The Respiratory Transition from Water to Air Breathing During Amphibian Metamorphosis[1]

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SYNOPSIS. Profound developmental changes occur in the morphology and physiology of the respiratory system of amphibians during the transition from strictly aquatic to dual aquatic-aerial breathing. This developmental transition usually involves modifications in ventilatory mechanisms and/or respiratory surfaces (*e.g.*, degeneration of gills, ventilation of functional lungs). Both the first appearance of obligate air breathing and the subsequent dependence upon it by amphibian larvae are sensitive to a variety of environmental stressors during critical developmental periods. These stressors include oxygen availability, ambient temperature, the risk of predation and mode of feeding.

INTRODUCTION

Bimodal breathing in animals has been studied for more than a century, with the last three decades seeing increasing numbers of studies on the physiology and anatomy of animals that breathe both air and water (see Johansen, 1970; Randall *et al.*, 1981; Little, 1983, 1990; Shelton *et al.*, 1986; Burggren and McMahon, 1988; Boutilier, 1990). Bimodally breathing animals have been studied both in their own right, as fascinating animals that have evolved often rather complex respiratory processes and structures to exploit simultaneously both water and air breathing, as well as paradigms for understanding the evolutionary transition from marine and/or fresh water to an amphibious or terrestrial existence.

Unfortunately, almost all of the data available to date describe the form and function of *adult* animals. While under-

standing the adult form of a species is certainly important, it is no more important than understanding any of the intermediate developmental stages, particularly since extensive natural selection occurs throughout development prior to maturation (Wassersug, 1975; Burggren, 1992).

While our knowledge of the developmental transition leading to bimodal breathing in adult fishes is truly "embryonic," somewhat more is known about the onset of air breathing (and thus the development of bimodal breathing) in aquatic amphibian larvae. The purpose of this article is to describe developmental changes in the respiratory system of amphibians during this transition from strictly aquatic to dual aquatic-aerial breathing. Though it will be necessary to describe some developmental events in aquatic larvae prior to the onset of obligate air breathing and in air breathing adults after metamorphosis, the emphasis of our discussion will be on the *transition* to air breathing in aquatic larvae. Also, our discussion will emphasize anuran and to a lesser extent urodele larvae, leaving apodan larvae virtually undescribed, reflecting the discrepancies in what is known about development within the various taxa.

FUNCTIONAL MORPHOLOGY OF THE WATER TO AIR BREATHING TRANSITION

Most amphibians, as either larvae or adults, use two basic mechanisms for gas exchange. The first mechanism involves simple diffusion of respiratory gases across the skin. This is a "passive" mechanism, in that ventilation of the skin is not required. The second mechanism is "active," in that muscular effort is involved in generating a flow of respiratory medium over the respiratory surfaces, which take the form of gills or lungs (with possibly a small contribution by buccopharyngeal surfaces in some species). In addition to active ventilation of the gills and lungs, water currents generated by body movements may augment cutaneous gas exchange in some species (see Feder and Burggren, 1985).

The developmental transition from water to air breathing is usually accompanied by modifications in ventilatory mechanisms and/or respiratory surfaces. The extent of these modifications depends greatly upon the species, and the developmental patterns they show. In ranid anurans, for example, gas exchange is achieved largely across cutaneous surfaces in early larvae, with probably a minor contribution from external tuft-like gills that contain a few capillary loops. Quickly these external gills are replaced by filamentous internal gills located on four gill arches. The paired branchial cavities that envelop these gills are ventilated by dorsoventral movements of the buccal floor (Gradwell 1972a, b), which generate a unidirectional flow of water in the mouth, through the gill sieve, and out the single opercular spout located on the animal's left side. This form of ventilation appears homologous with that of fishes. Even in amphibian larvae with well developed internal gills, the skin still makes an important contribution to gas exchange (Burggren and West, 1982; see below).

The transition from strictly water to combined water and air breathing is not necessarily coincidental with the loss of gills. As discussed below, some amphibians only begin to air breathe at metamorphic climax, at which time the internal gills degenerate. In many ranid larvae, however, air breathing begins in the early aquatic larval stages,

well before gill loss, and these larvae concurrently use skin, gills and lungs to breathe. Obviously, adequate lung development must precede or be concurrent with a developmental transition to respiration that involves pulmonary gas exchange. Structurally, the lungs of most anuran larvae are present very early in larval development, appearing initially as simple thin-walled sacs. In ranid larvae they begin to develop primary, secondary and tertiary septa beginning at Taylor-Kollros (TK) St. V (hind limb buds present), and are essentially adult in form in metamorphosing animals at TK St. XXII (Atkinson and Just, 1975; Burggren and Mwalukoma 1983; Dupré et al., 1985; Burggren, 1989). Aquatic anuran larvae ventilate their developing lungs using buccal pumping. While the mechanics of lung ventilation have been described for adult anurans (de Jongh and Gans, 1969; West and Jones, 1975; Jones, 1982; Vitalis and Shelton, 1990), studies on the functional transition from buccal movements designed to generate water flow over the gills to those designed to fill the lungs in aquatic amphibian larvae just beginning to breathe air are few (de Jongh, 1968). Some bufonids and *Ascaphus* only begin to air breathe at metamorphic climax, at which point the buccal pump and surrounding structures have taken on the adult morphology and presumably operate as in fully metamorphosed adults. Indeed, full lung development and ventilation is delayed until just before or during metamorphic climax in almost all stream-dwelling anuran larvae (Nodzenski et al., 1989).

In most amphibians, regardless of the extent to which gills and lungs develop and are ventilated, gas exchange across the highly vascularized skin accounts for a large proportion of total oxygen consumption and carbon dioxide elimination both before and after the onset of air breathing (see below). In most urodeles, the blood supply to the skin of the trunk is derived directly from the systemic vertebral arteries. In ranids, however, the skin of the trunk receives a dual supply from the segmental vertebral arteries and from the cutaneous artery, the latter derived from the pulmocutaneous artery that conveys blood with lower oxygen

content, thereby enhancing O_2 uptake across the skin.

Transitions in Gas Exchange Partitioning and Ventilatory Pattern

The respiratory transition from water to air breathing in amphibians is not a simple linear sequence from diffusion of gases across the body surface to aquatic gas exchange with gills to aerial gas exchange with lungs. Rather, for the greater part of larval development and adult life, most amphibian species exchange gases at two and even three sites simultaneously. In considering interspecific comparisons, the partitioning of gas exchange among multiple sites depends on natural selection pressures that have led to the evolution of enhanced performance of one site over another. Within an individual, the partitioning of gas exchange among multiple sites is influenced acutely by changes in O_2 and CO_2 levels and temperature, as well as predation, feeding modes and sexual behaviors.

Throughout much of their development, most amphibian larvae depend primarily upon aquatic exchange of both O_2 and CO_2. Prior to metamorphosis in anurans, cutaneous exchange of O_2 and CO_2 predominates even after "active" processes such as gill and lung ventilation begin (see Burggren and West, 1982; Burggren et al., 1983; Feder and Burggren, 1985). Larval Ambystoma tigrinum obtain 60% of their O_2 via aquatic gas exchange at 23°C (cutaneous and branchial) (Heath, 1976). The skin serves as the predominant site of CO_2 excretion in anurans, urodeles and apodans throughout all of larval development and adult life (Feder and Burggren, 1985). Some urodeles, notably the entire family Plethodontidae, exchange gases exclusively across the skin after metamorphosis, for the adults lack both gills and lungs.

External gills develop in embryonic urodeles and apodans, and develop before or shortly after hatching in anurans (Noble, 1931; Duellman and Trueb, 1986). In urodeles, external gills become more morphologically complex and are retained throughout larval development, as well as in neotenes (see Guimond and Hutchison, 1976; Malvin, 1989). In the large neotenic urodeles, the importance of the gills in O_2 exchange greatly varies, from 2.5% of total in Siren to 60% in Necturus at 25°C (Guimond and Hutchison, 1976). Comparable studies in other urodeles that undergo metamorphosis are lacking.

In most anurans, the morphologically simple external gills are replaced by far more complex internal gills (McIndoe and Smith, 1984). In Rana catesbeiana and Rana berlandieri, the gills contribute most significantly to O_2 and CO_2 exchange prior to the onset of metamorphosis (TK St. XVI), accounting for about 20–40% of O_2 and CO_2 exchange (Burggren and West, 1982; Burggren et al., 1983). However, in spite of the active ventilation of internal gills, the skin accounts for a greater percentage of total O_2 and CO_2 exchange than the gills throughout all of larval life (Burggren and West, 1982).

The transition to air breathing in many amphibians and urodeles begins with the appearance of occasional air breaths superimposed upon the basic larval pattern of aquatic (cutaneous + branchial) gas exchange. The specific time in development when lung ventilation begins varies widely in anurans, but occurs well prior to metamorphosis in many species (Feder, 1984; Burggren and Pinder, 1991). For example, air breathing has been observed in the earliest stages after hatching in ranid larvae (Wassersug and Seibert, 1975; Burggren et al., 1983; Feder, 1983a). In other anurans, lung ventilation does not begin until metamorphic climax (e.g., bufonids, Ascaphus), or in direct-developing frogs until development is complete and hatching occurs (Wassersug and Seibert, 1975).

Unlike in Xenopus, where air breathing is crucial for gas exchange even in very young larvae (Wassersug and Murphy, 1987), the infrequent air breaths of ranid larvae contribute little to meeting overall gas exchange needs at rest (Burggren and West, 1982; Burggren et al., 1983; Feder, 1983a). Indeed, when ranid larvae are prevented from surfacing, or when the lungs are surgically ablated, there is little effect on total $\dot{V}_{O_2}$ and only transient effects on gill ventilation frequency (Helff, 1931; Feder, 1983a; Infantino, 1992). Larval Rana pipiens have even been observed to complete metamorphosis

after the lungs have been surgically ablated (Helff, 1931). However, the lack of a significant role of lung ventilation under resting, normoxic conditions *does not* imply that the lungs are non-functional. Sharp increases in lung ventilation are observed during hypoxic and hypercapnic exposure (see below), as well as after intense activity (Quinn, 1982). In fact, in severe aquatic hypoxia pulmonary ventilation accounts for almost all of total O_2 uptake in *Rana berlandieri* at 25°C (Feder, 1983a).

In many urodele species, lung ventilation begins in larval stages. Pulmonary O_2 exchange accounts for 40% of total $\dot{V}_{O_2}$ at 23°C in larval *Ambystoma tigrinum* (Heath, 1976). In other primarily aquatic urodeles, pulmonary gas exchange plays a minor role in adults (Guimond and Hutchison, 1976), and presumably in larvae as well. Vestiges of the lungs appear as a depression on the floor of the embryonic esophagus in some lungless urodeles, but never fully develop to participate in gas exchange (see Burggren, 1989).

In the majority of adult anurans, urodeles and apodans, lung ventilation is the primary means of oxygen exchange. Little is known about the phylogenetic diversity of metamorphic transitions from the larval pattern, with O_2 exchange at multiple sites, to the adult pattern, in which O_2 exchange occurs primarily in the lungs in many species. The bullfrog *Rana catesbeiana* exhibits a rather abrupt transition from larval to adult lung ventilation pattern during metamorphic climax, as shown in Figure 1. Lung ventilation is infrequent in larvae younger than St. XXII in normoxic water, although lung ventilation frequency (f_L) increases during hypoxic and hypercapnic exposure (Burggren and West, 1982; Burggren and Doyle, 1986; Infantino, 1992). During the developmental transition from St. XXII to St. XXIV, resting f_L increases sharply, nearly 20-fold greater than the frequency just a few days earlier in development at 25°C. Air breathing behavior also changes. Prior to St. XXII, larvae swim to the surface to ventilate the lungs in a single inflation/deflation cycle in which air is captured through the mouth. Stage XXIII–XXIV marks the onset of a breathing pattern in which animals float at the surface with only the nares breaking the plane of the water's surface, a pattern which persists in post-metamorphic animals. The developmental transition in lung ventilation pattern occurs over the course of one to several hours at 25°C. Bouts containing more than one air breath are occasionally observed in froglets after periods of voluntary diving. Bout breathing becomes a more prominent feature of the ventilatory pattern when animals are exposed to hypoxia and/or hypercapnia, as has been previously reported for adult anurans (see Shelton *et al.*, 1986; Boutilier, 1990 for review).

ENVIRONMENTAL STRESSORS AND GAS EXCHANGE DURING THE WATER TO AIR BREATHING TRANSITION

The morphological and physiological transition from water breathing to air breathing in amphibian larvae is sensitive to a variety of environmental stressors during critical developmental periods. The effects of fluctuations in oxygen availability on gas exchange during the water to air transition have been studied extensively, because oxygen depletion is a common stressor in natural environments. Elevated carbon dioxide levels have been used to probe regulation of gas exchange experimentally, but environmental hypercapnia is relatively rare. Temperature has also been heavily studied because of its pervasive influence on physiological processes. Additional factors affecting gas exchange during the water to air transition include the risk of predation and mode of feeding.

Temperature

Temperature is one of the most fundamental of environmental stressors, altering almost all biological processes through its action on basic chemical reactions supporting physiological processes. Investigation of temperature effects on gas exchange in bimodal breathers have revolved primarily around changes in gas exchange partitioning and measurements of Q_{10}s for ventilation and metabolism. Generally, observations of adult bimodally breathing vertebrates, including air breathing fishes (*Amia, Lepisosteus*) and adult frogs and salamanders (*Xenopus, Amphiuma, Siren*), indicate that an
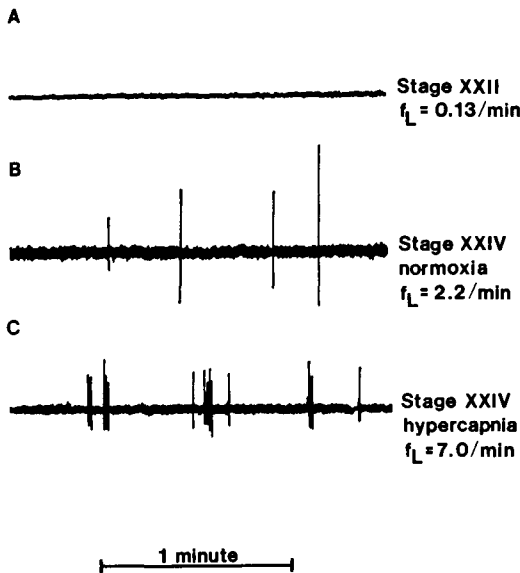
FIG. 1. Transition in lung ventilatory pattern during metamorphic climax in *Rana catesbeiana* (6.6 g at St. XXII, 25°C). Trace A shows lung ventilation movements recorded from the animal in normoxic water, measured by impedance pneumography. Lung ventilation is rare in larvae at this stage, leading to very low values for lung ventilation frequency (f_L), and no air breathing was recorded during the sampling period shown. With development to St. XXIV (one to two days at 25°C), breathing pattern changes fundamentally (Trace B), with frequent lung ventilation now characteristic. Bouts containing multiple lung ventilations were seen occasionally in animals breathing normoxic water, especially after free diving. The f_L increased during hypoxic and/or hypercapnic exposure, and bout breathing was characteristic of the response to these treatments (Trace C; P_{O_2} = 145 mmHg, P_{CO_2} = 8.1 mmHg).

increase in temperature usually produces a shift of oxygen uptake towards the lungs and away from aquatic gas exchange sites (see Burggren *et al.,* 1983 for references).

Temperature effects on gas exchange in larval amphibians appear to differ from those of adult air breathing fishes or amphibians, however. Amphibian larvae generally show little change in gas exchange partitioning between the gills/skin and the lungs as temperature changes. For example, in air breathing larvae of the frogs *Rana catesbeiana* and *Rana berlandieri*, gas exchange partitioning is only very slightly affected from 15–33°C, even though total

oxygen uptake and carbon dioxide elimination increases, with a Q_{10} of nearly 2 over this same temperature range (Burggren *et al.,* 1983).

Unknown, but of particular interest, is whether high temperatures and their attendant increased metabolic rates and decreased water oxygen contents will result in the onset of obligate air breathing at an earlier stage of development to escape this "functional hypoxia." Studies addressing this would be extremely interesting (though not trivial to interpret, due to the effect of temperature on growth rate, food assimilation and body mass, etc.). An additional consideration is that changes in ambient temperature may cause a functional shift between obligate air breathing and strictly water breathing in larvae. For example, larval *Ambystoma tigrinum* at 25°C breathe air every 3–8 min, but when maintained at 5°C (an ecologically relevant temperature experienced in winter) they reduce air breathing to once every 30 min or hour or eliminate it altogether (Burggren and Wood, 1981). The respiratory and acid-base physiology changes accordingly, reflecting either the unimodal or bimodal state of gas exchange.

Oxygen availability

The effects of hypoxia on respiration in metamorphosing amphibian larvae have been well documented, for both urodeles and anurans (see Burggren and Just, 1992; Infantino, 1992 for extensive discussion and references). The nature of the response depends upon whether hypoxic exposure is acute or chronic, and whether it is strictly aquatic or both aquatic and aerial. Acute aquatic hypoxia with continued availability of normoxic gas for air breathing is the most natural form of hypoxic stress, though amphibians living at high altitude would experience both aquatic and aerial hypoxia. In *Rana catesbeiana* aquatic hypoxia alone generates increases in both gill and then lung ventilation (West and Burggren, 1982; Burggren and Doyle, 1986; Infantino, 1992). Combined aerial and aquatic hypoxic intensifies these ventilatory responses (Infantino, 1992). Larvae chronically exposed to both

aquatic and aerial hypoxia exhibit hypertrophy of respiratory surfaces. The functional exchange surface of the gills increases, and the lungs assume greater internal partitioning and volume (cf., Bond, 1960; Burggren and Mwalukoma, 1983). Additionally, the skin capillary network becomes more dense (adding respiratory surface area) and the individual capillaries move closer to the skin's surface (reducing blood-environment diffusion distance for gases).

As is the case for studies on the influence of temperature on larval gas exchange, studies of how oxygen availability affects the morphological and physiological development of gas exchange organs have been directed primarily at larval amphibians that are already breathing air. The effects of chronic hypoxia on the developmental timetable for the onset of air breathing have not been studied systematically. Whether hypoxia could prematurely induce those genes that direct the development of the lungs and the physiological processes that lead to their ventilation remains unknown.

Predation

Another important environmental stress that has been implicated repeatedly in the multiple independent evolutions of air breathing in vertebrates is the presence of predators and prey items in the aquatic and/or aerial environment (Randall et al., 1981; Little, 1983, 1990; Graham, 1994). Being preyed upon and/or being a predator/forager impacts upon air breathing in two ways.

The transition to air breathing in an aquatic amphibian larva increases its risk of predation. Obviously, the onset of obligate air breathing means that repeated visits to the air-water interface must be made. Each air breath carries with it the risk of aquatic predation during movement through the water column, and of aerial predation when the surface is broached (Feder, 1983b). In an intriguing study that integrates behavioral ecology and respiratory physiology, Lannoo and Bachmann (1984) showed that larval *Ambystoma tigrinum* reduce air breathing frequency during the daylight hours, presumably also reducing the risk of aerial predation. Adult male newts have also

been shown to reduce air breathing frequency for "non-respiratory reasons," in this case to ensure maximum time of contact with females during the breeding season (Halliday and Sweatman, 1976). No specific alterations of air breathing related to predation have been described in anuran larvae, but they probably exist, since anuran larvae certainly suffer heavily from predators in both water (frogs, turtles, snakes) and air (herons, kingfishers, etc.) (Wassersug and Sperry, 1973). Certainly, air breathing fishes show a variety of respiratory behaviors both as individuals (single breaths rather than a bout of breathing) and as groups (e.g., synchronous rising to the surface for air breathing) that appears to minimize the risk of aerial predation (see Kramer et al., 1983; Kramer 1988).

Foraging (most anuran larvae) or searching for prey (urodele and apodan larvae) can also impact upon air breathing. Larval *Xenopus laevis* are obligate suspension feeders whose gills have prominent gill filters rather than respiratory filaments. This species experiences "functional conflicts" between feeding and aerial gas exchange under some environmental conditions (Seale et al., 1982; Feder et al., 1984). Aquatic foraging or predatory behavior often means that the larva leaves an otherwise safe hiding spot and enters into the water column. Air breathing would then introduce an additional risk of predation. However, movement into the water column also potentially involves changes in buoyancy. Short-term changes in buoyancy, in turn, depend upon adjustments in lung volume which can be affected by adjustments in the rate and/or depth of air breathing (see Burggren, 1989 for discussion). Returning to Lannoo and Bachmann's (1984) study on larval *Ambystoma tigrinum*, larvae presented with benthic food items reduced air breathing frequency and buoyancy compared with those that preyed on pelagic zooplankton in the middle of the water column. Further interactions between air breathing, buoyancy and risk of predation were evident, however. Air breathing frequency was relatively high (to increase lung volume and buoyancy) if pelagic zooplankton were present during the

day, but was higher still if pelagic zooplankton were present during the night, when the larva's own risk of aerial predation was reduced! Buoyancy changes associated with air breathing also weigh into evaluation of locomotion in anuran larvae (Wassersug and Feder, 1983). In larval *Xenopus laevis*, for example, the benefits from a respiratory viewpoint of air breathing may be offset by the greater cost of locomotion resulting from the increased buoyancy produced by the air-filled lungs.

Clearly, the potential exists for complex, dynamic interactions between buoyancy regulation, respiratory needs, feeding and predation risk in larval amphibians. More studies of an interdisciplinary nature by respiratory physiologists and behavioral ecologists are needed to understand fully the impact of environment on the transition to air breathing.

SUMMARY

Most amphibian larvae make the transition from strictly water breathing to combined water and air breathing before metamorphic climax. The transition from water to air breathing requires the development of functional lungs, possible anatomical modifications to the buccal pump for ventilating the lungs, and neurophysiological changes in the patterns of motor output to ventilatory muscles. During this transition, oxygen uptake (and carbon dioxide elimination, to a much lesser extent) shifts from skin and gills towards lungs.

While the presence or absence of respiratory structures and processes during amphibian development is genetically "predetermined" or "fixed," the timing of their first appearance, and their magnitude after they appear, may not be. For example, that bullfrogs develop lungs as larvae is an absolute. However, the time of onset of air breathing and the relative size of the lungs as they develop may be modified by environmental factors such as temperature, oxygen availability, or predation. Additional studies that elucidate the developmental malleability of the respiratory transition from water to air breathing using these factors as experimental variables will be informative, indeed.

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