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ONTOGENY OF REGULATION OF GILL AND LUNG VENTILATION IN THE BULLFROG, *RANA CATESBEIANA*

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Abstract. Gill and lung ventilatory frequencies at 20–23 °C were recorded in five different larval stages and in the adults of the bullfrog, *Rana catesbeiana* (n = 101, body mass 40 mg–90 g). Ventilatory frequencies in unanesthetized, unrestrained animals were determined (1) after normoxic acclimation, (2) during acute hypoxic and hyperoxic exposure, and (3) after brief but intense activity. Gill ventilation frequency (f_G) under normoxic, resting conditions was 110–120 cycles · min^{-1} immediately after hatching, but fell to and remained at 40–50 cycles · min^{-1} for the remainder of larval development. Activity caused a sharp decrease in f_G in newly hatched larvae, a sharp increase in larvae between stages IV–XIV, and no change in f_G in all older larval states. Hypoxia increased f_G in younger larvae up to developmental stage XIV, but had no effect upon f_G in older larvae. Lung ventilation was rare in normoxic, resting larvae up to stage X. Thereafter until metamorphosis lung ventilation frequency (f_L) was 2–6 breaths · h^{-1}, with f_L in adults being much higher at 1–3 breaths · min^{-1}. Activity did not affect f_L in any larval stage, but markedly increased f_L in adults. Hypoxia had no significant effect on mean f_L in larvae below stage XX. Mean values of f_L increased during acute hypoxic exposure in most adults, but these changes were not significant. Collectively, these data indicate that progressive larval development is accompanied by a decline in reflex regulation of branchial ventilation frequency well before reabsorption of gills occurred. At the same time, respiratory responses are 'transferred' to the lung prior to metamorphosis and the attendant increasing dependence on air breathing.

Amphibian Hypoxia Ontogeny
Gill Lung Ventilation

The early respiratory efforts of the mammalian fetus represent but physiological rehearsals for the transfer of respiratory processes at birth – they need not be effective responses since the respiratory needs of the fetus are met by the placenta. This situation of full respiratory support for the developing mammalian fetus until essentially adult pulmonary function develops is in stark contrast with that of many lower vertebrates.

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In many amphibians, for example, hatching from the egg occurs within a few weeks of fertilization, so survival as a completely free-living entity begins at a comparatively very immature stage of morphological and physiological respiratory development. In many amphibian species the respiratory needs of the larvae immediately after hatching are probably met largely by diffusion through the body wall (see Burggren, 1984). However, both increases in body mass and overall metabolic demand require the progressive development of more effective respiratory structures (gills, lungs) in these developing larvae, since there is no luxury of a placental analogue to provide gas exchange while the respiratory organs of the larva and adult are forming. These structures must develop while the larva is completely exposed to an environment that is potentially limiting to gas exchange.

A corollary of this precocious development of respiratory structures is the need for relatively early development of functional respiratory reflexes. Indeed, an increase in the frequency of gill and lung ventilation in response to aquatic hypoxia has been demonstrated approximately half way through larval development in ranid frogs and salamanders (for references see Feder, 1983a; West and Burggren, 1983). Interestingly, lung ventilation transiently inhibits gill ventilation in the frog larvae, indicating complex reflex interactions well before metamorphosis (West and Burggren, 1982). These responses apparently are mediated by both mechano- and chemoreceptors intimately associated with the lungs and gills (West and Burggren, 1983).

Unfortunately, no systematic study of amphibians has been made to determine at what embryonic or larval stage respiratory reflexes, in particular those related to oxygen availability, exercise and gill/lung interactions, first occur. It is also unknown how these reflexes change with development as respiration shifts from water breathing with gills towards air breathing with lungs. We emphasize that not only must reflexes associated with lung ventilation and perfusion *develop* as larval maturation proceeds, but the complex reflexes regulating buccal pumping for gill ventilation must be *modified* or *abolished* as the gills degenerate during metamorphosis. The ontogeny of respiratory control in amphibians thus represents an interesting case study in which one complex set of respiratory reflexes is necessarily superseded by another as a part of normal development.

In this study on *Rana catesbeiana* we describe the general developmental transitions in ventilatory frequency responses of the gills and lungs to resting, active and hypoxic/hyperoxic conditions.

Materials and methods

Measurements of gill and lung ventilation frequency in the bullfrog, *R. catesbeiana* were made at 6 different developmental stages (Taylor–Kollros scheme). Stage groups, with mean body mass, were as follows: I (40 mg), IV–VII (2.2 g), X–XIV (12.0 g), XVI–XIX (21.1 g), XX–XXIV (8.5 g), postmetamorphic adults (70.4 g). Adults were acquired from commercial suppliers, while all larval stages were captured in Hampshire County,

Massachusetts. The animals were maintained in the laboratory (20–23 °C, 12 : 12 photoperiod) for at least 1 week before experimentation, and were regularly fed either spinach (larvae) or flies and maggots (adults).

Animal preparation and protocol. Essentially, experimental techniques, protocols and analyses were very similar to those described in detail in a previous study on the ontogeny of cardiac regulation in larval and adult bullfrogs (Burggren and Doyle, 1986). The reader is directed to this study for additional information. Briefly, gill ventilation movements in unanesthetized Stage I larvae were monitored visually. In older developmental stages, fine electrodes consisting of #36–#45 gauge copper wire were inserted under MS-222 anesthesia on either side of the mouth. The electrodes were connected to a UFI 2991 Impedance Convertor, whose signal was further processed and displayed by a Narco MK-IV rectilinear recording system. This technique allowed direct recording of gill and lung ventilation movements in unrestrained animals as small as 1–2 g (Burggren and West, 1982). Animals were placed in a small, water-filled aquarium in which the ambient P_{O_2} could be varied at will (see Burggren and Doyle, 1986).

All animals were given at least 12 h under normoxic conditions to recover after electrode implantation. Following this recovery period, recordings were made under normoxic, resting conditions to generate the first of several 'control' sets of data. Ambient P_{O_2} was then decreased progressively over a 30 min period from 150 mm Hg to 90 mm Hg. Gill and/or lung ventilation frequencies almost always stabilized within 10–15 min at this new P_{O_2} level. After recording stabilized ventilation frequencies at 90 mm Hg, the water P_{O_2} was reduced to 60 mm Hg over 30 min, and the stabilized ventilation frequency at this new P_{O_2} recorded. This procedure was repeated once again at a P_{O_2} of 30 mm Hg, after which P_{O_2} was raised to >500 mm Hg for a final measurement of ventilation frequency in this experimental series. Following exposure to hyperoxia, animals were returned to normoxia (about 150 mm Hg) for 1–2 h.

Ventilation frequencies at the end of this second normoxic recovery period were not significantly different ($P > 0.1$, see below) from the resting, control rates recorded before hypoxic/hyperoxic treatment.

After this second normoxic acclimation period, each animal was mechanically stimulated to produce intense, sustained locomotor movements for 1 min duration. (Exhaustion occurs after approximately 5 min of such activity – Quinn and Burggren, 1983.) Recordings of ventilation frequency in response to activity were then begun immediately and continued for 15 min.

Statistical analyses. All data were expressed as mean $\pm$ 1 SE. The significance of 'treatment' effects (developmental stage of a given experimental condition, ambient P_{O_2} or activity for a given developmental group) was assessed with one-way Analysis of Variance (ANOVA). Where ANOVA revealed significant treatment effects, differences between individual means within a developmental group were subsequently assessed for significance with the 'T-method'. This procedure allows for unplanned multiple comparisons among pairs of means based on equal sample size, and generates a

minimum significant difference (MSD) by which two means must differ. A fiducial limit of 0.05 was adopted for all analyses.

Results

Gill ventilation

(1) *At rest.* The frequency of buccal pumping for gill ventilation (f_G) at rest was highly dependent upon developmental stage (fig. 1A). Immediately after hatching (Stage I), f_G was 100–120 cycles · min^{-1}. Interestingly, at this point in development the gills are still external extensions of the body wall and the buccal movements are not yet effectively ventilating the gills. However, with further larval development to Stage IV–VII, the gills became enclosed internally within paired branchial chambers. Concomitantly, f_G fell sharply to about 50 cycles · min^{-1}, a level characterizing resting f_G in all older larval stages (fig. 1A). At metamorphic climax, the gills degenerate and disappear. However, rhythmic buccal movements at a frequency of 40–80 cycles · min^{-1} persisted in the adult bullfrog.

Buccal cycles producing gill ventilation were usually continuous at rest in all larval stages examined, though spontaneous periods of apnea lasting from a few seconds to a few minutes were observed occasionally in most larvae. In larval stages that had begun lung ventilation (Stage XVI and beyond – see below), an air breath consistently produced a transient alteration of the pattern of gill ventilation (fig. 2A). Generally, within seconds of an air breath, f_G fell sharply by 10–20 cycles · min^{-1}, not returning to the pre-breath frequency for 1–3 min (fig. 2A). The amplitude of the impedance signal recorded from the buccal cavity varied in direct proportion with f_G. While this signal was not calibrated for amplitude of buccal movement, buccal pressures similarly decrease following an air breath in Stage XVI–XIX larvae of *R. catesbeiana* (West and Burggren, 1982).

(2) *Following activity.* Spontaneous, voluntary locomotion was observed in all developmental stages. In larvae immediately post-hatch, these periods of activity were relatively brief, and rarely caused any increase in f_G. In larvae from Stage IV and older, spontaneous activity was usually accompanied by a large increase in f_G (fig. 2B).

Experimentally induced activity of one min duration produced significant changes in f_G in most developmental stages examined (fig. 1A). While the pattern of change in gill ventilation frequency following induced activity was consistent within developmental stages, the response was rather complex when comparing the six developmental stages examined. In newly hatched Stage I larvae, induced activity decreased f_G by more than 40% ($P < 0.01$). Yet, induced activity in larvae of developmental Stages IV up to XIV had the opposite effect, increasing f_G by nearly 50%. At Stage XVI and beyond, however, induced activity produced no significant effect on f_G ($P > 0.10$).

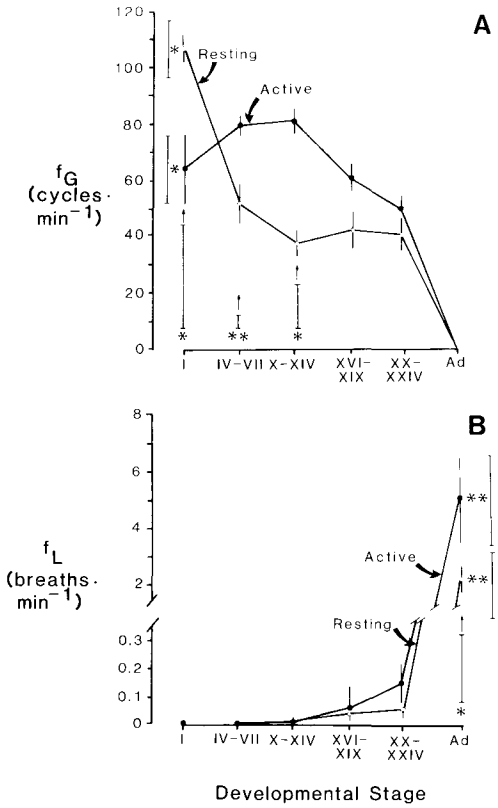

Fig. 1. Frequency of gill (A) and lung (B) ventilation as a function of developmental stage in unrestrained *R. catesbeiana*. Frequencies were measured in normoxia at rest (○) and immediately following 1 min of induced intense activity (●). Mean values ± 1 SE are presented. Number of animals used is 13,11,12,12,13 and 15 from Stage I through Adult, respectively. The results of one-way ANOVA between means for different developmental stages in either resting or active conditions are indicated by asterisks (* = $P < 0.05$, ** = $P < 0.01$) along the margin of the graph immediately beside each line. Also shown for each condition, as a vertical bar beside the asterisks, is the Minimum Significant Difference (MSD) for unplanned multiple comparisons, by which two means for any given treatment must differ if the two means are significantly different. Significant levels of ANOVA and MSD between resting and active means within a single developmental group are indicated immediately below the means for that group. See text for further details.

(3) *During changing O_2 availability.* Figure 3 presents gill (and lung) ventilation frequency as a function of environmental P_{O_2} in six different developmental groups. Changes in f_G in response to hypoxia and hyperoxia vary greatly as a function of development, with three general patterns emerging. Stage I larvae showed highly significant (ANOVA, $P < 0.001$) changes in buccal pumping rates in response to oxygen availability within one day of hatching, even though at this early stage the external gills are not yet actively ventilated by buccal movements. At a P_{O_2} of both 90 and 60 mm Hg,

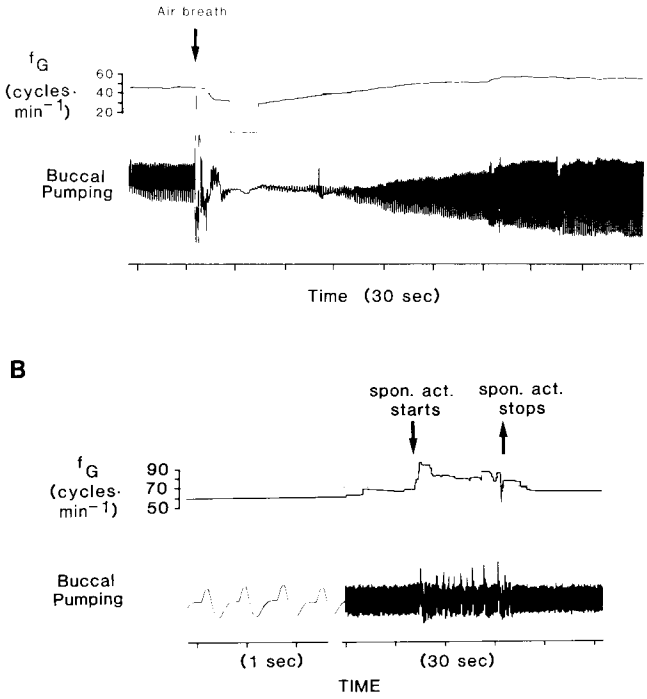

Fig. 2. Representative records of the buccal impedance signal associated with gill ventilation and of the instantaneous frequency of buccal pumping movements (fG) in unrestrained, normoxic larvae of *R. catesbeiana*. (A) Records from a 18.9 g, Stage XIX larva showing the effect of an air breath upon the buccal impedance signal and fG. (B). Records from a 15.0 g, Stage XIV larva showing the effect of spontaneous activity (swimming) upon the buccal impedance signal and fG.

fG was significantly elevated above normoxic levels ($P < 0.05$). However, severe hypoxia (about 30 mm Hg) had an apparent depressive effect on fG which fell to 1/2 of that rate during normoxia. Hyperoxia ($P_{O_2} > 500$ mm Hg) caused an almost total cessation of gill ventilation, indicating a strong hypoxic drive for gill ventilation.

In larvae of Stage IV–XIV, fG was also significantly affected by ambient P_{O_2} (ANOVA, $P < 0.05$), but the pattern was quite different from that of younger larvae (fig. 3). As ambient P_{O_2} declined, fG increased progressively, showing the highest rates at the lowest P_{O_2}. On the other hand, hyperoxia, which severely reduced fG in Stage I larvae, had no significant effect in these intermediate larval groups.

Larvae of Stage XVI and older showed yet a third pattern of gill ventilatory response to varying ambient O_2. Essentially, fG was no longer stimulated by decreasing ambient P_{O_2}, even at the lowest P_{O_2} tested (ANOVA, $P > 0.10$). Similarly, hyperoxia caused no change from the resting, normoxic fG.

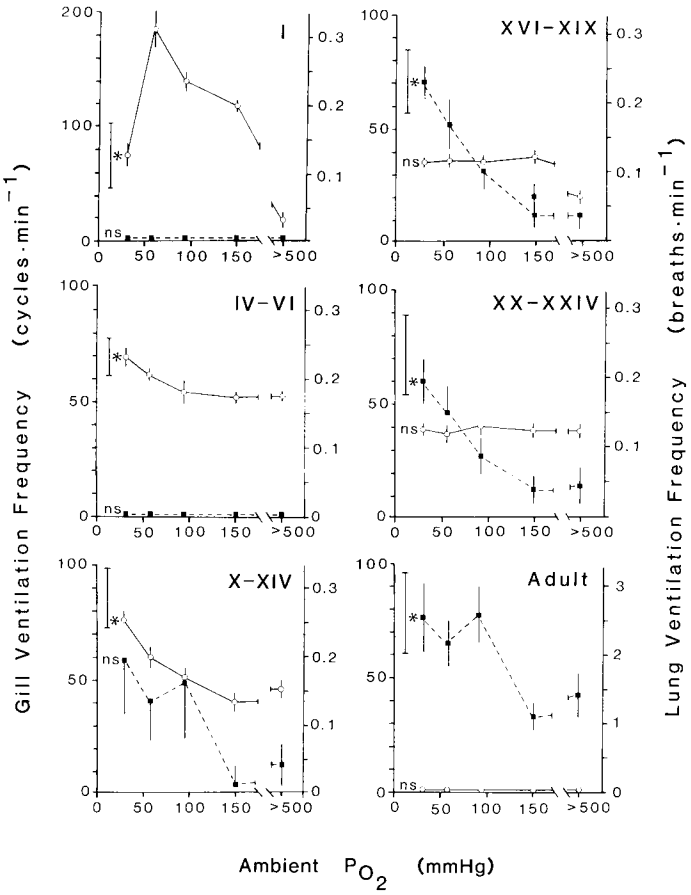

Fig. 3. Frequencies of gill ventilation (○———○) and lung ventilation (□- - - - - - - -□) during acute exposure to varying levels of ambient aquatic P_{O_2}. Statistical conventions are as in the legend for fig. 1. ns, not significant. See text for further details.

Lung ventilation.

(1) *At rest.* Air breathing frequency (f_L), like f_G, was highly dependent upon developmental stage ($P < 0.001$, fig. 1B). Air breathing under resting, normoxic conditions was a very rare occurrence up to Stage X, even though lungs have developed by at least Stage III. Even in Stages XVI–XXIV, an air breath occurred only about once every 15–20 min at 20 °C. After metamorphosis to the adult, f_L at rest increased greatly, ranging from 1–3 breaths · min^{-1}.

(2) *Following activity.* Neither spontaneous nor induced activity produced any significant effect ($P > 0.10$) upon resting f_L in any larval stage examined (fig. 1B), in spite of large changes in f_G.

In adults, however, f_L more than doubled immediately following induced activity (fig. 1B). Considerable variation in f_L existed between individual adults immediately following activity as well as during resting periods.

(3) *During changing O_2 availability.* Changes in F_L as a function of environmental P_{O_2} are presented in fig. 3. Even extreme hypoxia had no significant effect ($P > 0.10$) upon mean f_L in larvae at Stage VI or younger, with all ventilatory responses being manifested by adjustments in gill rather than lung ventilation. It is noteworthy, however, that moderate hypoxia in particular induced a considerable increase in f_L in a few individual larvae that showed some air breathing during normoxia, and stimulated the onset of air breathing in even a few Stage IV larvae that had previously shown no air breathing during normoxia.

By Stage IX of development, acute hypoxic exposure began consistently to produce a significant increase in f_L as well as f_G. All older larval stages showed a significant ($P < 0.05$) stimulation of f_L with reduced ambient O_2. Unlike for f_G, acute hyperoxic exposure had no significant effect upon f_L.

The effects of hypoxia and hyperoxia on f_L in adults were extremely variable when comparing individuals, as has been previously noted for adult anurans (Pinder and Burggren, 1986). Nonetheless, mean levels of f_L nearly doubled at P_{O_2} values of 90 and lower – these increases were statistically significant ($P < 0.05$).

Discussion

Regulation of branchial ventilation frequency. Gill ventilation frequencies in resting, normoxic *R. catesbeiana* changed in a marked and complex fashion as development proceeded from hatching to metamorphosis. Doubtlessly, these changes are a product of both a 'scaling' effect related strictly to the increase in body mass, which can be greater than three orders of magnitude from hatching to metamorphosis, as well as 'pure' developmental changes in morphology and physiology. In this context it is noteworthy that the pattern of decrease in resting cardiac frequency with larval development in *R. catesbeiana* (Burggren and Doyle, 1986) is very similar to the pattern of decrease of gill ventilation frequency observed in the present study. The ontogenetic changes in cardiac frequency, in fact, can be attributed largely to increasing body mass (Burggren, unpublished), assuming that the allometric relationship between f_H and body mass established for adult vertebrates also holds for larvae. In any event, the interactions between body mass, developmental stage and total oxygen uptake in larval anuran amphibians are extremely complex (Feder, 1982, 1983b), and it is not surprising to see these complexities reflected in rapidly changing patterns of ventilation and gas exchange of the three potential respiratory sites (skin, gills and lungs) (Burggren and West, 1982; fig. 1).

It is evident from this study that, even in morphologically very immature larvae of *R. catesbeiana*, the buccal mechanisms that ultimately produce ventilation of the gills

can be regulated in response to changes in environmental oxygen availability. Moreover, there is a strong hypoxic drive to gill ventilation frequency in newly hatched larvae, evidenced by the profound reduction in fG in hyperoxia. The developmental origin of these ventilatory responses to oxygen availability can be traced back to newly hatched larvae, even though at that early stage they have simple external gills that are ventilated only by body movements or ambient water currents rather than by buccal movements.

Newly hatched larvae showed a profound decrease in fG when ambient P_{O_2} fell below about 60 mm Hg. A similar sharp decrease in cardiac frequency occurs in newly hatched *R. catesbeiana* exposed to a similar level of ambient hypoxia (Burggren and Doyle, 1986). These responses may actually represent a direct, general depression of cardiorespiratory function during severe hypoxia in newly hatched larvae. Certainly, the strictly aquatic, newly hatched larvae of *Rana berlandieri* show considerably elevated whole body concentrations of lactate during severe hypoxic exposure, while later developmental stages apparently use a combination of increased branchial ventilation and lung ventilation to maintain aerobic respiration (Feder, 1983a). Ranid anuran larvae are generally thought of as highly tolerant to hypoxia in order to survive within thick jelly-like egg masses. Yet, recent measurements indicate that even the center of the egg masses of *Rana palustris* remain relatively well oxygenated until hatching of the eggs (Burggren, 1985), and so adaptations for surviving severe hypoxia may not have been strongly selected for in late embryonic and very early larval stages.

The period before incorporation of the gills into internal, paired branchial chambers ventilated by buccal movements lasts only a few days in ranid larvae. In all older developmental stages, buccal irrigation of the branchial chambers is crucial to maintain total oxygen uptake (Burggren and West, 1982). Modification of both buccal frequency and volume of water pumped per cycle, as distinct from pumping frequency, are a crucial part of the overall respiratory response to aquatic hypoxia in anuran larvae. In the present study, fG increased significantly with decreasing ambient P_{O_2} in all larvae up to about Stage XIV. In every developmental stage examined, however, significant increases in fG did not occur until ambient P_{O_2} fell below about 60 mm Hg. It is possible that the volume of water pumped per cycle increased at more moderate levels of hypoxia, but these two branchial variables generally change in synchrony in *R. catesbeiana* (Burggren and West, 1982). The oxygen affinity of the whole blood of larval *R. catesbeiana* is very high (P_{50} of 6–10 mm Hg, Pinder and Burggren, 1983), and it may require a relatively severe level of ambient hypoxia to begin to compromise blood oxygen transport and consequently stimulate gill ventilation.

In developmental stages of XVI and older, the failure of aquatic hypoxia to stimulate any reflex adjustment in fG does not necessarily indicate that the larvae are no longer sensitive to, or affected by, aquatic hypoxia, since hypoxia caused a profound stimulation of lung ventilation (see discussion below).

Intense activity is associated with an increase in both anaerobic and aerobic metabolism in larval anuran amphibians (Hillman and Lea, 1983; Quinn and Burggren, 1983; Wassersug and Feder, 1983). Although a complete developmental series for a given species has not previously been examined, increases in gill ventilation frequency

with activity have generally been reported for several specific developmental stages. The present study confirms these findings for the larvae of *R. catesbeiana*, but reveals several additional important effects that development apparently has on the gill ventilatory response to activity. Whereas larvae of Stages IV–XIV showed the anticipated increase in f_G immediately following intense activity, newly hatched larvae showed a sharp decrease in f_G to levels only about 60% of resting, normoxic values (fig. 1A). This depression, rather than the expected elevation, of f_G may represent a direct depression of ventilation due to the tissue and blood hypoxia that might reasonably be expected to accompany intense activity. This explanation is consistent with the depression of f_G and f_H in newly hatched larvae that occurs during ambient aquatic hypoxia (fig. 1A; Burggren and Doyle, 1986).

Unlike previous studies, the present study also records that intense activity had no significant effect upon f_G in larvae of Stage XVI or older, even though maximum total oxygen uptake in response to exercise continues to increase during development (Hillman and Lea, 1983). Clearly, the role of gill ventilation in maintaining oxygen uptake during either aquatic hypoxia or exercise peaks in the middle stages of larval development, declining thereafter as lung ventilation comes under increasing regulation (see below).

Regulation of lung ventilation. The developmental stage of anuran larvae at which air breathing actually begins is a point of some dispute for any given species, though the reasons for differing observations can probably be attributed largely to between-study variation in ambient temperature, oxygen availability, period of time allowed for acclimation, method of recording lung ventilation, etc. (see Burggren and West, 1982; Burggren *et al.*, 1983; Wassersug and Feder, 1983). In the present study, a few resting normoxic individuals began precocial lung ventilation in very early developmental stages, while others lagged well behind the developmental norm. Importantly, most larvae of Stages IX–XX, which showed low values of f_L at rest during normoxia, could be stimulated to much higher values by exposure to relatively mild aquatic hypoxia. The mean values that were presented in fig. 1B reveal, nonetheless, that normoxic, resting lung ventilation frequency generally increases very sharply after Stage XX. Unlike changes in f_G, this developmental change in f_L is apparently not related primarily to changes in body mass *per se*, since f_L is dramatically higher in post-metamorphic juveniles compared with late larval stages of the same body mass. The high resting levels of f_L in adults relative to pre-metamorphic larvae is no doubt related in part to the take-over by the lungs of that proportion of the oxygen uptake formerly achieved by the gills prior to metamorphosis (Burggren and West, 1982). It is important to emphasize, however, that the sharp rise in f_L at metamorphosis may also be related to both energetic and ecological considerations. Whereas post-metamorphic adult bullfrogs are generally at the water surface and thus can easily ventilate the lungs, air breathing in anuran larvae requires firstly, movement through the water column up to the surface and then, following an air breath, overcoming the increased buoyancy caused by filling the lungs (see Feder, 1984). Air breathing behavior is not only energetically expensive in terms

of locomotion, but also increases the risk of predation (see Feder, 1984, for discussion). Thus, there may have been 'non-physiological' phenomena which have selected against greater use of the lung in larval anurans possessing adequate gills.

Significant stimulation of lung ventilation frequency by exposure to aquatic hypoxia did not occur until development proceeded to Stage XVI. All larvae prior to this stage clearly were detecting aquatic hypoxia, since they modified gill ventilation appropriately. Even though lungs may be present and ventilated, pulmonary surface area is relatively small compared to that in later larval stages and in adults (Atkinson and Just, 1975). Changes in cardiovascular morphology favoring lung perfusion also occur relatively late in development (see Burggren, 1985). Thus, an increase in gill ventilation in response to hypoxia in early and middle larvae may be more cost-effective in terms of gas exchange than is an increase in pulmonary ventilation.

Activity has been reported to produce increases in air breathing frequency in both larval and adult anuran amphibians (for references see Wassersug and Feder, 1983). In the present study only post-metamorphic adults significantly elevated f_L in response to activity, with increases in f_G being the predominant respiratory response to activity in younger larvae. Curiously, then, larvae between Stage XVI and metamorphosis showed no significant elevation in either gill or lung ventilation frequency in response to brief intense activity. The present study cannot rule out that there were changes in pulmonary tidal volume (or branchial 'stroke' volume) and thus in total ventilation volume. However, changes in pulmonary ventilation volume in adult *Rana pipiens* are due primarily to changes in frequency rather than tidal volume (Pinder and Burggren, 1986). Alternatively, it is possible that 1 min of activity was insufficient to generate an internal hypoxia sufficient to trigger hyperventilation, though this is unlikely given the relatively low stamina of swimming larvae (Wassersug and Feder, 1983; Quinn and Burggren, 1983). Whatever the net change in ventilation volume of the gills or lungs of late larval stages, it is important to indicate that nearly 70% of the total oxygen uptake and over 85% of the total carbon dioxide elimination of late larval *R. catesbeiana* occurs not via gills or lungs, but rather via the skin (Burggren and West, 1982). Thus, factors in *Rana* that affect cutaneous exchange, such as changes in skin capillary recruitment or in skin ventilation by environmental water currents (Burggren and Moalli, 1983; Feder and Burggren, 1985, 1986; Burggren and Feder, 1986) could assist respiratory gas exchange during activity when neither f_G nor f_L are apparently increasing.

Co-ordination of ontogenetic changes in regulation of gill and lung ventilation frequency. Intuitively, adjustments in the regulation of ventilatory processes must occur with the developmental transition from a strictly aquatic, newly hatched larva to the primarily air breathing adult. Yet, how and when this adjustment occurs in development has not formerly been described for any vertebrate animal making this ontogenetic transition. The present study on *R. catesbeiana* has revealed that the effector component of the respiratory reflexes modulating ventilation frequency in response to aquatic hypoxia or intense activity is transferred from the gills to the lungs with relatively little developmental overlap. Acute adjustments in gill ventilation frequency dominate

early larval development, while it is primarily lung ventilation frequency that is modified during late larval development and in the adult.

Perhaps the most surprising finding of this study is that the onset of pulmonary regulation and the disappearance of branchial regulation do not occur progressively, but occur nearly simultaneously. Thus, only in the brief period in the middle of larval development do the animals show evidence of being in a transitional phase in which central motor output is distributed so as to generate changes in both gill and lung ventilation frequency. Similarly abrupt, non-progressive changes in the neural/hormonal regulation of cardiac frequency also occur in *R. catesbeiana* mid-way during larval development rather than primarily at metamorphosis (Burggren and Doyle, 1986). Together, these observations suggest that the commonly held view of metamorphic climax being the crucial period of morphological and physiological change in amphibians may require some re-evaluation.

Finally, it should be indicated that virtually nothing is known of the ontogeny of the afferent arm of the reflexes regulating gill and lung ventilation frequency. Whether branchial mechano- and chemo-receptors analogous or even homologous to those of fishes exist, let alone modulate ventilation, has yet to be described. Mechano- and chemo-sensitive pulmonary elements have been identified in larvae of *R. catesbeiana* of Stages XVII–XIX (West and Burggren, 1983), but whether these represent branchial structures incorporated into the lung vasculature during the profound developmental arrangement of the branchial vasculature, or whether they have arisen *de novo*, requires further investigation.

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