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RESPONSES TO ACUTE AQUATIC HYPOXIA IN LARVAE OF THE FROG *RANA BERLANDIERI*

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SUMMARY

The oxygen consumption of larvae of the frog *Rana berlandieri* Baird was reduced during exposure to aquatic hypoxia at 25 °C, and under severe hypoxia the larvae lost oxygen to the water. The larvae responded to aquatic hypoxia by increasing aerial oxygen consumption and lung ventilatory frequency, and also by altering their heart rate and gill ventilation frequency. Under severe or prolonged aquatic hypoxia without access to air, *Rana* larvae accumulated lactate. When prevented from breathing air, the larvae were unable to compensate fully by increasing their aquatic oxygen consumption. Body size or the interaction of body size and oxygen partial pressure significantly affected the aerial oxygen consumption, the total oxygen consumption and gill ventilation frequency, but did not affect other aspects of larval gas exchange. Anuran larvae resemble air-breathing fishes in some responses to aquatic hypoxia (e.g. increased dependence upon aerial oxygen uptake and changes in ventilatory frequencies), but are unusual in some ways (e.g. oxygen loss to the water). The interactions of body size and hypoxia are not sufficient to explain why so many anuran larvae without lungs are small.

INTRODUCTION

The respiratory patterns of amphibious vertebrates have attracted considerable attention in recent years (Johansen, 1970; Hughes, 1976; Randall, Burggren, Farrell & Haswell, 1981). A consensus among these and other studies is that the evolution of bimodal respiration was not a unique transitional event in the origin of terrestrial vertebrates from aquatic ancestors, but that it has evolved many times in response to adverse conditions commonly encountered by vertebrates in aquatic environments. Many adult fishes, amphibians and reptiles increase their dependence upon aerial oxygen uptake as aquatic oxygen declines, and show a variety of cardiac and respiratory responses that facilitate this transition. Obviously, the larvae of amphibians are of special interest in testing the generality of conclusions drawn mainly from adult vertebrates (Burggren & Wood, 1981; Burggren & West, 1982).

Although larval anurans (tadpoles) may encounter aquatic hypoxia in the field (Crump, 1981; Noland & Ultsch, 1981), aerial oxygen uptake of tadpoles has not been

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examined until recently (Feder, 1981*a*,*b*; Burggren & West, 1982; West & Burggren, 1982), perhaps because these organisms seem to be exclusively aquatic and their blood PCO_2, $[HCO_3^-]$, and pH are characteristic of water breathers (Just, Gatz & Crawford, 1973). Indeed, tadpoles have several respiratory surfaces that are well developed for aquatic gas exchange, including the gills, the vascularized opercula, and the skin (Strawinski, 1956). However, many tadpoles have lungs that they ventilate regularly, even at developmental stages well before the appearance of limbs (Just *et al.* 1973; Wassersug & Seibert, 1975). Moreover, tadpoles generally increase the frequency of pulmonary ventilation (f_{lung}) in response to aquatic hypoxia (Wassersug & Seibert, 1975). These morphological and behavioural observations prompted an examination of physiological responses to acute hypoxia in the larvae of a frog, *Rana berlandieri*, with particular emphasis on air-breathing.

In addition to examining the generality of vertebrate responses to hypoxia, a study of larval anurans may also provide insights into the scaling of the partitioning of oxygen uptake between air and water as well as the limitations imposed on gas exchange by body size. Because scaling of respiratory surface area with body size is allometric in amphibians, some large amphibians may face problems with diminished O_2 exchange capacity (Ultsch, 1976). Moreover, factors such as large size, an aquatic existence, high temperatures, activity, lunglessness and hypoxia may exacerbate respiratory supply problems, especially when amphibians must deal with two or more of these factors simultaneously (Ultsch, 1976; Feder, 1977). One possible result of these problems is that large larvae should depend more on aerial respiration than small ones. A second possible result (reviewed by Feder, 1977) is that amphibians lacking lungs would be unable to attain large body sizes. Indeed, a *prima facie* case for respiratory restriction to small size is evident in anuran larvae (Wassersug & Seibert, 1975). Bufonids (toads) lack lungs as larvae and metamorphose at relatively small sizes, even though adults of some bufonids (e.g. *Bufo alvarius, Bufo marinus*) are among the largest adult anurans. Generally, non-bufonid larvae, most of which have lungs, become larger than bufonid larvae before metamorphosis.

The present study examines (1) the significance of aerial gas exchange as a response to aquatic hypoxia in tadpoles of *Rana berlandieri*; (2) alternative responses to hypoxia, including increased gill ventilation and anaerobiosis; and (3) the effects of body size on the relative importance of aerial oxygen uptake. With a similar rationale, Burggren & West (1982) and West & Burggren (1982) have undertaken parallel studies of a congeneric species, *Rana catesbeiana*.

MATERIALS AND METHODS

Animals

The details of the origin and care of larvae appear elsewhere (Feder, 1981*a*). The experimental larvae were maintained at a constant temperature, 25 °C, on a constant photoperiod (LD 14: 10 centred at 13.00 local time) for at least 1 week before experimentation. All measurements were made under these conditions. The larvae used in the measurements of the rate of oxygen consumption ($\dot{V}O_2$) were not fed for 2 days before experimentation to minimize production of faeces and associat

crobial $\dot{V}O_2$ (Feder, 1981*a*). Even so, these larvae were not strictly post-absorptive as they continuously ingested faeces and detritus. The larvae in other experiments were not separated from food until 4 h before experimentation. All larvae were at developmental stages before emergence of the forelimbs [stages I–XV of Taylor & Kollros (1946)].

$\dot{V}O_2$ measurements

Closed-system respirometers were employed that could be flushed with water at a desired PO_2 and then sealed without disturbing larvae. These respirometers were thermostatted at 25 ± 0·1 °C. Respirometers were Erlenmeyer or round-bottomed flasks fitted with rubber stoppers. The respirometer volumes were between 100–300 ml. The respirometer volume, animal size and the number of animals per respirometer (1–5) were varied to achieve a similar ratio of animal volume to respirometer volume.

Experimental larvae were placed in respirometers and left overnight to become accustomed to these chambers; aerated water was flushed through the respirometers during this period. Before each measurement of aquatic $\dot{V}O_2$, respirometers were flushed with 750–1000 ml of water at a pre-determined PO_2. This water was sterilized and pre-treated with antibiotics to minimize microbial respiration (see Feder, 1981*a*), and its PO_2 was regulated by bubbling N_2 through it. After the flushing had been completed water samples were withdrawn from the open respirometers into glass syringes, and the respirometers were sealed at the beginning of measurements. The PO_2 of half of each sample was determined immediately. The remaining half was held in the syringe as a control for microbial $\dot{V}O_2$. A final sample was taken after 10–45 min had elapsed. The aquatic $\dot{V}O_2$ was calculated from the difference between the PO_2 of the final sample and the control sample, the elapsed time, the respirometer volume and the O_2 capacitance under experimental conditions.

The PO_2 was measured with a thermostatted No. 730 Clark microelectrode and Chemical Microsensor system (Transidyne General Corp., Ann Arbor, MI). The electrode was calibrated between each measurement with air-equilibrated water at 25 ± 0·1 °C.

This protocol was varied in three ways. (a) Animals were allowed no access to air, and the PO_2 was reduced continually throughout experimentation. (b) Animals were treated as in (a), but a magnetic stirring bar was included in the respirometer. The stirring bar was agitated during measurements (10–20 min), and animals responded by swimming vigorously and continuously. (c) Animals had access to air (approximately 10 ml) in the upper neck of a 281 ml round-bottomed flask. Both aerial and aquatic compartments were flushed before measurements. The aerial phase was sampled with gas-tight syringes and its PO_2 determined with a 0·5 ml Scholander analyser (Scholander, 1947). The dead space was filled with mercury to minimize error. The aerial $\dot{V}O_2$ was calculated from the change in aerial PO_2 and airspace volume. All calculations of aerial and aquatic $\dot{V}O_2$ were adjusted for diffusion from air to water; adjustments were based upon the apparent aerial O_2 consumption of a control respirometer containing no animals. The PO_2 of the aerial phase never declined below 130 Torr; the initial PO_2 of the water was reduced continually in nsecutive measurements.

Respiratory frequencies

Pulmonary ventilation frequency (f_{lung}), branchial ventilation frequency (f_{gill}), and heart rate (f_{heart}) were determined visually. The larvae were placed in experimental containers 4 h before observation. The PO_2 was measured with a YSI 54A O_2 meter (Yellow Springs Instruments, Antioch, OH), and was adjusted by bubbling N_2 through the water.

To determine the f_{lung}, the larvae were placed in individual 1000 ml Erlenmeyer flasks containing water at approximately the desired PO_2, and observed for 1 h. The PO_2 was measured after observations were complete. To measure f_{heart} and f_{gill} in larvae with access to air, larvae were placed in a screen cylinder (20 cm diameter × 20 cm height) within a larger aquarium. This arrangement allowed gassing of water but minimized physical disturbance of the larvae. Each tadpole was observed for 5–6 intervals of 1 min, and the frequencies recorded during these intervals were averaged. The body wall beneath the heart was surgically removed sufficiently to visualize the heart beat (Wassersug, Paul & Feder, 1981) in those animals undergoing determination of f_{heart}. This procedure apparently caused no more trauma to the larvae than the implantation of subcutaneous ECG electrodes (Wassersug *et al.* 1981; Burggren, Feder & Pinder, 1983). The f_{gill} was also measured in larvae held without access to air in a submerged, screen-covered box (see Wassersug *et al.* 1981). The PO_2 was reduced between each determination of f_{gill} and f_{heart}.

Lactate production

In one experiment, larvae were held in 300 ml screen cylinders within aerated aquaria for 4 h before experimentation. The PO_2 was then adjusted to experimental levels, the larvae were held at that PO_2 for 1 h, and the larvae were then killed for determination of lactate content. Feder (1981*a*) described the lactate determination procedure. The larvae were divided into two groups. In one group, the containers were submerged so that larvae had no access to air during the entire 5 h of experimentation. The other group had access to air during experimentation. An additional group of larvae were placed in screen containers, the containers were submerged overnight in aerated water, and the larvae were killed for determination of lactate the following morning.

To avoid lactate production due to handling of the larvae, morphometric measurements were made on the day before experimentation. These measurements were substituted into a multiple regression equation to calculate the dry mass of the larvae on the day in which lactate was determined (Feder, 1981*a*).

Tolerance of hypoxia

Larvae were confined without access to air in separate 37 ml compartments of a screen-covered box. After 4 h for equilibration, an observer reduced the PO_2 in the container and noted the 'critical activity point', at which larvae no longer responded to tactile stimuli.

Routine activity

Larvae were placed individually in a glass aquarium (50×20×20 cm) filled wi

rated water. After 4 h for equilibration, an observer noted all locomotor movements of the larvae and their duration. Observations were repeated for each larva after reduction of PO_2. A plastic screen shielded larvae from physical disturbance due to bubbling of N_2 and PO_2 measurements.

Statistical analysis

The larvae were weighed and measured as detailed by Feder (1981*a*). In some cases the $\dot{V}O_2$ data were transformed by expressing the $\dot{V}O_2$ as a percentage of the routine $\dot{V}O_2$ expected for *Rana berlandieri* larvae of similar size (Feder, 1982); the range of these data exceeded 100 % of expected routine $\dot{V}O_2$. The transformed $\dot{V}O_2$ data were analysed according to several statistical models, including linear, polynomial and logarithmic regressions (Mangum & Van Winkle, 1973), and two line segments with a common join point (Welch, 1979). The latter model accounted for the greatest proportion of the variance in the $\dot{V}O_2$, and was used subsequently.

Multiple stepwise regression was performed to determine how body size affected bimodal gas exchange. The $\dot{V}O_2$ (aerial, aquatic and total) of all larvae was first expressed as a proportion of the $\dot{V}O_2$ expected for an animal of mean size. This proportion was the dependent variable in the multiple regression. It was impossible to enter the PO_2 directly into the multiple regression because its effect on $\dot{V}O_2$ was not linear (Figs 1, 2); hence the $\dot{V}O_2$ expected for an animal of mean size ($\dot{V}O_2$M) was entered in its place as the first independent variable. Next, terms representing dry mass, and the interaction of $\dot{V}O_2$M and dry mass were entered in that order.

RESULTS

Aerial and aquatic $\dot{V}O_2$

Fig. 1 shows the response of *Rana* larvae to a decline in aquatic PO_2. (Hereafter, PO_2 refers exclusively to the PO_2 of the water.) Table 1 presents the equations for the line segments presented in Fig. 1. In normoxic water, aerial gas exchange averaged 18 % of the total $\dot{V}O_2$. As the PO_2 declined the aquatic $\dot{V}O_2$ decreased steadily, and animals lost O_2 to the water at low PO_2. By contrast, the aerial $\dot{V}O_2$ increased and actually came to account for more than 100 % of the total $\dot{V}O_2$. This increase, however, was insufficient to offset the decrement in aquatic $\dot{V}O_2$; hence the overall $\dot{V}O_2$ declined under hypoxia.

Also evident was substantial individual variation about this general pattern. For example, in one animal ('+', 144 mg dry mass) the aerial $\dot{V}O_2$ accounted for a much larger proportion of the total $\dot{V}O_2$ than in other animals (Fig. 1). Part of this variation may have been due to individual differences in routine activity (see below).

Body size had little effect on the aquatic $\dot{V}O_2$ of *Rana* larvae. Neither large nor small larvae showed a tendency to depart from the general relationships in Fig. 1. Hence, hypoxia affected the aquatic $\dot{V}O_2$ equally in all larvae, regardless of size. In statistical terms, body size and body size-PO_2 interaction (actually body size-$\dot{V}O_2$M interaction; see Materials and Methods) accounted for < 1 % of the variance in aquatic $\dot{V}O_2$ (Table 1). No effects were attributable to the modest variation in developmental stage the experimental larvae

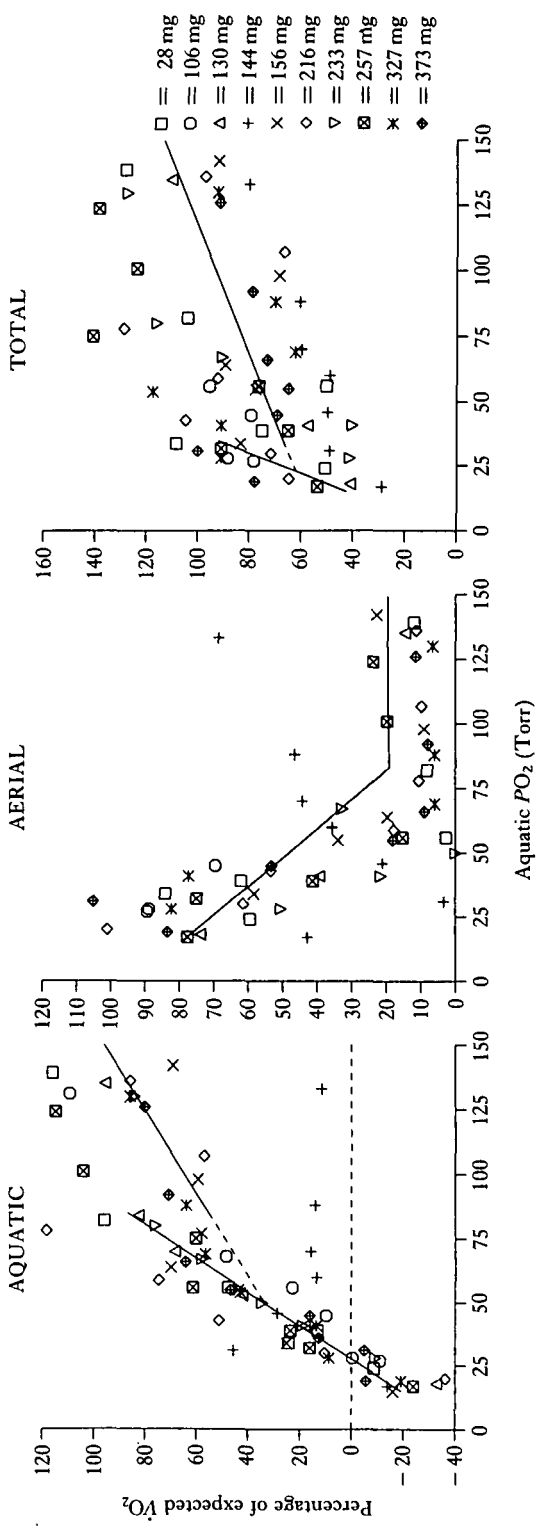

Fig. 1. Effect of body size and aquatic PO_2 on the aquatic, aerial and total $\dot{V}O_2$ in *Rana* larvae. The data are reported as a percentage of the total $\dot{V}O_2$ expected for larvae in normoxic water (Feder, 1982). The lines were fitted according to the method of Welch (1979); the dashed line represents an extension of the right line segment (Table 1) to the common join point. The equations for the lines are given in Table 1. When more than one larva were measured simultaneously, they were represented as a single larva of mean mass.

Table 1. *Effect of aquatic* P_{O_2} *on the aerial, aquatic and total* $\dot{V}_{O_2}$ *in* Rana berlandieri *larvae*

	Aerial	Aquatic	Total
n	55	67	55
Px	45	85	35
$P_{O_2} < Px$	$\dot{V}_{O_2}M = 92{\cdot}6 - 0{\cdot}89P_{O_2}$	$\dot{V}_{O_2}M = 1{\cdot}5P_{O_2} - 41{\cdot}9$	$\dot{V}_{O_2}M = 2{\cdot}5P_{O_2} + 5{\cdot}3$
$P_{O_2} > Px$	$\dot{V}_{O_2}M = 17{\cdot}7 - 0{\cdot}02P_{O_2}$	$\dot{V}_{O_2}M = 0{\cdot}6P_{O_2} + 1{\cdot}9$	$\dot{V}_{O_2}M = 0{\cdot}4P_{O_2} + 52{\cdot}8$
% of variance explained by:			
(A) $\dot{V}_{O_2}M$ (i.e. P_{O_2})	61 %	75 %	29 %
(B) Body size	< 1 %	< 1 %	3 %
(C) $\dot{V}_{O_2}M$-Body size interaction	6 %	< 1 %	11 %
Overall equation:	$\dot{V}_{O_2}$ (%) = 23·7 + 0·34A − 0·11B + 0·003C	$\dot{V}_{O_2}$ (%) = 0·92A + 0·01B + 0·004C − 2·3	$\dot{V}_{O_2}$ (%) = 2·33A + 0·57B − 0·006C − 116·41

Data were fitted to two line segments according to the method of Welch (1979). *Px* is the P_{O_2} that separates the left and right segments (see Fig. 1). The $\dot{V}_{O_2}M$ is the $\dot{V}_{O_2}$ expected for a larva of mean size, and is equivalent to the line segments depicted in Fig. 1. The overall equation relates the aerial, aquatic and total $\dot{V}_{O_2}$ of larvae (expressed as a proportion of the $\dot{V}_{O_2}M$) to the $\dot{V}_{O_2}M$ (A), body size (B), and $\dot{V}_{O_2}M$ − body size interaction (C).

By contrast, body size markedly affected the aerial $\dot{V}_{O_2}$ and the total $\dot{V}_{O_2}$ of the larvae (see Fig. 2). At normoxic P_{O_2}, the small larvae depended more upon aerial $\dot{V}_{O_2}$ than the large tadpoles. As the P_{O_2} declined, however, this relationship reversed and large larvae depended disproportionately upon aerial $\dot{V}_{O_2}$. The total $\dot{V}_{O_2}$ also reflected these trends (Fig. 2). Large larvae maintained a relatively equable total $\dot{V}_{O_2}$ throughout a wide range of P_{O_2}. Smaller tadpoles had a high relative total $\dot{V}_{O_2}$ in normoxic water but underwent a precipitous decline in total $\dot{V}_{O_2}$ as the P_{O_2} decreased. In statistical terms, body size accounted for little of the variance in aerial $\dot{V}_{O_2}$ and total $\dot{V}_{O_2}$, but the interaction of P_{O_2} and body size (i.e. $\dot{V}_{O_2}M$-body size interaction) explained 6 % and 11 % of the variation in the aerial $\dot{V}_{O_2}$ and the total $\dot{V}_{O_2}$, respectively (Table 1). Developmental stage had little effect on the aerial and total oxygen uptake.

In normoxic water, larvae without access to air were able to augment their aquatic $\dot{V}_{O_2}$ to compensate for the absence of aerial O_2 uptake (Fig. 3). This ability was size dependent. The total $\dot{V}_{O_2}$ (in $\mu l\,h^{-1}$) for these animals and for similar animals that had access to air (Feder, 1982) was compared with analysis of covariance. Access to air *per se* did not affect the total $\dot{V}_{O_2}$ ($F_{(1,66)} = 1{\cdot}12$; $P = 0{\cdot}29$); however, the interaction of body mass and access to air significantly altered the total $\dot{V}_{O_2}$ ($F_{(1,66)} = 4{\cdot}68$; $P = 0{\cdot}03$). In other words, both the large and the small larvae without access to air were able to achieve approximately 100 % of the expected routine $\dot{V}_{O_2}$; however, the small larvae (but not the large larvae) frequently exceeded 100 % of the routine $\dot{V}_{O_2}$.

By contrast, the total $\dot{V}_{O_2}$ of larvae without access to air decreased markedly under hypoxia (Fig. 3). This effect was equally evident in larvae of all sizes.

Fig. 3 also shows the aquatic $\dot{V}_{O_2}$ expected when larvae had access to air. As is evident from this Figure, the larvae without access to air did not exceed this level in

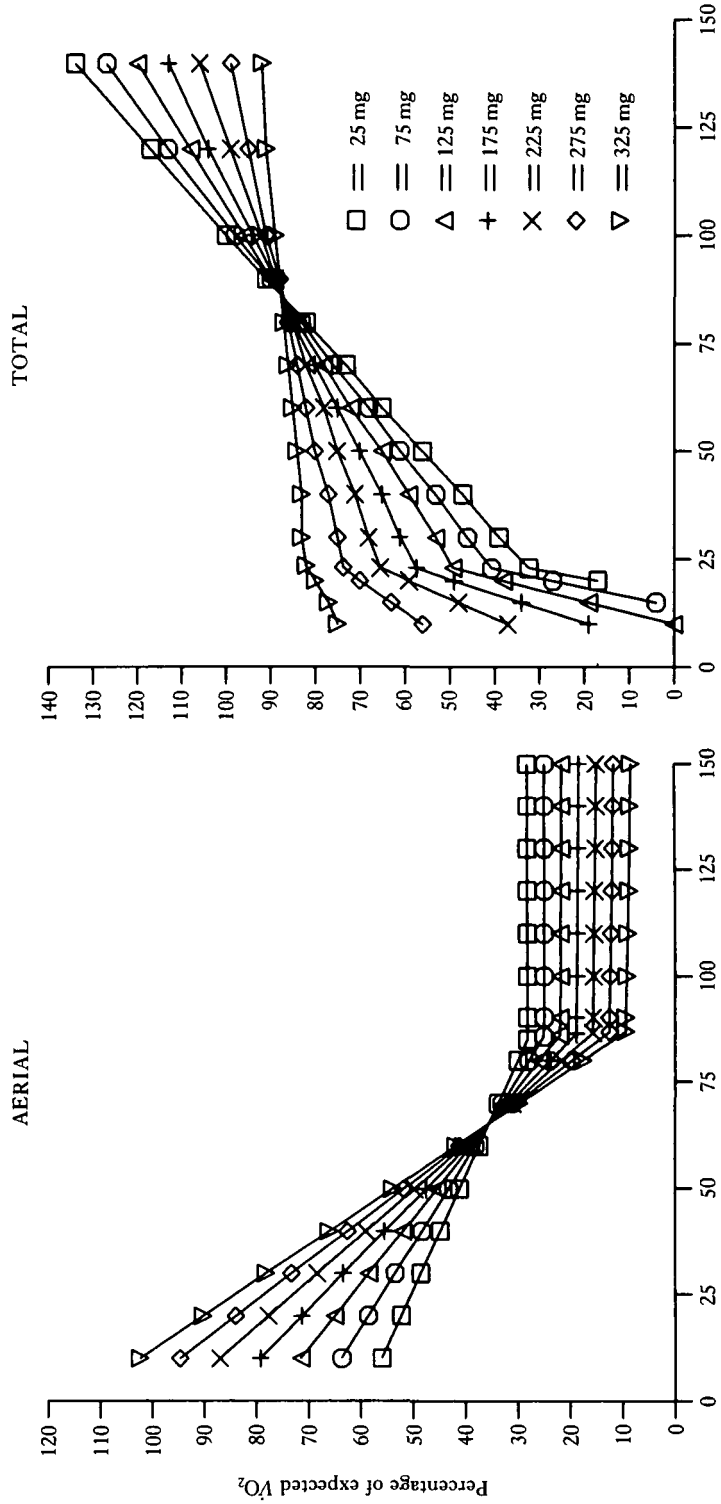

Fig. 2. Effect of the interaction of body size and aquatic PO_2 on the aerial and total $\dot{V}O_2$ of *Rana* larvae. This figure exhibits the 'overall regression' of Table 1, and was produced by substituting representative values of dry mass into the regression equation. The $\dot{V}O_2$ is plotted as in Fig. 1.

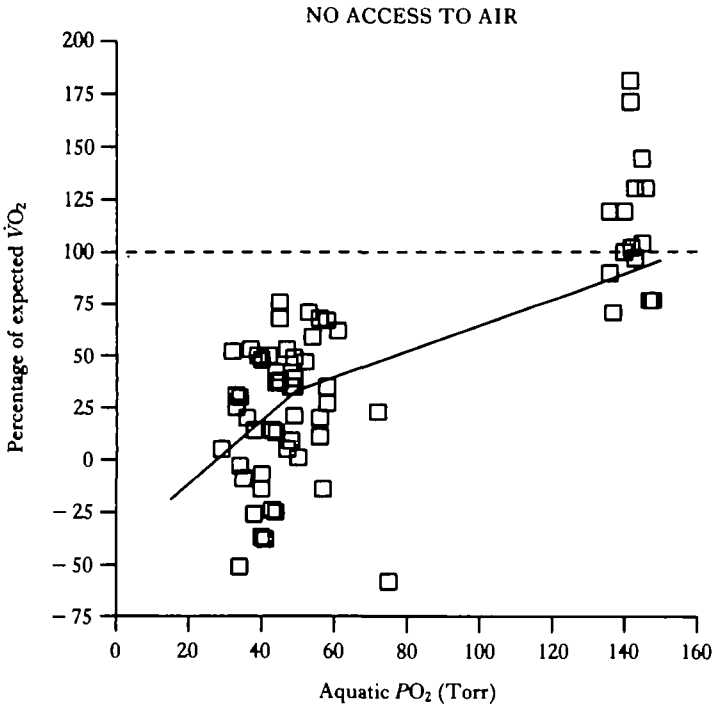

Fig. 3. Effect of aquatic PO_2 on the $\dot{V}O_2$ in exclusively aquatic *Rana* larvae. All air was excluded from the respirometer in which measurements were made. The $\dot{V}O_2$ is plotted as in Fig. 1. The total $\dot{V}O_2$ (in % of expected routine $\dot{V}O_2$) of these larvae was related to PO_2 (Torr) by the following equation:

$$\dot{V}O_2 = 0{\cdot}9PO_2 - 20{\cdot}3;$$

$r = 0{\cdot}76$; $n = 65$. Addition of polynomial terms to the regression improved the fit by less than 1 %. Body size and body size–PO_2 interaction had little effect on $\dot{V}O_2$; addition of these variables to the regression equation increased R^2 (the fraction of explained variance) by only 0·005 and 0·018, respectively. The solid line represents the relationship between aquatic $\dot{V}O_2$ and PO_2 in other larvae with access to air (Fig. 1).

hypoxic water. To determine whether this situation represented a voluntary reduction in the $\dot{V}O_2$ or a limitation of the aquatic gas exchanger, some animals without access to air were stimulated to vigorous activity. In 14 trials ($PO_2 = 44$–70 Torr; size = 13–596 mg dry mass) the $\dot{V}O_2$ averaged 80 % of the expected routine total $\dot{V}O_2$, and ranged from −22 % to 200 %. No correlation was evident between the $\dot{V}O_2$ (in % of expected levels) and either the PO_2 or body size. Obviously, some larvae extracted more O_2 from water than was evident in 'resting' measurements (Figs 1, 2). However, it is unclear whether this increase represented a physiological response to activity or inadvertent facilitation of ventilation and diffusion due to stirring of the water.

Respiratory behaviour

As indicated by the direct measurements of the total $\dot{V}O_2$ and the aerial $\dot{V}O_2$, the larvae breathed air at all PO_2 observed and increased their f_{lung} in response to low PO_2 (Fig. 4). The f_{lung} was unaffected by the PO_2 above approximately 50–65 Torr; below this range the f_{lung} increased in inverse proportion to the PO_2. This threshold

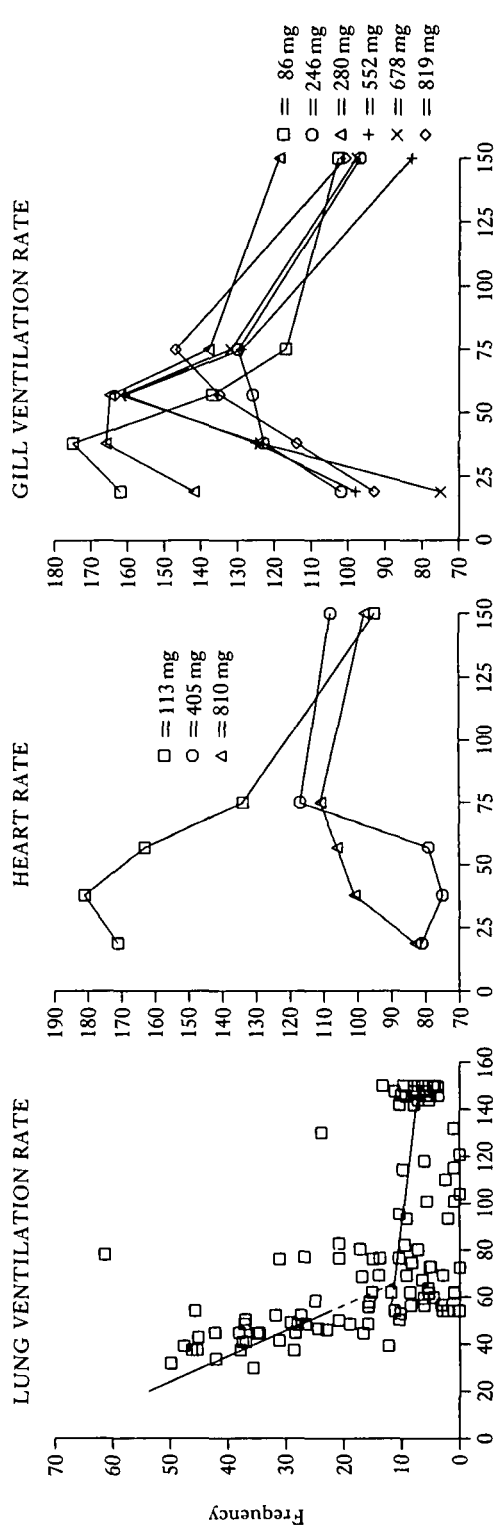

Fig. 4. Effect of aquatic PO_2 on the respiratory frequencies in *Rana* larvae. The ventilation rate (f_{lung}) is h^{-1}; the heart rate (f_{heart}) and the branchial ventilation frequency (f_{gill}) are both min^{-1}. Data relating f_{lung} to PO_2 were fitted to several models of regressions; a fit to two line segments explained the largest amount of the variation in f_{lung} ($R^2 = 56\%$). The equation for the left segment ($PO_2 < 53$ Torr) is f_{lung} (in h^{-1}) $= -0{\cdot}923\,PO_2$ (in Torr) $+ 72{\cdot}3$; the equation for the right segment is $f_{lung} = -0{\cdot}052\,PO_2 + 14{\cdot}8$. The dry mass of animals used in determinations of f_{heart} and f_{gill} is presented to the right of each figure; body size had a negligible effect on f_{lung}.

P_{O_2} was similar for both large and small larvae; also, larvae of all sizes showed similar changes in the f_{lung} in response to declining P_{O_2}.

The f_{heart} was observed in only three animals (Fig. 4). In these animals the f_{heart} increased and then decreased as the P_{O_2} declined.

Similarly, the f_{gill} increased from normoxic levels under moderate hypoxia, but declined from maximal levels under severe hypoxia (Fig. 4). The maximum f_{gill} was approximately twice the f_{gill} for animals in normoxic water.

The pattern of change in the f_{gill} was different for large and small larvae. In the smaller larvae, the curves relating the f_{gill} to the P_{O_2} were displaced towards lower P_{O_2}; i.e., the maximal f_{gill} occurred at relatively low P_{O_2} and the f_{gill} was relatively high, even at low P_{O_2}. This apparent trend was confirmed by calculating the P_{O_2} at the centroid of the polygon bounded by each f_{gill}-P_{O_2} curve in Fig. 4 and the X axis. Also analysed were similar measurements (not shown in Fig. 4) for seven additional small larvae. The central P_{O_2} was positively correlated with the size of larvae (Spearman's $r = 0{\cdot}587$; $P < 0{\cdot}05$).

The f_{gill} differed in larvae with and without access to air. In the larvae without access to air (Fig. 5), the f_{gill} was low in normoxic water and increased steadily as the P_{O_2} decreased (Friedman's analysis of variance; $P < 10^{-4}$). Thus the f_{gill} was not maximal at some intermediate P_{O_2}, as in the larvae with access to air. Moreover, the f_{gill} for exclusively aquatic larvae was significantly lower ($P < 0{\cdot}05$) than the f_{gill} for air-breathing larvae at moderate and high P_{O_2} (57–150 Torr), similar at 38 Torr ($P > 0{\cdot}75$), and

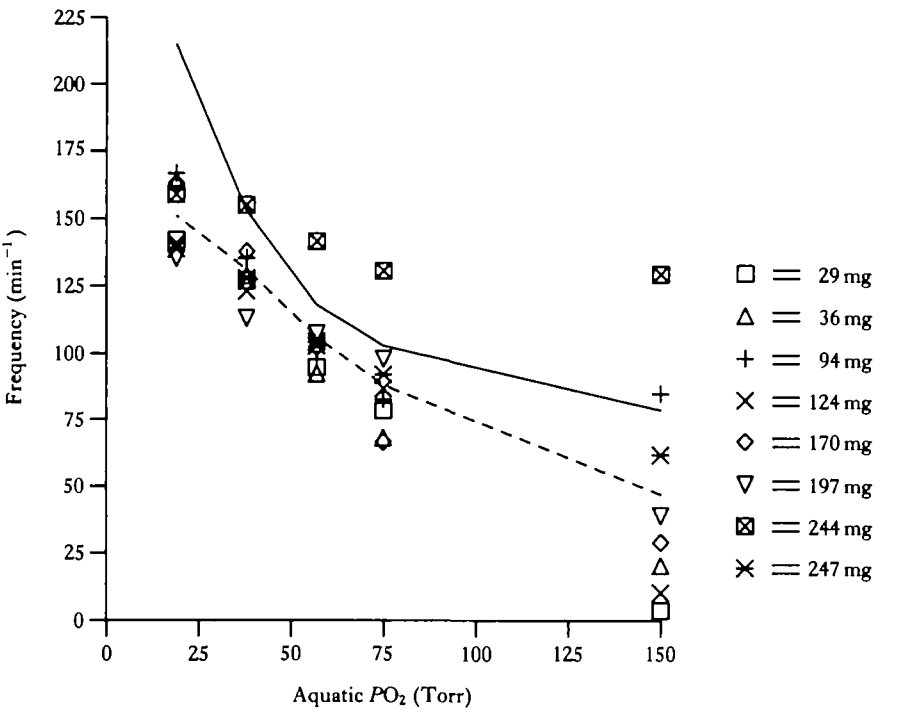

Fig. 5. Effect of aquatic P_{O_2} on the f_{gill} in *Rana* larvae without access to air. The broken line connects the mean f_{gill} at each P_{O_2}; the solid line connects the mean f_{gill} for 20 smaller larvae measured under slightly different conditions (see text).

significantly greater at 19 Torr ($P < 0{\cdot}05$; one way analysis of variance in each case).

Considerably smaller larvae ($n = 20$; mean = 4·5 mg; range = 1–8·6 mg dry mass) were also observed while without access to air in individual compartments of a plastic box (Wassersug *et al.* 1981). These larvae displayed significant increases in the f_{gill} with declining PO_2 ($P < 0{\cdot}001$) that were qualitatively similar to the pattern for larger tadpoles. However, the f_{gill} was relatively high in the small larvae, ranging from 111–167 % of the mean f_{gill} of larger larvae at each PO_2.

Routine movement

Larvae varied enormously in the amount of spontaneous activity. At any given PO_2, individual larvae differed in activity; some moved almost continually and others were quiescent. Frequently, individual larvae underwent different amounts of activity at each PO_2. No characteristic pattern of activity was associated with either large or small larvae.

Lactate concentration

Among the larvae exposed to aquatic hypoxia for 1 h (Table 2), only exclusively aquatic larvae at the lowest PO_2 (19 Torr) showed a significant elevation of whole-body lactate concentration ($P < 0{\cdot}01$; analysis of covariance with predicted dry mass as the covariate). The lactate concentration of bimodally breathing larvae at the same PO_2 was not elevated similarly. If the larvae at 19 Torr are excluded from the analysis, none of the remaining experimental groups, whether exposed to hypoxic PO_2, denied access to air, or both, differed significantly from one another in lactate concentration ($P = 0{\cdot}57$; analysis of covariance).

In exclusively aquatic larvae, exposure to moderate hypoxia ($PO_2 = 38$ Torr) for 3 h did not elevate the lactate concentration significantly as compared to the 1 h levels (Table 2). However, exposure to water at 38 Torr for 6 h resulted in a five-fold increase in the lactate concentration ($P < 0{\cdot}01$; analysis of covariance).

In the larvae held overnight in hypoxic water without access to air, the lactate

Table 2. *Effect of aquatic* PO_2 *on the whole-body lactate concentration in* Rana berlandieri *larvae*

PO_2 (Torr)	Duration of exposure (h)	Lactate concentration (mg/g dry mass) Access to air	Submerged
150	0	0·83 ± 0·27	1·05 ± 0·27[1]
75	1	1·57 ± 0·25	1·09 ± 0·08[1]
59	1	1·25 ± 0·38	1·13 ± 0·54[1]
38	1	1·13 ± 0·11	0·89 ± 0·14[1]
19	1	1·15 ± 0·34	6·84 ± 0·57[1]
38	3	—	1·35 ± 0·24[2] (5)
38	6	—	6·49 ± 1·41[2] (4)
150	16	—	0·97 ± 0·07[2](13)

[1] Submerged for 4 h in normoxic water before indicated exposure.
[2] Submerged overnight in normoxic water before indicated exposure.
The lactate concentration is in mg lactate/g predicted dry mass (Feder, 1981*a*), and is given as the mean ± S.E. Sample size is 6 unless otherwise noted in parentheses.

[redacted]ncentration was positively correlated with body size (Spearman's $r = 0{\cdot}65$). Although the correlation was significant ($P < 0{\cdot}05$), the lactate levels were comparable to concentrations in normoxic animals with continual access to air.

Hypoxia tolerance

With the exception of a single larva (182 mg dry mass) that succumbed at 38 Torr, the critical activity point ranged between 2–17 Torr PO_2 for larvae without access to air. The tolerance of hypoxia was unrelated to the body size. In three separate determinations, the correlation between the PO_2 at the critical activity point and the body size was not significant (Spearman's $r = 0{\cdot}07$, $0{\cdot}13$ and $0{\cdot}31$; $P > 0{\cdot}05$ in each case).

DISCUSSION

Environmental and comparative aspects of air-breathing

Most textbook accounts and many recent studies have characterized the tadpole as an exclusively aquatic stage that precedes the transition to air-breathing in anurans. As the present study clearly demonstrates, air-breathing contributes significantly to total oxygen uptake in the larvae of *Rana berlandieri* even in normoxia, and is the sole mode of oxygen acquisition under severe aquatic hypoxia. Similar results have been reported for larvae of *Rana catesbeiana* (Burggren *et al.* 1983) and *Xenopus laevis* (Feder, 1981*b*). Some tadpoles, in fact, live entirely outside the water for considerable lengths of time (Wassersug & Heyer, 1983). All of the above reports plus several accounts of air-breathing behaviour (Just *et al.* 1973; Wassersug & Seibert, 1975) refer in part to larvae before metamorphosis [Taylor & Kollros (1946) stages I–XV]. Thus, except in certain lungless tadpoles (Wassersug & Seibert, 1975), bimodal respiration is a common and significant feature of anuran larvae well before the transition to an adult morphology.

The occurrence of air-breathing in tadpoles need not imply that aquatic respiration is ineffective. In normoxic water at a variety of temperatures, anuran larvae use aquatic respiration (principally cutaneous gas exchange) to meet the majority of their oxygen demands (Burggren & West, 1982; West & Burggren, 1982; Burggen *et al.* 1983). In the present study, larvae in normoxic water without access to air were able to sustain the same or greater total $\dot{V}O_2$ than unrestrained larvae with access to air. The larvae of *Rana catesbeiana* in fact cease ventilating their lungs when in hyperoxic water (West & Burggren, 1982). Nonetheless, even though tadpoles can augment branchial O_2 uptake somewhat in response to moderate hypoxia (West & Burggren, 1982), hypoxia ultimately limits the effectiveness of aquatic O_2 uptake as the PO_2 gradient across the skin and gills declines.

In addition to air-breathing, the acute responses to aquatic hypoxia in tadpoles include direct avoidance of hypoxia (Costa, 1967); undirected movement that may lead larvae from hypoxic water or minimize the boundary layer about the skin (Hutchison, Haines & Engbretson, 1976); and changes in the branchial flow rate, the branchial O_2 extraction efficiency (West & Burggren, 1982), the f_{gill}, and the f_{heart}. Many of these responses attain their greatest magnitude at moderate hypoxia and diminish [redacted]der severe hypoxia, since they actually promote loss of O_2 to the water under the

latter condition. Anaerobiosis does not occur except in unusual circumstances. Th[illegible] the total $\dot{V}O_2$ evidently decreases in hypoxia, as it does in some fish (Burggren & Randall, 1978).

The pattern of responses to hypoxia in tadpoles is of special interest because the phylogenetic affinity of tadpoles is with adult anurans, which are predominantly air-breathers (Hutchison *et al.* 1976; Burggren & West, 1982), but the habitat and habits of tadpoles resemble those of fish in which air-breathing is supplementary. The anuran larvae examined in the present study and elsewhere (Feder, 1981*b*; Burggren & West, 1982; West & Burggren, 1982; Burggren *et al.* 1983) resemble air-breathing fishes in the general pattern of responses to hypoxia. The general pattern is one intermediate between strict metabolic conformity to aquatic PO_2 and strict metabolic regulation (McMahon, 1970; Hughes & Singh, 1971; Singh & Hughes, 1971; Graham, Kramer & Pineda, 1977; Gee & Graham, 1978; Stevens & Holeton, 1979). The pattern of increasing dependence upon aerial respiration as the aquatic PO_2 declines is also common in air-breathing fishes (Johansen, 1970; Randall, Burggren *et al.* 1981).

However, fishes and tadpoles typically differ in aspects of branchial ventilation and anaerobiosis. Although some species do not conform to this pattern, fishes respond primarily by increasing the buccal pump stroke volume, with increasing f_{gill} playing a secondary role (Johansen, Lenfant & Grigg, 1967; Hughes & Saunders, 1970; Cech & Wohlschlag, 1973; Hughes, 1973; Kerstens, Lomholt & Johansen, 1979; Holeton, 1980). By contrast, larvae of every anuran species studied to date vary their f_{gill} in response to changing respiratory needs (Wassersug & Seibert, 1975; Feder, 1981*a*,*b*; Wassersug *et al.* 1981; Burggren *et al.* 1983). Changes in branchial stroke volume may also occur, but are inconsistent in their prevalence and direction (West & Burggren, 1982; Burggren *et al.* 1983). A second difference concerns the end product of anaerobiosis. Lactate production is a common response to severe hypoxia in amphibians (Armentrout & Rose, 1971; Bennett & Licht, 1974; D'Eon, Boutilier & Toews, 1978; Gatz & Piiper, 1979; Feder, 1981*b*). By contrast, hypoxic fish produce relatively little lactate, and generally accumulate other end products of anaerobiosis (Hochachka, 1980). Given the circumstances under which lactate formation occurs in amphibians and the deleterious consequences of lactate formation, it appears that anaerobiosis in tadpoles is primarily a response of last resort or reflects the increasing stress and muscular activity in severe hypoxia, rather than a routine response to moderate hypoxia.

A more significant difference between anuran larvae and fishes is that the skin is a major site of organismal gas exchange in anuran larvae (Burggren & West, 1982; Burggren *et al.* 1983), while in fishes it is not (Kirsch & Nonnotte, 1977). Unlike the skin of fishes, which is covered by scales and a layer of mucus, tadpole skin is relatively thin and highly vascularized (Strawinski, 1956), as in most amphibians. This arrangement may facilitate cutaneous O_2 uptake in normoxia (Burggren & West, 1982), but evidently renders impossible the curtailment of O_2 loss to hypoxic water. Fishes may prevent most O_2 loss to hypoxic water simply through cessation of branchial gas exchange. This may occur behaviourally by reducing water flow over the gills or through evolution of a reduced branchial surface area (Randall, Burggren *et al.* 1981). Thus loss of O_2 to hypoxic water in fishes, while it may sometimes happen (Burggr[illegible]

9; Stevens & Holeton, 1979; Randall, Cameron, Daxboeck & Smatresk, 1981), is relatively uncommon. Many tadpoles also decrease branchial ventilation in response to severe aquatic hypoxia (West & Burggren, 1982; M. E. Feder, unpublished data for *Bufo woodhousei* and *Xenopus laevis*), but this response leaves cutaneous exchange unencumbered. One means of minimizing cutaneous O_2 loss is the shunting of blood away from the skin. However, although some adult amphibians can control the perfusion of the skin to alter gas exchange (Moalli, Meyers, Jackson & Millard, 1980), such control (if present at all) is ineffective in preventing O_2 loss in larvae of *Rana* and of other species (Feder, 1981*b*). Larvae are only able to offset aquatic O_2 loss by greatly increasing aerial ventilation. Thus in some circumstances anuran larvae that have lungs may be committed to aerial respiration to a far greater extent than are most air-breathing fishes.

Body size and air-breathing

The responses to hypoxia in the *Rana* larvae suggest that the lack of lungs *per se* does not restrict larvae, such as those of *Bufo* (see Introduction), to small size. For lunglessness to restrict anuran larvae to small sizes, the aquatic gas exchangers (gills and skin) should be inadequate in large larvae. If so, then large larvae should be more susceptible to hypoxia or show greater efforts at compensating for hypoxia than small larvae (Ultsch, 1976; Feder, 1977). Most data for the *Rana* larvae, however, were contrary to these predictions. The large and the small larvae showed no consistent differences in critical P_{O_2}, underwent equivalent depressions in $\dot{V}_{O_2}$ when denied access to air in hypoxic water, and were similarly tolerant of hypoxia. Although the large larvae had greater lactate concentrations than the small larvae after prolonged submergence in normoxic water, this difference was quantitatively negligible. Among *Rana* larvae with access to air, the large larvae seemed more effective in their use of bimodal gas exchange than the small larvae. The large larvae derived a relatively high proportion of their total $\dot{V}_{O_2}$ from aerial sources, and decreased their f_{gill} at a low P_{O_2} more readily than the small larvae. The relatively precise regulation of the total $\dot{V}_{O_2}$ in the large larvae (Fig. 2) reflected these differences.

No effects in the present study were attributable to the developmental stage (as opposed to body size). The absence of any ontogenetic trends may reflect no more than the similar developmental stages of the larvae chosen as experimental subjects. Other studies that intentionally varied the developmental stages of larvae have shown major ontogenetic changes in gas exchange associated with the development of lungs and the atrophy of gills (Burggren & West, 1982; West & Burggren, 1982).

Pulmonary respiration is obviously an important component of the responses to aquatic hypoxia in anuran larvae. Moreover, in larvae that can use their lungs, large larvae gain more benefit from lungs than do small larvae. Even so, in larvae that are exclusively aquatic, the disadvantages of lunglessness seem to affect larvae of all sizes equally. A small lungless larva, such as *Bufo*, would be no better off than a large tadpole without lungs, although both might be at a disadvantage compared to larvae with lungs. Hence, the small size of bufonid larvae may be related primarily to ecological and energetic considerations (Feder, 1976) rather than a direct sequence of lunglessness.

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REFERENCES

ARMENTROUT, D. & ROSE, F. L. (1971). Some physiological responses to hypoxia in the Great plains toad, *Bufo cognatus*. *Comp. Biochem. Physiol.* **39**A, 447–455.

BENNETT, A. F. & LICHT, P. (1974). Anaerobic metabolism during activity in amphibians. *Comp. Biochem. Physiol.* **39**, 319–327.

BURGGREN, W. W. (1979). Bimodal gas exchange during variation in environmental oxygen and carbon dioxide in the air breathing fish *Trichogaster trichopterus*. *J. exp. Biol.* **82**, 197–213.

BURGGREN, W. W., FEDER, M. E. & PINDER, A. W. (1983). Temperature and the balance between aerial and aquatic respiration in larvae of *Rana berlandieri* and *Rana catesbeiana*. *Physiol. Zool.* (in the press).

BURGGREN, W. W. & RANDALL, D. J. (1978). Oxygen uptake and transport during hypoxic exposure in the sturgeon, *Acipenser transmontanus*. *Respir. Physiol.* **34**, 171–183.

BURGGREN, W. W. & WEST, N. H. (1982). Changing respiratory importance of gills, lungs and skin during metamorphosis in the bullfrog, *Rana catesbeiana*. *Respir. Physiol.* **47**, 151–164.

BURGGREN, W. W. & WOOD, S. C. (1981). Respiration and acid-base balance in the tiger salamander, *Ambystoma tigrinum*: Influence of temperature acclimation and metamorphosis. *J. comp. Physiol.* **144**, 241–246.

CECH, J. J., JR. & WOHLSCHLAG, D. E. (1973). Respiratory responses of the striped mullet, *Mugil cephalus* (L.) to hypoxic conditions. *J. Fish Biol.* **5**, 421–428.

COSTA, H. H. (1967). Avoidance of anoxic water by tadpoles of *Rana temporaria*. *Hydrobiologia* **30**, 374–384.

CRUMP, M. L. (1981). Variation in propagule size as a function of environmental uncertainty for tree frogs. *Am. Nat.* **117**, 724–737.

D'EON, M. E., BOUTILIER, R. G. & TOEWS, D. P. (1978). Anaerobic contributions during progressive hypoxia in the toad, *Bufo marinus*. *Comp. Biochem. Physiol.* **60**A, 7–10.

FEDER, M. E. (1976). Lunglessness, body size, and metabolic rate in salamanders. *Physiol. Zool.* **49**, 398–406.

FEDER, M. E. (1977). Oxygen consumption and activity in salamanders: effect of body size and lunglessness. *J. exp. Zool.* **202**, 403–413.

FEDER, M. E. (1981*a*). Effect of body size, trophic state, time of day, and experimental stress on oxygen consumption of anuran larvae: an experimental assessment and evaluation of the literature. *Comp. Biochem. Physiol.* **70**A, 497–508.

FEDER, M. E. (1981*b*). Responses to acute aquatic hypoxia in larvae of amphibians, *Rana* and *Xenopus*. *Am. Zool.* **21**, 928.

FEDER, M. E. (1982). Effect of developmental stage and body size on oxygen consumption of anuran larvae: a reappraisal. *J. exp. Zool.* **220**, 33–42.

GATZ, R. N. & PIIPER, J. (1979). Anaerobic energy metabolism during severe hypoxia in the lungless salamander *Desmognathus fuscus* (Plethodontidae). *Respir. Physiol.* **38**, 377–384.

GEE, J. H. & GRAHAM, J. B. (1978). Respiratory and hydrostatic functions of the intestine of the catfishes *Hoplosternum thoracatum* and *Brochis splendens* (Callichthyidae). *J. exp. Biol.* **74**, 1–16.

GRAHAM, J. B., KRAMER, D. L. & PINEDA, E. (1977). Respiration of the air breathing fish *Piabucina festae*. *J. comp. Physiol.* **122**, 295–310.

HOCHACHKA, P. W. (1980). *Living Without Oxygen*. Cambridge: Harvard University Press.

HOLETON, G. F. (1980). Oxygen as an environmental factor of fishes. In *Environmental Physiology of Fishes*, (ed. M. A. Ali), pp. 7–32. New York and London: Plenum Press.

HUGHES, G. M. (1973). Respiratory responses to hypoxia in fish. *Am. Zool.* **13**, 475–489.

HUGHES, G. M. (1976). *Respiration of Amphibious Vertebrates*. London: Academic Press.

HUGHES, G. M. & SAUNDERS, R. L. (1970). Responses of the respiratory pumps to hypoxia in the rainbow trout (*Salmo gairdneri*). *J. exp. Biol.* **53**, 529–545.

HUGHES, G. M. & SINGH, B. N. (1971). Gas exchange with air and water in an air-breathing catfish, *Saccobranchus* (=*Heteropneustes*) *fossilis*. *J. exp. Biol.* **55**, 667–682.

HUTCHISON, V. H., HAINES, H. B. & ENGBRETSON, G. (1976). Aquatic life at high altitude: respiratory adaptations in the Lake Titicaca frog, *Telmatobius culeus*. *Respir. Physiol.* **27**, 115–129.

JOHANSEN, K. (1970). Air breathing in fishes. In *Fish Physiology*, Vol. 4, (eds W. S. Hoar & D. J. Randall), pp. 361–411. New York: Academic Press.

JOHANSEN, K., LENFANT, C. & GRIGG, G. C. (1967). Respiratory control in the lungfish, *Neoceratodus forsteri* (Krefft). *Comp. Biochem. Physiol.* **20**, 835–854.

JUST, J. J., GATZ, R. N. & CRAWFORD, E. C. (1973). Changes in respiratory functions during metamorphosis of the bullfrog, *Rana catesbeiana*. *Respir. Physiol.* **17**, 276–282.

[redacted]TENS, A., LOMHOLT, J. P. & JOHANSEN, K. (1979). The ventilation, extraction and uptake of oxygen in undisturbed flounders, *Platichthys flesus*: responses to hypoxia acclimation. *J. exp. Biol.* **83**, 169–179.

KIRSCH, R. & NONNOTTE, G. (1977). Cutaneous respiration in three freshwater teleosts. *Respir. Physiol.* **29**, 339–354.

MCMAHON, B. R. (1970). The relative efficiency of gaseous exchange across the lungs and gills of an African lungfish, *Protopterus aethiopicus*. *J. exp. Biol.* **52**, 1–15.

MANGUM, C. & VAN WINKLE, W. (1973). Responses of aquatic invertebrates to declining oxygen conditions. *Am. Zool.* **13**, 529–541.

MOALLI, R., MEYERS, R. S., JACKSON, D. C. & MILLARD, R. W. (1980). Skin circulation of the frog, *Rana catesbeiana*, distribution and dynamics. *Respir. Physiol.* **40**, 137–148.

NOLAND, R. & ULTSCH, G. R. (1981). The roles of temperature and dissolved oxygen in microhabitat selection by the tadpoles of a frog (*Rana pipiens*) and a toad (*Bufo terrestris*). *Copeia 1981*, 645–652.

RANDALL, D. J., BURGGREN, W. W., FARRELL, A. P. & HASWELL, M. S. (1981). *The Evolution of Air Breathing in Vertebrates*. Cambridge: Cambridge University Press.

RANDALL, D. J., CAMERON, J. N., DAXBOECK, C. & SMATRESK, N. (1981). Aspects of bi-modal gas exchange in the bowfin, *Amia calva L.* (*Actinopterygii*: *Amiiformes*). *Respir. Physiol.* **43**, 339–348.

SCHOLANDER, P. F. (1947). Analyzer for accurate estimation of respiratory gases in one-half cubic centimeter samples. *J. biol. Chem.* **167**, 235–250.

SINGH, B. N. & HUGHES, G. M. (1971). Respiration of an air-breathing catfish, *Clarias batrachus* (L.). *J. exp. Biol.* **55**, 667–682.

STEVENS, E. D. & HOLETON, G. F. (1979). The partitioning of oxygen uptake from air and water by erythrinids. *Can. J. Zool.* **56**, 965–969.

STRAWINSKI, S. (1956). Vascularization of respiratory surfaces in ontogeny of the edible frog, *Rana esulenta* [sic] L. *Zoologica Poloniae* **7**, 327–365.

TAYLOR, A. C. & KOLLROS, J. (1946). Stages in the normal development of *Rana pipiens* larvae. *Anat. Rec.* **94**, 7–24.

ULTSCH, G. R. (1976). Respiratory surface area as a factor controlling the standard rate of oxygen consumption of aquatic salamanders. *Respir. Physiol.* **26**, 357–369.

WASSERSUG, R. J. & HEYER, W. R. (1983). Morphological correlates of sub-aerial existence in leptodactylid tadpoles associated with flowing water. *Can. J. Zool.* (in the press).

WASSERSUG, R. J., PAUL, R. D. & FEDER, M. E. (1981). Cardiorespiratory synchrony in anuran larvae (*Xenopus laevis*, *Pachymedusa dacnicolor*, and *Rana berlandieri*). *Comp. Biochem. Physiol.* **70**A, 329–334.

WASSERSUG, R. J. & SEIBERT, E. A. (1975). Behavioral responses of amphibian larvae to variation in dissolved oxygen. *Copeia 1975*, 86–103.

WELCH, W. R. (1979). *Fitting Segmented Straight Lines: A Mini-manual for Use with the Minitab Computing System*. Madison: University of Wisconsin.

WEST, N. H. & BURGGREN, W. W. (1982). Gill and lung ventilatory responses to steady-state aquatic hypoxia and hyperoxia in the bullfrog tadpole (*Rana catesbeiana*). *Respir. Physiol.* **47**, 165–176.