Journal of FISH BIOLOGY

fsbi An International Society for Fish Biology

Journal of Fish Biology (2014) **84**, 682–704
doi:10.1111/jfb.12310, available online at wileyonlinelibrary.com

Acid–base and ion balance in fishes with bimodal respiration

R. B. Shartau* and C. J. Brauner

Department of Zoology, University of British Columbia, 6270 University Blvd., Vancouver, BC, V6T 1Z4 Canada

The evolution of air breathing during the Devonian provided early fishes with bimodal respiration with a stable O_2 supply from air. This was, however, probably associated with challenges and trade-offs in terms of acid–base balance and ionoregulation due to reduced gill:water interaction and changes in gill morphology associated with air breathing. While many aspects of acid–base and ionoregulation in air-breathing fishes are similar to water breathers, the specific cellular and molecular mechanisms involved remain largely unstudied. In general, reduced ionic permeability appears to be an important adaptation in the few bimodal fishes investigated but it is not known if this is a general characteristic. The kidney appears to play an important role in minimizing ion loss to the freshwater environment in the few species investigated, and while ion uptake across the gut is probably important, it has been largely unexplored. In general, air breathing in facultative air-breathing fishes is associated with an acid–base disturbance, resulting in an increased partial pressure of arterial CO_2 and a reduction in extracellular pH (pH_E); however, several fishes appear to be capable of tightly regulating tissue intracellular pH (pH_I), despite a large sustained reduction in pH_E, a trait termed preferential pH_I regulation. Further studies are needed to determine whether preferential pH_I regulation is a general trait among bimodal fishes and if this confers reduced sensitivity to acid–base disturbances, including those induced by hypercarbia, exhaustive exercise and hypoxia or anoxia. Additionally, elucidating the cellular and molecular mechanisms may yield insight into whether preferential pH_I regulation is a trait ultimately associated with the early evolution of air breathing in vertebrates.

Key words: air breathing; facultative; intracellular pH; ionoregulation; obligate; pH regulation.

INTRODUCTION

Acid–base and ion regulation are essential to vertebrate life, with the former inextricably linked with ion regulation through the need to maintain electroneutrality, according to strong ion difference theory (Stewart, 1983). Disruption of acid–base and ionoregulation can have dire consequences for cellular signalling and processes such as volume regulation, through to organ function and whole animal performance (Putnam & Roos, 1997). While acid–base and ionoregulation are reasonably well studied in a few species of water-breathing fishes (Evans *et al.*, 2005; Perry

*Author to whom correspondence should be addressed. Tel.: +1 604 379 4524; email: shartau@zoology.ubc.ca

& Gilmour, 2006; Gilmour & Perry, 2009), relatively little is known in fishes with bimodal respiration.

The evolution of air breathing during the Devonian provided early bimodal breathers with a stable and ample O_2 supply. Acid–base and ionoregulatory challenges probably arose as a consequence of air breathing due to the reduced gill:water interaction and the physical differences between air and water as respiratory media. More specifically, some of the differences between water and bimodal breathers that could potentially affect acid–base and ion regulation include changes in gill morphology, which alter ion exchange rates [*e.g.* pirarucu *Arapaima gigas* (Schinz 1822) (Gonzalez *et al.*, 2010)] and a reduction in gill surface area, and reduced gill ventilation hindering CO_2 excretion and thus pH balance [*e.g.* spotted gar *Lepisosteus oculatus* Winchell 1864 (Smatresk & Cameron, 1982)]. Furthermore, the nature of their habitat may impose constraints on acid–base and ion regulation because of the lack of available ions [*e.g.* Amazon sailfin catfish *Pterygoplichthys pardalis* (Castelnau 1855) (Brauner *et al.*, 2004*a*)] or extended stay out of water associated with aerial excursions [*e.g.* slender African lungfish *Protopterus dolloi* Boulenger 1900 (Wilkie *et al.*, 2007; Patel *et al.*, 2009*a*)].

The study of extant bimodal breathers is likely to provide insight into adaptations associated with this crucial but understudied aspect of the terrestrialization of vertebrates. The degree to which fishes with bimodal respiration depend upon air breathing varies dramatically. Obligate air breathers drown without access to air, and thus their air breathing is continuous (Graham, 1997). In facultative air breathers, aquatic respiration is sufficient to satisfy O_2 uptake under routine conditions, but air breathing is used to supplement aquatic O_2 uptake at higher temperatures, during and following exercise or during exposure to aquatic hypoxia (Graham, 1997; Lefevre *et al.*, 2014). The degree to which aerial respiration is relied upon in these different conditions is species specific and given that air breathing is thought to have evolved independently as many as 67 times, the effect on both acid–base and ionoregulation is likely to be diverse. This review will discuss what is known about acid–base regulation and ionoregulation in fishes with bimodal respiration by comparison with unimodal water-breathing fishes, then discuss how air-breathing fishes respond to acid–base and ionoregulatory challenges and then, finally, discuss future directions for research.

IONOREGULATION IN BIMODAL BREATHERS

Studies investigating the mechanisms of ionoregulation specifically in fishes capable of bimodal respiration are limited. In water-breathing fishes, ionoregulation is accomplished in the branchial mitochondrial-rich cells (MRCs; Perry, 1997; Evans *et al.*, 2005) and it is believed that bimodal breathers generally use similar mechanisms (Lin & Sung, 2003); however, during air breathing, gill:water interaction may become limited and it has been assumed that the gut and kidney may play a greater role in ionoregulation (Hochachka *et al.*, 1978; Brauner *et al.*, 2004*b*; Gonzalez *et al.*, 2010), as observed in terrestrial air breathers (Graham, 1997).

Bimodal breathers generally have reduced gill surface area relative to body mass compared with water breathers (Hughes & Morgan, 1973; Brauner & Berenbrink, 2007) and a higher density of MRCs on the gills (Brauner *et al.*, 2004*b*). Because

air breathers can secure O_2 extra-branchially (Graham, 1997), some of the design constraints on gill morphology associated with gas exchange may be relaxed, allowing the gills to take on an increased ionoregulatory role that otherwise could not occur because it would impair gas exchange. A comparison of branchial MRC localization in bimodal and water breathers found that the former were more likely to possess MRCs on both the filament and lamellae, while the latter tended to possess MRCs only on the filament (Lin & Sung, 2003). Lamellar proliferation of MRCs could increase blood–water diffusion distance and, thus, impair gas transfer (Lin & Sung, 2003) resulting in an osmorespiratory compromise (Nilsson, 1986; Sardella & Brauner, 2007). Given, however, that obligate air-breathing fishes are less dependent on branchial gas exchange than water breathers, gill remodelling could occur to enhance ion exchange without compromising gas exchange. The gills of *A. gigas*, an obligate air breather, undergo extensive remodelling with development. Initially, their gills have a similar appearance to a closely related water breather, the silver arowana *Osteoglossum bicirrhosum* (Cuvier 1829) where lamellae are clearly visible; however, with development, the interlamellar spaces become filled with cells, including MRCs, resulting in only column-shaped filaments with a high density of MRCs on the apical surface (Brauner *et al.*, 2004*b*). The reduced gill surface area in *A. gigas* does not necessarily reduce ion loss, because Gonzalez *et al.* (2010) found increased Na^+ loss in larger fish with reduced gill surface area. Why this occurred is unclear but was suggested to be a consequence of increased epithelial permeability due to changes in cell type density, as MRCs are more permeable than pavement cells. The findings in *A. gigas* are difficult to evaluate as reduced surface area along with increased Na^+ flux cannot explain the morphological changes alone; however, they may confer an advantage of decreased sensitivity to challenging water conditions, particularly to low pH environments (Gonzalez *et al.*, 2010). Further investigation is required to understand why these changes occur.

The activity and localization of ion transporters during ionoregulatory stress have been studied in a few freshwater air-breathing Anabantoidei. In pearl gourami *Trichopodus leeri* (Bleeker 1852), exposure to deionized water increased Na^+, K^+-ATPase (NKA) activity and MRC numbers in the anterior, but not posterior, gill arches, implying that the former may play a greater role in ionoregulation (Huang *et al.*, 2008). In moonlight gourami *Trichopodus microlepis* (Günther 1861), exposure to brackish water (salinity of 10) resulted in an increase in gill NKA and vacuolar-type H^+-ATPase (VHA) protein expression consistent with what has been observed in exclusive water-breathing fishes (Huang *et al.*, 2010). In a third species, dwarf gourami *Trichogaster lalius* (Hamilton 1822), exposure to low pH for 4 days resulted in the disappearance of lamellae in 30% of the fish (Huang & Lin, 2011), resulting in a gill morphology similar to *A. gigas* where the interlamellar space is filled with MRCs. This gill remodelling may also play a role in minimizing ionic stress associated with acidic or dilute environments in this species; however, further studies are required.

The African lungfishes (Protopteridae) have the ability to form cocoons during air exposure, which limits ion exchange with the environment. When *P. dolloi* and West African lungfish *Protopterus annectens* (Owen 1839) were exposed to air, a cocoon was formed but no disturbances in blood osmotic or ion balance were observed, despite large increases in plasma urea. In water, their body surface including the gills has relatively low ionic and water permeability, with rates of unidirectional

Na^+ and Cl^- influx that were 90% lower than values typically measured in similar sized freshwater fishes. This low influx was balanced by a similarly low rate of efflux (Wilkie *et al.*, 2007). Despite the reduced ionic flux of Na^+ and Cl^-, similarities in the overall pattern of ion balance exist between these fishes and water breathers based on the ion types, transporters and site of exchange (Patel *et al.*, 2009*a*, *b*). Reduced ionic permeability may be an adaptation to reduce energy expenditures during re-immersion following air exposure (Wilkie *et al.*, 2007). In general, reduced ionic permeability may appear to be an important adaptation in many bimodal fishes, a trait hypothesized to have been important in the transition of vertebrates from water to land (Ultsch, 1987, 1996).

The role of the kidney in ion regulation in water breathers is small relative to the gills (Evans *et al.*, 2005), but as the opportunity for ion exchange at the gills disappeared as vertebrates evolved onto land, it is expected that the kidney may take an increased ionoregulatory role in bimodal breathers (Cameron & Wood, 1978). Few studies have looked at renal involvement in ion regulation in bimodal breathers but Cameron & Wood (1978) were among the first to investigate kidney function between closely related water and air-breathing fishes [trahira *Hoplias malabaricus* (Bloch 1794) and jeju *Hoplerythrinus unitaeniatus* (Spix & Agassiz 1829)] and found no clear relationship between kidney function and air breathing. In *A. gigas*, the kidney is greatly enlarged compared to its water-breathing relative, *O. bicirrhosum* (Hochachka *et al.*, 1978). In *A. gigas*, renal Na^+–K^+-ATPase activity is greater than in its gills (Gonzalez *et al.*, 2010) and in the kidney of the *O. bicirrhosum* (Hochachka *et al.*, 1978). Additionally, urine:plasma ratios of Na^+ and Cl^- were one-tenth and one-hundredth, respectively, of the values for most freshwater fishes, indicating a much greater degree of ion conservation from the urine. These fishes are suggested to have adopted a more terrestrial mode of ion regulation, emphasizing renal conservation of salts, along with a greater dependency on diet for salt uptake, which may be beneficial in the ion-poor environment of the Rio Negro, Brazil (Gonzalez *et al.*, 2010). Similarly, two species of African lungfish (*P. dolloi* and *P. annectens*), which are able to aestivate for months without access to water (Perry *et al.*, 2008), also exhibited low urinary excretion rates for Na^+, Cl^- and Ca^{2+} and a higher water reabsorption rate than in rainbow trout *Oncorhynchus mykiss* (Walbaum 1792), a water breather (Patel *et al.*, 2009*b*). Increased use of renal ion regulation is probably a consequence of these fishes' habitat, ion-poor water or terrestrialization, rather than of air breathing *per se*. Bimodal breathers have access to new habitats, which are either ion-poor waters in the case of *A. gigas* or ion-free air in the case of a terrestrial lungfish; therefore, the gills become less effective at ion regulation and the focus becomes more on ion conservation. Ion regulation during the transition from water to land deserves further study, particularly the involvement of the kidney and gut, in order to gain insight into this evolutionary transition.

ACID–BASE BALANCE

The primary site of acid–base regulation in water-breathing fishes is gills, which typically account for *c*. 90% of total acid–base ion transport during pH compensation (Heisler, 1984; Evans *et al.*, 2005), with the kidney and intestine accounting for the remaining 10%. Nonetheless, some work on the renal mechanisms suggests a

larger role for the kidneys (Perry & Gilmour, 2006) and this may be especially true in bimodal breathers. Fishes, similar to other vertebrates, can minimize or compensate for an acid–base disturbance by either (1) direct transfer of acid–base relevant ions between the cell and blood, and the blood and the environment, (2) buffering with bicarbonate and non-bicarbonate buffers or (3) altering ventilation rate to modify blood PCO_2 and, thus, pH *via* the CO_2–HCO_3^- buffer system (Heisler, 1984; Evans *et al.*, 2005; Brauner & Baker, 2009). The principle mechanism of short-term acid–base compensation in terrestrial air breathers consists of the latter because blood PCO_2 is highly relative to environmental levels [*e.g.* *c.* 40 Torr *v.* <1 Torr (*c.* 5333 Pa *v.* 133 Pa) PCO_2], so considerable adjustment of blood pH can be accomplished through changes in ventilation. In water breathers, this mechanism is much less effective owing to the similarity of blood and environmental PCO_2 levels [*c.* 2–5 Torr *v.* <1 Torr (*c.* 267–667 Pa *v.* 133 Pa) PCO_2] (Heisler, 1984) and, thus, water-breathing fishes rely on buffering to minimize and direct transfer of acid–base relevant ions to compensate acid–base disturbances. The mechanisms through which bimodal fishes regulate acid–base status are generally thought to be similar to that of water-breathing fishes; however, changes in ventilation may be involved in some of these fishes (Perry *et al.*, 2005), and there may be an increased dependence on the kidney (Heisler, 1984; Ultsch, 1996; Graham, 1997).

THE ROLE OF BUFFERS IN ACID–BASE DISTURBANCES

Buffers minimize the magnitude of pH disturbances. Important buffers include bicarbonate (because of the CO_2–HCO_3^- system), phosphate buffers and haemoglobin (Hb) (due to the presence of histidine and their associated imidazole side chains that buffer H^+ at physiological pH). Fishes, in general, have lower blood and tissue buffer values than other vertebrates (Heisler, 1984). Within the blood, however, these values vary among fishes, with the more basal groups (chondrichthyans and basal actinopterygians) having higher blood buffer values than teleosts (Berenbrink *et al.*, 2005). Interestingly, several groups within the teleosts include fishes with bimodal respiration, such as the Siluriformes, Synbranchiformes, Cypriniformes (specifically Cobitoidea) and Gymnotiformes, which have secondarily increased Hb buffer values and reduced Root and Bohr effects (Berenbrink *et al.*, 2005). The Hb buffer values, and Hb–O_2 and CO_2 transport characteristics of a limited number of bimodal fishes have been studied, a consistent pattern appears to emerge. In Fig. 1, it can be seen that, in general, obligate air breathers possess the greatest Hb buffer values and smallest Bohr effects, whereas water breathers tend to have small Hb buffer values and large Bohr effects, while facultative air breathers fall in between those groups.

In water breathing and facultative air-breathers, the large Bohr effect and low buffer value indicate a tight coupling of O_2 and CO_2 transfer within the red blood cell at both the tissues and the gills (Wyman, 1964; Lapennas, 1983; Brauner & Randall, 1996). A low Bohr effect and high Hb buffer capacity, however, seen in the obligate air breathers (albeit only two species) indicate an uncoupling of O_2 and CO_2 at the level of the red blood cell that is probably associated with the spatial uncoupling of gas exchange, where O_2 uptake occurs primarily across the air-breathing organ (ABO) and CO_2 is excreted into the water, presumably across the gills (Brauner *et al.*, 2004*b*).

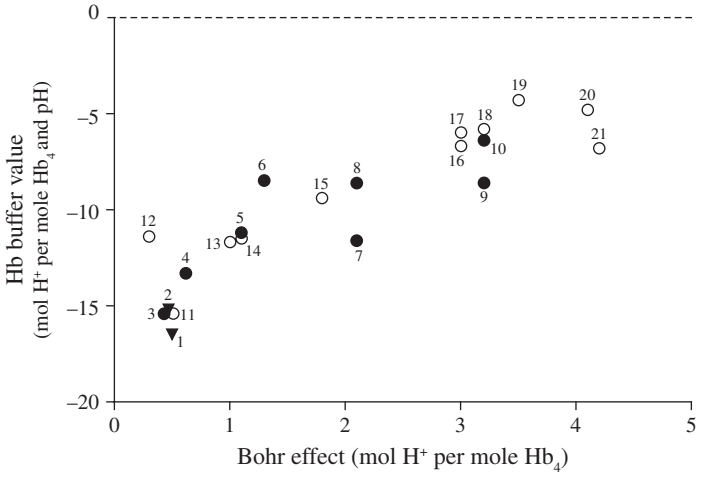

FIG. 1. Relationship between haemoglobin (Hb) buffer value and Bohr effect in bimodal and water-breathing fishes (●, facultative air breathers; ▼, obligate air breathers; ○, water breathers): 1, *Lepidosiren paradoxa* (Berenbrink *et al*., 2005); 2, *Protopterus aethiopicus* (Lenfant & Johansen, 1968); 3, *Polypterus senegalus* (Vokac *et al*., 1972); 4, *Neoceratodus forsteri* (Lenfant *et al*., 1966); 5, *Silurus glanis* (Berenbrink *et al*., 2005); 6, *Monopterus albus* (Berenbrink *et al*., 2005); 7, *Erpetoichthys calabaricus* (Berenbrink *et al*., 2005); 8, *Lepisosteus platyrhincus* (Berenbrink *et al*., 2005); 9, *Megalops cyprinoides* (Berenbrink *et al*., 2005); 10, *Amia calva* (Berenbrink *et al*., 2005); 11, *Latimeria chalumnae* (Wood *et al*., 1972); 12, *Scyliorhinus stellaris* (Berenbrink *et al*., 2005); 13, *Squalus acanthias* (Berenbrink *et al*., 2005); 14, *Mustelus asterias* (Berenbrink *et al*., 2005); 15, *Acipenser ruthenus* (Berenbrink *et al*., 2005); 16, *Oncorhynchus mykiss* (Berenbrink *et al*., 2005); 17, *Tinca tinca* (Berenbrink *et al*., 2005); 18, *Coryphaena hippurus* (Berenbrink *et al*., 2005); 19, *Thunnus obesus* (Berenbrink *et al*., 2005); 20, *Cyprinus carpio* (Berenbrink *et al*., 2005); 21, *Scleropages jardinii* (Berenbrink *et al*., 2005).

Within a fish, there is considerable variation in compartment buffer capacity. The blood and extracellular fluid comprise 20–25% of body water which, in fishes, has relatively low bicarbonate levels compared with other vertebrates (4–13 mM *v*. 15–40 mM) (Ultsch, 2012). In combination with low blood non-bicarbonate buffer values, due to a relatively low haematocrit, net transport of acid–base equivalents can have a large effect on the extracellular acid–base status in fishes (Heisler, 1984). The intracellular body compartments represent a much larger proportion of the body water (75–80%), are much more heterogeneous in nature and typically have a much higher buffer value. White muscle makes up the largest intracellular compartment in fishes (40–70% of the intracellular body fluids) and has buffer values about five-fold that of the extracellular compartment. Other intracellular compartments also have significantly greater buffer values than the extracellular compartment. Because of the large volume and high buffer value of the intracellular compartments, they may account for up to 90% of the total buffer capacity in fishes (Heisler, 1984) and this does not appear to differ in bimodal fishes (Heisler, 1982).

ACID–BASE RELEVANT ION EXCHANGE

The net transfer of acid–base equivalents between the fish and its environment is the primary means by which fishes can compensate for acid–base disturbances.

The underlying cellular and molecular mechanisms of acid–base ion transport at the gill are not well described but the putative acid–base transporters are the Na^+–H^+ exchangers (NHE), VHA coupled to apical membrane Na^+ channels (ENaC) and the HCO_3^-–Cl^- exchangers (Evans *et al.*, 2005). A general model has been developed for acid–base regulation in the water-breathing *O. mykiss*. Within the gill epithelium, MRCs are believed to be the primary site of acid–base regulation. Two populations of MRCs exist, those with peanut lectin agglutinin (PNA)-binding sites on their apical membranes (PNA^+ MRC) and those lacking such sites (PNA^- MRC). PNA^- MRCs are proposed to be responsible for acid excretion where it is believed that H^+ elimination occurs *via* an apical NHE or a VHA coupled to an apical ENaC. The result is hyperpolarization of the plasma membrane by transporting H^+ *via* VHA across the membrane, resulting in a favourable electrochemical gradient for diffusion of Na^+ *via* ENaC (Goss *et al.*, 1998; Gilmour & Perry, 2009). Net acid excretion is then achieved by the combined actions of apical H^+ efflux and basolateral HCO_3^- influx. Exchange of HCO_3^- is believed to occur *via* a HCO_3^-–Cl^- exchanger, such as those found in the solute carrier (SLC)4 or SLC26 family and by the Na^+–HCO_3^- co transporter (NBC, also found in the SLC4 family) (Evans *et al.*, 2005; Parks *et al.*, 2009; Perry *et al.*, 2009). The PNA^+ MRC is proposed to be responsible for base excretion in which apical membrane HCO_3^-–Cl^- exchanger links Cl^- uptake to HCO_3^- excretion. Apical membrane HCO_3^- efflux along with basolateral H^+ efflux, *via* a VHA, would result in net transepithelial base excretion (Gilmour & Perry, 2009). Using these membrane transporters, net acid–base equivalents can be transported from the blood to the environment to ensure pH homeostasis. Models involving similar transporters located in other acid and base secreting cell types have also been developed for zebrafish *Danio rerio* (Hamilton 1822) and dogfish *Squalus acanthias* L. 1758 (Gilmour & Perry, 2009). Few studies have been conducted to elucidate the mechanism of acid–base and ion exchange specifically in bimodal breathers; however, based on the limited data (Gonzalez *et al.*, 2001; Gilmour *et al.*, 2007; Patel *et al.*, 2009*a*), there appear to be many similarities with water breathers. This is clearly a topic in need of further study.

PATTERNS OF ACID–BASE REGULATION; INTRACELLULAR AND EXTRACELLULAR RESPONSES

In most fishes, a severe acid–base disturbance associated with severe hypoxia or anoxia, exhaustive exercise or exposure to elevated environmental CO_2 (hypercarbia) results in a reduction in extracellular pH (pH_E) and, if the disturbance is severe, a reduction in intracellular pH (pH_I). While all cells have some ability to regulate pH_I (Putnam & Roos, 1997), this capacity is limited and thus changes in pH_E and pH_I generally follow a similar qualitative pattern (Wood *et al.*, 1990; Wood & LeMoigne, 1991; Brauner & Baker, 2009). Typically, the reduction in pH_I is less than pH_E largely due to the greater buffer value of the tissues (Heisler, 1984). Recovery of pH_E occurs through the mechanisms described above with pH_I following a similar, if not slightly faster, recovery. This has been thought to represent the typical pattern of acid–base regulation in fishes for decades (Truchot, 1987; Brauner & Baker, 2009).

Recent studies, however, have elucidated a different pattern in some species where pH_I is tightly regulated despite large reductions in pH_E, termed preferential pH_I regulation (Brauner & Baker, 2009). This trait is best described in the white sturgeon

Acipenser transmontanus Richardson 1836, a basal water-breathing actinopterygian (Baker *et al.*, 2009, 2011; Huynh *et al.*, 2011; Baker & Brauner, 2012). When *A. transmontanus* are subjected to a severe respiratory acidosis by means of hypercarbia (6% CO_2), pH_E is depressed from 7·8 to 7·1 and is not compensated over 48 h (Baker *et al.*, 2009). pH_I of heart, muscle, brain, liver and white muscle, however, remain tightly regulated with no measurable depression, and more often a significant increase of up to 0·2 pH units within hours or days of exposure (Baker *et al.*, 2009, 2011). Preferential pH_I regulation has now been documented in five bimodal species, the marbled swamp eel *Synbranchus marmoratus* Bloch 1795 (Heisler, 1982; also see Table I), *P. pardalis* (Brauner *et al.*, 2004*a*; also see Table I), *L. oculatus*, longnose gar *Lepisosteus osseus* (L. 1758) and alligator gar *Atractosteus spatula* (Lacépède 1803) (unpubl. data), and has been proposed to be a general pattern in bimodal fishes (Brauner & Baker, 2009).

SPATIAL SEPARATION OF GAS EXCHANGE DURING AIR BREATHING AND EFFECTS ON ACID–BASE STATUS

In water-breathing fishes, both O_2 and CO_2 transfers occur across the gills. The capacitance coefficient for O_2 is much lower than that for CO_2 in water and, thus, if gill ventilation rate is sufficient for O_2 uptake, CO_2 is relatively easily excreted into the water. Consequently, water breathers have low blood PCO_2 levels relative to terrestrial air breathers (Truchot, 1987; Dejours, 1988). Bimodal breathers continue to rely upon the gills and skin for the majority of CO_2 excretion, despite a significant amount (and in some cases the majority) of O_2 uptake being across the ABO. This has been shown in several facultative air breathers (such as bowfin *Amia calva* L. 1766, *L. oculatus* and *S. marmoratus*) when breathing air (Graham, 1997). In obligate air-breathing fishes, *c.* 80% (or more) of total O_2 uptake occurs across the ABO while *c.* 80% of CO_2 eliminated is excreted into the water (Johansen & Lenfant, 1968; Johansen *et al.*, 1968, 1970; Lenfant & Johansen, 1968; Randall *et al.*, 1978*a*; Brauner & Berenbrink, 2007); however, *P. dolloi* appears to be an exception where CO_2 is primarily released at the lung (Perry *et al.*, 2005) and at higher temperatures the South American lungfish *Lepidosiren paradoxa* Fitzinger 1837 increases the amount of CO_2 excreted across its gills (Amin-Naves *et al.*, 2004). Thus, in general, there is spatial separation of O_2 uptake *via* the ABO and CO_2 excretion across the gills and skin to some degree in all fishes during bimodal respiration, the magnitude of which varies interspecifically and as a function of the internal and external environment. It also varies during development, as even obligate air breathers start their lives as exclusive unimodal water breathers (Graham, 1997; Brauner *et al.*, 2004*b*).

During air breathing in most bimodal breathers, access to both water and air is required for gas exchange. If fishes cannot release sufficient CO_2 while air breathing, either due to emersion, where the gills are no longer in contact with the water, or due to a reduction in gill ventilation associated with securing O_2 from their ABO, then arterial blood PCO_2 (P_ACO_2) will become elevated, reducing pH. Among facultative air breathers, an increased P_ACO_2 during air breathing has been observed in Australian lungfish *Neoceratodus forsteri* (Krefft 1870) (Lenfant *et al.*, 1966), marbled lungfish *Protopterus aethiopicus* Heckel 1851 (Lenfant & Johansen, 1968),

TABLE I. Acid–base status and blood gases of bimodal breathing fishes during various experimental conditions

Species	Experimental conditions	Time (h)	pH_E	PCO_2 (Torr)#	$[HCO_3^-]$ (mM)	PO_2 (Torr)#	Temperature (° C)
Lepidosiren paradoxa[a]	Water PCO_2 49 Torr	C	7·50	18	22	64	25
		1	7·23	37	23	75	
		3	7·13	39	21	87	
		6	7·23	47	24	88	
		24	7·26	42	28	94	
	Air and water PCO_2 49 Torr	C	7·71	18	22	58	25
		1	7·38	38	28	71	
		3	7·40	43	35	82	
		6	7·31	48	30	82	
		24	7·20	66	37	60	
Protopterus annectens[b]	1 h of acid infusion	C	7·62	9·8	17·2		25
		1	7·37	14	13·8		
		18	7·54	10·2	15·1		
	1 h of base infusion	C	7·54	12·4	18		25
		1	7·82	10·7	31		
		18	7·60	12·2	21		
Protopterus aethiopicus[c]	Air breath	0 min					20
		1 min	7·62			32	
		5 min	7·75			25	
	Air breath	25 min					
		26 min	7·74			33	
	Water PCO_2 37·5 Torr, start	46 min					
		49 min	7·78			25	
	Air breath	51 min					
		52 min	7·73			38	
	Air breath	68 min					
		69 min	7·70			36	

TABLE I. Continued

Species	Experimental conditions	Time (h)	pH_E	PCO_2 (Torr)#	$[HCO_3^-]$ (mM)	PO_2 (Torr)#	Temperature (° C)
	Water PCO_2 37·5 Torr, end	72 min					
	Air bubbling and air breath	81 min					
		92 min	7·58			36	
Neoceratodus forsteri[d]	In CO_2-free water		7·64	3·60	4·5	131	18
	Following 9 min forced air exposure		7·55	9	9		
	Following 39 min forced air exposure		7·28	18	11		
Erpetoichthys calabaricus[e]*	7·5 Torr P_ACO_2		7·56				25
	37·5 Torr P_ACO_2		7·00				
	67·5 Torr P_ACO_2		6·83				
	7·5 Torr P_ACO_2		7·65				
	37·5 Torr P_ACO_2		–				
	67·5 Torr P_ACO_2		6·87				
Polypterus senegalus[f]*	5 Torr P_ACO_2		7·74	7·0			30
	30 Torr P_ACO_2		7·00–7·35	13·6–15·4			
Lepisosteus oculatus[g]	7·5 Torr PCO_2	C	7·85	4	9	30	20
		1	7·60	7·5	9·2	30	
		4	7·62	9·4	11·7	30	
		8	7·65	9·0	12·8	32	
L. oculatus[g]†		C	7·76	4·8	8·6		20
	1 h after change to 30° C	1	7·62	9·0	9·1		30
	4 h after change to 30° C	4	7·61	8·8	7·9		30
	8 h after change to 30° C	8	7·62	8·0	8·3		30
	24 h after change from 30 to 20° C	72	7·75	6·2	7·9		20
Lepisosteus osseus[h]	Summer-acclimated fish**		7·440	13·2	10·2		25
	Winter-acclimated fish††		7·831	3·2	8·9		10

TABLE I. Continued

Species	Experimental conditions	Time (h)	pH_E	PCO_2 (Torr)#	$[HCO_3^-]$ (mM)	PO_2 (Torr)#	Temperature (° C)
Amia calva[i]	11 Torr PCO_2	C	7·96	4	13		
		3	7·60	10	13		
		6	7·51	12	13		
		24	7·70	11	16		
	45 Torr PCO_2	C	7·97	4	13		
		3	6·93	55	14		
		6	7·00	45	14		
		24	7·18	45	22		
Electrophorus electricus[j]	Air		7·55	27·7		21	28
	High PO_2 (450 Torr)		7·51	32·1		28·2	
	Low PO_2 (<70 Torr)		7·65	22·2		12·6	
	High CO_2 (~35 Torr)		7·45	33·3		22·7	
Arapaima gigas[k]‡		0 min	7·53	28		36	28–30
		1 min	7·58	26		40	
		3 min	7·59	26		38	
A. gigas[l]	40 Torr PCO_2 for 72 h§	C	7·70	16·5	33·3	33·6	28
		72	7·48	43·0	43·0	33·6	
	40 Torr PCO_2 for 72 h‖	C	7·58	26·5	40·2	32·9	
		72	7·53	34·1	46·7	26·8	
Synbranchus marmoratus[m]	Air breathing	96	7·45–7·60	23–29	22–26		28–30
	Water breathing	96	8·03–8·17	6–7	22–26		
White muscle pH	Air breathing	96	6·79–6·88		3·5–5·0		
	Water breathing	96	6·81–6·92		0·5–1·1		
Heart pH	Air breathing	96	7·06–7·30		8·5–12·3		
	Water breathing	96	7·07–7·32		1·8–3·7		

TABLE I. Continued

Species	Experimental conditions	Time (h)	pH_E	PCO_2 (Torr)#	$[HCO_3^-]$ (mM)	PO_2 (Torr)#	Temperature (° C)
Channa argus[n]	Access to water	C	7·52	15	15·8	25	25
	Air exposure	1	7·21	26	13·2	33	
	Air exposure	3	7·22	28	15·0	31	
	1 h post re-immersion	1	7·50	16	16·0	25	
Pterygoplichthys pardalis[o]	7 Torr PCO_2	0	7·79	3	7		28
		3	7·68	7	10		
		6	7·68	7	10		
		24	7·68	8	11		
	14 Torr PCO_2	0	7·88	3	7		
		3	7·35	14	11		
		6	7·45	12	12		
		24	7·53	12	16		
	42 Torr PCO_2	0	8·0	3	7		
		3	6·99	43	15		
		6	7·01	42	16		
		96	7·21	32	20		
	14 Torr PCO_2	0	7·63	5·5	9·8		
		6	7·39	16·9	15·3		
		24	7·42	14·9	15·3		
Red cell pH	14 Torr PCO_2	0	6·98				
		6	6·90				
		24	6·90				
Heart pH	14 Torr PCO_2	0	6·63		0·7		
		6	6·75		3·2		
		24	6·78		3·1		
Liver pH	14 Torr PCO_2	0	6·73		1·0		
		6	6·82		4·0		
		24	6·79		2·7		

TABLE I. Continued

Species	Experimental conditions	Time (h)	pH_E	PCO_2 (Torr)#	$[HCO_3^-]$ (mM)	PO_2 (Torr)#	Temperature (° C)
White muscle pH	14 Torr PCO_2	0	7·05		2·2		
		6	7·05		6·8		
		24	7·02		5·2		
	32 Torr PCO_2	0	7·70	4·5	8·8		
		6	7·23	33·4	20·6		
		24	7·32	31·9	25·9		
		72	7·29	31·9	22·7		
Red cell pH	32 Torr PCO_2	0	7·01				
		6	6·80				
		24	6·81				
		72	6·82				
Heart pH	32 Torr PCO_2	0	6·79		0·7		
		6	6·80		5·4		
		24	6·80		5·6		
		72	6·80		6·0		
Liver pH	32 Torr PCO_2	0	6·70		0·9		
		6	6·70		7·0		
		24	6·73		7·3		
		72	6·73		6·7		
White muscle pH	32 Torr PCO_2	0	7·05		1·9		
		6	7·02		13·0		
		24	7·05		13·3		
		72	7·10		15·9		
Hypostomus sp.[p]	Aerated water, P_WCO_2 0·22 Torr		7·54		0·21		
	Aerated water, P_WCO_2 40·90 Torr		6·89		9·0		
	Hypoxic water, P_WCO_2 0·22 Torr¶		8·19		1·24		
	Hypoxic water, P_WCO_2 40·90 Torr¶		7·18		16·1		

TABLE I. Continued

Species	Experimental conditions	Time (h)	pH_E	PCO_2 (Torr)#	$[HCO_3^-]$ (mM)	PO_2 (Torr)#	Temperature (° C)
Hoplerythrinus unitaeniatus[q]	Fish in water		7·807	11·09		30·96	27–28
	Fish in air + 20 min		7·532	22·20		24·73	
	Fish returned to water + 20 min		7·823	12·12		24·64	
Galaxias maculatus[r]		C	7·50				14
	1 h of aquatic hypoxia	1	7·17				
	1 h of emersion	2	7·25				
	5 h of emersion	6	7·22				

#, 1 Torr = 133·32 Pa. C, control conditions; pH_E, to blood pH (unless otherwise stated).

Experimental conditions: *, blood samples *in vitro*; †, effect of temperature on blood pH; ‡, changes in blood pH following an air breath; §, smaller *A. gigas c*. 67 g; ‖, larger *A. gigas c*.724 g; ¶, hypoxic water used to induce air breathing; **, frequent air breathing (<8 min between air breaths); ††, infrequent air breathing (>200 min between air breaths).

[a]Sanchez *et al.* (2005); [b]Gilmour *et al.* (2007); [c]Johansen & Lenfant (1968); [d]Lenfant *et al.* (1966); [e]Beitinger *et al.* (1985); [f]Vokac *et al.* (1972); [g]Smatresk & Cameron (1982); [h]Rahn *et al.* (1971); [i]Brauner & Baker (2009); [j]Johansen *et al.* (1968); [k]Randall *et al.* (1978*a*); [l]Gonzalez *et al.* (2010); [m]Heisler (1982); [n]Ishimatsu & Itazawa (1983); [o]Brauner *et al.* (2004*a*); [p]Wood *et al.* (1979); [q]Randall *et al.* (1978*b*); [r]Urbina & Glover (2012).

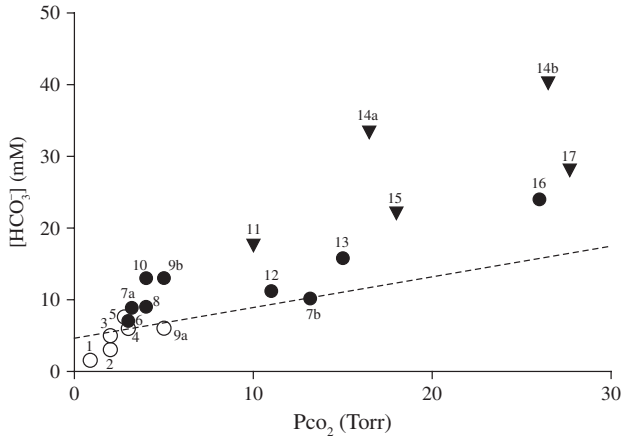

FIG. 2. Arterial plasma [HCO_3^-] as a function of blood PCO_2 in bimodal and water-breathing fishes (●, facultative air breathers; ▼, obligate air breathers; ○, water breathers). The blood buffer line of *Lepisosteus osseus* is indicated (- - - -) in order to provide an example of blood buffer capacity of a facultative bimodal breather during air breathing at 25° C (Rahn *et al*., 1971). Specific numbers with different letters either indicate the same species under different circumstances or closely related species. 1, *Leucoraja ocellata* at 12° C (Wood *et al*., 1990); 2, *Oncorhynchus mykiss* at 15° C (Wood & LeMoigne, 1991); 3, *Conger conger* at 17° C (Toews *et al*., 1983); 4, *Acipenser transmontanus* at 13° C (Baker *et al*., 2009); 5, *Scyliorhinus stellaris* at 17° C (Heisler *et al*., 1976); 6, *Pterygoplichthys pardalis* at 28° C (Brauner *et al*., 2004*a*); 7a, *Lepisosteus osseus* at 10° C when no air breathing occurs (Rahn *et al*., 1971); 7b, *L. osseus* at 25° C when frequent air breathing occurs (Rahn *et al*., 1971); 8, *Lepisosteus oculatus* at 30° C (Smatresk & Cameron, 1982); 9a, *Hoplias malabaricus* at 28–32° C (Cameron & Wood, 1978); 9b, *Hoplerythrinus unitaeniatus* at 28–32° C (Cameron & Wood, 1978); 10, *Amia calva* at 24·5° C (Randall *et al*., 1981); 11, *Protopterus annectens* at 25° C (Gilmour *et al*., 2007); 12, *Neoceratodus forsteri* at 18° C (Lenfant *et al*., 1966); 13, *Channa argus* at 25° C (Ishimatsu & Itazawa, 1983); 14a, *Arapaima gigas* smaller fish at 28° C (Gonzalez *et al*., 2010); 14b, *A. gigas* larger fish at 28° C (Gonzalez *et al*., 2010); 15, *Lepidosiren paradoxa* at 25° C (Sanchez *et al*., 2005); 16, *Synbranchus marmoratus* at 28–30° C (Heisler, 1982); 17, *Electrophorus electricus* at 28–30° C (Johansen *et al*., 1968).

L. oculatus (Smatresk & Cameron, 1982), northern snakehead *Channa argus* (Cantor 1842) (Ishimatsu & Itazawa, 1983), *H. unitaeniatus* (Randall *et al.*, 1978*b*), armoured catfish *Hypostomus* sp. (Wood *et al.*, 1979), *S. marmoratus* (Heisler, 1982) and *A. calva* by Johansen *et al.* (1970), but not by McKenzie & Randall (1990); in these fishes, a reduction in pH_E was also observed in conjunction with an increased P_ACO_2 (Table I). As fishes become more dependant on air breathing (*i.e.* facultative *v*. obligate air breathers), P_ACO_2 and plasma [HCO_3^-] rise (Ultsch, 1996) (Fig. 2). The increase in plasma [HCO_3^-] is higher than it would be expected strictly due to increased P_ACO_2 because, as Fig. 2 shows, most bimodal breathers have [HCO_3^-] above what would be expected based only on the buffer capacity of the blood [whole blood buffer capacity of *L. osseus* at 25° C is used as an example (Rahn *et al.*, 1971)]. The increased [HCO_3^-] occurs in conjunction with a reduction in plasma Cl^-, indicating that these animals live in a continued state of partially compensated respiratory acidosis (Brauner *et al.*, 2004*b*). The increased [HCO_3^-] may be accomplished through HCO_3^- uptake from environment and internal sources [*e.g.* kidney (Gonzalez *et al.*, 2010) and dermal bone (Janis *et al.*, 2012)].

Air breathing in a facultative air breather is not always associated with respiratory acidosis. In *A. calva* air exposed for 5 days, while blood PCO_2 increased, there

was no change in pH_E (McKenzie & Randall, 1990). In *Hypostomus* sp. induced to breathe air by exposure to aquatic hypoxia, there was no reduction in pH_E despite a large increase in P_ACO_2 from 3 to 20 Torr (*c*. 400 to 2666 Pa), pH_e compensation occurred *via* an increase in plasma [HCO_3^-] (from 2 to 13 mM) (Wood *et al.*, 1979), presumably in exchange for Cl^-, which is the 'typical' pattern observed in water-breathing fishes (Brauner & Baker, 2009). The more common pattern is that air breathing in facultative air breathers is associated with an uncompensated reduction in pH_E (Truchot, 1987; Table I). In *S. marmoratus* induced to air breathe through exposure to aquatic hypoxia, P_ACO_2 increased from 6 to 26 Torr (*c*. 800 to 3466 Pa), pH_E fell by 0·6 pH units and there were no changes to plasma [HCO_3^-] (Heisler, 1982), indicating a complete lack of pH_E compensation. As mentioned above, pH_I of white and heart muscle was not reduced following 4–5 days of air breathing (the only time point measured), despite a large decrease in pH_E. In fact, pH_E was well below the blood buffer line, probably associated with net tissue acid extrusion. This study was the first to demonstrate preferential pH_I regulation indicating that tissues have the ability to regulate their pH independent of pH_E, a trait that had not been previously been observed in vertebrates but may be a common strategy in air-breathing fishes (Brauner & Baker, 2009).

PREFERENTIAL pH_I REGULATION DURING HYPERCARBIA

While preferential pH_I regulation has not been observed in other bimodal fishes during air breathing, it has been observed in fishes exposed to hypercarbia where a respiratory acidosis similar to, or greater in magnitude than, those observed during air breathing. Brauner *et al.* (2004*a*) investigated the response of *P. pardalis* to various degrees of hypercarbia and found that at 7, 14 and 32 Torr (*c*. 933, 1867 and 4266 Pa) PCO_2, pH_E was uncompensated despite an acidosis of 0·2, 0·6 and 1·0 pH units. During exposure to 14 and 32 Torr PCO_2 for up to 72 h, pH_I of heart, liver and white muscle did not change despite a drop in pH_E of over 0·5 pH units (Table I). Preferential pH_I regulation of the heart appears to confer protection of cardiac function at high CO_2 tensions (7·5% CO_2) *in situ* (Hanson *et al.*, 2009). Recent findings on *L. oculatus*, *L. osseus* and *A. spatula* show that preferential pH_I regulation occurs in the heart, liver, brain and white muscle during exposure to 6% CO_2 (unpubl. data). There is also indirect evidence of preferential pH_I regulation in *A. calva* (Brauner & Baker, 2009) and *L. paradoxa* (Sanchez *et al.*, 2005; Brauner & Baker, 2009) during exposure to hypercarbia. All studies have observed preferential pH_I regulation during sustained respiratory acidosis in bimodal respiring fishes when both pH_E and pH_I have been measured.

ACID–BASE REGULATION DURING METABOLIC DISTURBANCES

Few studies have investigated how bimodal breathers compensate for metabolic acid–base disturbances that are the result of endogenous acid or base production. Compensation for a metabolic pH disturbance induced by acid or base infusion has been examined in *P. annectens* (Table I). Gilmour *et al.* (2007) found that following the development of a metabolic acidosis or alkalosis, *P. annectens* used both

respiratory (*i.e.* changes in ventilation rate) and metabolic pathways to restore pH_E; a response that differs from most fishes studied to date. Following acid infusion, respiratory compensation appeared to be the primary mechanism for pH_E recovery as both pulmonary and branchial ventilation increased to enhance CO_2 excretion; there was a small but insignificant increase in net acid excretion *via* the gills (Gilmour *et al.*, 2007). Following base infusion, there was a significant reduction in branchial ventilation (reducing CO_2 excretion) and net base excretion *via* the gills and skin associated with pH_E recovery. Interestingly, there was no change in pulmonary ventilation following base infusion. The authors suggest that these lungfish have characteristics of both terrestrial air breathers in that they can alter ventilation to regulate pH, and water breathers as metabolic compensation was accomplished to some degree at the gills and skin. The role of the kidney in net acid–base excretion was minimal (Gilmour *et al.*, 2007). This is the only study to date to investigate the relative involvement of respiratory *v*. metabolic compensation in response to pH disturbances in bimodal breathers, an area clearly worthy of further investigation.

Air breathing during exercise in facultative air breathers (*A. calva* and *L. oculatus*) may be important for satisfying the increased metabolic demand associated with exercise (Farmer & Jackson, 1998; McKenzie *et al.*, 2012) which would delay recruitment of anaerobiosis and thus minimize acid–base disturbances associated with a given level of exercise intensity. In two facultative air breathers, *A. calva* (Gonzalez *et al.*, 2001) and *L. oculatus* (Burleson *et al.*, 1998), however, air breathing following exhaustive exercise delayed pH recovery, probably associated with an air-breathing induced elevation in PCO_2 (Burleson *et al.*, 1998; Gonzalez *et al.*, 2001). In *L. oculatus*, blood PCO_2 remained elevated for 2–4 h post-exercise, whereas in most water breathers PCO_2 returns to normocapnic values after 0·5 h post-exercise (Burleson *et al.*, 1998). In *A. calva*, recovery of the acid–base disturbance following exhaustive exercise was complete within 4 h without access to air, while a significant acidosis remained even following 8 h in fish with access to air. In another facultative air breather, Pacific tarpon *Megalops cyprinoides* (Broussonet 1782), recovery from strenuous exercise as indicated by lactate removal was faster in those without access to air (Wells *et al.*, 2007). Together, these studies suggest that recovery from an exhaustive exercise-induced acidosis appears to require aquatic respiration for CO_2 removal and exchange of net acid–base equivalents across the gills to remove the acid load. While there was no mortality associated with exhaustive exercise in these bimodal fishes, it is well known that fish, such as *O. mykiss*, can experience post-exercise mortality rates of up to 40% (Wood *et al.*, 1983). The cause of post-exercise mortality is unknown but is thought to be associated with a severe intracellular acidosis (suggested to be white muscle) incurred during exercise as decreased pH_E does not appear to be related to mortality (Wood *et al.*, 1983; Milligan & Wood, 1986; Burleson *et al.*, 1998). The only study in which tissue pH_I has been measured following exhaustive exercise in a bimodal fish is in *P. pardalis*. Despite a significant reduction in pH_E of 0·25 pH units and a 12 fold increase in plasma lactate 2 h following exhaustion, there were no changes in pH_I of heart, liver or brain (T. S. H. Harter, R. B. S. Shartau, D. W. B. Baker, D. C. J. Jackson, A. L. V. Val & C. J. B. Brauner, unpubl. data). *Pterygoplichthys pardalis* were also assessed for their ability to regulate pH during more severe conditions of 1 h aquatic anoxia where lactate increased 18 fold over control values. Three hours following exposure,

pH_E had decreased by 0·5 pH units while no changes in pH_I of heart, liver or brain occurred, suggesting preferential pH_I regulation may be able to protect against both metabolic (T. S. H. Harter, R. B. S. Shartau, D. W. B. Baker, D. C. J. Jackson, A. L. Val & C. J. B. Brauner, unpubl. data) and respiratory acidosis (Brauner *et al.*, 2004*a*). Thus, if preferential pH_I regulation is a general trait among bimodal fishes, this may confer a reduced sensitivity to certain stressors, an area clearly worthy of further investigation.

CONCLUSION AND FUTURE DIRECTIONS

Air-breathing fishes are commonly found in tropical or sub-tropical fresh waters that may resemble the conditions that ancestral basal euteleostomi fishes encountered in the Devonian when invading fresh water: ion-poor and prone to hypoxia and hypercarbia. Regulation of pH_E during exposure to hypercarbia through a net increase in plasma $[HCO_3^-]$ in exchange for $[Cl^-]$ probably represents the basal condition for acid–base regulation in fishes (Brauner & Baker, 2009). This process, however, appears to be limited to a PCO_2 of 15–20 Torr (*c*. 2000–2666 Pa; 2–2·5%) during short-term exposure to hypercarbia due to an apparent bicarbonate threshold (Heisler, 1984; Brauner & Baker, 2009), while it is not uncommon for CO_2 levels in tropical environments to approach 60 Torr (*c*. 8000 Pa 8% CO_2). Furthermore, net plasma $[HCO_3^-]$–$[Cl^-]$ exchange is also limited in the ion-poor waters (low Na^+, Cl^-, HCO_3^- and Ca^{2+}; Larsen & Jensen, 1997) that are characteristic of the Amazon Basin. Thus, preferential pH_i regulation may have evolved to overcome these types of limitations, providing exceptional CO_2 tolerance and providing a mechanism to deal with the air-breathing induced respiratory acidosis (Brauner & Baker, 2009) that appears to be relatively common among extant facultative air-breathing fishes. Preferential pH_I regulation has only been identified in five air-breathing fishes to date; however, limited data indicate that other air-breathing fishes do not compensate pH_E following a respiratory acidosis, suggesting preferential pH_I regulation may be more widespread than currently known and may represent a common characteristic of bimodal fishes (Brauner & Baker, 2009).

There are clearly large voids in the understanding of ionoregulation and acid–base regulation in bimodal fishes. These are too numerous to mention here, but the following are some areas worthy of further investigation based upon the above discussion. Reduced ionic permeability appears to be an important adaptation in the few bimodal fishes investigated, determining whether this is a general characteristic among bimodal fishes would prove interesting. The role of the kidney in ionoregulation should be investigated further, and the role of the gut remains virtually unstudied. The specific cellular and molecular mechanisms associated with ionoregulation and acid–base regulation in these tissues also remains largely unstudied with the exception of just a few species. The relative involvement of respiratory *v*. metabolic compensation (at both the gills and kidney) in response to pH disturbances in bimodal breathers is an area bound to reveal interesting findings. Finally, determining whether preferential pH_I regulation confers reduced sensitivity to all types of acid–base disturbances among bimodal fishes and elucidating the cellular and molecular mechanisms involved are areas for research that are timely and largely unexplored.

The work was supported by a Natural Sciences and Engineering Research Council (NSERC) of Canada Discovery grant to C.J.B. and an NSERC CGS-D3 to R.B.S. The authors would like to thank two anonymous reviewers and the editor for valuable suggestions.

References

Amin-Naves, J., Giusti, H. & Glass, M. L. (2004). Effects of acute temperature changes on aerial and aquatic gas exchange, pulmonary ventilation and blood gas status in the South American lungfish, *Lepidosiren paradoxa*. *Comparative Biochemistry and Physiology A* **138,** 133–139.

Baker, D. W. & Brauner, C. J. (2012). Metabolic changes associated with acid–base regulation during hypercarbia in the CO_2-tolerant chondrostean, white sturgeon (*Acipenser transmontanus*). *Comparative Biochemistry and Physiology A* **161,** 61–68.

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*. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology* **296,** R1868–R1880.

Baker, D. W., Hanson, L. M., Farrell, A. P. & Brauner, C. J. (2011). Exceptional CO_2 tolerance in white sturgeon (*Acipenser transmontanus*) is associated with protection of maximum cardiac performance during hypercapnia in situ. *Physiological and Biochemical Zoology* **84,** 239–248.

Beitinger, T. L., Pettit, M. J. & Hutchison, V. H. (1985). Oxygen transfer characteristics of the blood of reedfish, *Erpetoichthys calabaricus*. *Comparative Biochemistry and Physiology* **82,** 553–558.

Berenbrink, M., Koldkjaer, P., Kepp, O. & Cossins, A. R. (2005). Evolution of oxygen secretion in fishes and the emergence of a complex physiological system. *Science* **307,** 1752–1757.

Brauner, C. J. & Baker, D. W. (2009). Patterns of acid-base regulation during exposure to hypercarbia in fish. In *Cardio-Respiratory Control in Vertebrates: Comparative and Evolutionary Aspects* (Glass, M. L. & Wood, S. C., eds), pp. 43–63. Berlin: Springer-Verlag.

Brauner, C. J. & Berenbrink, M. (2007). Gas transport and exchange. In *Primitive Fishes, Fish Physiology* (McKenzie, D. J., Farrell, A. P. & Brauner, C. J., eds), pp. 213–282. New York, NY: Elsevier.

Brauner, C. J. & Randall, D. J. (1996). The interaction between oxygen and carbon dioxide movements in fishes. *Comparative Biochemistry and Physiology A* **113,** 83–90.

Brauner, C. J., Wang, T., Wang, Y., Richards, J. G., Gonzalez, R. J., Bernier, N. J., Xi, W., Patrick, M. & Val, A. L. (2004*a*). Limited extracellular but complete intracellular acid-base regulation during short-term environmental hypercapnia in the armoured catfish, *Liposarcus pardalis*. *Journal of Experimental Biology* **207,** 3381–3390.

Brauner, C. J., Matey, V., Wilson, J. M., Bernier, N. J. & Val, A. L. (2004*b*). Transition in organ function during the evolution of air-breathing; insights from *Arapaima gigas*, an obligate air-breathing teleost from the Amazon. *Journal of Experimental Biology* **207,** 1433–1438.

Burleson, M., Shipman, B. & Smatresk, N. (1998). Ventilation and acid-base recovery following exhausting activity in an air-breathing fish. *Journal of Experimental Biology* **201,** 1359–1368.

Cameron, J. N. & Wood, C. M. (1978). Renal function and acid-base regulation in two Amazonian erythrinid fishes: *Hoplias malabaricus*, a water breather, and *Hoplerythrinus unitaeniatus*, a facultative air breather. *Canadian Journal of Zoology* **56,** 917–930.

Dejours, P. (1988). *Respiration in Water and Air: Adaptations, Regulation and Evolution*. Amsterdam: Elsevier.

Evans, D. H., Piermarini, P. M. & Choe, K. P. (2005). The multifunctional fish gill: dominant site of gas exchange, osmoregulation, acid-base regulation, and excretion of nitrogenous waste. *Physiological Reviews* **85,** 97–177.

Farmer, C. G. & Jackson, D. C. (1998). Air-breathing during activity in the fishes *Amia calva* and *Lepisosteus oculatus*. *Journal of Experimental Biology* **201,** 943–948.

Gilmour, K. M. & Perry, S. F. (2009). Carbonic anhydrase and acid-base regulation in fish. *Journal of Experimental Biology* **212,** 1647–1661.

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*. *Journal of Experimental Biology* **210,** 1944–1959.

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*. *Physiological and Biochemical Zoology* **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*. *Physiological and Biochemical Zoology* **83,** 322–332.

Goss, G. G., Perry, S. F., Fryer, J. N. & Laurent, P. (1998). Gill morphology and acid-base regulation in freshwater fishes. *Comparative Biochemistry and Physiology A* **119,** 107–115.

Graham, J. B. (1997). *Air Breathing Fishes: Evolution, Diversity, and Adaptation*. San Diego, CA: Academic Press.

Hanson, L. M., Baker, D. W., Kuchel, L. J., Farrell, A. P., Val, A. L. & Brauner, C. J. (2009). Intrinsic mechanical properties of the perfused armoured catfish heart with special reference to the effects of hypercapnic acidosis on maximum cardiac performance. *Journal of Experimental Biology* **212,** 1270–1276.

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. *Journal of Experimental Biology* **99,** 9–28.

Heisler, N. (1984). Acid-base regulation in fishes. In *Fish Physiology*, Vol. 10 (Hoar, W. S. & Randall, D. J., eds), pp. 315–401. San Diego, CA: Academic.

Heisler, N., Weitz, H. & Weitz, A. M. (1976). Hypercapnia and resultant bicarbonate transfer processes in an elasmobranch fish (*Scyliorhinus stellaris*). *Bulletin Européen de Physiopathologie Respiratoire* **12,** 77–86.

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. *Canadian Journal of Zoology* **56,** 820–832.

Huang, C.-Y. & Lin, H.-C. (2011). The effect of acidity on gill variations in the aquatic air-breathing fish, *Trichogaster lalius*. *Comparative Biochemistry and Physiology A* **158,** 61–71.

Huang, C.-Y., Lee, W. & Lin, H.-C. (2008). Functional differentiation in the anterior gills of the aquatic air-breathing fish, *Trichogaster leeri*. *Journal of Comparative Physiology B* **178,** 111–121.

Huang, C. Y., Chao, P. L. & Lin, H. C. (2010). Na^+/K^+-ATPase and vacuolar-type H^+-ATPase in the gills of the aquatic air-breathing fish *Trichogaster microlepis* in response to salinity variation. *Comparative Biochemistry and Physiology A* **155,** 309–318.

Hughes, G. M. & Morgan, M. (1973). The structure of fish gills in relation to their respiratory function. *Biological Reviews* **48,** 419–475.

Huynh, K. T., Baker, D. W., Harris, R., Church, J. & Brauner, C. J. (2011). Capacity for intracellular pH compensation during hypercapnia in white sturgeon primary liver cells. *Journal of Comparative Physiology B* **181,** 893–904.

Ishimatsu, A. & Itazawa, Y. (1983). Blood oxygen levels and acid-base status following air exposure in an air-breathing fish, *Channa argus*: the role of air ventilation. *Comparative Biochemistry and Physiology A* **74,** 787–793.

Janis, C. M., Devlin, K., Warren, D. E. & Witzmann, F. (2012). Dermal bone in early tetrapods: a palaeophysiological hypothesis of adaptation for terrestrial acidosis. *Proceedings of the Royal Society B* **279,** 3035–3040.

Johansen, K. & Lenfant, C. (1968). Respiration in the African lungfish *Protopterus Aethiopicus*: II. Control of breathing. *Journal of Experimental Biology* **49,** 453–468.

Johansen, K., Lenfant, C., Schmidt-Nielsen, K. & Petersen, J. (1968). Gas exchange and control of breathing in the electric eel, *Electrophorus electricus*. *Zeitschrift für vergleichende Physiologie* **61,** 137–163.

Johansen, K., Hanson, D. & Lenfant, C. (1970). Respiration in a primitive air breather, *Amia calva*. *Respiration Physiology* **9,** 162–174.

Lapennas, G. N. (1983). The magnitude if the Bohr coefficient: optimal for oxygen delivery. *Respiration Physiology* **54,** 161–172.

Larsen, B. K. & Jensen, F. B. (1997). Influence of ionic composition on acid-base regulation in rainbow trout (*Oncorhynchus mykiss*) exposed to environmental hypercapnia. *Fish Physiology and Biochemistry* **16,** 157–170.

Lefevre, S., Domenici, P. & McKenzie D.J. (2014). Swimming in air breathing fishes. *Journal of Fish Biology* **84,** 661–681.

Lenfant, C. & Johansen, K. (1968). Respiration in the African lungfish *Protopterus aethiopicus*: I. Respiratory properties of blood and normal patterns of breathing and gas exchange. *Journal of Experimental Biology* **49,** 437–452.

Lenfant, C., Johansen, K. & Grigg, G. C. (1966). Respiratory properties of blood and pattern of gas exchange in the lungfish *Neoceratodus forsteri* (Krefft). *Respiration Physiology* **2,** 1–21.

Lin, H.-C. & Sung, W.-T. (2003). The distribution of mitochondria-rich cells in the gills of air-breathing fishes. *Physiological and Biochemical Zoology* **76,** 215–228.

McKenzie, D. J. & Randall, D. J. (1990). Does *Amia calva* aestivate? *Fish Physiology and Biochemistry* **8,** 147–158.

McKenzie, D. J., Steffensen, J. F., Taylor, E. W. & Abe, A. S. (2012). The contribution of air breathing to aerobic scope and exercise performance in the banded knifefish *Gymnotus carapo* L. *Journal of Experimental Biology* **215,** 1323–1330.

Milligan, C. L. & Wood, C. M. (1986). Intracellular and extracellular acid-base status and H^+ exchange with the environment after exhaustive exercise in the rainbow trout. *Journal of Experimental Biology* **123,** 93–121.

Nilsson, S. (1986). Control of gill blood flow. In *Fish Physiology: Recent Advances* (Nilsson, S. & Holmgren, S., eds), pp. 86–101. London: Croom Helm.

Parks, S. K., Tresguerres, M. & Goss, G. G. (2009). Cellular mechanisms of Cl^- transport in trout gill mitochondrion-rich cells. *American Journal of Physiology* **296,** R1161–R1169.

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*. *Journal of Fish Biology* **75,** 862–884.

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*. *Comparative Biochemistry and Physiology A* **152,** 149–157.

Perry, S. F. (1997). The chloride cell: structure and function in the gills of freshwater fishes. *Annual Review of Physiology* **59,** 325–347.

Perry, S. F. & Gilmour, K. M. (2006). Acid-base balance and CO_2 excretion in fish: unanswered questions and emerging models. *Respiratory Physiology and Neurobiology* **154,** 199–215.

Perry, S. F., Gilmour, K. M., Swenson, E. R., Vulesevic, B., Chew, S. F. & Ip, Y. K. (2005). An investigation of the role of carbonic anhydrase in aquatic and aerial gas transfer in the African lungfish *Protopterus dolloi*. *Journal of Experimental Biology* **208,** 3805–3815.

Perry, S. F., Euverman, R., Wang, T., Loong, A. M., Chew, S. F., Ip, Y. K. & Gilmour, K. M. (2008). Control of breathing in African lungfish (*Protopterus dolloi*): a comparison of aquatic and cocooned (terrestrialized) animals. *Respiratory Physiology and Neurobiology* **160,** 8–17.

Perry, S. F., Vulesevic, B., Grosell, M. & Bayaa, M. (2009). Evidence that SLC26 anion transporters mediate branchial chloride uptake in adult zebrafish (*Danio rerio*). *American Journal of Physiology* **297,** R988–R997.

Putnam, R. W. & Roos, A. (1997). Intracellular pH. In *Handbook of Physiology*, Vol. 14 (Hoffman, J. & Jamieson, J., eds), pp. 389–440. Oxford: Oxford University Press.

Rahn, H., Rahn, K. B., Howell, B. J., Gans, C. & Tenney, S. M. (1971). Air breathing of the garfish (*Lepisosteus osseus*). *Respiration Physiology* **11,** 285–307.

Randall, D. J., Farrell, A. P. & Haswell, M. S. (1978a). Carbon dioxide excretion in the pirarucu (*Arapaima gigas*), an obligate air-breathing fish. *Canadian Journal of Zoology* **56,** 977–982.

Randall, D. J., Farrell, A. P. & Haswell, M. S. (1978b). Carbon dioxide excretion in the jeju, *Hoplerythrinus unitaeniatus*, a facultative air-breathing teleost. *Canadian Journal of Zoology* **56,** 970–973.

Randall, D. J., Cameron, J. N., Daxboeck, C. & Smatresk, N. (1981). Aspects of bimodal gas exchange in the bowfin, *Amia calva* L. (Actinopterygii: Amiiformes). *Respiration Physiology* **43,** 339–348.

Sanchez, A. P., Giusti, H., Bassi, M. & Glass, M. L. (2005). Acid-base regulation in the South American lungfish *Lepidosiren paradoxa*: effects of prolonged hypercarbia on blood gases and pulmonary ventilation. *Physiological and Biochemical Zoology* **78,** 908–915.

Sardella, B. A. & Brauner, C. J. (2007). The osmo-respiratory compromise in fish: the effects of physiological state and the environment. In *Fish Respiration and Environment* (Fernandes, M. N., Rantin, F. T., Glass, M. L. & Kapoor, B. G., eds), pp. 147–165. Enfield, NH: Science Publishers.

Smatresk, N. J. & Cameron, J. N. (1982). Respiration and acid-base physiology of the spotted gar, a bimodal breather. II. Responses to temperature change and hypercapnia. *Journal of Experimental Biology* **96,** 281–293.

Stewart, P. A. (1983). Modern quantitative acid–base chemistry. *Canadian Journal of Physiology and Pharmacology* **61,** 1444–1461.

Toews, D. P., Holeton, G. F. & Heisler, N. (1983). Regulation of the acid-base status during environmental hypercapnia in the marine teleost fish *Conger conger*. *Journal of Experimental Biology* **107,** 9–20.

Truchot, J.-P. (1987). *Comparative Aspects of Extracellular Acid-Base Balance*. Berlin: Springer.

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. *Palaeogeography Palaeoclimatology Palaeoecology* **123,** 1–27.

Ultsch, G. R. (2012). Metabolism, gas exchange, and acid-base balance of giant salamanders. *Biological Reviews* **87,** 583–601.

Urbina, M. & Glover, C. (2012). Should I stay or should I go?: physiological, metabolic and biochemical consequences of voluntary emersion upon aquatic hypoxia in the scaleless fish *Galaxias maculatus*. *Journal of Comparative Physiology B* **182,** 1057–1067.

Vokac, Z., Ahmed, N. E. D. & Abdel Magid, A. M. (1972). The *in vitro* respiratory properties of blood of the primitive fish *Polypterus senegalus*. *Journal of Experimental Biology* **57,** 461–469.

Wells, R. M. G., Baldwin, J., Seymour, R. S., Christian, K. A. & Farrell, A. P. (2007). Air breathing minimizes post-exercise lactate load in the tropical Pacific tarpon, *Megalops cyprinoides* Broussonet 1782 but oxygen debt is repaid by aquatic breathing. *Journal of Fish Biology* **71,** 1649–1661.

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*): ionoregulation and osmoregulation in a fish out of water. *Physiological and Biochemical Zoology* **80,** 99–112.

Wood, C. M. & LeMoigne, J. (1991). Intracellular acid-base responses to environmental hyperoxia and normoxic recovery in rainbow trout. *Respiration Physiology* **86,** 91–113.

Wood, S. C., Johansen, K. & Weber, R. E. (1972). Haemoglobin of the coelacanth. *Nature* **239,** 283–285.

Wood, S. C., Weber, R. E. & Davis, B. J. (1979). Effects of air-breathing on acid-base balance in the catfish, *Hypostomus sp*. *Comparative Biochemistry and Physiology A* **62,** 185–187.

Wood, C. M., Turner, J. D. & Graham, M. S. (1983). Why do fish die after severe exercise? *Journal of Fish Biology* **22,** 189–201.

Wood, C. M., Turner, J. D., Munger, R. S. & Graham, M. S. (1990). Control of ventilation in the hypercapnic skate *Raja ocellata*: II. Cerebrospinal fluid and intracellular pH in the brain and other tissues. *Respiration Physiology* **80,** 279–298.

Wyman, J. (1964). Linked functions and reciprocal effects in hemoglobin: a second look. *Advances in Protein Chemistry* **19,** 223–286.