# 7

# Chemoreceptive Control of Ventilation in Amphibians and Air-Breathing Fishes

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*Abstract*

*Ventilation is a critically important process in providing $O_2$ to the respiratory surfaces and removing $CO_2$ from them. When either environmental gas composition or tissue demands change, then adjusting ventilation through rate and amplitude modifications is the most direct response to ensure respiratory gas exchange. In amphibious vertebrates breathing both air and water and using a suite of respirator structures (which can include skin, external gills, internal gills, lungs, gas bladders and intestines), the process of ventilatory adjustment can be complex indeed. The present review examines the morphology, physiology and evolutionary biology of ventilatory responses to altered $O_2$ and $CO_2$ levels in amphibians and air-breathing fishes. Additionally, the vital role in modulating ventilatory responses of both centrally and peripherally located chemoreceptors and mechanoreceptors, as investigated by in vitro and in vivo methods, is examined. Finally, this analysis concludes by posing an extensive list of areas in lower vertebrate respiratory control deserving future investigation.*

## Introduction

Many vertebrates exploit some combination of aquatic and aerial gas exchange to provide $O_2$ uptake and $CO_2$ elimination. In fishes, exploitation of aerial gas exchange has evolved independently many times, involving a variety of air breathing organs (for general reviews see Johansen, 1970; Randall et al., 1981; Little, 1983; Graham, 1997; Maina, 2002). Indeed, air-breathing occurs in at least 49 known families of fish (Graham, 1997). In the Amphibia, a large proportion of the more than 6000

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amphibian species dwell in water (e.g. the anuran amphibians and especially frogs) using their lungs for aerial gas exchange and their skin for aquatic gas exchange. From a developmental perspective, almost all air-breathing fishes and amphibians exhibit early *embryonic/larval stages that are strictly aquatic and use solely water for gas exchange*, but subsequently undergo a fascinating and complex developmental transition that includes the capacity for air breathing.

The term "bimodal breather" has been used extensively in describing various amphibious vertebrates, but some confusion as to the meaning of this term still persists. "Mode" is typically defined as "..a way of doing something...", hence "biomodal" refers to *two* ways of doing something. It follows, then, that "bimodal gas exchange" refers to the two ways in which gas exchange is achieved, not the two respiratory media (water, air) that are used (Figure 1). Thus, here we use the term "biomodal" to mean that two different respiratory structures are used.[1] This may at first seem like a trivial semantic diversion. Consider, however, that many amphibious vertebrates, at some stage in their development, are actually *tri*modal breathers that us various combinations of skin *plus* gills *plus* lungs to breath both water (skin and/or gills) and air (skin and/or lungs). In many respects, trimodal breathing represents a much more complex respiratory

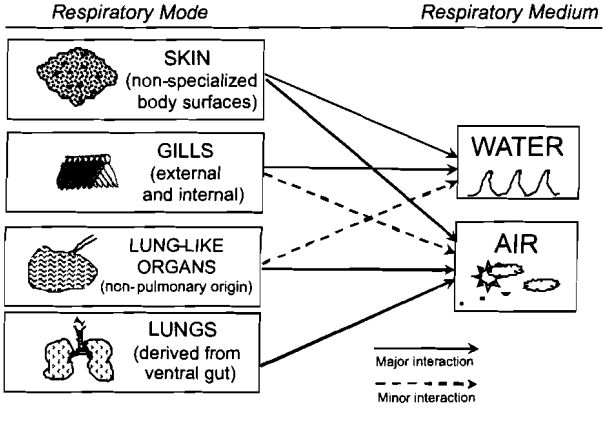


**Figure 1:** Interrelationships between modes of breathing with various respiratory organs and the two respiratory media—water and air. Many amphibious vertebrates use combinations of respiratory modes during their developmental life cycle, as well as concurrently as adults. The skin is the only respiratory organ that can serve equally well when either water- or air-exposed. Note that in some species of air- breathing fishes the gills do not entirely collapse when air exposed, and can still participate in some degree of gas exchange. Similarly, some non-pulmonary air-breathing organs in fishes can continue to exchange gas at slow rates even when water-filled (e.g. the labyrinth organ of labyrinthodontid fishes).

[1] It could be argued that "diffusion" and "convection" are indeed also "modes" of gas exchange, but here we shall confine the use of mode to structure rather than process.

situation compared with the far simpler respiratory circumstances of animals that use almost exclusively either gills or lungs. Indeed, the non-linear developmental transition of many amphibians from breathing only with skin (early larvae) → skin + gill breathing → skin + gill + lung breathing → skin + lung breathing (adults) has been used a physiological model for complexity change and its analysis (Burggren and Monticino, 2005; Burggren, 2006).

Tremendous variety is to be found in the combinations of various modes of breathing in amphibious vertebrates. A review of this material is beyond the scope of this chapter. However, evident from Figure 1 is that in any vertebrate using multiple respiratory modes and multiple respiratory media, the control of ventilation process is potentially quite complicated. In this chapter we will discuss the chemoreceptive control of air breathing in amphibious vertebrates using multiple modes of gas exchange. To begin this process, let us first briefly consider from a ventilatory control point of view both the physico-chemical characteristics of the respiratory media as well as the nature respiratory modes (structures).

## The Respiratory Media

Water and air differ enormously in attributes important to the process of gas exchange: density, viscosity and oxygen capacitance. These three factors interact to make breathing water a very different process from breathing air. Because water is heavier, more viscous and has a much lower $O_2$ capacity than air, animals actively breathing water—that is, using muscle power to generate a flow of water over their respiratory surfaces—will have to pump a 30-40 times greater volume of water than an air breather would have to pump of air. Thus, the cost of breathing water in aquatic fishes, while apparently quite variable, is certainly much more expensive than in vertebrates that breathe air (see Randall, 1970; Steffenson and Lomholt, 1983; Maina, 2002). Consequently, there is an additional energetic burden on aquatic vertebrates to ensure that ventilation is carefully monitored and regulated. Also, because aquatic vertebrates like fishes necessarily ventilate their gills with a high volume of water—and because $CO_2$ has a much greater capacitance coefficient for $CO_2$ than $O_2$, metabolically produced $CO_2$ is quickly washed out of the blood. Typically, fishes have a venous blood $PCO_2$ of less than 1 kPa, compared with much higher values typically in the range of 5-8 kPa for terrestrial, air-breathing animals.

For equivalent molar quantities of $CO_2$ entering a given volume of water or air, the very high solubility of $CO_2$ in water means that the increase in measurable $PCO_2$ in air will be much higher than the $PCO_2$ increase in water. In other words, elimination of a large amount of $CO_2$ into water will produce only very small increases in $PCO_2$ in the exhalant water stream. The addition of $CO_2$ into water will result in a variable degree of fall in the pH of exhalent water, because the actual change in water pH for a given molar quantity of $CO_2$ eliminated from the blood is dependent upon the

exhalent water's buffer capacity. While some aquatic species are tolerant of only a very narrow suite of water characteristics, others can range more freely and experience a significant range of water quality, including buffer capacity. Thus, monitoring of either $PCO_2$ or pH in exhalent water for the purposes of regulation of aquatic ventilation will be unreliable for an aquatic animal.

As a consequence of these physico-chemical differences in air and water, aquatic vertebrates have evolved ventilatory control systems that predominantly affect the uptake of $O_2$ (Smatresk, 1990; Taylor et al., 1999; Florindo et al., 2004; Vulesevic and Perry, 2006). Ventilatory changes for $CO_2$ elimination are rarely necessary, though fishes do have some ventilatory responses to aquatic hypercarbia (e.g. Perry and McKendry, 2001). Body fluid pH in strictly water-breathing vertebrates is maintained in large part by the controlled elimination of $H^+$ and $HCO_3^-$ ions, since the high solubility of $CO_2$ in water makes untenable retention of $CO_2$ in the blood to be "blown off" in a regulated fashion. In contrast, in terrestrial air-breathing animals air is relatively inexpensive to metabolically pump through lungs or lung-like organs, and $O_2$ is in abundance. Minute-to-minute ventilatory control thus tends to center around elimination of $CO_2$ to maintain of appropriate body fluid pH levels, though internal or, more rarely, environmental hypoxia can nonetheless profoundly stimulate ventilation.

Gas exchange and ventilatory control complexity reaches a zenith in amphibious, bimodal breathers that have to face concurrently both the advantages and disadvantages of air and water as a respiratory medium. We will return to this topic after considering the respiratory organs, themselves.

## Modes of Gas Exchange: The Respiratory Structures of Vertebrates

Collectively, amphibians and air-breathing fishes show examples using all four major categories of respiratory structure of vertebrates: skin, gills, non-pulmonary air-breathing organs, and lungs. Many species, either as larvae or adults, show combinations of exchangers as either bimodal or trimodal breathers.

### *Skin*

All animals have some capacity for gas exchange via their generalized body surface (skin). Even in heavily scaled fishes or furry mammals there is measurable $O_2$ uptake and $CO_2$ elimination via the skin. In some lightly scaled or scale-less aquatic fishes and in primarily aquatic amphibians, cutaneous gas exchange can account for up to 40% of $O_2$ uptake and, in amphibians, even larger proportions of $CO_2$ elimination (Feder and Burggren, 1985). The rest of the gas exchange in these bimodal or tri-modal breathers occurs by gills, ABOs or lungs. In terrestrial vertebrates with thin, relatively moist skin (e.g. toads), there is a reduced role of the skin in $O_2$ uptake, which occurs primarily via pulmonary routes, but the skin retains importance in $CO_2$ elimination.

There is an historical, dominant view in the literature that cutaneous gas exchange "just happens"—that is, it cannot be regulated *per se* and instead, gas exchange across the generalized body surface merely reflects the partial pressure gradients for gases between blood and surrounding air or water. While both the relative role of the skin in gas exchange declines as metabolic rate increases and the transcutaneous partial pressure gradients are certainly of pre-eminent importance (e.g. Pinder et al., 1991), recent experiments on terrestrial toads have shown that, overall, cutaneous blood flow, as well as regionalized capillary recruitment and derecruitment, can actively regulate cutaneous gas exchange (Burggren and Vitalis, 2004) to an extent not previously appreciated. Skin of aquatic vertebrates can also be actively ventilated via behavioral mechanisms. One of the biggest obstacles to effective cutaneous gas exchange is the build-up of a boundary layer of stagnant water adjacent to the skin, essentially increasing the diffusion distance for respiratory gases. By positioning the body in a current of water or by actively swimming or creating other body movements, fishes and aquatic amphibians can disrupt these boundary layers and increase the efficiency of cutaneous gas exchange.

Whether there is localized sensory monitoring of changes in tissue $O_2$ and $CO_2$ that reflexly alter either cutaneous perfusion or activities that "ventilate" the skin is currently unknown.

### *Gills*

The structure and respiratory function of gills has been extensively documented in fishes (for recent reviews and entry into the extensive literature see Maina, 2002; Olson, 2002; Wilson and Laurent, 2002; Evans et al., 2005). Briefly, in all but the most primitive fishes, the branchial arches (typically 4 to 6 pairs depending on genus) are enclosed in paired internal branchial chambers. Buccal pumping drives large volumes of water across the gills in a direction counter to that of the blood flow within the individual gill filaments. Gill ventilation is carefully tuned to oxygen demands via sensory feedback involving receptors located within the branchial chambers, on the gill surfaces, or internally in excurrent (arterialized) blood (see Chapter 1, this volume). The gills of larval and neotenous amphibians have been much less examined compared to fish gills (Malvin, 1989; Pinder and Burggren, 1986; Maina, 2002).

External gills contribute to gas exchange in early developmental stages in both fishes and amphibians, and persisting in adults in a few neotonous amphibian species. Since these gills are not enclosed in a ventilated, internal chamber, they are faced with the issues of boundary layer build-up as skin. However, once again behavioral activities enhancing gas exchange involve orientation of the body in currents or, in the case of some amphibians with external gills, doing "pushups" to wave the gills and break up boundary layers in hypoxic water. Generally, gills are solely instruments of aquatic gas exchange, collapsing to a fraction of their original surface area when removed from the buoying effect of water and exposed to air. However, the gills of a few amphibious

fishes that venture on to land (e.g. *Periopthalmus, Boleophthalmus)* have mechanical spacers that hold apart the individual filaments and allow some continuing aerial gas exchange (see Graham, 1997).

### *Non-pulmonary Air-Breathing Organs*

Non-pulmonary air-breathing organs (ABOs) are found in the air-breathing fishes excluding the lungfishes, which have true lungs (see below). ABOs are found in many shapes and forms in air-breathing fishes (Randall et al., 1981; Graham, 1997). They have evolved as both *de novo* structures (e.g. labyrinth organs of the gourami, *Trichogaster trichopterus* and the Siamese fighting fish, *Betta splendens*), and through partial modification of organs used for other purposes (e.g. the hindgut of the African weather loach, *Misgurnus anguillacaudatus* or the swim bladder of the arapaima, *Airpaima gigas*). Lung-like ABOs, which can be quite elaborate with an alveolar-like structure, always retain a residual gas volume are never exposed to water. However, labyrinth organs in epibranchial chambers alternate between being water and air filled, and the gut breathers must accommodate both air and regular gastrointestinal contents.

ABOs tend to be regularly ventilated, with the ventilation rate increasing with higher metabolic demand, increasing temperature (which heightens the metabolic rate), and with decreasing environmental $O_2$ levels. The reflex mechanisms by which air ventilation in ABOs of air-breathing fishes are regulated are not nearly as well categorized as for amphibians or aquatic fishes, as will be discussed below.

### *Lungs*

True lungs are found only in the lungfishes (*Lepidosiren, Neoceratodus, Protopterus)* and in tetrapod vertebrates including, of course, the amphibians. These structures are ventrally derived outgrowths of the esophagus, a definition that differentiates them from swim bladders that might otherwise occupy the same region of the body cavity and have a similar structure to primitive lungs. Lungs are also perfused by arteries derived from the branchial arch VI, comprising a pulmocutaneous artery in amphibians and a pulmonary artery in lungfishes. Having made this embryological/anatomical distinction, the mechanisms of ventilation of the lungs of lower vertebrates are quite similar to those of ABOs derived from swim bladders. Rather than a diaphragmatic mechanism as in mammals, for example, the lungs are ventilated by positive pressure produced by buccal gas compression in both lungfishes (McMahon, 1969; DeLaney and Fishman, 1977) and amphibians (Shoemaker et al., 1992; Jorgensen, 2000; Vasilakos et al., 2006), though some dispute still exists over the precise mechanics and patterns of gas flow (see Fernandes et al., 2005). The interior structure of the lungs of amphibians and lungfishes is quite variable, but generally they are more secular than the more highly alveolarized lungs of reptiles and mammals.

Having reviewed the respiratory structures of bimodally breathing air-breathing fishes and amphibians, now let us turn to how the specific role of chemoreceptors in the regulation of their ventilation.

# Chemoreceptors and Ventilatory Control in Amphibians

## Sensory Systems for Ventilation Regulation

The role of the respiratory system of amphibians is to maintain appropriate respiratory gas composition within the circulating body fluids. To achieve this task, information on respiratory gases, acid-base status, and ventilatory performance transduced by various $O_2$- and $CO_2$-sensitive chemoreceptors and mechanoreceptors must reach the brain, especially the medulla. It is in this central nervous system structure where the respiratory rhythm is both formed and modulated, generating appropriate respiratory behaviors to meet the tissues' gas exchange requirements. Sensing both the external and internal environment is the key to effective regulation of ventilation. Thus amphibians, not surprisingly, show a variety of sensory receptors that monitor both peripheral and central changes in respiratory gases and pH. The general subject of chemoreceptors in amphibians has previously been reviewed in depth (Smatresk, 1990; West and Van Vliet, 1992; Kusakabe, 2002; Reid, 2006; Gargaglioni and Milsom, 2007), and our intent here is to provide a general overview.

### *Respiratory Tract Chemoreceptors*

**Pulmonary Stretch Receptors.** Pulmonary stretch receptors deliver dynamic information on the extent of lung deflation and inflation to the brain stem via the vagus nerve. Generally, amphibian pulmonary stretch receptors are stimulated by a dynamic increase in lung volume or pulmonary wall tension, which in turn increases expiration and inhibits inspiration (West and Van Vliet, 1992; Wang et al., 1999; Reid et al., 2000; Sanders and Milsom, 2001; Reid, 2006; Gargaglioni and Milsom, 2007). These receptors are divided into three groups. The first group responds to the *degree* of lung inflation, and can be viewed as a pulmonary volume receptor. The second group of phasic pulmonary stretch receptors is stimulated by the *rate* of inflation, increasing their firing frequency when the rate of stretch increases. Individual receptors in the last, distinct, group actually respond to both rate and extent of stretch (Milsom and Jones, 1977; Kinkead and Milsom, 1996; Reid, 2006). Reid and West (2004) investigated the role of phasic pulmonary stretch receptor (rate-sensitive) in ventilation in the cane toad, *Bufo marinus*, using tidally ventilation instead of the more commonly used unidirectional ventilation method. Efferent neural recording of trigeminal nerve activity showed that stimulation of the phasic pulmonary stretch receptor increased overall breathing frequency.

While pulmonary stretch receptors in amphibians are primarily responsive to their distortion when pulmonary volume changes, these receptors also respond to increasing intrapulmonary $CO_2$ levels by decreasing their firing rate (Milsom and Jones, 1977; Reid et al., 2000; Reid and West, 2004; Reid, 2006; Gargaglioni and Milsom, 2007). The interaction between these two kinds of stimuli is responsible for the overall respiratory input from the lungs to the brainstem in the bullfrog (Sanders and Milsom, 2001; Reid, 2006). Indeed, the $CO_2$-sensitive stretch receptor in amphibians may represent the archetype for specialized $CO_2$ receptors found in higher vertebrates (Milsom and Jones, 1977; Milsom, present volume).

**Olfactory $CO_2$ Chemoreceptors.** Olfactory receptors of amphibians are also $CO_2$ sensitive, and they respond to elevated $CO_2$ levels by sending inhibitory afferent signals that ultimately inhibit breathing, which is likely to be a defensive mechanism (Getchell and Shepherd, 1978; Sakakibara, 1978; Coates and Ballam, 1990). The information is conveyed via the olfactory nerve, since transection of that nerve eliminates the $CO_2$ response (Coates, 2001). The population of $CO_2$-sensitive olfactory receptors is relatively rare. In the salamander, only 1 to 2% of olfactory receptors responded to 5% $CO_2$ while the remainder were stimulated by odorants (Getchell and Shepherd, 1978). The response of these receptors showed dose-dependent increases for $CO_2$ levels from 0.5 to 10% in the bullfrog (Coates and Ballam, 1990). Carbonic anhydrase (CA), a family of enzyme catalyzing the hydration of $CO_2$, was found to participate in the $CO_2$ sensing mechanism in amphibian olfactory epithelium. Coates et al. (1998) reported that CA immunoactivity was localized mainly in the dorsal and ventral regions, where 23 out of 1222 sites examined responding to 5% $CO_2$. Inhibition of the enzyme (CA) by acetazolamide attenuated the response by 65%. These findings support the evidence of the rare presence of $CO_2$-sensitive olfactory receptors found in salamanders and indicate the role of CA in $CO_2$ detection in olfactory epithelium (Coates et al. 1998).

**Other Receptors in the Respiratory Tract.** In addition to pulmonary stretch receptors and olfactory $CO_2$ receptors, narial mechanoreceptors can be identified that are sensitive to water. They prevent water from entering the respiratory tract by inhibiting ventilation upon submergence. The feedback from this type of receptor also contributes to the overall output of breathing frequency (West and Van Vliet, 1992). Taste cells are also sensitive to water; however, no evidence has shown its relationship to the ventilation of animals. A population of water-sensitive receptors is also located in the glottis and pharynx, and inhibits lung ventilation during swallowing and water entry (West and Van Vliet, 1992).

## *Arterial Chemoreceptors*

Hypoxic stimulation of lung ventilation in adult anuran amphibians is mediated primarily by peripheral $O_2$-sensitive receptors that monitor arterial blood. At least two locations have been identified for these chemoreceptors. The first is the carotid labyrinth, which is a highly vascular plexus located in the bifurcation of the common

carotid artery forming the internal and external carotid arteries (Kusakabe, 2002). Though similar in many respects to the mammalian carotid body, these structures are not homologous. Both chemo- and baroreceptor functions have been confirmed for the carotid labyrinth through electrophysiological recording (Van Vliet and West, 1992) and nerve ablation (Jones and Chu, 1988).

Arterial $O_2$-sensitive chemoreceptors are also located in the aortic arch. Injection of sodium cyanide and perfusion with hypoxic or hypoxic-hypercapnic solutions result in discharge of the receptors within the aortic arch, indicating the presence of $O_2$ chemoreceptors (Van Vliet and West, 1992). However, the aortic chemoreceptor has received lesser attention as compared to the carotid labyrinth in amphibian.

Receptors within the pulmonary vasculature also participate in chemoreception for ventilation. Injection of cyanide into the pulmonary arterial circulation causes fictive hyperventilation, suggesting the presence of pulmonary arterial $O_2$-sensitive receptors (Wang et al., 2004). Denervation of the recurrent laryngeal nerves innervating the baroreceptor within the pulmocutaneous arteries caused a threefold increase in pulmonary blood flow and increased net transcapillary fluid flux, suggesting that pulmocutaneous baroreceptors protect the anuran lung by regulating pulmonary blood flow (Smits et al., 1986). Neuroepithelial bodies are also plentiful in amphibian lungs (Goniakowska-Witalińska, 1997). These structures, which are located mainly in the ciliated epithelium of the apical part of the septa, may also play a role in chemoreception involving intrapulmonary gas composition.

### *Afferent and Efferent Innervation*

Receptors providing environmental cues for ventilatory regulation are distributed throughout the lungs, as well as some locations in the central arterial circulation. Afferent information bound for the medulla (the site of respiratory rhythm generation) occurs via a variety of nerves carrying sensory fibers from these receptors including cranial nerves I, V, IX and X (see Table 1).

Modulation of chemoreceptor performance occurs via efferent ("motor") innervation. The carotid labyrinth of anurans is innervated by neurons containing regulatory neuropeptides thought to modulate chemoreceptor sensitivity and vascular tone (Kusakabe et al., 1995; Kusakabe, 2002). The neuroepithelial bodies in the lungs of amphibians also receive efferent innervation, but the physiological significance of this neural innervation has not been identified (Goniakowska-Witalińska, 1997).

### *Central Nervous System and Ventilatory Chemoreception*

**1 Hypoxia.** To the best of our knowledge there is no evidence for the existence of an $O_2$ receptor in the central nervous system that directly monitors changes in brain blood $PO_2$ and induces physiological responses, such as hyperventilation. However, some cell groups are thought to participate in the pathways responding to hypoxia.

**Table 1:** Afferent innervation of structures bearing chemo- and mechanoreceptors regulating gas exchange in amphibians.

| Anatomical Structure(s) | Cranial Nerve Carrying Afferent Fibers | Reference |
|---|---|---|
| • Nares<br>• Olfactory epithelium | Cranial nerve I<br>Cranial nerve V | Sakakibara, 1978<br>West and Van Vliet, 1992<br>Coates, 2001 |
| • Tongue | Cranial nerve IX | Inoue, 1978<br>West and Van Vliet, 1992 |
| • Pharynx<br>• Glottis<br>• Lungs<br>• Carotid labyrinth | Cranial nerve IX<br>Cranial verve X | West and Burggren, 1983<br>Van Vliet and West , 1986<br>West and Van Vliet, 1992<br>Kusakabe, 2002 |

The nucleus isthmi (NI), a mesencephalic structure located between midbrain and the cerebellum, inhibits the hypoxic ventilatory response in toads, *Bufo paracnemis*, by inhibiting the increase in tidal volume that would normally accompany hypoxia. It shows the regulatory role of structures in the CNS in the hypoxic hyperventilatory response. Glutamate and nitric oxide (NO) may be two of the possible candidates that mediate this inhibitory effect (Gargaglioni and Branco, 2004).

**$CO_2$.** Despite multiple locations for $CO_2$ chemorecptors in amphibians, the $CO_2$-sensitive receptors present in the ventral medulla of the central nervous system, which arise in late larval development, are considered the dominant sensory site for $CO_2$ chemoreception in amphibians as well as other tetrapods (Smatresk and Smits, 1991; West and Van Vliet, 1992; Torgerson et al., 1997; Taylor et al., 2003). Stimulation of these receptors by high $PCO_2$ and low pH caused both an increase in ventilation frequency and tidal volume (West and Van Vliet, 1992; Wang et al., 1999). Central chemosensitivity to $CO_2$ and pH is enhanced by a 9-day-exposure to hypercapnia (3.5% $CO_2$) as investigated by both *in vivo* monitoring of breathing frequency and *in vitro* neural recording from brainstem-spinal cord preparations in an adult anuran, *Bufo marinus* (Gheshmy et al., 2006)

In mammals, several sites within the central nervous system exhibit $CO_2$ chemoreception, including the nucleus tractus solitarius, the locus coeruleus, the midline medullary raphe, the ventral respiratory group, the fastigal nucleus, and the retrotrapezoid nucleus (Feldman et al., 2003). Among these many structures, only the locus coeruleus (LC) has been described in amphibians (Noronha-de-Souza, et al., 2006). In the adult toad, *Bufo schneideri*, lesions in the LC diminish the hyperventilatory response to hypercarbia, and injection of acidic solution into the LC induces hyperventilation (Noronha-de-Souza et al., 2006). Increased immunoreactivity of c-fos after exposure to 5% $CO_2$ indicates that the nucleus was activated by hypercarbia.

In addition to the inhibitory effect on hypoxic hyperventilation, the nucleus isthmi (NI) has a similar inhibitory effect to hypercapnia-induced hyperventilation. The

NI differentiates during metamorphosis when the transition of branchial ventilation to pulmonary ventilation occurs. Chemical lesion of the NI enhanced hypercarbic hyperventilation, demonstrating the inhibitory role of the NI when respiratory stimulus is high (Gargaglioni and Branco, 2004). As mentioned earlier, the NI does not function as a direct sensor for $CO_2$ or pH in the CNS, because lesions in the NI do not affect resting breathing frequency (Gargaglioni and Branco, 2004).

## Brain Respiratory Centers

The two different ventilatory acts of frogs—the more frequent and rhythmic buccal ventilation and the more irregular and stronger lung ventilation—appear to be generated by two distinct coupled central pattern generators (CPGs) (Wilson et al., 2002; Vasilakos et al., 2006). Pulmonary respiratory rhythms in amphibians originate from central pattern generators located in the medulla (see McLean et al., 1995; Perry et al., 1995; Milsom et al., 1999). Unlike CPGs in mammals and birds, the CPGs in adult amphibians do not provide for a constant pulmonary ventilation rhythm, but rather generate motor output for frequent but irregularly spaced breaths. Input from sites in the dorsal brainstem caudal to the optic chiasma clusters breaths into small groupings. Segmental generators in the medulla produce the primary rhythm, and are subsequently entrained to create the typical intermittent pattern of pulmonary ventilation (Reid et al., 2000). Nitric oxide provides excitatory input to the bullfrog's CPGs in the brainstem (Hedrick et al., 1998; Hedrick and Morales, 1999; Harris et al., 2002).

Buccal respiratory rhythms in adult amphibians are modulated by activity in both caudal and rostral levels of the brainstem (Wilson et al., 2002). The buccal oscillator is coupled to the pulmonary oscillator via chloride-mediated, opiod-sensitive mechanisms (Vasilakos et al., 2006).

Modulation of the normal ventilation pattern in adult amphibians is based on sensory input that occurs via several brain structures. Elevation of the respiratory drive results in tegmental and medullary inputs that modify the burst pattern of motor output to respiratory muscles (Reid et al., 2000). The nucleus isthmi, a mesencephalic structure situated between the cerebellum and the roof of the midbrain, is thought to modify the hypoxic and hypercapnic drives (Kinkead et al., 1997; Gargaglioni and Branco, 2004), a process involving both glutamate, nitric oxide , substance P, etc. (Perry et al., 1995; Gargaglioni and Branco, 2004).

The developmental changes in the location, anatomy, neurochemistry and function of amphibian central pattern generators have been investigated because of both the usefulness of the model in understanding lower vertebrate ventilation patternsits used as a model for considering the evolution of CPGs. This interesting subject is beyond the scope of this review, but the reader is referred to recent reviews by (Gdovin et al. 1999; Straus, 2000; Hedrick et al., 2005)

## Regulation of Ventilation by Chemoreceptors

Ventilatory responses in amphibians represent a complex integration of input from pulmonary stretch receptors, olfactory chemoreceptors and intrapulmonary and arterial chemoreceptors (Figure 2). Not surprisingly, then, there is no "standard" hypoxic or hyepercapnic response. Thus, observation of the nature of a hypoxic or hypercanpic drive based on data from the popular brainstem model is perhaps more useful in teasing apart the "internal wiring" of the brainstem than it may be in describing the actual responses of the whole animals. The ventilatory response of fictive lung breathing in brainstem preparations was briefly discussed above, and it is not our intention to review these CNS responses (for reviews see Milsom et al., 1999; Reid, 2006). Here we will focus on *in vivo*, whole animal responses.

### *Ventilatory Responses to Lung Inflation and Hypoxia*

Intact, conscious amphibians typically exhibit a strong hypoxic drive for both buccal and pulmonary ventilation, a finding long recognized for anurans (e.g. Babak, 1911; Smyth, 1939; reviewed by West and van Vliet, 1992; see also Branco and Glass, 1995; Hou and Huang, 1999). Not only does inspiration of hypoxic gas stimulate ventilation, but hyperoxia actually inhibits ventilation. In the toad *Bufo marinus*, hyperoxia inhibits ventilation even though hypercapnia and respiratory acidosis ensues (Toews and Kirby, 1985; West et al, 1987), indicating that the hypoxic drive can dominate in controlling ventilation in this toad. A typical finding in studies showing hypoxic stimulation of ventilation is that not only is pulmonary minute ventilation increased, but the *pattern* of ventilation changes as a consequence of inspiration of hypoxic gas (Pinder and Burggren, 1986; West and Van Vliet, 1992; Kinkead and Milsom, 1994; Gardner et al., 2000; Gargaglioni and Branco, 2000; Gargaglioni et al., 2002).

Does inspiration of hypoxic gas stimulate lung ventilation through reduction of arterial $PO_2$ or reduction of arterial blood oxygen concentration? Ventilatory responses to hypoxia persist independently from changes in blood $O_2$ carrying capacity in *Bufo paracnemis* (Wang et al., 1994; Andersen et al., 2003), indicating that blood-facing receptors are monitoring $PO_2$.

Hypoxic ventilatory responses appear to have a seasonal component in some anurans. In *Bufo paracnemis*, toads that respond vigorosly to hypoxia at 25°C during summer show no hypoxic response at 25°C in winter, despite the fact that blood gases showed no seasonal effect (Bicego-Nahas et al., 2001), suggesting that seasonal effects are affecting some aspect of the chemoreceptors or the integration of the information they provide to the CNS. *Rana catesbeiana* shows enhancement of temperature-dependent hypoxic ventilatory responses in winter, and reduction in summer, with intermediate responses in spring and autumn (Rocha and Branco, 1998)

The neotenous axolotl, *Ambystoma mexicanum*, provides an interesting perspective in an "adult" amphibian (or at least one that is no longer developing) that ventilates

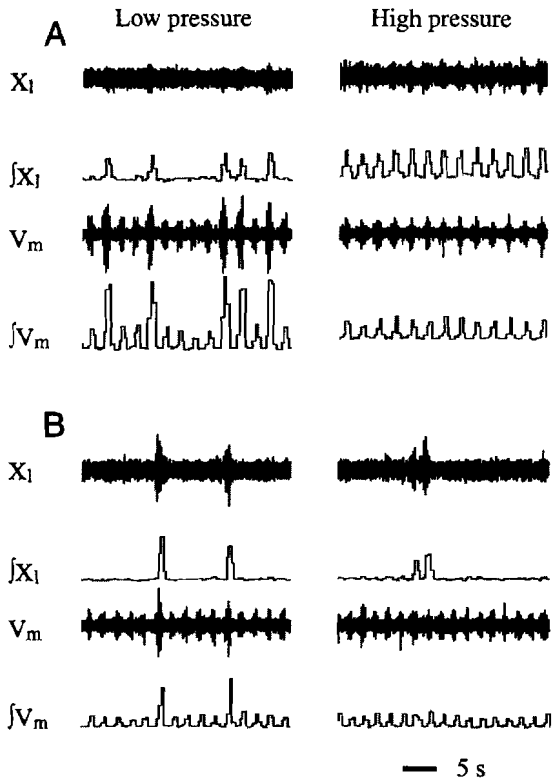


**Figure 2:** Electroneurograms representing fictive breathing recorded from the laryngeal branch of the vagus nerve ($X_l$) and the mandibular branch of the trigeminal nerve ($V_m$) in unidirectionally ventilated, decerebrate bullfrogs, *Rana catesbeiana*. In A, frogs were ventilated with air, while in B animals are ventilated with 3% $CO_2$ in air. "Low pressure" corresponds to 1 $cmH_2O$, while "high pressure" corresponds to 5 $cmH_2O$. Note that stimulation of stretch receptors by increased ventilation pressure in the lungs suppressed $V_m$ burst amplitude such that fictive lung ventilations (taller spikes in $V_m$ recordings) became indistinguishable from fictive buccal oxcillations (shorter spickes). $CO_2$ stimulated absolute fictive lung ventilation, primarily by reducing apnea length rather than breathing depth. These experiments show the complex nature of the interactions between mecho- and chemoreceptors in modulating the central rhythm generators in anuran amphibians (from Sanders and Milsom, 2001).

with both gills and lungs. Hypoxia stimulated ventilation rate of both the gills and the lungs, as did infusion of NaCN into the ventilatory stream or the arterial bloodstream (McKenzie and Taylor, 1966). Interestingly, norepinephrine stimulated gill ventilation but not lung ventilation rates. The axolotl thus shows similar ventilatory responses to larval amphibians.

The caecilian *Typhlonectes natans* shows an interesting suite of ventilatory responses to hypoxia, differing somewhat from other amphibians, in that aquatic hypoxia affects neither breathing frequency nor mechanics (Gardner et al., 2000). Yet, aerial hypoxia increases ventilation frequency as in other amphibians,

The salamander *Desmognathus fuscus* responds to hypoxic exposure with an increase in buccal pumping, even though as adults they lack lungs (Sheafor et al., 2000), similarly to how lunged salamanders would respond, though the role of this buccal hyperventilation in the observed maintenance of oxygen uptake in milder hypoxia is unknown.

### *Ventilatory Responses to Hypercapnia*

Interpretation of hypercapnic responses in amphibians is confounded by the considerable capacity for cutaneous $CO_2$ elimination. With the potential for $CO_2$ loss across the skin, arterial $PCO_2$ values will be lower for a given inspired $PCO_2$ than in reptiles, birds or mammals, for example. Short of concurrently measuring blood $PCO_2$ and acid-base parameters along with ventilation, quantitative determination of the sensitivity of the pulmonary hypercapnic response—and certainly any comparison with similar exposure in reptiles, for example—is problematic.

Anuran amphibians typically respond to elevations in aerial $CO_2$ with increased lung ventilation (see reviews by West and Van Vliet, 1992; Reid, 2006) resembling terrestrial tetrapod vertebrates (see other chapters, this volume). Most urodeles, however, show little or no ventilatory responsive to hypercapnia, and lung ventilation frequency is not correlated with arterial $PCO_2$ (see West and Van Vliet, 1992). The predominantly skin-breathing salamander *Cryptobranchus alleganiensis* responds to aquatic hypercapnia with an increase in pulmonary ventilation (Boutilier and Toews, 1981), more like anurans. In caecilians, where independent and combined exposure to aerial and aquatic hypoxia has been determined, aquatic rather than aerial hypercapnia is the more potent ventilatory stimulant (Gardner et al., 2000).

Similar to the hypoxic ventilatory response in anuran amphibians, there is seasonal variation in the extent of the hyperventilation stimulated by hypercapnia, with winter bullfrogs (*Rana catesbeiana*) showing a temperature-independent muting of the ventilatory response to 3-5% inspired $CO_2$ (Bicego-Nahas and Branco, 1999).

## Chemoreceptors and Intermittent Ventilation

Amphibians are typically intermittent lung breathers (see Boutilier, 1984; Smatresk, 1990; Feder and Burggren, 1992; Taylor et al., 1999; Reid and West, 2004). Apneic periods (essentially, diving in aquatic species) range from a few seconds to literally hours, depending upon species, metabolic rate, and temperature. Understanding the dynamics of control of intermittent ventilation in non-endothermic vertebrates has vexed researchers for decades (Gottlieb and Jackson, 1976; Burggren and Shelton,

1979; Boutilier and Shelton, 1986; West et al., 1989; Milsom, 1991; Kinkead and Milsom, 1996), as they have tried to understand how the lung ventilation is reflexly stimulated. Compounding the analysis is the fact that amphibians are bi- or trimodal breathers, which provides a whole additional layer of complexity of chemoreceptive control.

The simplest hypothesis for what triggers the initiation of air breathing is that here exist regulatory set-point(s) for "acceptable" blood $PO_2$, $PCO_2$ or $[H^+]$. When a threshold level is crossed (increased $PCO_2$ or decreased $PO_2$ or pH), then lung ventilation is triggered. This "threshold hypothesis" has much appeal, fitting in with the steady-state, homeostatic view of ventilatory control in mammals and birds, which typically are constant breathers that experience relatively little variation in blood gases and acid-base status. Unfortunately, analyses of blood gases and pH during bouts of intermittent breathing in amphibians reveal only a moderate correlation between a short-term specific threshold (e.g. $PO_2$ 15 kPa or pH 7.45) and the onset of lung ventilation following an apneic period in anuran amphibians (e.g. Coelho and Smatresk, 2003; Boutilier and Shelton, 1986). Feedback from intrapulmonary chemoreceptors may be more influential in terminating or initiating apneic episodes, but Kinkead and Milsom (1996) report an indirect modulatory effect rather than a direct control of the intermittent breathing pattern by such receptors.

One of the confounding factors in understanding how the internal respiratory environment influences intermittent breathing in amphibians may lie in the large contribution of cutaneous gas exchange to total gas exchange in most amphibians. In the adult bullfrog, for example, the skin accounts for approximately 10-25% of total $O_2$ uptake and up to 80% of total $CO_2$ elimination (Gottlieb and Jackson, 1976; Burggren and West, 1982). Intracardiac admixture of systemic venous blood draining the skin with systemic venous blood from non-cutaneous systemic vascular beds will elevate arterial $PO_2$ and reduce arterial $PCO_2$ an effect that may grow as the apneic period progresses. This diminishes the signal for arterial blood-monitoring chemoreceptors (e.g. aortic arch, carotid labyrinth) that would normally occur during interruption of pulmonary ventilation in a strictly lung breather.

Clearly, the additional study of factors—both peripheral and CNS - terminating apnea in intermittent breather is highly warranted.

## Development of Chemoreceptive Ventilatory Control in Amphibians

Almost all amphibians begin life with embryonic/larval stages that are almost entirely aquatic. Later in their life cycle, they develop from bimodal (skin, gills) into trimodal breathers with the addition of pulmonary ventilation. The changing importance of respiratory organs during development, evident from gas exchange partitioning studies, has been investigated in many anuran species (see Burggren and West; 1982; Burggren

and Just, 1992; de Souza and Kuribara, 2006). Given the considerable ontogenetic restructuring of gas exchange organs and their perfusion that also happens to occur, not surprisingly the sites of chemoreceptors and mechanoreceptors provide sensory feedback involved in the regulation of ventilation also change following larval development.

### *Ventilatory Responses to Lung Inflation and Hypoxia*

Most studies on the development of the respiratory regulatory system have focused on anuran larvae ("tadpoles"), which have become popular models for probing vertebrate respiratory development (Reid and Milsom, 1998; Gdovin et al., 1999; Straus, 2000; Wassersug and Yamashita, 2000; Straus et al., 2001). In early larval stages, anurans respond to aquatic hypoxia by increasing buccal pumping frequency, which in turn increase irrigation of the internal gills (see Burggren and Just, 1992). This response is evident as early as the Taylor-Kollros stage I, even before the appearance of internal gills (Burggren and Doyle, 1986). As lungs develop, pulmonary gas exchange is also increased by hypoxic stimulation. The sensory system involved in the hypoxic stimulation of gill ventilation appears to involve receptors at two locations. The larvae of bullfrogs (*Rana catesbeiana*) as early as stage V through stage XIX show rapid response (from 1.3 to 3.3 sec depending on stages) to inhalation of hypoxic or hyperoxic water or water laced with sodium cyanide (NaCN), a stimulant of $O_2$-sensitive receptors (Jia and Burggren, 1997a; Straus et al., 2001). This response is subsequently abolished by the removal of the first gill arch (Figure 3, Jia and Burggren, 1997b). Neurophysiological recordings have subsequently been made from $O_2$-sensitive neurons on the first gill arch of bullfrog tadpoles (Strauss et al., 2001). A second, slower hypoxic response to inhalation of hypoxic water (varying from 7.7 to 19 sec) persists after the first gill arch has been removed, indicating another population of more centrally located $O_2$-sensitive receptors in larval anurans (West and Van Vliet, 1992; Jia and Burggren, 1997b). The specific location and structure of these "non-branchial" receptors has not been identified.

The carotid labyrinth is an important site of chemoreception in adult amphibians, as discussed above, but in the anurans *Rana catesbeiana* and *Xenopus laevis*, and the urodele *Ambystoma tiginnum*, this structure is not fully developed until the completion of metamorphosis (Malvin, 1985, 1989; Kusakabe, 2002). The adrenergic cells of the branchial shunt vessels in larval *Ambystoma tigrinum* may also be the site of arterial blood chemoreceptors (Malvin and Dail, 1986).

*In vitro* characterization of the respiratory neural output from the brain stem of pre-metamorphic bullfrog (stages VIII to XVI) shows that neither the gill nor lung fictive ventilation frequency is affected by severe hypoxia (Winmill et al., 2005). This indicates the absence of central $O_2$-sensitive receptors for stimulating ventilation during late larval development and supports the notion of arterial $O_2$ receptors in anuran larvae. However, direct evidence indicating the existence of peripheral $O_2$-sensitive receptors reflexly affecting both gill and lung ventilation is still lacking.

The increase in gill ventilation frequency in response to aquatic hypoxia in larval anuran amphibians quickly diminishes as lung ventilation begins and becomes progressively more important to $O_2$ consumption. Eventually hypoxic branchial ventilatory responses and branchial ventilation itself then disappears with subsequent development, and is replaced by the typical ventilatory responses of the adults (West and Burggren, 1982; Burggren and Doyle, 1986).

### *Pulmonary Stretch (Mechano-)Receptors*

In addition to $O_2$- and $CO_2$-sensitive receptors, pulmonary mechanoreceptors are also involved in regulation of gill ventilation in anuran larvae. Gill ventilation frequency

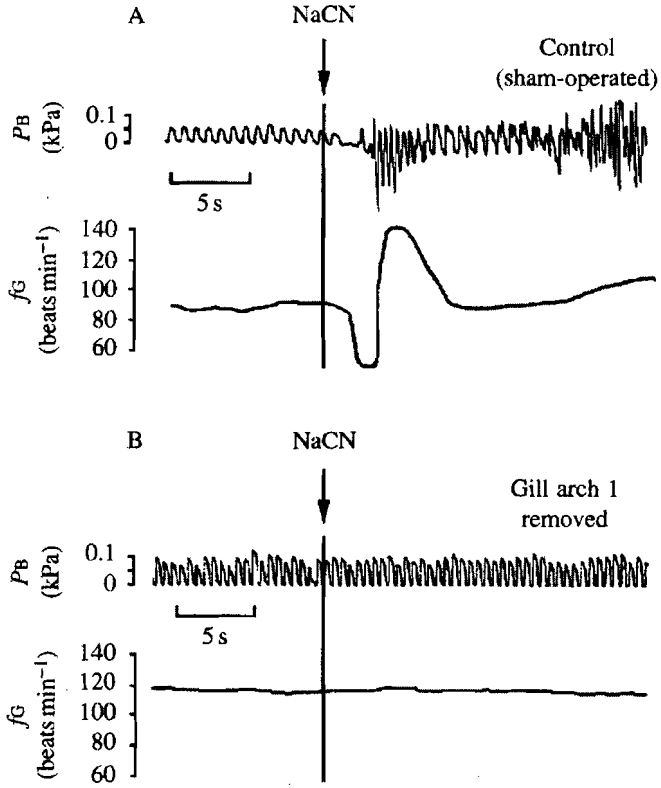


**Figure 3:** Effect on branchial ventilation of the injection of water containing 0.5% NaCN into the inhalent water stream in an unanesthetized stage VI larva of the bullfrog (*Rana catesbeiana*). (A) Control animal with intact gill arch 1. (B) Larva following surgical removal of gill arch 1. Note that the rapid (within 2 sec) onset of the response to NaCN is completely eliminated with removal of gill arch 1 (from Jia and Burggren, 1997b).

typically decreases following a single air breath in the larvae of *Rana catesbeina* (Figure 4). In bullfrog larvae at stage XVII-XIX, artificial inflation of lungs with nitrogen, air or oxygen temporarily reduces gill ventilation frequency (West and Burggren, 1983). This finding is supported by the study on decerebrate larvae at the same developmental stage (Gdovin et al., 1998). Larvae at stage XVI-XIX showed reduced gill ventilation frequency following lung inflation by cranial nerve VII recording and electromyogram of the buccal levator muscle (Gdovin et al., 1998). After the initial decrease in gill ventilation frequency, lung inflation with nitrogen subsequently increased gill ventilation; on the other hand, initial oxygen inflation was subsequently followed by a reduction in gill ventilation (West and Burggren, 1983). These experiments suggest that input from the pulmonary stretch receptor initially causes a reflex reduction in gill ventilation frequency, while the longer term changes resulting from nitrogen or oxygen inflation are mediated by input from $O_2$-sensitive chemoreceptors in the lungs or pulmonary vessels. These spatio-temperal interactions of chemo- and mechanoreceptors in larval stages likely ensure optimal $O_2$ acquisition from both respiratory media after the pulmonary system has developed, but before the gills undergo developmentally associated apoptosis. These interactions between chemo- and mechanoreceptors can also help minimize the loss of $O_2$ from blood through the gills into surrounding water when environmental aquatic hypoxia reverses the $PO_2$ gradient across the branchial membranes.

### *$CO_2$-Sensitive Chemoreceptors*

The location of central $CO_2$-sensitive chemoreceptors in larval anuran amphibians has been demonstrated *in vitro*. Hypercapnia stimulates fictive gill ventilation in stage X to XIX bullfrog larvae. After stage XX, perfusion of the brain stem with hypercapnic solution increasingly stimulates fictive lung ventilation (Torgerson et al., 1997). The locations of $CO_2$-sensitive receptors are within the ventral medulla: chemical and protease lesions at specific sites localized these chemoreceptors to be adjacent to the origin of cranial nerves V and X (Taylor et al., 2003). There is as yet no evidence for the presence of peripheral $CO_2$-receptors on the internal gills in larval amphibian during early development, in contrast to the presence of these in air-breathing fishes (see below).

In summary, amphibian anuran larvae respond to both hypoxia and hypercapnia by increasing gill ventilation frequency in the early stages and then show a developmental transition to predominant adjustments in lung ventilation. The location of receptors sensing ambient $O_2$ level is on the first branchial arch in early development, along with some other likely sites, such as the aorta or brain stem. The peripheral $O_2$-sensitive chemoreceptors migrate during development from the gill arch(es) to the carotid labyrinth following the completion of metamorphosis. $CO_2$-sensitive chemoreceptors are found within ventral medulla.

The investigation of the development of ventilatory control in amphibians has been heavily focused on anuran larvae. While the cardiovascular anatomical and physiological

development of urodeles (salamanders) has been characterized (see Malvin, 1985, 1989), we know relatively little about the extent to which the development of respiratory regulation in anurans maps onto salamanders and newts.

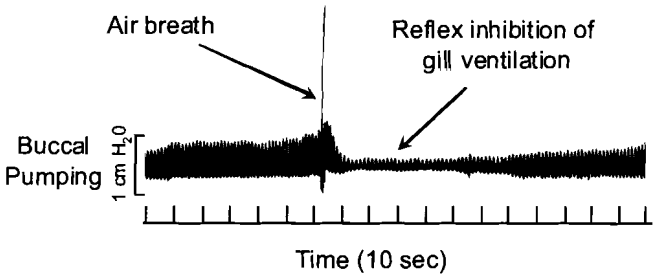


**Figure 4:** A single air breath results in a reflex inhibition of gill ventilation in an unrestrained larva (St TK XIX) of the bullfrog, *Rana catesbeiana* (after West and Burggren, 1983).

# Chemoreceptive Control in Air-Breathing Fishes

In addition to internal gills ventilated by a conventional piscine buccal pump, air-breathing fishes typically possess various methods for exploiting air-breathing, including non-pulmonary air-breathing organs (ABOs) or, in the case of the lungfishes, true lungs (see above)(Figure 1). Like their gills, the ABOs/lungs of air-breathing fishes have sensory innervation, allowing transmission of chemo- and mechanoreceptor information from these organs to the CNS, and allowing motor control over ventilation of the gills (and perhaps even neuromodulation of the sensors themselves), as we will now consider.

## Chemoreception in Air-Breathing Fishes

Although a systematic examination of chemoreceptors in air-breathing fishes is lacking, several studies of both an *in vivo* and *in vitro* nature reveal their existence in both central and peripheral locations.

### *Central Chemoreception*

The appearance of central $CO_2$/pH chemoreception is often linked to terrestriality related to air breathing and the associated elevated venous blood $PCO_2$, as previously mentioned. However, air-breathing fishes also possess central $CO_2$/pH chemoreception. In *in vitro* brainstem preparations, fictive air-breathing frequency increased following hypercarbia in superfusing solution in the long nose gar, *Lepisosteus osseus* (Wilson et al., 2000; Remmers et al., 2001). In the South American lungfish, *Lepidosiren*

*paradoxa*, the reduction of pH in the solution perfusing the isolated fourth cerebral ventricle increased lung ventilation and breathing frequency (Sanchez et al., 2001). These data suggest the presence of central acid-base and $CO_2$ receptors in a few species of air-breathing fishes. However, there is insufficient evidence and too few species examined to conclude that central $CO_2$/pH chemoreception evolves concurrent with the evolution of air-breathing in fishes.

### *Peripheral Chemoreception*

As in water-breathing fishes and larval amphibians, the branchial $O_2$ chemoreceptors of air-breathing fishes monitor gas composition near the gills and control the net level of ventilation—i.e. ventilation of gills and ABOs (Smatresk, 1990). On the other hand, some species, such as lungfish, rely on internal arterial receptors for regulating respiratory and cardiovascular behavior in response to hypoxia or hypercapnia (Perry et al., 2005). We now consider peripheral chemoreception of air-breathing fishes.

Cranial nerve denervation has been a direct method to test peripheral chemoreceptive control, despite confounding side effects such as stress, metabolic depression and decreased arterial $PO_2$ (Graham, 1997; McKenzie et al., 1991). Denervation of cranial nerves IX and X had no effect on air-breathing responses to aquatic hypoxic conditions in the bowfin, *Amia calva*. Thus, the $O_2$-sensitive chemoreceptor responsible for increasing air-breathing frequency does not appear to reside on the gills of *Amia calva* (Hedrick and Jones, 1999). However, pseudobranch ablation in the same species abolished the air-breathing responses to aquatic hypoxia, indicating that the $O_2$-sensitive chemoreceptors may be located on the pseudobranch instead of gills (McKenzie et al., 1991). Mechanical movement and compression of the gas bladder of *Amia calva* stimulates a ventilatory response, indicating the likely presence of a stretch receptor (Hedrick and Jones, 1999). The African lungfish (*Protopterus dolloi*) is another species that relies only on internal $O_2$ chemoreceptor, because only aerial hypoxia induced the secretion of catecholamines and cardiorespiratory responses (Perry et al., 2005). The long nose gar (*Lepisosteus osseus*) also possesses internal chemoreceptors for $O_2$. A decrease in arterial $PO_2$ and injection of NaCN into the ventral aorta both stimulated air-breathing frequency; however, air-breathing frequency also increased as $O_2$ level in air bladder fell, suggesting peripheral ventilatory control mechanism also exists in this species (Smatresk et al., 1986).

In addition to $O_2$-sensitive chemoreceptors, lungfish may also have peripheral $CO_2$/pH-sensitive receptors. Lung ventilation increased by 20% in hypercarbia (6.5 KPa in both water and air) when the cerebral ventricular system was superfused with normocarbic solution in the South American lungfish, *Lepidosiren paradoxa* (Amin-Naves et al., 2007).

Innervation of the ABOs has also been studied in the Indian catfish, *Heteropneustes fossilis*, the Asian catfish, *Pangasius hypophthalmus*, and the Nile bichir, *Polypterus bichir*

*bichir*. Expression of various neuropeptides was found in the air-breathing organs of these species (Mauceri et al., 2005; Zaccone et al., 2007). Neuroendocrine cells and their innervation have been located in the lungs of *Protopterus aethiopicus, Amia calva, Polypterus delhezi, Polypterus ornatipinnis* and *Polypterus bichir bichir* (Zaccone et al., 1989, 1995, 2007). Immunoreactivity of several neuropeptides was found in these cells. The role of these transmitters may be autonomic control of circulation and respiration. However, the relative importance and significance of these signals to the respiratory responses of air-breathing fishes is still enigmatic, and additional studies are needed to link the morphology, function and innervation of the neuroendocrine cells.

## Ventilatory Responses in Air-Breathing Fishes

Precise regulation of the ventilation of both gills and ABOs has been studied in numerous species of air-breathing fishes (see for example Randall et al., 1981; Graham, 1997; Brauner et al., 2004). In contrast to ventilatory chemoreception in amphibians, where there is considerable conformity in regulatory patterns, amongst the air- breathing fishes there appear to be three major groupings: aquatic hypoxia driven, aerial hypoxic driven, and a hybrid pattern of responses evident in the lungfishes. This information is grouped and summarized here according to the diverse ventilatory responses to hypoxia or hypercapnia in air and water.

### *Ventilation Driven Primarily by Aquatic Hypoxia*

The first group of air-breathing fishes, primarily responsive to aquatic hypoxia, comprises both facultative and obligatory air-breathers. As an example, aquatic hypoxia below 6.5 KPa stimulates both gill ventilation and air-breathing frequency in the South American tamoatá, *Hoplosternum littorale* (Affonso and Rantin, 2005) and the jeju, *Hoplerythrinus unitaeniatus* (Oliveira et al., 2004). Also in this first category is the gourami, *Trichogaster trichopterus*, an obligate air-breather from South-East Asia that has a labyrinth organ contained within a suprabranchial chamber. This species also responds to both aquatic and aerial hypoxia ($PO_2$ ~7 KPa) and aquatic hypercapnia ($PCO_2$ ~3 KPa) by increasing air-breathing frequency. Hypoxia also increases $O_2$ uptake by the labyrinth (Burggren, 1979). In the bowfin, *Amia calva*, air breaths are categorized into two types. The first includes exhalation followed by inhalation, and it is stimulated by both aerial and aquatic hypoxia. The second is characterized by inhalation only, which may be used for regulating gas bladder volume and buoyancy (Hedrick and Jones, 1999). Low aquatic $O_2$ partial pressure is the main stimulant driving these respiratory responses, and the branchial chemoreceptor is the predominant sensor eliciting these responses. This group of responses for air-breathing fishes resemble those observed in water-breathing teleost fishes (see Jonz and Nurse, Chapter 1) and larval anuran amphibians (e.g. Burggren and Just, 1992; Straus, 2000).

### *Ventilation Driven Primarily by Aerial Hypoxia*

The second group of air-breathing species includes fishes such as the mudskipper, *Periophthalmodon schlosseri* and the Australian desert goby, *Chlamydogobius eremius*. Unlike the first group of air-breathing fishes that respond primarily to aquatic hypoxia, the species in this latter group respond only modestly, if at all, to $PO_2$ changes in water. Rather, they increase gill and ABO ventilation frequency markedly in response to aerial hypoxia. The mudskipper, for example, responds to aerial hypoxia by increasing both air-breathing frequency and tidal volume of the vascularized buccopharyngeal cavity (Aguilar et al., 2000). The desert goby decreases its opercular movements in aerial hypoxia as severe as a $PO_2$ of ~2 KPa. In a (futile) attempt to cope with experimental severe aerial hypoxia, this species relies more on the bubbles in buccal cavity for $O_2$ acquisition, increasing the percentage of total $O_2$ consumption via buccal bubbles during aerial hypoxic exposure (Thompson and Withers, 2002).

### *Ventilation Driven by both Aquatic and Aerial Hypoxia—the Lungfishes*

The last functionally-categorized group of air-breathing fishes is represented by the lungfish. The Sarcopterygii are characterized by true lungs resembling those of amphibians. They have reduced gills (especially the anterior-most arches) and generally share similar ventilatory control mechanism and responses with tetrapod vertebrates. Lung ventilation is stimulated by both aerial and arterial hypoxia (below 7 KPa) in the South American lungfish, *Lepidosiren paradoxa* and the African lungfish, *Protopterus dolloi* (Sanchez et al., 2001; Perry et al., 2005). Aquatic hypoxia has little or no effect on pulmonary ventilatory rate of these species. However, the Australian lungfish, *Neoceratodus forsteri*, responds to aquatic hypoxia (3 kPa) with increased branchial ventilation and air-breathing frequency (Fritsche et al., 1993).

The difference in response among lungfish species may be due to the relative importance of air breathing. The Australian lungfish is a facultative air breather that begins using lung ventilation in aquatic hypoxia. The other two species are obligate airbreathers with reduced gill surface area (Johansen, 1970; Graham, 1997; Fritsche et al., 1993; Sanchez et al., 2001). The respiratory regulatory system of *Neoceratodus* may rely more on the signals from water, it being more critical as a respiratory medium for maintaining normal aerobic metabolism in this more aquatic species. However, the existence of branchial $O_2$-sensitive chemoreceptors in the American and African lungfishes cannot be excluded until such time as more direct loss-of-function experiments on gills—e.g. denervation of cranial nerves IX and X—are completed.

The major differences in hypoxic ventilatory responses discussed above likely reflect differences in habitat rather than fall into any sort of strict taxonomic pattern. Air-breathing fishes of the Amazon Basin and many South-East Asian habitats experience both hypoxia and hypercapnia on a daily and seasonal basis due to alternating cycles of photosynthesis, respiration, decay of vegetation, flooding, etc. In contrast, the desert goby and lungfish live in temporary ponds and may be completely out of water during

the dry season. The mudskipper resides in mud burrows filled with extremely hypoxic water during low tide, where they survive in the environment by solely using aerial gas exchange (Aguilar et al., 2000; Sanchez et al., 2001; Thompson and Withers, 2002). Heavy utilization of aerial gas exchange may have contributed to the loss of aquatic gas sensing in these species. Such adaptation to their habitat makes the chemoreceptive control of ventilation in this group more similar to adult anurans, despite the fact that no evidence has pointed out the actual location of their $O_2$-sensitive chemoreceptors. Also, little work has been done regarding the existence of fish-like (peripheral) or amphibian-like (central) $CO_2$ chemoreception in these species.

# Conclusions

## General Trends

Air-breathing fishes and amphibians occupy a fascinating functional transition point in the evolution of terrestrial tetrapods from their aquatic fish-like ancestors. Not surprisingly, considerable attention has been paid to the chemoreceptors that regulate ventilation, as well as the ventilatory responses themselves. Perhaps reflecting the extreme diversity of the air-breathing habit in air-breathing fishes and amphibians, there are relatively few general lessons that can be derived with certainty from both interpretation of existing studies and planning of future ones. However, a few key principles do emerge:

1. the more aquatic in nature the animal, the greater is the tendency to have sophisticated receptors for, and to respond primarily to, changes in $O_2$ levels in the interior milieu;
2. as a transition to air-breathing and terrestrial life develops, the greater is the likelihood of having $CO_2$-/pH-sensitive receptors that participate in regulation of ventilation;
3. central chemosensitivity is a highly conserved trait, evident in all semi-amphibious and amphibious animals.;
4. despite the difference in exact location of $O_2$-sensitive receptors, $O_2$ chemoreception remains peripherally located while $CO_2$ chemoreception turns centrally during evolution.

## Unanswered Questions/Future Experiments

When our understanding of a subject like chemoreception in amphibious vertebrates is so incomplete, not surprisingly several areas ripe for future experimentation emerge, including:

1. the role of daily and seasonal influences on chemoreception and ventilatory control, particularly in those animals that live in environments with large changes in temperature, pH, $CO_2$ and $O_2$ levels;

2. the specific location and morphological/neurophysiological characterization of chemoreceptors—both central and peripheral—in air-breathing fishes and amphibians;
3. explanation of the lack of hypercapnic ventilatory responses in animals whose isolated brainstems prove to be exquisitely sensitive to $CO_2$ and pH;
4. the role, if any, of *efferent* innervation of chemoreceptors, and the associated extent of neuromodulation that might occur;
5. better understanding of the developmental changes in chemoreception, brought into an explicit "evo-devo" context;
6. the interaction of chemo- and mechano-receptors in the regulation of both aquatic and aerial gas exchange;
7. the effect of chronic hypoxia and hypercarbia (hypercapnia) on ventilatory behavior in bimodal breathers, especially during development when physiological plasticity may be at its greatest.
8. whether in animals heavily exploiting the cutaneous gas exchange the general body surface has respiratory chemo-receptors involved in facilitating behaviors or processes.

Since a strong interest exists in the evolution of chemoreception, as reflected in the many chapters considering this subject in the present volume, a final perspective is that investigators be encouraged to take a truly comparative, multi-species, systematic approach. Currently, we are typically faced with attempting to fit into a patchy mosaic of emerging information the results of an *in vivo* study of branchial denervation here, and an *in vitro* investigation of brainstem responses there, most likely carried out on distantly related species. The most rapid progress will come when, instead, a systematically robust and taxonomically relevant suite of species is concurrently investigated by the same investigators under the same experimental conditions with the same techniques. The rewards of such an approach, though demanding, will be manifold.

## Acknowledgements

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## Notes

1. It could be argued that "diffusion" and "convection" are indeed also "modes" of gas exchange, but here we shall confine the use of mode to structure rather than process.

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