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REVIEW PAPER

Breaking wind to survive: fishes that breathe air with their gut

J. A. Nelson

Department of Biological Sciences, Towson University, Towson, MD 21252-0001, U.S.A.

Several taxonomically disparate groups of fishes have evolved the ability to extract oxygen from the air with elements of their gut. Despite perceived difficulties with balancing digestive and respiratory function, gut air breathing (GAB) has evolved multiple times in fishes and several GAB families are among the most successful fish families in terms of species numbers. When gut segments evolve into an air-breathing organ (ABO), there is generally a specialized region for exchange of gases where the gut wall has diminished, vascularization has increased, capillaries have penetrated into the luminal epithelium and surfactant is produced. This specialized region is generally separated from digestive portions of the gut by sphincters. GAB fishes tend to be facultative air breathers that use air breathing to supplement aquatic respiration in hypoxic waters. Some hindgut breathers may be continuous, but not obligate air breathers (obligate air breathers drown if denied access to air). Gut ABOs are generally used only for oxygen uptake; CO_2 elimination seems to occur *via* the gills and skin in all GAB fishes studied. Aerial ventilation in GAB fishes is driven primarily by oxygen partial pressure of the water (PO_2) and possibly also by metabolic demand. The effect of aerial ventilation on branchial ventilation and the cardiovascular system is complex and generalizations across taxa or ABO type are not currently possible. Blood from GAB fishes generally has a low blood oxygen partial pressure that half saturates haemoglobin ($p50$) with a very low erythrocytic nucleoside triphosphate concentration [NTP]. GAB behaviour in nature depends on the social and ecological context of the animal as well as on physiological factors.

Key words: ABO; facultative, continuous air breathing; GAB; hypoxia.

GENERAL AQUATIC HABITATS AND FISH RESPIRATION

Although oxygen is not a requirement for life, fishes have followed an evolutionary path that requires oxygen as a terminal electron acceptor under most conditions. Some fish species can survive extended periods in anoxic water (Nilsson, 2001), but all fishes are obligate aerobes and require O_2 to complete their life cycle. Oxygen availability in water is very dependent on environmental conditions, but always far less abundant than in air. Thus, the evolution of air breathing is a frequent event in the vertebrate lineage, possibly occurring independently as many as 70 times (Graham, 2011*a*).

Tel.: +1 410 704 3945; email: jnelson@towson.edu

Aquatic hypoxia and anoxia develop due to various combinations of (1) aquatic respiratory rates exceeding photosynthetic rates, (2) poor to no mixing at the aerial–aquatic interface, (3) poor light penetration due to shading or turbidity and (4) isolation of water parcels (Junk, 1984; Wetzel, 2001; Diaz & Breitburg, 2009). These conditions occur most frequently in fresh water, so it is probably not a coincidence that most air-breathing fishes as well as most gut air-breathing (GAB) fishes are freshwater fishes (Graham, 1997). In temperate to polar regions, the most common scenarios resulting in hypoxia and anoxia involve isolation of the water body from the oxygen-rich atmosphere. Other temperate fish habitats subject to hypoxia include soft sediments that do not mix well with the water column and swamp habitats. Hypoxic and anoxic waters form in tropical waters through a diversity of processes that generally deplete oxygen faster and are harder to characterize than temperate systems (Junk, 1984). The combinations of (1) high temperature, (2) nutrient-rich waters, (3) dense terrestrial vegetation that can block sunlight and wind, (4) dense surface vegetation that can not only block sunlight and wind but can also contribute photosynthetic oxygen to the water and (5) a dense biota that can influence the water chemistry as much as physical factors create a mosaic of hypoxic and anoxic aquatic habitats across the tropics. It is common for tropical waters of the Amazon flooded forest (varzea) to have their top 10 cm be the only predictable aquatic oxygen source for months at a time (Val & Almeida-Val, 1995). It is also not uncommon to find waters that cycle between near oxygen saturation conditions during the day and complete anoxia at night (Junk, 1984). Anthropogenic climate disruption is predicted to expand the prevalence of hypoxic zones, possibly expanding global niche space for air-breathing fishes (Keeling *et al.*, 2010).

EVOLUTION OF AIR BREATHING IN FISHES

Graham (2011*a*) reports 400 air-breathing fish species distributed amongst 50 fish families, but these numbers are certainly underestimated due to poorly described tropical GAB families (Graham, 1999). The Neotropical Loricariidae is the most diverse siluriform family and occupies most hypoxia-prone habitats in the Neotropics. Loricariids comprise 83 genera with over 825 nominal species, 709 of which are considered valid (Armbruster, 2006). Although air breathing is not synapomorphic in this family (Armbruster, 1998), most loricariids will facultatively breathe air upon exposure to hypoxia using their stomach as an air-breathing organ (ABO) (Gee, 1976; Graham, 1997) or show morphological evidence of air breathing (Armbruster, 1998). As the loricariids and other speciose GAB families such as the Callichthyidae and Trichomycteridae and potential GAB families such as Scoloplacidae (Armbruster, 1998) are examined in more detail, it is likely that the number of extant air-breathing fish species will exceed one thousand. Of the documented air-breathing species, members of the families listed in Table I have been confirmed to use endodermally derived elements of their gut (*e.g*. oesophagus, stomach or intestine) as an ABO. As the siluriforms listed in Table I are among the poorest described taxonomically and are already listed at 1026 species by the All Catfish Species Inventory (Sabaj *et al.*, 2009) with an estimated 305 newly discovered species yet to be described,

Table I. Families of fishes that have been confirmed to use endodermally derived portions of their gut to extract oxygen from air. The data for species numbers were drawn from the All Catfish Species Inventory (Sabaj *et al.*, 2009) for all siluriform species and from FishBase (Froese & Pauly, 2011) for all other families. Adapted from Nelson & Dehn (2011)

Family	Gut region used for air breathing	Number of species in that family	Estimated number of undescribed species
Umbridae	Oesophagus	5	0
Blenniidae	Oesophagus	420	?
Loricariidae	Stomach	673	205
Trichomycteridae	Stomach	176	55
Cobitidae	Intestine	110	?
Callichthyidae	Intestine	177	45

?, no estimate of undescribed species found.

fishes that use their gut to breathe air could eventually account for the majority of all air-breathing fish species.

The tetrapod lung may have been the original ABO in fishes, possibly appearing as early as in placoderms of the Devonian period (Perry, 2007) and is still in use by extant dipnoans, polypterids and tetrapods. The evolution of an unpaired, dorsal gas bladder connected to the pharynx *via* a pneumatic duct (physostomous condition), not homologous to the tetrapod lung (Perry, 2007), apparently canalized ABO development for some time. Evolution of air breathing early in the actinopterygian radiation exclusively exploited the gas bladder as an ABO (Graham, 1997, 2011*a*). Once the pneumatic duct was lost in some lineages of modern teleosts (physoclistous condition) or the swimbladder became encased in bone (as in the loricarioids; Schaefer & Lauder, 1986), natural selection acted on other body parts to obtain aerial O_2. Thus, a diversity of ABOs evolved in teleosts, including the alimentary tract that is the subject of this review (Graham, 1997, 2011*a*).

GAB fishes fall into two general categories: (1) facultative air breathing (FAB), which refers to those animals that only breathe air when oxygen in the aquatic medium does not meet biological requirements or they are emersed, and (2) continuous air breathing (CAB), which refers to those animals that do not suffocate when denied access to air but still breathe air continuously when allowed access to the surface, even in normoxic water (Graham, 1997; Chapman & McKenzie, 2009). A GAB fish that drowns when denied access to air (obligate air breather) has yet to be described.

EVOLUTION OF GUT AIR-BREATHING ORGANS

Considering the phylogenies of the families in Table I, each evolution of the gut proper as an ABO appears to be a unique evolutionary event (Fig. 1). Armbruster (1998) suggests, based on morphological evidence mapped onto the phylogenies of Schaefer & Lauder (1986) and de Pinna (1993), that there may have been five independent origins of GAB just within the loricariids and scoloplacids. This would bring the total number of independent GAB evolution events to 10. This proclivity for GAB to evolve suggests some selective advantages to using the gut for this purpose.

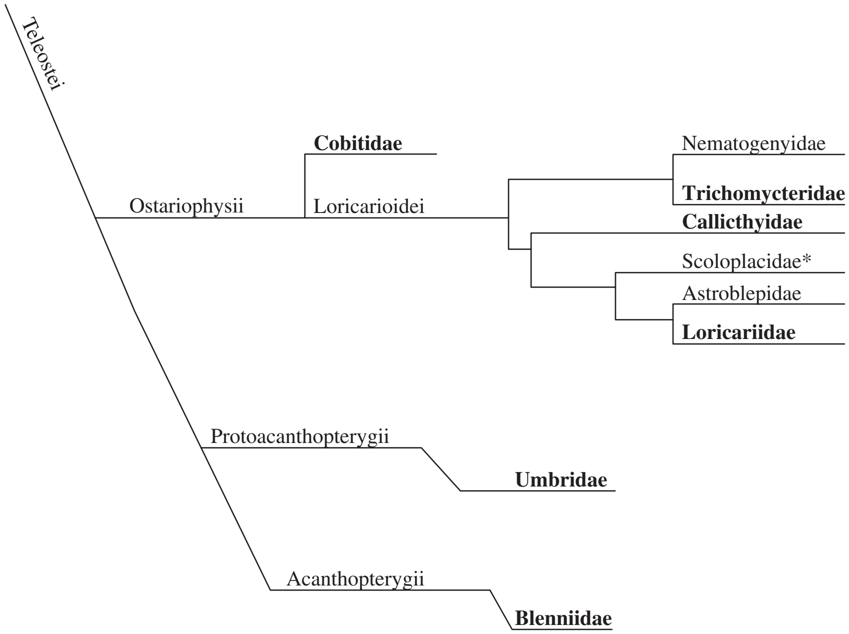

FIG. 1. Phylogeny of known and suspected gut air-breathing (GAB) fish families. Families in bold font have at least one species documented to extract oxygen from the air with their gut. *, Family suspected of breathing air with their gut based on histology. Other families shown have no species that have been demonstrated to breathe air. Ostariophysan relationships were drawn from the All Catfish Species Inventory (Sabaj *et al.*, 2009).

Aside from obtaining access to atmospheric oxygen, which is true of any ABO, the question arises as to why the gut has evolved so frequently into an ABO. One argument could be that the nutrient transport function of the gut could predispose it to gas transport by diffusion. This hypothesis would be more credible if all GAB fishes used their intestines (the site of most nutrient uptake), to breathe air, or if GAB fishes did not require substantial morphological remodelling of their gut to breathe air (see below).

A second hypothesis for the frequent evolution of GAB is that the infrastructure for ventilation was already present to transport food and fill physostomous gas bladders. This same morphology and physiology can be used to fill the buccal cavity and pharyngeal region with air and thus does not explain why some fishes evolved transport of the air to the gut as opposed to only exploiting the head region as an ABO.

As many of the GAB families tend to live in close association with the substratum, buoyancy may hold the key to understanding why GAB has evolved so frequently. Air in the head region would make the head more buoyant than the centre of mass and tend to lift it off the substratum away from benthic food and possibly increase visibility to predators or prey. As Gee (1976) discussed for buoyancy in general, asymmetric head buoyancy could also make it difficult to hold position in lotic waters. Displacing air to the gut would bring it closer to the centre of mass and minimize buoyancy asymmetries. Support for this hypothesis comes from GAB fishes that hold on to air even after the oxygen is depleted (Gee & Graham, 1978; McMahon & Burggren, 1987). Gee (1976) found that in a number of air-breathing loricarioids, the buoyancy attributable to respiratory air in the gut far surpassed buoyancy attributable to air in the gas bladder. Thus, buoyancy attributable to respiration represents a significant component of an animal's buoyancy budget and could be envisioned as a target for selection. Certainly for the loricariids, where many species use an oral sucker to remain inverted under logs or rocks, a gut ABO would seem advantageous to a head region ABO. A head region ABO would presumably require extensive vascularization of both dorsal and ventral buccal or pharyngeal surfaces to accommodate extraction of oxygen in both normal and inverted positions, respectively. Thus, buoyancy and positioning issues may explain the frequent evolution of gut ABOs.

PERCEIVED CHALLENGES IN USING THE GUT AS AN ABO

Among the perceived challenges of GAB are that digestive function could be compromised. Vertebrate digestion is generally an anaerobic process (van Soest, 1994) and many GAB fishes are herbivorous or detritivorous and presumably require an anaerobic gut to facilitate energy extraction from fermentative processes (Clements *et al.*, 2009). Thus, oxygenation of gut regions could compromise anaerobic microbial activity. Additionally, the regions of the gut specialized for respiration are not thought to be involved in either the secretory or the absorptive facets of digestion (Persaud *et al.*, 2006), although there is some evidence for nutrient absorption in the ABO regions of two GAB fishes (Podkowa & Goniakowska-Witalińska, 2002; Gonçalves *et al.*, 2007). Thus, allocating gut regions to respiration may require additional structure or modifications to existing structure to accomplish adequate digestion.

Conversely, food and digestive secretions in the gut could compromise gas exchange by the ABO. The thin epithelium necessary for efficient diffusive gas exchange would seemingly be vulnerable to damage by the acid, alkali, digestive enzymes and dietary items that are normal constituents of the vertebrate gut. In addition, the physical presence of food, protective mucus and digestive juices would tend to increase the diffusive distance for oxygen, potentially limiting oxygen uptake in a gut ABO. Finally, use of the gut as an ABO seemingly places digestion and respiration in competition for available blood (Fig. 2). Generally, full perfusion of the fish gut only occurs post-prandially in water-breathing fishes (Thorarensen & Farrell, 2006). Although ventilation–perfusion matching has not been studied in GAB fishes, presumably perfusion of gut ABOs is maximized when fresh air is present as in other air-breathing fishes (Burggren & Johansen, 1986). This could divert blood from digestive regions of the gut during air breathing, thus prolonging or compromising assimilation of dietary components.

MORPHOLOGICAL INDICATORS OF THE GUT AS AN ABO

Gut ABOs have morphological changes consistent with reduced digestive function and facilitation of gas exchange. Morphological features that are indicative of increased gas exchange include (1) increased vascularization, (2) capillaries

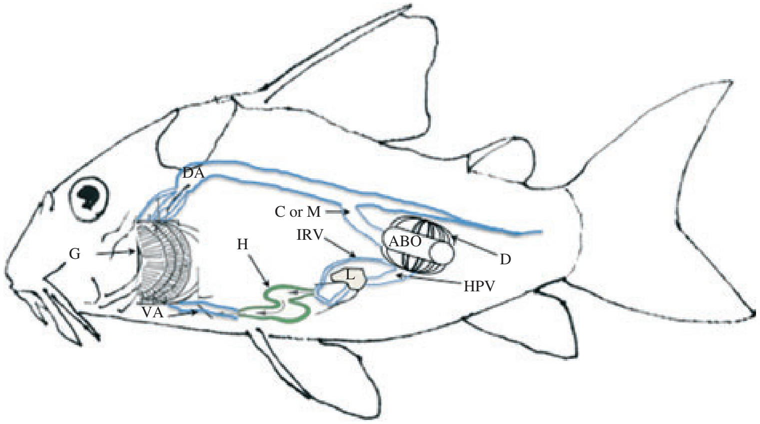

Fig. 2. Circulatory design of gut air-breathing (GAB) fishes. Composite drawing incorporating the major features of the different GAB fishes. Blood supply to the more anterior gut air-breathing organs (ABO) (*e.g. Dallia* and *Ancistrus*) is *via* the coeliac artery, whereas the more posterior intestinal ABOs tend to be supplied by the anterior mesenteric artery (*e.g. Misgurnus*) or directly from the dorsal aorta (*e.g. Callichthys* and *Hoplosternum*). ABO effluent travels back to the heart *via* the hepatic portal vein (Cobitidae) or the interrenal vein (all others). C or M, coeliac or anterior mesenteric artery; D, direct connection to the dorsal aorta through multiple vessels; DA, dorsal aorta; G, gills; H, heart; HPV, hepatic portal vein; IRV, interrenal vein; L, liver; VA, ventral aorta.

embedded in the epithelium with a concomitant reduced blood-lumen distance, (3) increased luminal surface area, (4) presence of surfactant-producing lamellar bodies and (5) presence of muscular sphincters isolating the respiratory region from other gut regions.

OESOPHAGUS BREATHERS

One umbrid (Crawford, 1971, 1974) and one blenniid (Laming *et al.*, 1982; Pelster *et al.*, 1988) are reported to use their oesophagus as a respiratory organ (Table I). The umbrid is the Alaska blackfish *Dallia pectoralis* Bean 1880, which has a highly vascularized swimbladder, but without capillaries that penetrate the inner epithelial lining. In contrast, the oesophagus of *D. pectoralis* is also highly vascularized, but with extensive capillary penetration into the epithelial lining. Crawford's (1974) histological analysis of *D. pectoralis* revealed an oesophageal blood–air barrier of <1 mm, similar to that reported for the ABO gas bladders of the bowfin *Amia calva* L. 1766 and the longnose gar *Lepisosteus osseus* (L. 1758) (Crawford, 1971; Table II). Gas absorption in *D. pectoralis* is probably limited to the oesophagus because of a stricture between the oesophagus and stomach and because there is no epithelial capillary penetration and gastric glands are present in the latter (Crawford, 1974).

Histological observation of the oesophagus of the shanny *Lipophrys pholis* (L.1758), an intertidal species of northern Europe (Laming *et al.*, 1982), revealed extensive vascularization, longitudinal folding and separation from the buccopharynx and stomach by sphincters. Laming *et al.* (1982) did not report a blood–air diffusion distance, but capillaries were reported to be embedded in the oesophageal folds and were described as 'superficial'.

Table II. Selected air–blood diffusion distances and capillary densities in purported respiratory mucosal epithelium of gut air-breathing fishes (modified from Nelson & Dehn, 2011)

Respiratory region of the gut	Family	Species	Air–blood diffusion distance	Capillary density	Source
Oesophagus	Umbridae	*Dallia pectoralis*	<1 µm		Crawford (1971)
Stomach	Loricariidae	*Ancistrus multispinis*	0·6 µm	7/100 µm	Satora (1998)
		Hypostomus plecostomus	0·25–2·02 µm; arithmetic mean ± s.e. = 0·86 ± 0·00 µm	3–4 100 μm^{-1}	Podkowa & Goniakowska-Witalińska (2003)
		Pterygoplichthys anisitsi	Harmonic mean = 0·40–0·74 µm; arithmetic mean ± s.e. = 1·52 ± 0·07 µm		Cruz *et al.* (2009)
Intestine	Callichthyidae	*Hoplosternum thoracatum*	1–2 µm		Huebner & Chee (1978)
		Corydoras aeneus	0·24–3·00 µm		Podkowa & Goniakowska-Witalińska (2002)
	Cobitidae	*Lepidocephalichthys guntea*	0·86–1·08 µm	0·15 μm^{-2}	Yadav & Singh (1980)

STOMACH BREATHERS

Members of the Neotropical catfish families Loricariidae and Trichomycteridae use the stomach as an ABO. Attenuation of gastric activity in favour of oxygen uptake may have been feasible in these groups because of their exceptionally long, coiled intestines (Armbruster, 1998; Nelson *et al.*, 1999, 2007; Delariva & Agostinho, 2001; Podkowa & Goniakowska-Witalińska, 2003) that could potentially compensate for reduced stomach digestive function. Carter & Beadle (1931) first described the ABO function of the loricariid stomach in *Pterygoplichthys ambrosettii* (Holmberg 1893). Subsequently, stomach air breathing has been documented in multiple genera of loricariids (*e.g. Hypostomus*, *Liposarcus*, *Pterygoplichthys* and *Panaque*) (Carter & Beadle, 1931; Carter, 1935; Satora, 1998; Souza & Intelizano, 2007; Oliveira *et al.*, 2001; Podkowa & Goniakowska-Witalińska, 2003; Nelson *et al.*, 2007; Cruz *et al.*, 2009) and in trichomycterids (Cala, 1987). Armbruster (1998) conducted a survey of gut gross morphology in loricarioids, in which he reports a range of modifications of the stomach for air breathing, ranging from mere enlargement of the stomach to varying degrees of stomach vascularization to air-filled diverticula branching off the stomach. Reports of capillaries embedded within the stomach mucosa include the genera *Ancistrus* (Satora, 1998; Satora & Winnicki, 2000), *Liposarcus* (Oliveira *et al.*, 2001), *Hypostomus* (Podkowa & Goniakowska-Witalińska, 2003), *Pterygoplichthys* (Cruz *et al.*, 2009), *Ancistrus*, *Hypostomus*, *Peckoltia*, *Pterygoplichthys* and *Megaloancistrus* (Souza & Intelizano, 2007). Diffusional distances between the stomach lumen and these capillaries are consistent with respiratory function (Table II).

Regional localization of respiratory function in some stomach ABOs is apparent from the distribution of digestive glands. Both *Ancistrus* (Satora, 1998) and *Pterygoplichthys* (Cruz *et al.*, 2009) have gastric glands, but with lower density in the corpus compared to the cardiac and pyloric regions. In *Ancistrus*, capillaries were only embedded in the mucosal epithelia in the corpus (Satora, 1998), further targeting the corpus as the site of O_2 uptake. Cruz *et al.* (2009) report that *Pterygoplichthys* has also reduced gastric gland density in the corpus region, coincident with a high degree of longitudinal folding of the mucosa. Other authors report the complete absence of digestive glands in the ABO stomach of loricariids (Carter, 1935; Oliveira *et al.*, 2001). Surfactant-producing lamellar bodies have also been documented in the stomach epithelial cells and gastric glands of *Ancistrus* (Satora & Winnicki, 2000), *Hypostomus* (Podkowa & Goniakowska-Witalińska, 2003) and *Pterygoplichthys* (Cruz *et al.*, 2009).

INTESTINAL BREATHERS

The respiratory intestines of cobitids and callichthyids are generally described as thin walled, translucent and highly vascularized. In cobitids, this is approximately the distal two-thirds of the intestine (Yadav & Singh, 1980; McMahon & Burggren, 1987; Moitra *et al.*, 1989), whereas in callichthyids approximately the posterior half of the intestine is involved in gas exchange (Huebner & Chee, 1978; Kramer & McClure, 1980; Podkowa & Goniakowska-Witalińska, 2002). Capillary penetration into the intestinal mucosa is reported from *Misgurnus* (Jasinski, 1973), *Hoplosternum* (Huebner & Chee, 1978) and *Corydoras* (Podkowa & Goniakowska-Witalińska, 2002; Table II). Surfactant-producing lamellar bodies in the respiratory intestinal epithelia have been described in *Misgurnus* (Jasinski, 1973) and *Corydoras* (Podkowa & Goniakowska-Witalińska, 2002). The amphibious Chilean clingfish *Sicyases sanguineus* Müller & Troschel 1843 (Gobiesocidae) has also been reported to breathe air with their intestines (Graham, 2011*b*), but there is no histological or physiological confirmation of this.

CIRCULATORY MODIFICATIONS ASSOCIATED WITH GUT ABO EVOLUTION

Capillaries of the gut would normally occupy the lamina propria, basolateral to the gut epithelium. This position apparently produced insufficient rates of diffusive oxygen uptake as gut ABOs evolved, so a general histological finding across all gut ABOs is that selection has favoured capillaries positioned in the epithelial layer, but not directly abutting the luminal surface of the gut. Table II presents estimated diffusional distances for gut ABOs from a number of histological studies. The ranges reported fall within those of other fish non-gut ABOs and are in the range of mammalian lung diffusive distances (Weibel, 1984). Capillary densities of gut ABOs are typical for fish ABOs in general (Podkowa & Goniakowska-Witalińska, 2002, 2003; Cruz *et al.*, 2009). Cruz *et al.* (2009) found the diffusing capacity of the *Pterygoplichthys* (Loricariidae) stomach ABO to be higher than for most other fish ABOs, but lower than published values for the lungs of the South American lungfish *Lepidosiren paradoxa* Fitzinger 1837 and the marbled lungfish *Protopterus aethiopicus* Heckel 1851 and substantially below estimates for lungs of same-sized mammals (Weibel, 1984).

The potential for air-breathing fishes to lose oxygen to hypoxic waters across their gills has been reviewed extensively (Graham, 1997, 2011*c*). The general circulatory plan of fishes with gut air-breathing organs (GABO) is shown in Fig. 2. Because oxygenated venous blood emanating from the ABO next encounters capillaries in the gills (or the liver and then the gills; Cobitidae), oxygen could diffuse to the water across the lamellar epithelium if the water oxygen partial pressure ($P\text{O}_2$) is below that of the blood. Thus, shunting of the blood away from lamellae during air breathing and aquatic hypoxia should be favoured by natural selection. Histological evidence for the possibility of shunting has been presented for *Hypostomus* (Fernandes & Perna, 1995), *Dallia* (Crawford, 1971) and *Lepidocephalus* (Cobitidae) (Yadav & Singh, 1980). Blood chemistry studies showing CO_2 retention in *Hypostomus* during air breathing (Wood *et al.*, 1979) are indicative of shunting, but there has yet to be a direct physiological demonstration of blood being shunted away from the gills of a GAB fish while breathing air.

Venous return from the ABO is either *via* the hepatic portal circulation (Cobitidae) or the systemic circulation (all others) (Carter & Beadle, 1931; Graham, 1997; Fig. 2). The systemic return is probably the more efficient of the two because bypassing the capillary beds of the liver will avoid a pressure drop in the freshly oxygenated blood and will facilitate a more global distribution of oxygen (Fig. 2). Systemic venous return would also expose the ABO effluent to more direct suction from the heart during cardiac relaxation (Olson, 1994).

Arterial blood takes different paths to the ABO depending on the location. Although all GABOs are supplied with blood from branches of the dorsal aorta (Fig. 2), the more anterior gut ABOs tend to be supplied by branches of the coeliac artery, *e.g. Dallia* (Crawford, 1971) and *Pterygoplichthys* (Carter & Beadle, 1931), whereas the more posterior intestinal ABOs tend to be supplied by the anterior mesenteric artery, *e.g. Misgurnus* (McMahon & Burggren, 1987), or directly from the dorsal aorta, *e.g. Callichthys* and *Hoplosternum* (Callichthyidae) (Carter & Beadle, 1931).

PHYSIOLOGY OF GAB

ABO VENTILATION

There appears to be no systematic studies on the mechanics of GABO inflation. Inspiration in GABO fishes is generally described as gulping, presumably a negative buccal cavity pressure derived from buccal and perhaps opercular cavity expansion as found in other air-breathing fishes (Liem, 1989). The air is then presumably driven posteriorly with positive pressure from buccal cavity contraction with closed oral and opercular valves, as detailed for lungs and gas bladder ABOs (Liem, 1989). Less is also known about expiration and air transport through the gut in GAB fishes, but the stomach and oesophageal breathers exhale by eructation, whereas the intestinal breathers exhale *via* flatulence (Graham, 2011*b*). Crawford (1971) suggests that oesophageal skeletal musculature may be involved in expelling gas from the *Dallia* oesophagus; however, there is no confirmation. Gradwell (1971) discusses three possible mechanisms of exhalation in *Plecostomus*: (1) hydrostatic pressure in conjunction with elastic recoil and appropriate valving, (2) contraction of visceral smooth muscle of the respiratory stomach and (3) positive abdominal cavity pressure developed through contraction of the rectus abdominis skeletal muscle, but there was no experimental differentiation amongst these alternatives.

Fishes with intestinal ABOs (Callichthyidae and Cobitidae) transport air the entire length of the gut and exhale through their anus (Gee & Graham, 1978; McMahon & Burggren, 1987). While this unidirectional transport of air minimizes respiratory dead space and probably improves the efficiency of gas exchange, it raises a new problem of co-ordinating transport of air and digesta. Both these families have a transitional zone between the digestive and respiratory portions of their intestine (McMahon & Burggren, 1987; Persaud *et al.*, 2006) that is richly endowed with smooth muscle and appears to function in compacting the digesta and possibly encasing it in mucus. This compacted digesta would then minimally interfere with gas exchange in the respiratory (posterior) segment of the intestine. Persaud *et al.* (2006) present evidence in callichthyids that air transport is necessary for normal digesta transport and that the amount of visceral smooth muscle in the respiratory portion of the intestine is insufficient for peristaltic transport of air. Thus, Persaud *et al.* (2006) propose that the positive pressure generated by the buccal and opercular pump provides the force for inspiration, air transport, digesta transport across the respiratory zone of the intestine and expiration in callichthyids.

AQUATIC GILL VENTILATION WHILE AIR BREATHING

The general pattern of branchial ventilation in fish exposed to hypoxia is to defend arterial PO_2 (oxygen regulation) by increasing gill ventilation (Holeton, 1980; Fig. 3). This is manifested by either increases in ventilatory frequency (f_V; Affonso & Rantin, 2005) or tidal volume V_T (Nelson *et al.*, 2007) with the latter more common (Mattias *et al.*, 1998; Fig. 3). As air-breathing fishes start using atmospheric oxygen, energy conservation would dictate that gill ventilation should diminish or stop. Fishes expend around 10% of their resting metabolic rate in ventilating their gills (Holeton, 1980; Glass & Rantin, 2009), and although air-breathing fishes generally still use their gills or skin to eliminate CO_2 (Johansen, 1970), the potential loss of O_2 to the water across the gill lamellae would seem to make aquatic ventilation in hypoxic water a poor physiological strategy. Indeed, fishes from one of the GAB taxa (*Lipophrys*) have been reported to cease branchial ventilation upon leaving the water (Laming *et al.*, 1982), although Pelster *et al.* (1988) claim that ventilation continues during emersion. The situation is also unclear in other GAB fishes. Some investigators have reported cessation of branchial ventilation in loricariids (Gradwell, 1971; Wood *et al.*, 1979), but these were visual observations and appear to have been erroneous. Graham (1983) reports a 20% drop in gill f_V in air-breathing *Ancistrus* when acclimated to hypoxic water, but Gee & Graham (1978) report a 33% increase in *Hoplosternum* gill f_V when induced to increase its frequency of air breathing through aquatic hypoxia and Affonso & Rantin (2005) report a 31% increase in hypoxic *Hoplosternum* gill f_V breathing air. Finally, both foregut breathers, *e.g.* *Hypostomus* (Nelson *et al.*, 2007), and hindgut breathers, *e.g.* *Misgurnus* (McMahon & Burggren, 1987), have been reported to not change their rate of aquatic ventilation when air breathing is induced with aquatic hypoxia. V_T has not been measured in GAB fishes while air breathing but the conclusion based on f_V measurements alone is that reduced gill ventilation while air breathing is not a general strategy GAB fishes have employed to either save energy or reduce O_2 loss to hypoxic water.

GAS EXCHANGE AND METABOLIC RATE IN GAB FISHES

Attempts to characterize the gas composition of GABOs date to the 19th century (Jobert, 1877), yet there is a surprisingly small amount of information on gas compositions and changes with time in GABOs. A general finding is that CO_2 is not released *via* the ABO. This observation holds for both foregut breathers, *e.g. Pterygoplichthys* (Carter & Beadle, 1931; Graham, 1983), and hindgut breathers, *e.g. Misgurnus* (McMahon & Burggren, 1987). The high capacitance of water for CO_2 (Schmidt-Nielsen, 1997) means that CO_2 will readily diffuse across gill epithelia or permeable skin in fishes submerged in low carbon dioxide partial pressure ($P\mathrm{CO}_2$) water. Blennies are the only GAB fishes that are classified as amphibious and *L. pholis* has had its plasma $P\mathrm{CO}_2$ measured when emersed (Pelster *et al.* 1988). They report a 53% rise (from 2·43 to 3·71 torr) in plasma $P\mathrm{CO}_2$ in emersed *L. pholis* but did not report ABO $P\mathrm{CO}_2$. Based on this limited physiology, GABOs do not appear to be significant organs of CO_2 excretion.

Almost without exception, GAB fishes exposed to hypoxia do not become hypometabolic when allowed access to air and instead retain oxygen regulation and normal levels of activity (Fig. 3). Rates of air breathing are thus complex functions of the factors that contribute to metabolic rate as well as ecological and behavioural factors. Metabolic rate in fishes depends on size, species and multiple additional factors such as social situation, stress level, diet, temperature and water chemistry (Nelson & Chabot, 2011). As this information is only sporadically available for GAB fishes, it is not possible to generalize ABO ventilation rates, respiratory partitioning (the fraction of aerial *v.* aquatic respiration) or rates of oxygen uptake from the ABO across GAB fishes. Graham's (1983) study of *Ancistrus* at 25° C and McMahon & Burggren's (1987) study of *Misgurnus* at 20° C give the most detailed accountings of changes in ABO gas composition over time.

OESOPHAGEAL ABO GAS EXCHANGE

Crawford (1971) showed that three individual *D. pectoralis* maintained normal metabolic rates below an $[O_2]$ of $2\,\mathrm{mg\,l^{-1}}$ by supplementing aquatic respiration with air breathing. Likewise, metabolic rates of *L. pholis* stayed relatively constant through cycles of immersion and emersion and skin oxygen consumption was minor, suggesting that the GABO is able to maintain normal resting rates of oxygen consumption in emergent members of this species (Laming *et al.*, 1982).

STOMACH ABO GAS EXCHANGE

Cala *et al.* (1990) report that the trichomycterid *Eremophilus* must increase its air-breathing frequency to survive aquatic hypoxia, but there have been no studies of actual gas exchange in this family. Loricariids follow the general FAB pattern (Fig. 3): as environmental $[O_2]$ drops, metabolic rate is regulated with aquatic ventilation until a species- and environment-specific oxygen partial pressure of the water ($P\mathrm{O}_2$) between 25 and 60 torr is reached (Graham & Baird, 1982; Graham, 1983;

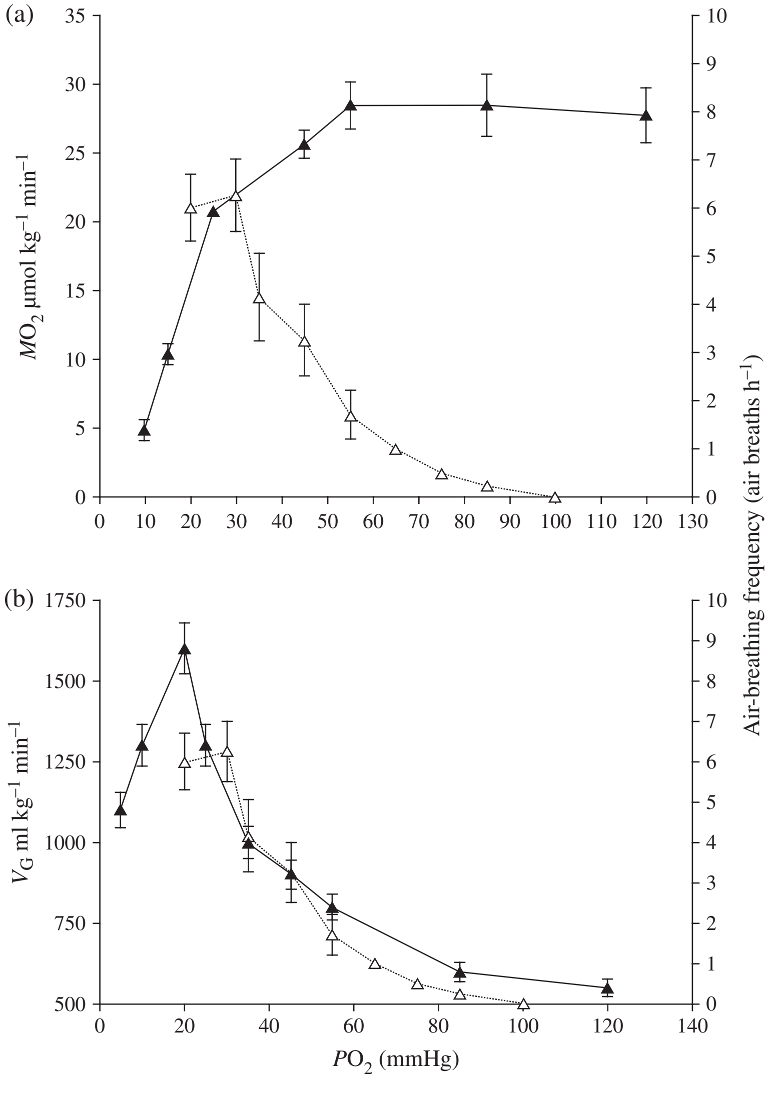

FIG. 3. (a) Air-breathing frequency (△) of *Hypostomus regani* ($n = 50$) exposed to progressive hypoxia (measured as oxygen partial pressure, $P\text{O}_2$) and allowed free access to the surface and aquatic mass-specific oxygen consumption ($M\text{O}_2$; ▲) of *H. regani* ($n = 17$) exposed to progressive hypoxia but denied access to the surface. Values are mean ± S.E. (b) Gill ventilation volume (V_G; ▲) of *H. regani* exposed to progressive hypoxia over a period of 9 h and denied access to the surface compared with the air-breathing frequency data (△) from (a). Values are mean ± S.E. Both from Mattias *et al.* (1998) and Nelson *et al.* (2007).

Nelson *et al.*, 2007). Below this $P\text{O}_2$, animals supplement aquatic oxygen uptake with stomach air breathing (Gee & Graham, 1978; Graham & Baird, 1982; Graham, 1983; Mattias *et al.*, 1998; Nelson *et al.*, 2007). Graham (1983) measured a progressive decline in [O_2] of ABO gas with breath hold length in *Ancistrus*. Oxygen uptake from the ABO can also be inferred from the subatmospheric gut oxygen levels reported by Carter & Beadle (1931) for *Pterygoplichthys* and Nelson *et al.* (2007) for *Hypostomus*. *Ancistrus* met increased metabolic demands in hypoxic water by increasing (f_V; air breaths (V_T) remained constant throughout changes in demand (Graham, 1983). Interestingly, *Ancistrus* acclimated to hypoxia by expanding the size of its ABO by 25% and increasing the efficiency of ABO oxygen extraction (Graham, 1983). Most authors report no air breathing from loricariids in normoxic water; however, MacCormack *et al.* (2006) report surfacing (presumably air breathing) behaviour that was independent of environmental [O_2] in telemetered *Glyptoperichthys*.

INTESTINAL ABO GAS EXCHANGE

Based on the available information, both the callichthyids and cobitids appear to be continuous, but not obligate air breathers (Gee & Graham, 1978; Kramer & McClure, 1980; McMahon & Burggren, 1987), although Wu & Chang (1945) claim that *Misgurnus* abandons CAB at low temperature. This implies that some other aspect of air ingestion such as buoyancy (Gee, 1976), digesta transport (Persaud *et al.*, 2006) or enhanced scope for activity (Gee & Graham, 1978; Almeida-Val & Farias, 1996) improves fitness (or has done so in the past). As representatives from both families follow the FAB pattern of responding to aquatic hypoxia with increased ventilation of their ABO to regulate metabolic rate (Gee & Graham, 1978; McMahon & Burggren, 1987), it is parsimonious to argue that these other factors were secondary to aquatic hypoxia in driving the evolution of intestinal respiration. McMahon & Burggren (1987) found that when *Misgurnus* was confronted with aquatic hypoxia, it increased the rate of intestinal ventilation entirely through increases in f_V. Changes in aerial V_T were not involved, similar to Graham's (1983) findings for stomach-breathing *Ancistrus*. Three species of callichthyids also increase f_V with progressive aquatic hypoxia (Gee & Graham, 1978; Kramer & McClure, 1980; Affonso & Rantin, 2005). McMahon & Burggren (1987) estimated a *c*. 70% turnover of ABO with each subsequent breath in *Misgurnus* intestines. Interestingly, they report no correlation between gas composition of the ABO and breath interval durations ranging from 5 min to1 h, suggesting that the available oxygen is extracted from the air rapidly with very little subsequent gas exchange. Interestingly, Jucá-Chagas (2004) reports that the intestinal breathing *Hoplosternum* can extract more oxygen per unit body mass from an air breath than either a gas bladder breathing erythrinid (*Hoplerythrinus*) or the lungfish *L. paradoxa*.

VENTILATORY DRIVE IN GUT AIR BREATHERS

Gee & Graham (1978) and McMahon & Burggren (1987) showed through manipulations of the aquatic and aerial medium that intestinal air breathers do not sense the chemical composition of the gas in the ABO to set ABO ventilation, although ABO volume may play a role (Gee & Graham, 1978). The main factors that appear to set the rate of aerial ventilation in GAB fishes are PO_2 and metabolic rate (Kramer & McClure, 1980; Graham & Baird, 1982; McMahon & Burggren, 1987). McMahon & Burggren (1987) report a modest sensitivity of *Misgurnus* (Cobitidae) f_V to water PCO_2 as do Graham & Baird (1982) for *Ancistrus* and *Hypostomus*, but the primary drive to ventilation is chemosensitivity to PO_2 as evidenced by the inverse relation of f_v with PO_2 in all species measured. This accords well with the finding that ABO ventilation can be controlled by external O_2 chemoreceptors on the gills for the more studied gas bladder breathing fishes *Lepisosteus* (Smatresk, 1986), *Amia* (McKenzie *et al.*, 1991) and *Hoplerythrinus* (Oliveira *et al.*, 2004). Graham & Baird (1982) reported no change in the threshold PO_2 for air breathing to commence even after hypoxia acclimation had produced improvements in the size and extraction efficiency of the ABO. This result is also suggestive of the chemosensation of environmental PO_2 eliciting this response. In contrast, Brauner *et al.* (1995) demonstrated an inverse relation between f_v and pH and a direct relationship between f_v and water hydrogen sulphide (HS^{-1}) concentration in *Hoplosternum*. These authors suggested that these latter two variables (indicative of water anoxia) may be as important as PO_2 in setting rates of ABO ventilation in nature.

Rates of aerial ventilation in GAB fishes are also sensitive to metabolic demand, either deduced from differences in size or activity or experimentally manipulated by changes in temperature. McMahon & Burggren (1987) reported a linear increase in ABO ventilation between 10 and 30° C (Q_{10} *c*. 2 for 10–20° C and Q_{10} *c*. 1·5 for 20–30° C) for *Misgurnus* and Graham & Baird (1982) reported a steady increase in f_v between 20 and 30° C (Q_{10} *c*. 1·4) for *Ancistrus*. Sloman *et al.* (2009) found that smaller *Hoplosternum* with a higher mass-specific metabolic rate had a higher PO_2 air-breathing threshold with progressive hypoxia than larger animals when held in isolation. This relationship disappeared in groups, casting doubt as to its field relevance, but still suggestive of the idea that metabolic demand may help drive ABO ventilation. Conversely, Mattias *et al.* (1998) found no relationship between body mass and air-breathing threshold in 50 *Hypostomus regani* (Ihering 1905) over a 600 g size range nor did Perna & Fernandes (1996) for *Hypostomus* over an 83 g size range. Activity also appears to correlate positively with air-breathing frequency in CAB callichthyids (Gee & Graham, 1978; Kramer & McClure, 1980).

CARDIOVASCULAR RESPONSE TO GAB

Although there is a rich literature on cardiovascular responses to air breathing in fishes, most of this literature relates to dipnoans and several other large (mostly gas bladder ABO) species (Graham, 1997, 2011*c*); there is very less cardiac, and no vascular, information on GAB fishes breathing air. GAB fishes generally exhibit an almost immediate reflex bradycardia when exposed to hypoxia as shown for *Hypostomus* by Nelson *et al.* (2007) (Fig. 4). This is presumably the same generalized, although not universal, vagally mediated reflex hypoxic bradycardia seen in water-breathing fishes (Taylor, 1992). This bradycardia appears to have a metabolic component, as Nelson *et al.* (2007) report a more profound hypoxic bradycardia at 30° C than at either 20 or 25° C in *Hypostomus*. A significant bradycardia without metabolic rate changes (Nelson *et al.*, 2007) suggests that the falling heart rate (f_H) may be compensated for with increases in stroke volume similar to water-breathing fishes (Stecyk & Farrell, 2002).

The precept of ventilation–perfusion matching dictates that inflation of the ABO by air-breathing fishes in hypoxic water will produce an increase in cardiac output coincident with diversion of blood to the ABO (Johansen, 1970). This is probably why some air-breathing fishes manifest an immediate post-breath tachycardia (Graham, 1997; although pharmacological blockade of post-breath tachycardia had no

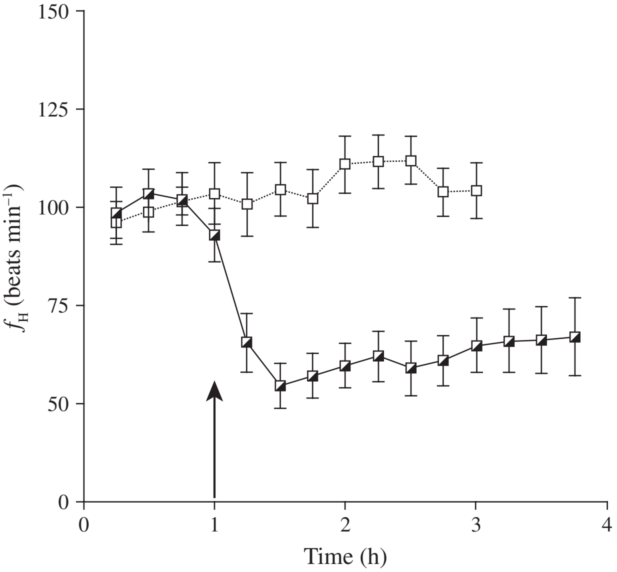

FIG. 4. Heart rate (f_H) in *Hypostomus regani* exposed to hypoxia (oxygen partial pressure, $PO_2 = 20$ mmHg for 3 h; $n = 12$; ◪) or kept normoxic ($PO_2 = 155$ mmHg for 3 h; $n = 9$; □). Values are mean ± S.E. →, initiation of hypoxia. Mean heart rate was significantly lower in the hypoxia-exposed animals throughout the exposure period (MANOVA, $P < 0.001$), although individual animals would briefly elevate their heart rate back to control levels or even higher after surfacing to breathe air.

effect on oxygen uptake in a gas bladder breathing fish; McKenzie *et al.*, 2007). The only pre and post-breath heart rates published for GAB fishes are for loricariids. Nelson *et al.* (2007) report an average 34% increase in heart rate immediately post-breath in *Hypostomus*, similar to the 32% air-breath tachycardia reported by Graham (1983) for *Ancistrus*.

BLOOD CHEMISTRY OF GAB FISHES

There is an extensive literature on the blood chemistry of GAB fishes, often collected at one time of the year from a single location (Powers *et al.*, 1979). These types of measurements have been the basis for speculation about the action of natural selection on haemoglobin, but as Graham (1997) points out, without standardized collection conditions or methodology for analysing haemoglobin, these adaptive scenarios are entirely speculative. Graham (1997) provides an extensive review of the blood chemistry of all air-breathing fishes, for which, much of the information comes from GAB fishes.

From an oxygen transport perspective, there are two major issues for the blood of a GAB fish. First, there is the problem of venous admixture with the (presumably saturated) blood leaving the ABO (Fig. 2) and, second, the potential loss of O_2 to hypoxic water at the gills. A GAB fish that emerges into air has the additional challenge of blood CO_2 accumulation. The haemoglobins of GAB can be generally characterized as having a moderate to low blood oxygen partial pressure that half saturates haemoglobin ($p50$) with coincident low erythrocyte nucleoside triphosphate concentration [NTP] (Powers *et al.*, 1979; Graham, 1997; Marcon *et al.*, 1999). As many of the GAB fishes acclimate to hypoxia by increasing blood oxygen affinity at least partially through reductions in erythrocyte [NTP] (Graham, 1983, 1985; Wilhelm & Weber, 1983; Val *et al.*, 1990), these published values will be dependent on the animals' environmental history prior to blood collection. The high blood oxygen affinity makes sense considering the general occupancy of hypoxic aquatic habitats by these fishes. On the other hand, the evolutionary prediction for air breathing is a right shift of haemoglobin–oxygen affinity to facilitate oxygen delivery as oxygen is now readily available in the ABO. Riggs (1979) summarized an extensive data set of Amazonian fishes and concluded that there was no evidence for right shifting in air-breathing fishes, although Morris & Bridges (1994) expanded that data set and provided some modest evidence for right shifting of haemoglobin–oxygen affinity across all air-breathing fishes.

The blood of GAB fishes can be further generalized as having a moderate to strong Bohr effect and no Root effect (Focesi *et al.*, 1979; Powers *et al.*, 1979; Bridges *et al.*, 1984). The Bohr effect appears maladaptive, at least in one genus, *Liposarcus*, that does not tightly regulate its plasma pH (Brauner *et al.*, 2004). When *Liposarcus* are acidotic, the Bohr effect would exacerbate the desaturation of haemoglobin in ABO effluent and increase the potential loss of O_2 to hypoxic water during gill transit (Fig. 2). The general absence of a Root effect in the GAB fishes is consistent with their general reduction in swimbladder function and the lack of a choroid rete (Gee, 1976; Schaefer & Lauder, 1986; Berenbrink, 2011).

The blood of GAB fishes can also be characterized, with the exception of *Lipophrys* (Bridges *et al.*, 1984), as having a high volume of erythrocytes (haematocrit), with a coincident high blood haemoglobin concentration (Graham, 1997). These values are also subject to acclimation in some loricariids (Graham, 1983, 1985; Val *et al.*, 1990), although Graham (1985) reports no acclimation of these factors in one callichthyid (*Hoplosternum*) and one loricariid (*Loricaria*). *Hypostomus* exposed to 3 h of hypoxia or 8 h of graded hypoxia had significantly higher [Hb] and smaller erythrocytes that contained more haemoglobin per erythrocyte than normoxic animals (Fernandes *et al.*, 1999; Nelson *et al.*, 2007). This result is most likely due to the hypoxic animals releasing immature erythrocytes from the spleen to enhance oxygen transport. Val *et al.* (1990) also reported a higher cell haemoglobin concentration in another loricariid exposed to hypoxia for 30 days or captured from hypoxia-prone habitats. Weber *et al.* (1979), however, reported cell swelling and decreased cell haemoglobin concentrations in loricariids exposed to hypoxia for 4–7 days, suggesting that there may not be a generalized loricariid or GAB blood chemistry response to hypoxia. Although GAB fishes have multiple haemoglobin genes, there is presently no evidence that air-breathing fishes adjust the relative expression of haemoglobin isoforms in response to hypoxia or air breathing (Almeida-Val *et al.*, 1999).

BEHAVIOURAL ECOLOGY OF GAB

Environmental oxygen availability should be a potent determinant of the ecology and behaviour of GAB fishes (Kramer, 1987). Predator–prey dynamics are undoubtedly a function of environmental [O_2] whether a fish is a water, air or bimodal breather (Domenici *et al.*, 2007). The increased aquatic ventilation required in hypoxic habitats will increase an animal's energy requirements and potentially its concealment from both potential predators and prey. Certainly, the diminished scope for activity under hypoxia (Chabot & Claireaux, 2008) could compromise predator–prey performance; however, the diminished feeding activity and scope for growth in hypoxic water (Chabot & Claireaux, 2008) may be just as important on longer time scales.

The evolution of air breathing solved some of these hypoxia issues for GAB fishes but offered new challenges (Kramer, 1987). Air breathing can expose animals to new predation regimes. In a direct test of this hypothesis, Kramer *et al.* (1983) showed that air-breathing fishes (one GAB fish) were more vulnerable to predation from a striated heron *Butorides striatus* when forced to breathe air than water-breathing fishes under similar conditions. Kramer & McClure (1980) suggested that hypoxic GAB fishes are less likely to surface the deeper they are, and Power (1984) provides evidence from the field that GAB loricariids will avoid shallow waters where they are vulnerable to avian predation despite the presence of abundant food. Several species of GAB fishes also engage in synchronized air-breathing behaviour (Kramer & Graham, 1976; Gee & Graham, 1978; Sloman *et al.*, 2009). These results suggest that evolution of GAB may have necessitated concomitant evolution of anti-predator behaviours to compensate for the increased visibility of surfacing. Indeed, many GAB fishes have adopted a nocturnal lifestyle where they are less likely to be visible to visual predators. Boujard *et al.* (1990) demonstrate nocturnal maxima of activity, feeding and air breathing in *Hoplosternum* that very quickly tracked experimental changes to the timing of the daylight cycle. Likewise, MacCormack *et al.* (2006) report only nocturnal surfacing (presumably air breathing) behaviour in the loricariid (*Glyptoperichthys*).

Considering the potential interactions between digestive and respiratory function when the gut is used for both, there is surprisingly little information on the partitioning between feeding activity and aerial respiration in GAB fishes. Persaud *et al.* (2006) found that two callichthyids would stop eating when denied access to air even in normoxic water and propose that air breathing is essential for digesta transport. Nelson *et al.* (2007) found no difference between fed and starved *Hypostomus* in their propensity to breathe air under hypoxia. Kramer & Braun (1983) report that air-breathing frequency after feeding in *Corydoras* (Callichthyidae) is variably dependent on PO_2. Above 50% saturation, there was a decrease in air-breathing frequency after feeding, whereas at a PO_2 of 44 torr there was no change and at a PO_2 of 24 torr there was an increase in air-breathing frequency (Kramer & Braun, 1983). Certainly, the understanding of how GAB fishes balance the digestive and respiratory functions of their guts is in its infancy.

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