Control of air-breathing in fishes: Central and peripheral receptors

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ABSTRACT

This review considers the environmental and systemic factors that can stimulate air-breathing responses in fishes with bimodal respiration, and how these may be controlled by peripheral and central chemoreceptors. The systemic factors that stimulate air-breathing in fishes are usually related to conditions that increase the O2 demand of these animals (e.g. physical exercise, digestion and increased temperature), while the environmental factors are usually related to conditions that impair their capacity to meet this demand (e.g. aquatic/aerial hypoxia, aquatic/aerial hypercarbia, reduced aquatic hydrogenic potential and environmental pollution). It is now well-established that peripheral chemoreceptors, innervated by cranial nerves, drive increased air-breathing in response to environmental hypoxia and/or hypercarbia. These receptors are, in general, sensitive to O2 and/or CO2/H+ levels in the blood and/or the environment. Increased air-breathing in response to elevated O2 demand may also be driven by the peripheral chemoreceptors that monitor O2 levels in the blood. Very little is known about central chemoreception in air-breathing fishes. The data suggest that central chemosensitivity to CO2/H+ is more prominent in sarcopterygians than in actinopterygians. A great deal remains to be understood about control of air-breathing in fishes, in particular to what extent control systems may show commonalities (or not) among species or groups that have evolved air-breathing independently, and how information from the multiple peripheral (and possibly central) chemoreceptors is integrated to control the balance of aerial and aquatic respiration in these animals.

1. Introduction

An adequate supply of O2 to meet metabolic demands is essential for life of aerobic organisms; any impairment can compromise performance and even be fatal, depending upon the physiological adaptations of the species concerned. Water has a low capacitance for O2, containing only a few milligrams per litre, and has low diffusion constants for dissolved gases, such that hypoxia can be a common condition in aquatic environments (Richards et al., 2009). Therefore, organisms that breathe water can often be challenged to meet their demands for O2 (Carter, 1931; Rahn, 1966; Driedzic and Hochachka, 1978; Kramer and Mehegan, 1981; Kramer and McClure, 1982; Randall, 1982; Kramer, 1987; Díaz and Breitburg, 2009).

Air is much richer in oxygen and fishes were the first vertebrates to evolve adaptations to breathe air, the fossil record dates to the late Silurian, ~ 420 million years ago. This predates the conquest of land by the ancestors of tetrapods, which evolved from lobe-finned fishes (Sarcopterygii) that resembled the extant Dipnoi (Panchen, 1980; Little, 1983, 1990; Gordon and Olson, 1994; Long, 1995; Shultze and Trueb, 1991; Amemiya et al., 2013). Aquatic hypoxia or periodic emersion (caused by tidal oscillations or by unfavorable environmental conditions), are considered the two main circumstances that exerted evolutionary pressure for selection of aerial respiration in fishes (Inger, 1957; Johansen, 1976; Graham et al., 1978; Randall et al., 1981; Horn and Gibson, 1988; Sayer and Davenport, 1991; Graham, 1997). However, it is important to emphasize that these were probably not the only selective pressures involved in the evolution of air-breathing, because it is a very diverse adaptation found in over 40 families, such that it seems to have evolved independently on numerous occasions (Randall et al., 1981; Graham, 1997; Hsia et al., 2013). All air-breathing fishes are
Aquatic surface respiration (ASR) is a common adaptation of unimodal water-breathing fishes, that apparently helps to maintain O2 uptake and aerobic metabolism during aquatic hypoxia. It consists of rising to the surface to ventilate the gills with the uppermost layer of water in contact with air, where there is constant diffusion of gases from the atmosphere (Kramer and Mehegan, 1981; Kramer and McClure, 1982). This adaptation is a reflex triggered by low O2 availability and is believed to have led towards the evolution of true air-breathing in fishes (Shingles et al., 2005; Florindo et al., 2006; Chapman and McKenzie, 2009; Richards, 2011). It is postulated that the ASR reflex may have favored the selection of air-breathing based on the following hypotheses: (1) ASR allowed individuals to survive in waters under severe hypoxia; (2) some individuals inadvertently came into contact with the air during ASR (e.g. by inhaling it), which provided conditions for O2 uptake from the air across vascularized epithelia, making it possible to select them as air-breathing organs (ABO) (Burggren, 1982; Gee and Gee, 1995); and (3) ASR exposes the animals to predation, so individuals with the capacity to gulp and hold air could protect themselves from predators by temporarily returning to the depths, hence further favoring the evolution of adaptations for true aerial respiration (Kramer et al., 1983; Smith and Kramer, 1986; Gee and Gee, 1995; Shingles et al., 2005; Chapman and McKenzie, 2009).

Despite being a phylogenetically ancient specialization, aerial respiration retains great importance in the natural history of many extant fish species, especially in tropical freshwaters that can become hypoxic due to reduced O2 capacitance at high temperatures (Rahn, 1966; Kramer et al., 1978; Graham, 1997; Diaz and Breitburg, 2009; Pörtner and Lannig, 2009), a condition that may be intensified by climate global change (Lehtonen, 1996; Diaz, 2001; Diaz and Breitburg, 2009). In addition to its importance in O2-poor environments, air-breathing behavior in fishes can also support O2 to support aerobic activities such as exercise or digestion, although this has received less research attention than responses to hypoxia (Johansen, 1970; Gee and Graham, 1978; Stevens and Holton, 1978; Dejours, 1994; Brauner et al., 1995; Graham, 1997; Seymour et al., 2004, 2007; McKenzie et al., 2012; Lefevre et al., 2014; Blasco et al., 2017).

Fishes possess specialized chemosensitive cells, located in the central and/or peripheral nervous system, capable of monitoring the partial pressure of O2 (P02) or CO2 (Pco2), as well as the hydrogenionic potential (pH), in the blood and/or the environment (internally and/or externally oriented chemoreceptors) (see Hará, 1992; Milsom, 2012 for reviews). These chemoreceptors modulate a wide range of behavioral and physiological adjustments that favor the survival of fishes during situations of hypoxia/hypoxaemia (i.e. reduced levels of O2 in the environment or in the body, respectively) and hypercapnia/hypercapnia (i.e. increased levels of CO2 in the environment or in the body, respectively), and air-breathing behavior is a prime example (Smatresk et al., 1986; McKenzie et al., 1991a; Milsom et al., 2002; Florindo et al., 2004, 2006; Boijink et al., 2010; Lopes et al., 2010; Milsom, 2012; Zeraik et al., 2013; Beloé et al., 2015). There are differences in the location, distribution and orientation of these chemoreceptors among species, and their functions may vary depending on the specificity of these cells – such characteristics of fish chemoreceptors and their respective physiological influences have been previously reviewed by Gilmore (2001), Perry and Gilmore (2002), Gilmore and Perry (2006), Milsom (2012), Perry and Abdallah (2012), Porteus et al. (2012) and Zachar and Jonz (2012).

The number of studies on the role of chemoreceptors in controlling air-breathing in fishes is still modest, especially regarding central chemoreceptors. However, peripheral chemoreceptors appear to be more involved in air-breathing modulation than central chemoreceptors, as in many species studied to date the disruption of afferent nervous pathways attenuates or abolishes this behavior in response to aquatic hypoxia or hypercapnia (Hedrick et al., 1991; McKenzie et al., 1991a; Boijink et al., 2010; Lopes et al., 2010; Beloé et al., 2015). Thus, given this overall context, this review evaluates current knowledge about systemic and environmental factors that stimulate air-breathing in fishes, and the role of chemoreceptors in the reflex control of this behavior.

2. Stimulators of reflex air-breathing responses

Several factors stimulate air-breathing behaviors in fishes, which can be classified as either “systemic” or “environmental” (Fig. 1). Systemic factors are states of the organism that increase O2 demand for aerobic metabolism, in particular warming, exercise and digestion, which then stimulate overall ventilatory activity. Environmental factors, on the other hand, are external conditions that increase the animals’ O2 demand; challenge respiratory gas exchange, and/or damage the fragile epithelium of the gill lamellae. This includes warming, hypoxia, hypercapnia, pH and pollutants. In species that are obligate air-breathers such factors will alter the intensity of aerial respiration whereas, in facultative air-breathers, such factors can trigger the air-breathing behavior as well as modulate its intensity.

2.1. Systemic factors

The O2 demands of fishes increase considerably during physical exercise, and this leads to an increase in air-breathing frequency in all species studied to date (Smatresk, 1988; Lefevre et al., 2014). Although this response would be expected in obligate air-breathers, it also occurs in all facultative species that have been studied, namely Amia calva,
Clarias gariepinus, Gymnotus carapo, Hoplosternum littorale, Lepisosteus oculatus, Megalops cyprinoides, Neoceratodus forsteri, and Pangasianodon hypophthalmus (Grigg, 1965; Gee and Graham, 1978; Farmer and Jackson, 1998; Seymour et al., 2004, 2007; McKenzie et al., 2012; Lefevre et al., 2013; Blasco et al., 2017). Food consumption also causes a transient increase in metabolic rate and O₂ demand, which can be extremely pronounced in some ectotherms (Secor, 2009; McCue, 2006). There is evidence that obligate air-breathing fishes increase aerial respiration when feeding in normoxic water, but this phenomenon is also expected to occur in facultative air-breathing fishes (Pandian and Vivekanandan, 1976; Lefevre et al., 2012).

An increase in temperature is a combined systemic/environmental factor that stimulates air-breathing behavior in fishes because it increases the demand for O₂ in the tissues and requires increased O₂ delivery (Johansen et al., 1976; Rahn et al., 1971; Horn and Riggs, 1973; Gee, 1980; Graham and Baird, 1982; Glass et al., 1986; Johnston and Dunn, 1987; McMahon and Burggren, 1987; Smatresk, 1988; Clarke and Johnston, 1999; Geiger et al., 2000; Silva et al., 2017). Indeed, warming also lowers O₂ solubility in water, which reduces aquatic PₐO₂ and additionally contributes to stimulate air-breathing behavior (Rahn, 1966; Johansen et al., 1970; Graham and Baird, 1982; McMahon and Burggren, 1987). Nevertheless, Gee (1980), Glass et al. (1986) and Geiger et al. (2000) performed experiments in which water temperature was manipulated without affecting its PₐO₂ levels (normoxia preserved by bubbling pure O₂ in the water when necessary), and observed an increase in air-breathing frequency associated with temperature elevation in Channa argus, Megalops atlanticus and Umbra limi – demonstrating that the stimulatory influence of temperature on this behavior is not a mere consequence of aquatic hypoxia.

The mechanisms by which air-breathing is stimulated by increased O₂ demand are not fully understood (Lefevre et al., 2014, 2016). The increase in air-breathing frequency could supplement aquatic gas exchange, because many bimodal breathing fishes have reduced gill surface areas, with lower O₂ extraction capacity than unimodal water-breathing species (Eduardo et al., 1979; Lomholt and Johansen, 1979; Graham, 1983; Rantin et al., 1992; Fernandes and Rantin, 1994; Fernandes et al., 1994; Graham, 1997; Oliveira et al., 2004; Belão et al., 2011). This argument cannot, however, explain why some bimodal breathing fishes can sustain similar aerobic scopes by aquatic respiration alone, if they are denied access to air during forced exercise (McKenzie et al., 2012; Lefevre et al., 2014, 2016).

The response may in fact be an inescapable chemoreflex, driven by internal chemoreceptors that monitor blood O₂ levels (Lefevre et al., 2014; McKenzie et al., 2016). It has been suggested that the spontaneous aperiodic episodes of air-breathing that are observed in many facultative air-breathing fishes in normoxia, when external O₂ chemoreceptors should be quiescent, are driven by the internal chemoreceptors: as blood O₂ levels fall after an air-breath, this eventually stimulates another breath (Shelton et al., 1986; Smatresk, 1988). There is some indirect evidence for this ‘internal’ respiratory drive because, in the spiny catfish C. gariepinus, individuals with higher intrinsic metabolic rates and O₂ demands spontaneously breathed more air in normoxia (McKenzie et al., 2016). When fishes exercise at increasing intensity, this progressively depletes venous blood of O₂ (Stevens and Randall, 1967; Farrell and Clutterham, 2003). In bimodal breathing fishes, this venous hypoxaemia may cause inescapable chemoreflexive stimulation of air-breathing, that increases with intensity as they exercise harder (McKenzie et al., 2012; Lefevre et al., 2014). This is an area that deserves further investigation.

2.2. Environmental factors

Fishes with facultative aerial respiration (both Actinopterygii and Sarcopterygii) typically shift from gill ventilation to air-breathing as O₂ levels in water fall. This transition has been described in numerous species, namely A. calva, Anabas testudineus, Ancistrus chagresi, C. gariepinus, Erpetoichthys calabaricus, Heteropneustes fossilis, Hoploerythrinus unitaeniatus, H. littorale, Hypostomus plecostomus, Hypostomus regani, L. oculatus, Lepisosteus osseus, M. atlanticus, M. cyprinoides, N. forsteri, P. hypophthalmus, Rhinelepis strigosa and Synbranchus marmoratus. The response is based on a chemoreflex, capable of providing sufficient O₂ to maintain aerobic metabolism during aquatic hypoxia, and the transition from water to air-breathing can be elicited either by external or internal [if environmental hypoxia induces hypoxaemia] O₂ chemoreceptors (Hughes and Singh, 1970; Johansen et al., 1970; Hughes and Singh, 1971; Gee and Graham, 1978; Graham and Baird, 1982; Smatresk and Cameron, 1982; Graham and Baird, 1984; Pettit and Beitinger, 1985; Smatresk, 1986; Fritsche et al., 1993; Takasu, 1994; Brauner et al., 1995; Mattias et al., 1998; Geiger et al., 2000; Kind et al., 2002; Seymour et al., 2004; Affonso and Rantin, 2005; McKenzie et al., 2007; Lopes et al., 2010; Belão et al., 2011; Lefevre et al., 2011; Belião et al., 2015; Thomsen et al., 2017). It is interesting that, regarding obligate air-breathing fishes, this response varies among actinopterygians (e.g. Electrophorus electricus exhibits no change in air-breathing frequency during aquatic hypoxia while Trichogaster trichopterus exhibits an increase) (Burggren, 1979; Johansen et al., 1968) but not among sarcopterygians (aquatic hypoxia does not change air-breathing frequency in Lepidostrodon paradoxa, Protopterus aethiopicus and Protopterus dolloi) (Johansen and Lenfant, 1968; Sanchez et al., 2001a; Perry et al., 2005a). It is conceivable that obligate sarcopterygians do not possess externally oriented O₂ chemoreceptors in the gills (Lahir et al., 1970; Perry et al., 2005a; Silva et al., 2017). However, the actinopterygian and sarcopterygian species just cited, as well as the obligate air-breathing teleosts C. argus and Monopterus cuchia, show an increase in air-breathing frequency when exposed to aerial hypoxia, which may have been triggered by hypoxaemia through internally oriented chemoreceptors or by external chemoreceptors that monitor the PₐO₂ of air in the ABO (Johansen et al., 1968; Johansen and Lenfant, 1968; Burggren and Johansen, 1974; Burggren, 1979; Glass et al., 1986; Sanchez et al., 2001a; Zacco et al., 2003, 2006; Perry et al., 2005a; Silva et al., 2011, 2017). Nonetheless, the facultative sarcopterygian N. forsteri did not exhibit this response after the injection of nitrogen into the lung (Johansen et al., 1967).

Aquatic hypercapnia can increase the frequency of aerial respiration in several species of fish with obligatory or facultative air-breathing, such as A. chagresi, E. calabaricus, H. unitaeniatus, H. plecostomus, L. paradoxa, N. forsteri, P. aethiopicus, P. dolloi, S. marmoratus and T. trichopterus (Johansen, 1966; Johansen et al., 1967; Johansen and Lenfant, 1968; Burggren and Johansen, 1979; Smatresk, 1986; Glass et al., 1986; Sanchez et al., 2001a; Zacco et al., 2003, 2006; Perry et al., 2005a; Silva et al., 2011, 2017). However, hypercapnia decreases or even reverses the CO₂ concentration gradient between water and blood, leading to an increase in plasma PₐCO₂. The accumulation of CO₂ can then produce a respiratory acidosis that hinders blood O₂ transport through Bohr and Root effects, potentially challenging O₂ supply (Perry and Kinkead, 1989; Randall et al., 2014). In bimodal breathers, the accumulation of CO₂ may be alleviated by direct elimination of this gas to the atmosphere (Lefevre et al., 2016). This could explain why air-breathing reflexes can be triggered by internal and/or external chemoreceptors sensitive to CO₂/H⁺ (Bojink et al., 2010; Milsom, 2012). Nonetheless, in A. calva, infusions of NH₄HCO₃ into the dorsal aorta caused large increases in plasma PₐCO₂ and stimulated gill ventilation, but had no effect on air-breathing, indicating that aerial respiration is not stimulated by internal CO₂ in this species (McKenzie et al., 1991b).
Table 1
Systemic and environmental factors and its effects on air-breathing behavior in various species of fish.

<table>
<thead>
<tr>
<th>Species</th>
<th>Physical Exercise</th>
<th>Digestion</th>
<th>Increased Temperature</th>
<th>Aquatic Hypoxia</th>
<th>Aerial Hypoxia</th>
<th>Aquatic Hypercarbia</th>
<th>Aerial Hypercarbia</th>
<th>Reduced Environmental / Blood pH</th>
<th>Contaminants (H₂S)</th>
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Note: (A) Actinopterygii; (S) Sarcopterygii; (H₂S) Hydrogen sulfide; (+) Stimulation; (-) Inhibition; (nc) No change; (blank) No data available.

References: 1 Smith (1930); 2 Grigg (1965); 3 Johansen (1966); 4 Jesse et al. (1967); 5 Johansen et al. (1967); 6 Johansen and Lenfant (1968); 7 Johansen et al. (1968); 8 Hughes and Singh (1970); 9 Johansen et al. (1970); 10 Hughes and Singh (1971); 11 Rahn et al. (1971); 12 Horn and Riggs (1973); 13 Lombolt and Johansen (1974); 14 Pandian and Vivekanandan (1976); 15 Gee and Graham (1978); 16 Babiker (1979); 17 Burggren (1979); 18 Gee (1980); 19 Graham and Baird (1982); 20 Smatresk and Cameron (1982); 21 Graham and Baird (1984); 22 Petit and Beiting (1985); 23 Glass et al. (1986); 24 Smatresk (1986); 25 McMahon and Burggren (1987); 26 McKenzie et al. (1991); 27 Pittsche et al. (1993); 28 Takasusuki (1994); 29 Brauner et al. (1995); 30 Farmer and Jackson (1996); 31 Mattias et al. (1996); 32 Geiger et al. (2000); 33 Sanchez and Glass (2001); 34 Sanchez et al. (2001); 35 Kind et al. (2002); 36 Seymor et al. (2004); 37 Afonso and Rantin (2005); 38 Sanchez et al. (2005); 39 Perry et al. (2005); 40 McKenzie et al. (2007); 41 Seymour et al. (2007); 42 Perry et al. (2008); 43 Bojilka et al. (2010); 44 Lopes et al. (2010); 45 Belio et al. (2011); 46 Lefevre et al. (2011); 47 Silva et al. (2011); 48 Lefevre et al. (2012); 49 McKenzie et al. (2012); 50 Lefevre et al. (2013); 51 Belio et al. (2015); 52 Blasco et al. (2017); 53 Silva et al. (2017); 54 Thomsen et al. (2017).
Several studies demonstrated that aerial hypercarbia can also increase air-breathing frequency in some air-breathing fishes, such as C. gutiaripinus, E. electricus, L. paradoxa, P. aethiopicus, Protoperus annectens and T. trichopterus (Smith, 1930; Johansen et al., 1968; Delaney et al., 1974, 1976; Delaney et al., 1977; Babiker, 1979; Burggren, 1979; Smatresk, 1988; Sanchez et al., 2005), while other studies showed that it can lead to a decrease (P. aethiopicus and P. dolloi) (Jesse et al., 1967), or even no changes (in L. oculatus, L. paradoxa and P. dolloi) (Smatresk and Cameron, 1982; Sanchez and Glass, 2001; Perry et al., 2008). Although many of these air-breathing responses may reveal direct chemosensitivity to CO2, some of them may be an indirect effect of reductions in blood O2 levels consequent to a respiratory acidosis. Also, the divergent responses may be explained by interspecific differences in the existence, orientation and function of O2 and CO2/H+ chemoreceptors, or by differences in CO2 diffusivity at the gills and ABO of these species. The divergent results may also reflect differences in the CO2 concentrations to which the animals were submitted, or even interspecific variation in the thresholds for CO2 concentrations in water or air that stimulate air-breathing.

The pH of water influences several physiological processes in fishes, including respiratory gas exchange and the excretion of nitrogenous wastes (Willie and Wood, 1991, 1996; Saha et al., 2002). Water pH is inversely proportional to water Pco2, and a reduction in environmental pH may lead to a respiratory acidosis that compromises O2 uptake in these animals (Perry and Kinkead, 1989; Perry et al., 1989; Lin and Randall, 1990; Willie and Wood, 1996). Therefore, to avoid this effect, fishes with aerial respiration show an increase in the frequency of this behavior when in contact with water with reduced pH (Brauner et al., 1995). In A. calvu, infusions of HCl into the dorsal aorta caused significant declines in blood pH and O2 content and elicited air-breathing responses. When, however, the animals were held in hypoxic water, the infusions only caused a decline in pH and there were no air-breathing responses. This was taken to indicate that there was no direct sensitivity of aerial respiration to plasma pH in that species (McKenzie et al., 1991b). Due to the intrinsic relationship between pH and CO2 concentration in water, it can also be difficult to separate responses to pH from those to Pco2. Brauner et al. (1995) found, however, that an increase in air-breathing frequency in H. littorale was a direct consequence of elevated water acidity and not of higher aquatic Pco2. Furthermore, despite the possible influence of pH and CO2 on affinity of hemoglobin for O2, the changes in respiratory patterns in H. littorale were probably not mediated by internal O2 chemoreceptors but by internal or external CO2/H+ chemoreceptors, because this species exhibited practically no Root effect (Willmer, 1934; Brauner et al., 1995).

Finally, another environmental factor that can stimulate air-breathing in fishes is aquatic pollution. Contaminants in aquatic environments can be of anthropic origin or even natural, such as the hydrogen sulfide (H2S) that is mainly produced by bacterial sulfate reduction in sediments and anaerobic decomposition of organic matter (Jorgensen, 1984). These compounds can stimulate aerial respiration in fishes for a variety of reasons. Hydrogen sulfide, for example, can reduce the affinity of hemoglobin for O2 and impair the electron transport chain reaction by binding to cytochrome-c oxidase (Bagarinio and Vetter, 1989, 1992; Volkel and Berenbrink, 2000; Affonzo et al., 2002, 2004), which may lead to both hypoxiaemia and an impaired ability to produce ATP, that in turn triggers an increase in air-breathing frequency (Brauner et al., 1995; Affonzo and Rantin, 2005). An alternative explanation would be that these animals use air-breathing as a strategy to partially and temporarily uncouple themselves from contaminated water (Brauner et al., 1995). Other pollutants, on the other hand, may irritate the gill epithelium of the animals, inducing changes in gill morphology (such as an increase in the number of interlamellar cells, cell hyperplasia and a greater production of mucus) that inhibits both xenobiotics absorption and branchial gas exchange (Hayton and Barron, 1990; Laurén, 1991; Laurent and Perry, 1991; Alazemi et al., 1996; Biagini et al., 2009) – a situation that may increase the requirement for air breathing. The influence of all environmental and systemic factors on air-breathing behavior in facultative and obligate air-breathing fishes are summarized in Table 1.

3. Peripheral receptors mediating air-breathing responses

According to Milsom (2012), the location of peripheral chemoreceptors (gills, orobranchial cavity or elsewhere) and their orientation (external water or internal blood) are highly variable among fishes, whether unimodal or bimodal breathers. Their response modality (O2 or CO2) and the reflex cardiorespiratory responses they engender (changes in gill ventilation rate or amplitude, heart rate, systemic vascular resistance, ASR or air-breathing) are also highly variable among species. Considering unimodal and bimodal breathers, there is a trend whereby the receptors involved in triggering changes in heart rate and gill ventilation rate in response to hypoxia and hypercarbia are preferentially located in the gills, whereas those that produce increases in gill ventilation amplitude are more extensive, often also being found in extrabranchial locations. Also, the distribution of CO2-sensitive chemoreceptors in the gills tends to be more restricted than O2-sensitive chemoreceptors, and the location of the CO2 receptors may differ from the O2 receptors.

Milsom (2012) proposed that most unimodal water breathers primarily increase respiratory amplitude during hypoxia. Bimodal breathing species may, however, reduce gill ventilation in aquatic hypoxia while they increase reliance on air-breathing (see Hughes and Shelton, 1962; Shelton et al., 1986; Perry et al., 2009 for reviews).

3.1. O2 chemoreceptors

During the dry season in tropical regions, fishes can be confined for weeks or even months to hypoxic and hypercarbic water. Aerial respiration is a common adaptation in fish species in these ecosystems (Dedarahi and Tripathi, 1976; Kramer et al. 1978; Glass et al., 1986; Graham, 1997). The ecological success of these fishes will depend on, amongst other things, their ability to sense O2 in the environment and rapidly engage the metabolic, cardiovascular and ventilatory adjustments that match O2 supply to their demand (Fritsche and Nilsson, 1993). This, in turn depends on the central interaction of a variety of sensory inputs, including inputs from chemoreceptors that monitor external (water) and internal (blood) gas tensions and acid-base balance (Milsom, 1997; Perry and Gilmour, 2002).

The primary sites of peripheral O2 sensing in fish appear to be the gills (including the pseudobranch in those species that possesses one) and orobranchial cavity (Laurent and Roygueu, 1972; Randall and Jones, 1973; Butler et al., 1977; Daxboeck and Holeton, 1978; Smith and Davie, 1984; Smatresk et al., 1986; Burleson and Smatresk, 1990; McKenzie et al., 1991a; Burleson and Milsom, 1993; Sundin et al., 1999, 2000; Milsom et al., 2002). Chemoreceptors in the orobranchial cavity are innervated by branches of the Vth (trigeminal) and/ or VIIth (facial) cranial nerves, those on the pseudobranch by branches of the VIIth and/or IXth (glossopharyngeal) cranial nerves, and those on the gill arches by branches of the IXth and/or Xth (vagus) cranial nerves (Butler et al., 1977; Burleson et al., 1992; Milsom et al., 2002; Reid et al., 2005). Some of these chemoreceptors respond only, or preferentially to changes in external (water) O2, others respond only, or preferentially, to changes in internal (blood) O2 and some respond to both (Milsom and Brill, 1986; Burleson and Milsom, 1993).

Highly sensitive mechanisms to monitor O2 and acid-base balance are important for the survival of all vertebrate species. In fishes, histological and neurophysiological evidence indicates that this requirement is primarily filled by endoderm-derived neuroepithelial cells (NECs) in the gills (Porteus et al., 2012; Zachar and Jonz, 2012; Hockman et al., 2017), however, other types of cells (which have not yet been characterized, but are neural crest-derived) may also be involved in this function (Hockman et al., 2017). So, as the predominant
putative $O_2/CO_2/H^+$ chemoreceptors in fishes, NECs are mainly located on the gill filaments and secondary lamellae of all branchial arches and are innervated by afferent fibers of the central nervous system (Bailly et al., 2009; Porteus et al., 2012). Also, these cells may contain several neurotransmitters in their vesicles such as serotonin, acetylcholine, catecholamines, nitric oxide, hydrogen sulfide, leu-5-enkephalin, met-5-enkephalin and neuropeptide Y (Zaccone et al., 1992; Burleson et al., 2002; Zaccone et al., 2003; Jonz and Nurse, 2003; Jonz et al., 2004; Coolidge et al., 2008; Porteus et al., 2015; Zaccone et al., 2017) – although serotonin-containing NECs located along the gill filaments are the most abundant and are the only type that has been found in all fishes studied to date (Bailly et al., 2009; Porteus et al., 2012).

NECs are involved in local and central control of branchial functions through the paracrine production of serotonin and their synaptic relationships with the sympathetic and intrinsic branchial nervous systems (Bailly et al., 2009; Zaccone et al., 2017). In cell culture, some NECs respond immediately, and in a dose-dependent manner, to changes in local PO2. Thus, the hypoxic stimulation of cell NECs seems to initiate the adaptive cardiorespiratory reflexes, which allows for O2 uptake and delivery to meet metabolic demands (Zachar and Jonz, 2012). Different responses can be triggered by distinct types of NECs, and it is not yet clear whether interspecific differences in the location of the NECs or in the responses triggered by each type of NECs might be attributable to differences in lifestyle (active versus sluggish fish), habitat (hypoxia tolerant versus intolerant fish) or phylogeny (Milsom et al., 1999; Perry and Gilmour, 2002; Reid et al., 2005; Coolidge et al., 2008).

There is considerable amount of data concerning the O2 chemoreceptors that drive gill versus air-breathing reflexes in bimodal breathing fishes, or the cardiovascular adjustments that accompany these. The most commonly observed adjustments to hypoxia/hypoxaemia is a decrease in gill ventilation once air-breathing is initiated (Johansen et al., 1970; Singh, 1976; Randall et al., 1981; Smatresk and Cameron, 1982; Smatresk, 1986; Shelton et al., 1986; McKenzie et al., 1991a; Graham, 1997). Also, a hypoxic bradycardia, which is a hallmark in most fishes, gives way to a tachycardia following each air breath, often accompanied by increases in cardiac output and perfusion of the air-breathing organ (Johansen, 1966; Johansen et al., 1968; Singh and Hughes, 1973; Jordan, 1976; Axelsson et al., 1988; Skaals et al., 2006; McKenzie et al., 2007; Nelson et al., 2007; Lopes et al., 2010; Belão et al., 2011; Iversen et al., 2011; Teixeira et al., 2015).

Sodium cyanide (NaCN) injections, which mimic hypoxic/hypoxaemia, have been used to elucidate the relative roles of externally versus internally O2-sensing chemoreceptors in such responses. In L. osseus, external NaCN stimulate air-breathing but not gill ventilation rate and gill ventilation amplitude (Smatresk, 1986; Smatresk et al., 1986). In A. calva, external NaCN stimulates air-breathing, gill ventilation rate and gill ventilation amplitude – although the stimulation of air-breathing occurs only when internal O2 chemoreceptors are costimulated (McKenzie et al., 1991a). Similarly, in L. osseus, when blood PO2 levels are low, stimulation of external O2 chemoreceptors increases air-breathing events even further (Smatresk et al., 1986; Smatresk, 1988). Internal injections of NaCN stimulate air-breathing, gill ventilation rate and gill ventilation amplitude in L. osseus (Smatresk, 1986; Smatresk et al., 1986), but display no effect on air-breathing in A. calva (McKenzie et al., 1991a). In no instance did stimulation of either group of O2 receptors in this manner produce a decrease in gill ventilation in these animals. In L. osseus, however, when internal PO2 levels were low, simultaneous stimulation of external O2 chemoreceptors eliminated the increase in gill ventilation (Smatresk et al., 1986; Smatresk, 1988). Complete branchial denervation (gills and pseudobranch) eliminates the cardiorespiratory responses to hypoxia/hypoxaemia in L. osseus and A. calva (Smatresk, 1988, 1989; McKenzie et al., 1991a).

In a study performed by Belão et al. (2015) in C. gariepinus, both external and internal NaCN injections revealed that O2 chemoreceptors mediating hypoxic gill ventilatory responses (gill ventilation rate and amplitude) are internally oriented, while O2 chemoreceptors mediating cardiovascular responses (heart rate) are both externally and internally oriented. Moreover, external and internal NaCN injections in fish that underwent a bilateral excision of the first gill arch, indicated that the gill ventilation rate responses were receptor-mediated predominantly in the first pair of gill arches, but the gill ventilation amplitude and heart rate responses were mediated by receptors located in all the gill arches (Belão et al., 2015). Still in C. gariepinus, air-breathing responses were predominantly mediated by external and internal O2 receptors in the first pair of branchial arches (Belão et al., 2015). In the facultative air-breathing fish H. unitaeniatus, the O2 receptors involved in eliciting cardiorespiratory responses to hypoxia are present in all gill arches (Lopes et al., 2010). In this species, there is evidence that externally and internally oriented O2 chemoreceptors mediate gill ventilation and cardiovascular responses, while internally oriented O2 chemoreceptors triggers air-breathing responses – however, as in A. calva, the stimulation of externally oriented O2 chemoreceptors induces air-breathing only when internally oriented O2 chemoreceptors are stimulated together (Lopes et al., 2010). Smatresk et al. (1986) hypothesized that the internally oriented O2 chemoreceptors set the level of hypoxic drive and are the main stimulators of air-breathing, but that input from externally oriented O2 chemoreceptors may modulate the threshold for this behavioral response, and most actinopterygians studied to date seem to corroborate this hypothesis.

Regarding sarcopterygians, there is one report that external nicotine injections stimulate air-breathing in the African lungfish (P. aethiopicus) (Johansen and Lenfant, 1968), but such kind of external stimuli has more often failed to trigger this behavior in dipnoans (Johansen and Lenfant, 1968; Sanchez et al., 2001a; Perry et al., 2005a). On the other hand, internal NaCN injections triggered air-breathing responses in P. aethiopicus (Lahiri et al., 1970), as well as exposure to aerial hypoxia did in L. paradoxus, P. aethiopicus and P. dolloi (Johansen and Lenfant, 1968; Sanchez et al., 2001a; Perry et al., 2005a; Silva et al., 2011, 2017). As pulmonary NECs were already found in lungfish (Zaccone et al., 1989, 1997; Kemp et al., 2003), it is possible that the exposure to aerial hypoxia stimulated air-breathing in these animals via external O2 chemoreceptors in the lungs rather than internal O2 chemoreceptors – however, at least in the case of P. aethiopicus, such air-breathing response is eliminated by complete gill denervation (Lahiri et al., 1970). The location, orientation and innervation of peripheral O2-sensitive chemoreceptors involved in the control of gill ventilation rate, gill ventilation amplitude and air-breathing in fishes with bimodal respiration are summarized in Tables 2–4, respectively.

### 3.2. $CO_2/H^+$ chemoreceptors

Acute exposure of fish to aquatic hypercapnia typically elicits significant increases in gill ventilatory amplitude and/or gill ventilation rate, resulting in an increase in total gill ventilation (Janssen and Randall, 1975; Thomas and LeRuz, 1982; Smith and Jones, 1982; Reid et al., 2000; Burleson and Smatresk, 2000; Perry and McKendry, 2001; McKendry and Perry, 2001; McKendry et al., 2001; Gilmour, 2001; Milsom et al., 2002; Perry and Reid, 2002). This is usually accompanied by a decrease in heart rate (Kent and Peirce, 1978; Perry et al., 1999; Sundin et al., 2000; Reid et al., 2000; Crocker et al., 2000; McKendry and Perry, 2001; McKendry et al., 2001; Perry and Reid, 2002) and increase in systemic vascular resistance (Perry et al., 1999; McKendry and Perry, 2001). There is strong evidence that these responses arise from the stimulation of specific CO2/H+$^+$ chemosensitive NECs and are not dependent on changes in water or blood O2 concentration (Butler and Taylor, 1971; Reid et al., 2000; Sundin et al., 2000; Heisler et al., 1988; Graham et al., 1990; Kinkead and Perry, 1991; Milsom, 1995a,b; Perry and Gilmour, 1996; Gilmour, 2001; Burleson and Smatresk, 2000; Milsom, 2012).

At present, the cardiorespiratory responses to $CO_2/H^+$ in fish are
believed to arise primarily from receptors distributed throughout the gill arches innervated by the IXth and Xth cranial nerves. It is clear that these receptors in the gills monitor the CO₂ in the water, but it is not clear whether they also respond to changes in the CO₂ of arterial blood. There is evidence to suggest both that they do (Janssen and Randall, 1975; Wood and Perry, 1985; Perry and Wood, 1989; Aota et al., 1990; Wood and Mugner, 1994; Gilmour and Perry, 1996) and do not (Mckenzie et al., 1991b; Perry et al., 1999; Reid et al., 2000; McKenzie et al., 2001; Perry and McKendry, 2001; McKenzie and Perry, 2002; Gilmour et al., 2005). Data suggest that the receptors responding to external stimuli respond specifically to changes in CO₂ in the water (not pH) (Neville, 1979; Thomas and Le Ruz, 1982; Sundin et al., 2000; Reid et al., 2000), while to the extent that there is evidence for ventilatory responses arising from receptors responding to internal stimuli, there is a better correlation between the changes in ventilation and changes in arterial pH rather than arterial CO₂ (Heisler et al., 1988; Graham et al., 1990; Wood et al., 1990; McKenzie et al., 1991b; Wood and Mugner, 1994).

Mckenzie et al. (1991a,b) observed that dorsal aortic infusions of HCl elicit air-breathing reflexes in A. calva, however, it is possible that internal H⁺-sensitive receptors are not involved in the control of this response because air-breathing was only triggered when blood O₂ content decreased below normoxic levels along with blood pH in these animals. The data of Boijink et al. (2010) indicate that the chemoreceptors eliciting gill ventilatory responses to hypercarbia in H. unitaeniatus are exclusively branchial (located primarily on the first gill arch), externally oriented, and respond specifically to changes in CO₂ and not H⁺. In this species, high levels of aquatic pCO₂ depressed gill ventilation and stimulated air-breathing. The chemoreceptors involved in stimulating air-breathing in response to hypercarbia also appeared to be branchial, distributed across all gill arches and responded specifically to changes in aquatic pCO₂. This would suggest that chemoreceptor groups with different orientations – external water versus internal blood – are involved in eliciting air-breathing responses to hypercarbia and hypoxia (respectively) in H. unitaeniatus. With respect to sarcopterygians, Amin-Naves et al. (2007a) demonstrated that L. paradoxa presents peripheral CO₂/H⁺ chemoreceptors capable of modulating pulmonary ventilation, but did not report data on the location, orientation and innervation of these receptors.

As previously commented, aquatic hypercarbia induces an increase in gill ventilation in most air-breathing fishes (Johansen and Lenfant, 1968; Perry et al., 2008; Boijink et al., 2010). Yet, in some species (such as H. unitaeniatus), if the increases in aquatic pCO₂ are large enough, they can inhibit gill ventilation and stimulate air-breathing (Johansen et al., 1967; Graham and Baird, 1982; Graham, 1997; Sanchez and Glass, 2001; Sanchez et al., 2005; Boijink et al., 2010). In other species, however, increases in aquatic pCO₂ are without effect on gill ventilation (Johansen, 1966; Todd, 1972; McMahon and Burggren, 1987) or air-breathing (Johansen et al., 1968; Lombolt and Johansen, 1974). Just as with aquatic pCO₂, bimodal breathing fishes exhibit a wide range of air-breathing responses to increasing levels of pCO₂ in inspired air, such as an increase (Smith, 1936; Johansen et al., 1968; Delaney et al., 1974, 1976; Delaney et al., 1977; Babiker, 1979; Burggren, 1979; Smatresk, 1988; Sanchez et al., 2005), a decrease (Jesse et al., 1967), or no change (Smatresk and Cameron, 1982; Sanchez and Glass, 2001; Perry et al., 2008).

The equivocal nature of the data raises questions about the existence and role of peripheral internally oriented CO₂/H⁺ sensitive chemoreceptors in driving gill ventilation or air-breathing in facultative and obligate air-breathing fishes. Clearly much remains to be investigated to resolve this issue. To date, this has not been deeply investigated in any air-breathing species and it will be intriguing to study the extent to which similar trends appear in air-breathing primitive actinopterygians and sarcopterygians. To provide an overview, the location, orientation and innervation of peripheral CO₂/H⁺ sensitive chemoreceptors involved in the control of gill ventilation rate, gill ventilation amplitude and air-breathing in fishes with bimodal respirations are summarized in Tables 2–4, respectively.

### Table 2
Location, orientation and innervation of peripheral O₂- and CO₂/pH-sensitive chemoreceptors involved in gill ventilation rate responses.

<table>
<thead>
<tr>
<th>Species</th>
<th>Receptor Location</th>
<th>Orientation</th>
<th>Innervation</th>
<th>References</th>
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<tr>
<td><strong>O₂ Chemoreceptors</strong></td>
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<td></td>
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<tr>
<td>Amia calva (A)</td>
<td>Pseudobranch + All gill arches</td>
<td>E + I</td>
<td>VII, IX, X</td>
<td>McKenzie et al. (1991a)</td>
</tr>
<tr>
<td>Clarias gariepinus (A)</td>
<td>All gill arches</td>
<td>I</td>
<td>IX, X</td>
<td>Beliao et al. (2015)</td>
</tr>
<tr>
<td>Hoploerythrus unitaeniatus (A)</td>
<td>All gill arches</td>
<td>E + I</td>
<td>IX, X</td>
<td>Lopes et al. (2010)</td>
</tr>
<tr>
<td>Lepisosteus osseus (A)</td>
<td>All gill arches</td>
<td>I</td>
<td>IX, X</td>
<td>Smatresk (1986); Smatresk et al. (1986); Smatresk (1988, 1989)</td>
</tr>
<tr>
<td>Pangasiodon hypophthalmus (A)</td>
<td>First gill arch + ?</td>
<td>E + I</td>
<td>IX, X</td>
<td>Thomsen et al. (2017); V.A. Armelin, M.T. Teixeira, M.T. Thomsen, L.H. Florindo, M. Bayley, and T. Wang, unpublished data.</td>
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<tr>
<td><strong>CO₂/H⁺ Chemoreceptors</strong></td>
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<td>Hoploerythrus unitaeniatus (A)</td>
<td>First gill arch</td>
<td>E</td>
<td>IX, X</td>
<td>Boijink et al. (2010)</td>
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Note: (A) Actinopterygii; (S) Sarcopterygii; (E) External – water – orientation; (I) Internal – blood – orientation; (?) Unknown; (VII) Facial nerve; (IX) Glossopharyngeal nerve; (X) Vagus nerve.

### Table 3
Location, orientation and innervation of peripheral O₂- and CO₂/pH-sensitive chemoreceptors involved in gill ventilation amplitude responses.

<table>
<thead>
<tr>
<th>Species</th>
<th>Receptor Location</th>
<th>Orientation</th>
<th>Innervation</th>
<th>References</th>
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<tr>
<td>Amia calva (A)</td>
<td>Pseudobranch + All gill arches</td>
<td>E + I</td>
<td>VII, IX, X</td>
<td>McKenzie et al. (1991a)</td>
</tr>
<tr>
<td>Clarias gariepinus (A)</td>
<td>All gill arches</td>
<td>I</td>
<td>IX, X</td>
<td>Beliao et al. (2015)</td>
</tr>
<tr>
<td>Hoploerythrus unitaeniatus (A)</td>
<td>All gill arches + Extrabranchial</td>
<td>E + I</td>
<td>IX, X, ?</td>
<td>Lopes et al. (2010)</td>
</tr>
<tr>
<td>Lepisosteus osseus (A)</td>
<td>All gill arches</td>
<td>I</td>
<td>IX, X</td>
<td>Smatresk (1986); Smatresk et al. (1986); Smatresk (1988, 1989)</td>
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<tr>
<td>Pangasiodon hypophthalmus (A)</td>
<td>First gill arch + ?</td>
<td>E + I</td>
<td>IX, X</td>
<td>Thomsen et al. (2017); V.A. Armelin, M.T. Teixeira, M.T. Thomsen, L.H. Florindo, M. Bayley, and T. Wang, unpublished data.</td>
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<td>IX, X</td>
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</tr>
</tbody>
</table>

Note: (A) Actinopterygii; (S) Sarcopterygii; (E) External – water – orientation; (I) Internal – blood – orientation; (?) Unknown; (VII) Facial nerve; (IX) Glossopharyngeal nerve; (X) Vagus nerve.
4. Central receptors mediating air-breathing responses

The presence of central CO₂/H⁺ chemoreceptors in fishes, especially in the air-breathing fishes, has been investigated because of the important role of these receptors in the control of ventilation in terrestrial vertebrates. In the descendants of the Sarcopterygii, the tetrapods, pulmonary ventilation is produced by a central pattern generator, whose activity is modulated by central and peripheral CO₂/H⁺ chemoreceptors (Branco et al., 1992, 1993; Milsom, 1995a,b; Noronha-de-Souza et al., 2006). Conversely, in Actinopterygii, there is little evidence for central chemoreceptors sensitive to CO₂/H⁺ influencing aerial respiration. Indeed, there is no evidence for the presence of such receptors in *A. calva* and *Dallia pectoralis* (Hedrick et al., 1991; Hoffman et al., 2009).

Another study, on an isolated brainstem preparation, showed that *L. osseus*, a primitive bony fish with bimodal respiration, possesses central sensitivity to CO₂/H⁺ that control air-breathing frequency but not gill ventilation rate (Wilson et al., 2000). It was observed that the isolated brainstem autonomously produces motor patterns that resemble that of gill ventilation and air-breathing, and that manipulations of the P_{CO₂}/H⁺ levels in the brainstem produced directly proportional changes in the frequency of air-breathing motor pattern (Wilson et al., 2000). This was the first evidence of a central air-breathing pattern generator and central respiratory chemosensitivity to CO₂/H⁺ in an actinopterygian fish with aerial respiration. Wilson et al. (2000) speculated that the origin of the central rhythm generator for aerial respiration occurred before the divergence of the actinopterygian and sarcopterygian lineages.

Other evidence of the existence of central chemoreceptors in fishes was reported by Sanchez et al. (2001b) and Amin-Naves et al. (2007b) in the obligate sarcopterygian *L. paradoxa*. These studies demonstrated that lung ventilation increased substantially when cerebrospinal fluid (CSF) P_{CO₂} was raised from 21 to 42 mmHg, while pH of CSF was held constant at a normal control value of 7.45. Likewise, a decrease in the pH of the CSF from 7.45 to 7.20 caused a large increase in ventilation at a constant P_{CO₂} (normocarbic value = 21 mmHg). When associated, however, low pH and high P_{CO₂} in CSF (pH = 7.10 and P_{CO₂} = 42 mmHg) reduced ventilation. Consequently, the combined effect of a decrease in pH and increase in P_{CO₂} of CSF is not additive to ventilation (Amin-Naves et al., 2007b). These results are similar to those of previous studies in toads, wherein the perfusion of the fourth cerebral ventricle with acidic, hypercapnic and acidic-hypercapnic CSF elicited hyperventilation (Smatresk and Smits, 1991; Branco et al., 1992, 1993) – in these animals, the combined effect of low pH and high P_{CO₂} in CSF was not additive to ventilation as well (Smatresk and Smits, 1991).

Finally, although there is evidence for central chemosensitivity to CO₂/H⁺ in one air-breathing holostean (Wilson et al., 2000), it is generally considered that peripheral gill receptors are the predominant sites for detecting CO₂/H⁺ in actinopterygian fishes. In sarcopterygians, on the other hand, central CO₂/H⁺ chemoreceptors appear to have a more prominent role as in tetrapods (Burleson and Smatresk, 2000; Reid et al., 2000; Sundin et al., 2000; Gilmour, 2001; McKenzie et al., 2001; Perry and Reid, 2002; Amin-Naves et al., 2007a).

5. Conclusions and research perspectives

This review indicates that there is overall understanding of how peripheral and central chemoreceptors can drive cardiorespiratory reflexes in fishes with bimodal respiration. That is, peripheral receptors, innervated by cranial nerves, drive increased air-breathing in response to environmental hypoxia and hypercapnia. These receptors are, in general, sensitive to sensitive to O₂ and/or CO₂/H⁺ levels in the blood and/or the environment. Increased O₂ demand also stimulates air-breathing, which may be a reflex driven by reduced O₂ levels in the blood, as the evidence for peripheral sensitivity to increased plasma P_{CO₂} remains equivocal. Air-breathing fishes also possess peripheral receptors that stimulate cardiac and gill ventilatory responses to hypoxia and hypercapnia, such as those found in unimodal water-breathing species but, in bimodal breathers, these receptors can sometimes cause responses that are different from the general unimodal pattern. Beyond these generalities, however, the existing data indicate that bimodal breathers show a great deal of interspecific variation in their reflex responses to dissolved gases, which is consistent with the fact that air-breathing has evolved independently on multiple occasions. There is the need to increase research in this area to see whether common patterns emerge across taxonomic groups. For example, to understand how information from the multiple different internally and externally oriented receptors is integrated to modify the relative intensity of aerial versus aquatic respiration. There is also a need for further research into central sensitivity to CO₂ and H⁺ in sarcopterygian and actinopterygian fishes. Although it appears to exist in bony fishes, more work is required to understand what role it plays in reflex cardioventilatory responses in conscious animals.

Finally, there is also now evidence that bimodal breathing fishes may breathe air for reasons that, at first sight, seem uncoupled from stimulation of peripheral or central receptors. McKenzie et al. (2016) found that, in normoxia, the intensity of spontaneous air-breathing was linked to individual boldness in a facultative air-breather, *C. gariepinus*, in a manner that was independent of their O₂ demand. Killen et al. (2018) found that aggression among individuals was a stronger driver of air-breathing than was oxygen demand in groups of *C. gariepinus*. It is
not clear whether these drivers of air breathing do in fact involve stimulation of peripheral receptors that monitor blood O2 in the interbreath interval (Shelton et al., 1986), with both and/or aggressive individuals perhaps having a lower threshold for stimulation of a surfacing response. It is also possible, however, that once the reflex circuits for air-breathing have evolved, higher order central mechanisms may subsequently evolve that can influence the intensity of the behavior.

References


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