R., 1961. Le problème hydrique et son contrôle hypophysaire chez le Protoperle. *Ann. Faculty Sci. Dakar* 6, 183–201.
- Goncalves, A.J., Castro, L.F.C., Pereira-Wilson, C., Coimbra, J., Wilson, J.M., 2007. Is there a compromise between nutrient uptake and gas exchange in the gut of *Misgurnus anguillicaudatus*, an intestinal air-breathing fish? *Comp. Biochem. Physiol. Part D Genomics Proteomics* 2, 345–355.
- Gonzalez, R.J., Milligan, L., Pagnotta, A., McDonald, D.G., 2001. Effect of air breathing on acid-base and ion regulation after exhaustive exercise and during low pH exposure in the bowfin, *Amia calva*. *Physiol. Biochem. Zool.* 74, 502–509.
- Gonzalez, R.J., Brauner, C.J., Wang, Y.X., Richards, J.G., Patrick, M.L., Xi, W., Matey, V., Val, A.L., 2010. Impact of ontogenetic changes in branchial morphology on gill function in *Arapaima gigas*. *Physiol. Biochem. Zool.* 83, 322–332.
- Graham, J.B., 1997. *Air-Breathing Fishes*. Academic Press, San Diego, pp. 1–299.
- Graham, J.B., Lee, H.J., 2004. Breathing air in air: in what ways might extant amphibious fish biology relate to prevailing concepts about early tetrapods, the evolution of vertebrate air breathing, and the vertebrate land transition? *Physiol. Biochem. Zool.* 77, 720–731.
- Gray, J., 1928. The role of water in the evolution of the terrestrial vertebrates. *J. Exp. Biol.* 6, 26–31.
- Greco, A.M., Gilmour, K.M., Fenwick, J.C., Perry, S.F., 1995. The effects of softwater acclimation on respiratory gas transfer in the rainbow trout *Oncorhynchus mykiss*. *J. Exp. Biol.* 198, 2557.
- Guppy, M., 2004. The biochemistry of metabolic depression: a history of perceptions. *Comp. Biochem. Physiol. Part B: Biochem. Molec. Biol.* 139, 435–442.
- Guyton, J.S., 1935. The structure of the nephron in the South American lungfish, *Lepidosiren paradoxa*. *Anat. Rec.* 63, 213–229.
- Hall, F.G., 1924. The functions of the swimbladder of fishes. *Biol. Bull.* 47, 79–124.
- Halpin, P.M., Martin, K.L.M., 1999. Aerial respiration in the salt marsh fish *Fundulus heteroclitus* (Fundulidae). *Copeia* 743–748.
- Harter, T.S., Shartau, R.B., Baker, D.W., Jackson, D.C., Val, A.L., Brauner, C.J., 2014. Preferential intracellular pH regulation represents a general pattern of pH homeostasis during acid-base disturbances in the armoured catfish, *Pterygoplichthys pardalis*. *J. Comp. Physiol. B* 184, 709–718.
- Heisler, N., 1982. Intracellular and extracellular acid-base regulation in the tropical freshwater teleost fish *Synbranchus marmoratus* in response to the transition from water breathing to air breathing. *J. Exp. Biol.* 99, 9–28.
- Heisler, N., 1984. In: Gills, Anonymous (Eds.), *Acid-Base Regulation in Fishes*. Academic Press, New York, pp. 315–401.
- Heisler, N., Forcht, G., Ultsch, G.R., Anderson, J.F., 1982. Acid-base regulation in response to environmental hypercapnia in two aquatic salamanders, *Siren lacertina* and *Amphiuma means*. *Respir. Physiol.* 49, 141–158.
- Hermes-Lima, M., 2004. Oxygen in biology and biochemistry: the role of free radicals. In: Storey, K.B. (Ed.), *Functional Metabolism: Regulation and Adaptation*. Wiley-Liss, Hoboken, pp. 319–368.
- Hochachka, P.W., Moon, T.W., Bailey, J., Hulbert, W.C., 1978. The osteoglossid kidney: correlations of structure, function, and metabolism with transition to air breathing. *Can. J. Zool.* 56, 820–832.
- Hulbert, W.C., Moon, T.W., Bailey, J., Hochachka, P.W., 1978a. The occurrence and possible significance of chloride-like cells in the nephron of Amazon fishes. *Can. J. Zool.* 56, 833–844.
- Hulbert, W.C., Moon, T.W., Hochachka, P.W., 1978b. The erythrinid gill: correlations of structure, function, and metabolism. *Can. J. Zool.* 56, 814–819.
- Hulbert, W.C., Moon, T.W., Hochachka, P.W., 1978c. The osteoglossid gill: correlations of structure, function, and metabolism with transition to air breathing. *Can. J. Zool.* 56, 801–808.
- Hung, C.Y.C., Tsui, K.N.T., Wilson, J.M., Nawata, C.M., Wood, C.M., Wright, P.A., 2007. Rhesus glycoprotein gene expression in the mangrove killifish *Kryptolebias marmoratus*; exposed to elevated environmental ammonia levels and air. *J. Exp. Biol.* 210, 2419.
- Hung, C.Y.C., Galvez, F., Ip, Y.K., Wood, C.M., 2009. Increased gene expression of a facilitated diffusion urea transporter in the skin of the African lungfish (*Protopterus annectens*) during massively elevated post-terrestrialization urea excretion. *J. Exp. Biol.* 212, 1202.
- Iftikar, F.I., Patel, M., Ip, Y.K., Wood, C.M., 2008. The influence of feeding on aerial and aquatic oxygen consumption, nitrogenous waste excretion, and metabolic fuel usage in the African lungfish *Protopterus annectens*. *Can. J. Zool.* 86, 790–800.
- Isaia, J., 1984. In: Hoar, G.W.S., Randall, D.J. (Eds.), *Water and Nonelectrolyte Permeation*. Academic Press, New York, pp. 1–38.
- Ishimatsu, A., 2012. Evolution of the cardiorespiratory system in air-breathing fishes. *Aqua-BioSci. Monogr.* 5, 1–28.
- Janis, C.M., Farmer, C., 1999. Proposed habitats of early tetrapods: gills, kidneys, and the water-land transition. *Zool. J. Linnean Soc.* 126, 117–126.
- Jenny, J.P., Francus, P., Normandeau, A., Lapointe, F., Perga, M.E., Ojala, A., Schimmelmann, A., Zolitschka, B., 2016. Global spread of hypoxia in freshwater ecosystems during the last three centuries is caused by rising local human pressure. *Glob. Change Biol. Bioenergy* 22, 1481–1489.
- Johannsson, O.E., Bergman, H.L., Wood, C.M., Laurent, P., Kavembe, D.G., Bianchini, A., Maina, J.N., Chevalier, C., Bianchini, L.F., Papah, M.B., Ojoo, R.O., 2014. Air breathing in Magadi tilapia *Alcolapia grahami*, under normoxic and hyperoxic conditions, and the association with sunlight and reactive oxygen species. *J. Fish Biol.* 84, 844–863.
- Johansen, K., 1972. Heart and circulation in gill, skin and lung breathing. *Respir. Physiol.* 14, 193–210.
- Johansen, K., Hanson, D., Lenfant, C., 1970a. Respiration in a primitive air breather, *Amia calva*. *Respir. Physiol.* 9, 162–174.
- Johansen, K., Hanson, D., Lenfant, C., 1970b. Respiration in a primitive air breather, *Amia calva*. *Respir. Physiol.* 9, 162–174.
- Karnaky, K.J., Degnan, K.J., Zadunaisky, J.A., 1977. Chloride transport across isolated opercular epithelium of killifish: a membrane rich in chloride cells. *Science* 195, 203–205.
- Kobayashi, H., Pelster, B., Scheid, P., 1990. CO_2 back-diffusion in the rete aids O_2 secretion in the swimbladder of the eel. *Respir. Physiol.* 79, 231–242.
- Konno, N., Hyodo, S., Yamaguchi, Y., Matsuda, K., Uchiyama, M., 2010. Vasotocin/V2-type receptor/aquaporin axis exists in African lungfish kidney but is functional only in terrestrial condition. *Endocrinology* 151, 1089–1096.
- Kramer, D.L., 1978. Ventilation of the respiratory gas bladder in *Hoplerthrinus unitaeniatus* (Pisces, Characidae, Erythrinidae). *Can. J. Zool.* 56, 931–938.
- Kramer, D.L., Lindsey, C.C., Moodie, G.E.E., Stevens, E.D., 1978. The fishes and the aquatic environment of the central Amazon basin, with particular reference to respiratory patterns. *Can. J. Zool.* 56, 717–729.
- Kregel, K.C., Zhang, H.J., 2006. An integrated view of oxidative stress in aging: basic mechanisms, functional effects, and pathological considerations. *Am. J. Physiol. Regul. Integ. Comp. Physiol.* 292, R18–R36.
- Kristensen, T., Rosseland, B.O., Kiessling, A., Djordevic, B., Massabau, J.C., 2010. Lack of arterial PO_2 downregulation in Atlantic salmon (*Salmo salar* L.) during long-term normoxia and hyperoxia. *Fish Physiol. Biochem.* 36, 1087–1095.
- Laurent, P., Perry, S.F., 1991. Environmental effects on fish gill morphology. *Physiol. Zool.* 64, 4–25.
- LeBlanc, D.M., Wood, C.M., Fudge, D.S., Wright, P.A., 2010. A fish out of water- gill and skin remodeling promotes osmo- and ionoregulation in the mangrove killifish, *Kryptolebias marmoratus*. *Physiol. Biochem. Zool.* 83, 942–949.
- Lee, P.J., Choi, A.M.K., 2003. Pathways of cell signaling in hyperoxia. *Free Radic. Biol. Med.* 35, 341–350.
- Lefevre, S., Bayley, M., McKenzie, D.J., Craig, J.F., 2014. Editorial: air-breathing fishes. *J. Fish Biol.* 84, 547–553.
- Lin, H.C., Sung, W.T., 2003. The distribution of mitochondria-rich cells in the gills of air-breathing fishes. *Physiol. Biochem. Zool.* 76, 215–228.
- Lushchak, V.I., 2011. Environmentally induced oxidative stress in aquatic animals. *Aquat. Toxicol.* 101, 13–30.
- Lushchak, V.I., 2014. Free radicals, reactive oxygen species, oxidative stress and its classification. *Chem. Biol. Interact.* 224, 164–175.
- Lushchak, V.I., Bagnyukova, T.V., 2007. Hypoxia induces oxidative stress in tissues of a goby, the rotan *Perccottus glenii*. *Comp. Biochem. Physiol. B: Biochem. Molec. Biol.* 148, 390–397.
- Lushchak, V.I., Lushchak, L.P., Mota, A.A., Hermes-Lima, M., 2001. Oxidative stress and antioxidant defenses in goldfish *Carassius auratus* during anoxia and reoxygenation. *Am. J. Physiol. Regul. Integ. Comp. Physiol.* 280, R100–R107.
- Lushchak, V.I., Bagnyukova, T.V., Husak, V.V., Luzhna, L.I., Lushchak, O.V., Storey, K.B., 2005a. Hyperoxia results in transient oxidative stress and an adaptive response by antioxidant enzymes in goldfish tissues. *Int. J. Biochem. Cell Biol.* 37, 1670–1680.
- Lushchak, V.I., Bagnyukova, T.V., Lushchak, O.V., Storey, J.M., Storey, K.B., 2005b.

- Hypoxia and recovery perturb free radical processes and antioxidant potential in common carp (*Cyprinus carpio*) tissues. *Int. J. Biochem. Cell Biol.* 37, 1319–1330.
- Marshall, W.S., 1977. Transepithelial potential and short-circuit current across the isolated skin of *Gillichthys mirabilis* (Teleostei: gobiidae), acclimated to 5% and 100% seawater. *J. Comp. Physiol.* 114, 157–165.
- Marshall, W.S., 1995. Transport processes in isolated teleost epithelia: opercular epithelium and urinary bladder. In: Wood, C.M., Shuttleworth, T.J. (Eds.), *Cellular and Molecular Approaches to Fish Ionic Regulation*. Academic Press, San Diego, New York, pp. 1–23.
- Marshall, W.S., Bellamy, D., 2010. The 50 year evolution of *in vitro* systems to reveal salt transport functions of teleost fish gills. *Comp. Biochem. Physiol. Part A* 155, 275–280.
- Marshall, W.S., Bryson, S.E., Wood, C.M., 1992. Calcium transport by isolated skin of rainbow trout. *J. Exp. Biol.* 166, 297–316.
- Marshall, W.S., Bryson, S.E., Burghardt, J.S., Verbost, P.M., 1995. Ca²⁺ transport by opercular epithelium of the freshwater adapted euryhaline teleost, *Fundulus heteroclitus*. *J. Comp. Phys. B* 165, 268–277.
- Martin, K.L., Bridges, C.R., 1999. Respiration in water and air. In: Horn, M.H., Martin, K.L., Chotkowski, M.A. (Eds.), *Intertidal Fishes: Life in Two Worlds*. Academic Press, New York, pp. 54–78.
- McCormick, S.D., Hasegawa, S., Hirano, T., 1992. Calcium uptake in the skin of a freshwater teleost. *Proc. Nat. Acad. Sci.* 89, 3635–3638.
- Metcalfe, N.B., Alonso-Alvarez, C., 2010. Oxidative stress as a life-history constraint: the role of reactive oxygen species in shaping phenotypes from conception to death. *Funct. Ecol.* 24, 984–996.
- Milsom, W.K., 2012. New insights into gill chemoreception: receptor distribution and roles in water and air breathing fish. *Respir. Physiol. Neurobiol.* 184, 326–339.
- Monaghan, P., Metcalfe, N.B., Torres, R., 2009. Oxidative stress as a mediator of life history trade-offs: mechanisms, measurements and interpretation. *Ecol. Lett.* 12, 75–92.
- Morris, S.M., Albright, J.T., 1981. Superoxide dismutase, catalase, and glutathione peroxidase in the swim bladder of the physoclistous fish, *Opsanus tau*. *L. Cell Tiss. Res.* 220, 739–752.
- Morris, S.M., Albright, J.T., 1984. Catalase, glutathione peroxidase, and superoxide dismutase in the rete mirabile and gas gland epithelium of six species of marine fishes. *J. Exp. Zool.* 232, 29–39.
- Muusse, B., Marcon, J., Van den Thillart, G., Almeida-Val, V., 1998. Hypoxia tolerance of Amazon fish respirometry and energy metabolism of the cichlid *Astronotus ocellatus*. *Comp. Biochem. Physiol. Part A* 120, 151–156.
- Nelson, J.A., 2014. Breaking wind to survive: fishes that breathe air with their gut. *J. Fish Biol.* 84, 554–576.
- Nonnotte, G., Nonnotte, L., Kirsch, R., 1979. Chloride cells and chloride exchange in the skin of a sea-water teleost, the shanny (*Blennius pholis* L.). *Cell Tiss. Res.* 199, 387–396.
- Oduleye, S.O., 1977. Unidirectional water and sodium fluxes and respiratory metabolism in the African lung-fish, *Protopterus annectens*. *J. Comp. Physiol.* 119, 127–139.
- Ojeda, J.L., Icardo, J.M., Wong, W.P., Ip, Y.K., 2006. Microanatomy and ultrastructure of the kidney of the African lungfish *Protopterus dolloi*. *Anat. Rec. Part A: Discov. Mol. Cell. Evol. Biol.* 288, 609–625.
- Ojeda, J.L., Wong, W.P., Ip, Y.K., Icardo, J.M., 2008. Renal corpuscle of the African lungfish *Protopterus dolloi*: structural and histochemical modifications during aestivation. *Anat. Rec.* 291, 1156–1172.
- Patel, M., Iftikar, F.I., Leonard, E.M., Ip, Y.K., Wood, C.M., 2009a. Ionoregulatory physiology of two species of African lungfishes, *Protopterus dolloi* and *Protopterus annectens*. *J. Fish Biol.* 75, 865–884.
- Patel, M., Iftikar, F.I., Smith, R.W., Ip, Y.K., Wood, C.M., 2009b. Water balance and renal function in two species of African lungfish *Protopterus dolloi* and *Protopterus annectens*. *Comp. Biochem. Physiol. Part A* 152, 149–157.
- Pelster, B., 1997. Buoyancy at depth. In: Randall, D., Farrell, A.P. (Eds.), *Deep-Sea Fish*. Academic Press, San Diego, pp. 195–237.
- Pelster, B., 2011. Swimbladder function and buoyancy control in fishes. In: Farrell, A.P. (Ed.), *Encyclopedia of Fish Physiology: From Genome to Environment*. Academic Press, San Diego, pp. 526–534.
- Pelster, B., 2015. Swimbladder function and the spawning migration of the European eel *Anguilla anguilla*. *Front. Physiol.* 5, 486.
- Pelster, B., Wood, C., Speers-Roesch, B., Driedzic, W.R., Almeida-Val, V., Val, A., 2015. Gut transport characteristics in herbivorous and carnivorous serrasalmid fish from ion-poor Rio Negro water. *J. Comp. Physiol. B* 185, 225–241.
- Pelster, B., Giacomini, M., Wood, C.M., Val, A.L., 2016. Improved ROS defense in the swimbladder of a facultative air-breathing erythrinid fish, jeju, compared to a non-air-breathing close relative, traira. *J. Comp. Physiol. B* 186, 615–624.
- Pelster, B., Wood, C.M., Jung, E., Val, A.L., 2018. Air-breathing behavior, oxygen concentrations, and ROS defense in the swimbladder of two erythrinid fish, the facultative air-breathing jeju, and the non-air-breathing traira during normoxia, hypoxia and hyperoxia. *J. Comp. Physiol. B* 188, 437–449.
- Perna, S.A., Fernandes, M.N., 1996. Gill morphology of the facultative air-breathing loriciariid fish, *Hypostomus plecostomus* (Walbaum) with special emphasis on aquatic respiration. *Fish Physiol. Biochem.* 15, 213–220.
- Perry, S.F., Gilmour, K.M., 2006. Acid-base balance and CO₂ excretion in fish: unanswered questions and emerging models. *Respir. Physiol. Neurobiol.* 154, 199–215.
- Perry, S.F., Wood, C.M., 1985. Kinetics of branchial calcium uptake in the rainbow trout: effects of acclimation to various external calcium levels. *J. Exp. Biol.* 116, 411.
- Perry, S.F., Braun, M.H., Noland, M., Dawdy, J., Walsh, P.J., 2010. Do zebrafish Rh proteins act as dual ammonia-CO₂ channels? *J. Exp. Zool. A: Ecol. Genet. Physiol.* 313A, 618–621.
- Piiper, J., 1965. Physiological equilibria of gas cavities in the body. In: Fenn, W.O., Rahn, H. (Eds.), *HandBook of Physiology, Respiration*, vol. 2. American Physiological Society, Bethesda, Maryland, pp. 1205–1218.
- Piiper, J., Humphrey, H.T., Rahn, H., 1962. Gas composition of pressurized, perfused gas pockets and the fish swim bladder. *J. Appl. Physiol.* 17, 275–282.
- Ramos, C.A., Fernandes, M.N., da Costa, O.T.F., Duncan, W.P., 2013. Implications for osmorepiratory compromise by anatomical remodeling in the Gills of *Arapaima gigas*. *Anat. Rec.* 296, 1664–1675.
- Randall, D.J., Baumgarten, D., Malyusz, M., 1972. The relationship between gas and ion transfer across the gills of fishes. *Comp. Biochem. Physiol. Part A* 41, 629–637.
- Randall, D.J., Burggren, W.W., Farrell, A.P., Haswell, M.S., 1981a. The Evolution of Air-breathing in Vertebrates. Cambridge University Press, Cambridge, pp. 1–133.
- Randall, D.J., Cameron, J.N., Daxboeck, C., Smatresk, N., 1981b. Aspects of bimodal gas exchange in the bowfin, *Amia calva* L. (Actinopterygii: amiiformes). *Respir. Physiol.* 43, 339–348.
- Richter, T., Zglinicki, T., 2007. A continuous correlation between oxidative stress and telomere shortening in fibroblasts. *Exp. Gerontol.* 42, 1039–1042.
- Ritola, O., Kiuru, T., Koponen, K., Molsa, H., Hanninen, O., Lindström-Seppä, P., 1999. Rainbow trout (*Oncorhynchus mykiss*) exposed to oxygen supersaturation and handling stress: plasma cortisol and hepatic glutathione status. *Acta. Biol. Hung.* 50, 215–227.
- Ritola, O., Livingstone, D.R., Peters, L.D., Lindström-Seppä, P., 2002. Antioxidant processes are affected in juvenile rainbow trout (*Oncorhynchus mykiss*) exposed to ozone and oxygen-supersaturated water. *Aquaculture* 210, 1–19.
- Robertson, L.M., Val, A.L., Almeida-Val, V., Wood, C.M., 2015. Ionoregulatory aspects of the osmorepiratory compromise during acute environmental hypoxia in 12 tropical and temperate teleosts. *Physiol. Biochem. Zool.* 88, 357–370.
- Ross, S.W., Dalton, D.A., Kramer, S., Christensen, B.L., 2001. Physiological (antioxidant) responses of estuarine fishes to variability in dissolved oxygen. *Comp. Biochem. Physiol. C Toxicol. Pharmacol.* 130, 289–303.
- Sawyer, W.H., 1966. Diuretic and natriuretic responses of lungfish (*Protopterus aethiopicus*) to arginine vasotocin. *Am. J. Physiol.* 210, 191–197.
- Sawyer, W.H., 1970. Vasopressor, diuretic, and natriuretic responses by lungfish to arginine vasotocin. *Am. J. Physiol.* 218, 1789–1794.
- Sawyer, W.H., Pang, P.K.T., 1980. Neurohypophysial peptides and epithelial sodium transport in fishes. In: Lahlou, B. (Ed.), *Epithelial Transport in Lower Vertebrates*. Cambridge University Press, Cambridge, pp. 331–336.
- Sawyer, W.H., Simpson, P.A., Sawyer, M.K., 1976. Renal responses of Australian lungfish to vasotocin, angiotensin II, and NaCl infusion. *Am. J. Physiol.* 231, 593–602.
- Sawyer, W.H., Uchiyama, M., Pang, P.K.T., 1982. Control of renal functions in lungfishes. *Fed. Proc.* 41, 2361–2364.
- Sayer, M.D., 2005. Adaptations of amphibious fish for surviving life out of water. *Fish Fish.* 6, 186–211.
- Schneebauer, G., Hanel, R., Pelster, B., 2016. *Anguillicola crassus* impairs the silvering-related enhancements of the ROS defense capacity in swimbladder tissue of the European eel (*Anguilla anguilla*). *J. Comp. Physiol. B* 186, 867–877.
- Schrader, M., Fahimi, H.D., 2008. The peroxisome: still a mysterious organelle. *Histochem. Cell Biol.* 129, 421–440.
- Scott, G.R., Matey, V., Mendoza, J.A., Gilmour, K.M., Perry, S.F., Almeida-Val, V.M.F., Val, A.L., 2017. Air breathing and aquatic gas exchange during hypoxia in armoured catfish. *J. Comp. Physiol. B* 187, 117–133.
- Shartau, R.B., Brauner, C.J., 2014. Acid–base and ion balance in fishes with bimodal respiration. *J. Fish Biol.* 84, 682–704.
- Shartau, R.B., Baker, D.W., Crossley, D.A., Brauner, C.J., 2016a. Preferential intracellular pH regulation: hypotheses and perspectives. *J. Exp. Biol.* 219, 2235–2244.
- Shartau, R.B., Crossley, D.A., Kohl, Z.F., Brauner, C.J., 2016b. Embryonic common snapping turtles (*Chelydra serpentina*) preferentially regulate intracellular tissue pH during acid–base challenges. *J. Exp. Biol.* 219, 1994–2002.
- Sies, H., 2015. Oxidative stress: a concept in redox biology and medicine. *Redox Biol.* 4, 180–183.
- Slovan, K.A., Slovan, R.D., De Boeck, G., Scott, G.R., Iftikar, F.I., Wood, C.M., Almeida-Val, V.M., Val, A.L., 2009. The role of size in synchronous air breathing of *Hoplosternum littorale*. *Physiol. Biochem. Zool.* 82, 625–634.
- Smatresk, N.J., 1986. Ventilatory and cardiac reflex responses to hypoxia and NaCN in *Lepisosteus osseus*, an air-breathing fish. *Physiol. Zool.* 59, 385–397.
- Smatresk, N.J., Cameron, J.N., 1982. Respiration and acid–base physiology of the spotted Gar, a bimodal breather: I. Normal values, and the response to severe hypoxia. *J. Exp. Biol.* 96, 263–280.
- Smith, H.W., 1931. Observations on the African lung-Fish, *Protopterus aethiopicus*, and on evolution from water to land environments. *Ecology* 12, 164–181.
- Smith, H.W., 1959. *From Fish to Philosopher. The Story of Our Internal Environment*. Revised Edition. Ciba, Chicago, USA.
- Smith, J.J., Aitchison, J.D., 2013. Peroxisomes take shape. *Nat. Rev. Mol. Cell Biol.* 14, 803–817.
- Smith, N.F., Talbot, C., Eddy, F.B., 1989. Dietary salt intake and its relevance to ionic regulation in freshwater salmonids. *J. Fish Biol.* 35, 749–753.
- Stiffler, D.F., 1988. Cutaneous exchange of ions in lower vertebrates. *Amer. Zool.* 28, 1019–1029.
- Stiffler, D.F., Graham, J.B., Dickson, K.A., Stöckmann, W., 1986. Cutaneous ion transport in the freshwater teleost *Synbranchus marmoratus*. *Physiol. Zool.* 59, 406–418.
- Storey, K.B., 1996. Oxidative stress: animal adaptations in nature. *Braz. J. Med. Biol. Res.* 29, 1715–1733.
- St-Pierre, J., Tattersall, G.J., Boutilier, R.G., 2000. Metabolic depression and enhanced O₂ affinity of mitochondria in hypoxic hypometabolism. *Am. J. Physiol. Regulat. Integ. Comp. Physiol.* 279, R1205–R1214.
- Sturla, M., Masini, M.A., Prato, P., Grattarola, C., Uva, B., 2001. Mitochondria-rich cells in gills and skin of an African lungfish, *Protopterus annectens*. *Cell Tissue Res.* 303, 351–358.

- Sturla, M., Paola, P., Carlo, G., Angela, M., Maria, U.B., 2002. Effects of induced aestivation in *Protopterus annectens*: a histomorphological study. *J. Exp. Zool. Part A: Ecol. Genet. Physiol.* 292, 26–31.
- Summarwar, S., Verma, S., 2012. Study of biomarkers of physiological defense against reactive oxygen species during environmental stress. *Int. J. Life Sci. Bt Pharm. Res.* 1, 198–205.
- Tahara, E.B., Navarete, F.D.T., Kowaltowski, A.J., 2009. Tissue-, substrate-, and site-specific characteristics of mitochondrial reactive oxygen species generation. *Free Radic. Biol. Med.* 46, 1283–1297.
- Tripathi, R.K., Mohindra, V., Singh, A., Kumar, R., Mishra, R.M., Jena, J.K., 2013. Physiological responses to acute experimental hypoxia in the air-breathing Indian catfish, *Clarias batrachus* (Linnaeus, 1758). *J. Biosci.* 38, 373–383.
- Turrens, J.F., 2003. Mitochondrial formation of reactive oxygen species. *J. Physiol. (Paris)* 552, 335–344.
- Uchiyama, M., Maejima, S., Yoshie, S., Kubo, Y., Konno, N., Joss, J.M., 2012. The epithelial sodium channel in the Australian lungfish, *Neoceratodus forsteri* (Osteichthyes: dipnoi). *Proc. Biol. Sci.* 279, 4795–4802.
- Uchiyama, M., Konno, N., Shibuya, S., Nogami, S., 2015. Cloning and expression of the epithelial sodium channel and its role in osmoregulation of aquatic and estivating African lungfish *Protopterus annectens*. *Comp. Biochem. Physiol. Part A* 183, 1–8.
- Ultsch, G.R., 1987. The potential role of hypercarbia in the transition from water-breathing to air-breathing in vertebrates. *Evolution* 41, 442–445.
- Ultsch, G.R., 1996. Gas exchange, hypercarbia and acid-base balance, paleoecology, and the evolutionary transition from water-breathing to air-breathing among vertebrates. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* 123, 1–27.
- Val, A.L., Almeida-Val, V.M.F., 1995. *Fishes of the Amazon and Their Environment*. Springer-Verlag, Berlin, Heidelberg, New York.
- van Ginneken, V., van den Thillart, G., 2009. Metabolic depression in fish measured by direct calorimetry: a review. *Thermochim. Acta* 483, 1–7.
- Vize, P.D., 2004. Homeric view of kidney evolution: a reprint of H.W. Smith's classic essay with a new introduction. *Anat. Rec.* 277, 344–354.
- Wake, M.H., 1986. Urogenital morphology of dipnoans, with comparisons to other fishes and to amphibians. *J. Morphol.* 190 (S1), 199–216.
- Welker, A.F., Moreira, D.C., Campos, E.G., Hermes-Lima, M., 2013. Role of redox metabolism for adaptation of aquatic animals to drastic changes in oxygen availability. *Comp. Biochem. Physiol. A: Mol. Integr. Physiol.* 165, 384–404.
- Wilkie, M.P., Morgan, T.P., Galvez, F., Smith, R.W., Kajimura, M., Ip, Y.K., Wood, C.M., 2007. The African lungfish (*Protopterus dolloi*): osmoregulation and ionoregulation in a fish out of water. *Physiol. Biochem. Zool.* 80, 99–112.
- Wood, C.M., 1991. Branchial ion and acid-base transfer in freshwater teleost fish: environmental hyperoxia as a probe. *Physiol. Zool.* 64, 68–102.
- Wood, C.M., Bucking, C., 2011. The role of feeding in salt and water balance. In: Grosell, M., Farrell, A.P., Brauner, C.J. (Eds.), *The Multifunctional Gut of Fish*. Elsevier, Amsterdam, pp. 166–212.
- Wood, S.C., Weber, R.E., Davis, B.J., 1979. Effects of air-breathing on acid-base balance in the catfish, *Hypostomus* sp. *Comp. Biochem. Physiol. Part A* 62, 185–187.
- Wood, C.M., Walsh, P.J., Chew, S.F., Ip, Y.K., 2005. Greatly elevated urea excretion after air-exposure appears to be carrier-mediated in the slender lungfish (*Protopterus dolloi*). *Physiol. Biochem. Zool.* 78, 893–907.
- Wood, C.M., Pelster, B., Giacomini, M., Sadauskas-Henrique, H., Almeida-Val, V.M., Val, A.L., 2016. The transition from water-breathing to air-breathing is associated with a shift in ion uptake from gills to gut: a study of two closely related erythrinid teleosts, *Hoplerythrinus unitaeniatus* and *Hoplias malabaricus*. *J. Comp. Physiol. B* 186, 431–445.
- Wright, P.A., Turko, A.J., 2016. Amphibious fishes: evolution and phenotypic plasticity. *J. Exp. Biol.* 219, 2245–2259.
- Wright, P.A., Wood, C.M., 2009. A new paradigm for ammonia excretion in aquatic animals: role of Rhesus (Rh) glycoproteins. *J. Exp. Biol.* 212, 2303–2312.
- Zadunaisky, J.A., 1984. The chloride cell: the active transport of chloride and the paracellular pathways. In: Hoar, W.S., Randall, D. (Eds.), *Fish Physiology*, vol. XB. Academic Press, Orlando, pp. 129–176.
- Zheng, W., Wang, Z., Collins, J.E., Andrews, R.M., Stemple, D., Gong, Z., 2011. Comparative transcriptome analyses indicate molecular homology of zebrafish swimbladder and mammalian lung. *PLoS ONE* 6, e24019.
- Zimmer, A.M., Wright, P.A., Wood, C.M., 2017. Ammonia and urea handling by early life stages of fish: a review. *J. Exp. Biol.* 220, 3843–3855.