J. Exp. Biol. 105, 191–203 (1983)
Printed in Great Britain © The Company of Biologists Limited 1983

RESPIRATION DURING CHRONIC HYPOXIA AND HYPEROXIA IN LARVAL AND ADULT BULLFROGS (*RANA CATESBEIANA*)

I. MORPHOLOGICAL RESPONSES OF LUNGS, SKIN AND GILLS

BY WARREN BURGGREN AND ANGANILE MWALUKOMA

Department of Zoology, University of Massachusetts, Amherst, MA 01003-0027, U.S.A.

(*Received 2 August 1982 – Accepted 2 March 1983*)

SUMMARY

Larval and adult bullfrogs, *Rana catesbeiana* (Shaw), were exposed to 28 days of normoxia (P_{O_2} 150 mmHg), hypoxia (P_{O_2} 70–80 mmHg) or hyperoxia ($P_{O_2} > 275$ mmHg) at 20–23 °C, after which the following morphological measurements were made: (1) mass, thickness, capillary mesh density and blood-water barrier of the skin; (2) mass, volume, cava density and blood-gas barrier of lungs; and, for the larvae, (3) arch length, filament density and size, and blood-water barrier of the gills.

Chronic hypoxia induced profound morphological changes in the gas exchange organs of larvae, but not of adults. In tadpoles, the skin became thinner, with a doubling of capillary mesh density and a halving of the blood-water barrier. The gas diffusion barrier of the lungs remained unchanged, but the lung volume and density of the lung wall cava both increased significantly. The internal gills showed a marked enlargement upon hypoxic exposure, both in numbers of gill filaments and size of each filament. The blood-water barrier remained unchanged. Chronic hyperoxia, unlike chronic hypoxia, caused no significant changes in the morphology of the gas exchange organs of larvae.

Chronic exposure to hypoxia or hyperoxia failed to produce any significant morphological changes in adult bullfrogs.

These data indicate that the great morphological plasticity of larvae, culminating in metamorphosis, also extends to profound adjustments in the gas exchange organs when oxygen transfer becomes limited, a response lacking in adults.

INTRODUCTION

The great majority of studies on the effects of hypoxia on gas exchange processes in ectothermic vertebrates have been confined to responses to acute hypoxia. Respiratory homeostasis during acute hypoxic exposure is usually maintained by increases in the convective flow of air or water through gas exchange organs (see Shelton, 1970; Dejours, 1981, for reviews). However, given a sufficient length of hypoxic exposure, morphological and biochemical changes can complement or even supersede such initial physiological responses. Since chronic hypoxia in aquatic habitats may occur

Key words: Bullfrog, hypoxia, acclimation.

during periods of reproductive activity or developmental change in animals, an ability to evoke adjustments maintaining oxygen delivery to the tissues over the longterm may have important consequences for survival.

It is thus not surprising that frog or salamander larvae raised in chronic aquatic hypoxia develop branchial hypertrophy (Babák, 1907; Drastich, 1925; Bond, 1960; Guimond & Hutchinson, 1976). However, for some amphibian larvae the lungs and/or skin, not the gills, may be the most important sites of respiration (see Burggren & West, 1982). Yet changes in skin or lung morphology specifically related to chronic aquatic hypoxia (and hyperoxia) have yet to be investigated in amphibians. Larvae normally undergo major morphological change leading up to metamorphosis, whereas the potential for tissue differentiation is comparatively reduced in adults. Thus chronic hypoxic or hyperoxic exposure might produce quite different morphological responses in the respiratory organs of larval compared with adult *Rana catesbeiana*.

This study, and a companion paper (Pinder & Burggren, 1983), begins an investigation of the morphological, physiological and biochemical responses to chronic hypoxia and hyperoxia in the larvae and adults of the bullfrog, *Rana catesbeiana*. In this particular report, morphological changes of the gas exchange organs induced by chronic hypoxia and hyperoxia are quantified.

MATERIALS AND METHODS

Twenty-two adult bullfrogs (mean mass 97 ± 50 g, mean ±1 S.D.) and 28 tadpoles (mean mass 15·7 ± 4·6 g) of developmental stages XV–XX (after Taylor & Kollros, 1946) were captured locally, and maintained on liver and fish (adults) or spinach and lettuce (tadpoles). Both populations experienced a maximum weight loss of about 5 % body weight over the experimental period of 4 weeks.

Adults and larvae were each divided into three distinct populations, with body weight (and in the case of tadpoles, developmental stage) randomly distributed between populations. Each population was maintained in a separate gas-tight chamber (volume = 40 l) at 20–23 °C. The P_{O_2} of water and gas was maintained at appropriate levels by constantly passing air/N_2 or air/O_2 mixtures through air stones inserted through the wall of each chamber.

The first population pair of adults and tadpoles, which served as the controls, were maintained under normoxic conditions, i.e. air-saturated water covered by an atmosphere of air (P_{O_2} about 150 mmHg). The second pair of populations constituted the hypoxic group, in which both gas and water phases were maintained at a P_{O_2} between 70 and 80 mmHg (Table 1). Acute exposure to these P_{O_2} levels produces hyperventilation of gills and lungs in bullfrog tadpoles (Burggren & West, 1982) and the adults of other anurans (see Boutilier & Toews, 1977). The final pair of populations consisted of hyperoxic groups, in which both gas and water phases of the chamber were maintained at a P_{O_2} above 275 mmHg. Oxygen partial pressures of both gas and water phase were monitored daily, and varied less than 10 mmHg on a day to day basis. However, both frogs and tadpoles were 'eased into' chronic hypoxia or hyperoxia by steadily increasing or decreasing P_{O_2} to the desired level over the course of the first 4 days of the experiment. This O_2 level was then maintained for a total elapsed exposure of 25–28 days.

Table 1. *The P_{O_2} of water and overlying gas in the experimental chambers in which larval and adult bullfrogs were kept for 4 weeks*

Population	Respiratory medium	P_{O_2} (mmHg)
Normoxic adults	gas	150 ± 4
	water	149 ± 5
Normoxic larvae	gas	151 ± 2
	water	148 ± 8
Hypoxic adults	gas	75 ± 25
	water	73 ± 15
Hypoxic larvae	gas	79 ± 22
	water	70 ± 22
Hyperoxic adults	gas	260 ± 76
	water	277 ± 53
Hyperoxic larvae	gas	387 ± 163
	water	430 ± 131

Values are means (± 1 S.E.) of 28 daily measurements.

Animals were then killed, the heart immediately exposed, and a blood sample taken into a heparinized syringe for haematological analyses (see Pinder & Burggren, 1983). A catheter was tied into the ventricle and the atria were opened. Heparinized phosphate buffer (pH 7·7, sucrose added to produce 340 mosmol kg^{-1} H_2O) containing India ink was then flushed through the circulation until the skin was uniformly darkened by the ink.

The following dissections/observations were then made, in the order presented.

In situ *lung volume and lung morphometrics*

The trachea of the undissected, freshly-killed frog was occlusively catheterized through the mouth. The catheter was connected to a T, with one arm attached to a glass, gas-tight syringe, and the other *via* non-compliant tubing to a calibrated Narco P-1000B pressure transducer attached to a Narco MK-IV chart recorder. Initially, the tap was opened to air and the body wall was compressed, causing collapse and emptying of the lungs. The tap was then closed and known volumes of air from the syringe were injected into the lungs until a static lung pressure of 6·0 cmH_2O was achieved, which is a pressure common during buccal filling in adult *Rana* (West & Jones, 1975).

The lungs of larval *Rana catesbeiana*, and to a lesser extent the adults, are heavily pigmented, so even ink-filled capillaries in the lung walls are not distinguishable. However, when the lungs were dissected out, opened and flattened lumen side up under a coverslip, the primary lung septa were observed to form discrete invaginated cava approximately 40–70 μm in diameter. The number of these cava within a defined field of view was counted and expressed as lung wall cava · cm^{-2}. Pieces of lung tissue approximately 1 mm^2 were then fixed and embedded and sections made (see below). The mean minimum length of the gas diffusion pathway between capillary blood and lung gas was determined by using a calibrated monocular micrometer to measure the direct distance between the lung wall epithelial cells and the edge of the capillary lumen. An average of the five smallest diffusion pathways observed was calculated for each tissue specimen.

Branchial morphometrics of larvae

The internal gills of larval *Rana* consist of four pairs of gill arches. Each arch bears two rows of approximately 8–30 discrete gill filaments. The gill filaments are highly branched giving rise to a variable number of 'finger-like' projections, each 50–100 μm in length and 10–30 μm in diameter (Figs 5, 6). These projections contain blood channels, and constitute the major gas exchange surface of the gills.

Excised gill arches from the left side were suspended in buffer for examination under a dissecting microscope (20–50× magnification). The following measurements were then made on each gill arch (III–VI) from the left side of the tadpole: arch length, number of filaments per arch (both rows counted), the height of each filament along one entire filament row, the cumulative filament height (the measured row ×2 to account for both rows on the arch) and the extreme height and extreme width of the filament occupying the centre position along the filament.

Individual branchial filaments were fixed and the mean minimum length of the diffusion pathway between water and capillary blood determined as described above.

Skin morphometrics

Skin both from the tail and the lateral surface of the flank (tadpole), or lateral surface of the hindlimbs and lateral surface of the flank (adult), was dissected out and transferred to a saline bath. The skin pieces were trimmed to a 1 cm square, smoothed out on a microscope slide and covered with a glass coverslip. The ink-filled capillaries were usually clearly visible under 100–400× magnification. Capillary density was measured and expressed as the number of capillary 'meshes', i.e. the number of tissue spaces completely enclosed by interconnecting capillaries (see Czopek, 1965), counted in a defined field of view, and converted to number of capillary meshes · mm^{-2}. After examination, the 1 cm^2 piece of skin was blotted dry and weighed. Pieces of tissue approximately 1 mm^2 were then fixed and skin thickness and the mean minimum diffusion pathway between the outer layer and capillary blood was measured as described above.

Tissue fixation

Excised tissues were fixed in 2 % phosphate buffered glutaraldehyde, post-fixed in 1 % phosphate buffered osmium tetroxide, and stained in uranyl acetate. Both fixative and post-fixative were adjusted with glucose to an osmolality of 325–340 mosmol kg^{-1} H_2O to minimize tissue shrinkage. Some small amount of tissue shrinkage may have persisted, but in any event the data analysis focused on relative changes between experimental treatments. Standard ethanol dehydration was

Fig. 1. Lung morphometrics in larval (dashed lines) and adult (unbroken lines) bullfrogs as a function of chronic exposure to normoxia, hypoxia or hyperoxia. Mean values ±1 S.E. are given. Numbers of adult frogs contributing to each mean are as follows: normoxia, 7; hypoxia, 9; hyperoxia, 6. Numbers of larvae contributing to each mean are as follows: normoxia, 7; hypoxia, 14; hyperoxia, 7. The letters (NS = not significant) or numbers beside each set of lines refers to the value for P, i.e. significance level, for an ANOVA of data groups for the three oxygen levels. Means which are different from the control (normoxic) means are indicated by one ($P<0{\cdot}05$) or two ($P<0{\cdot}01$) asterisks.

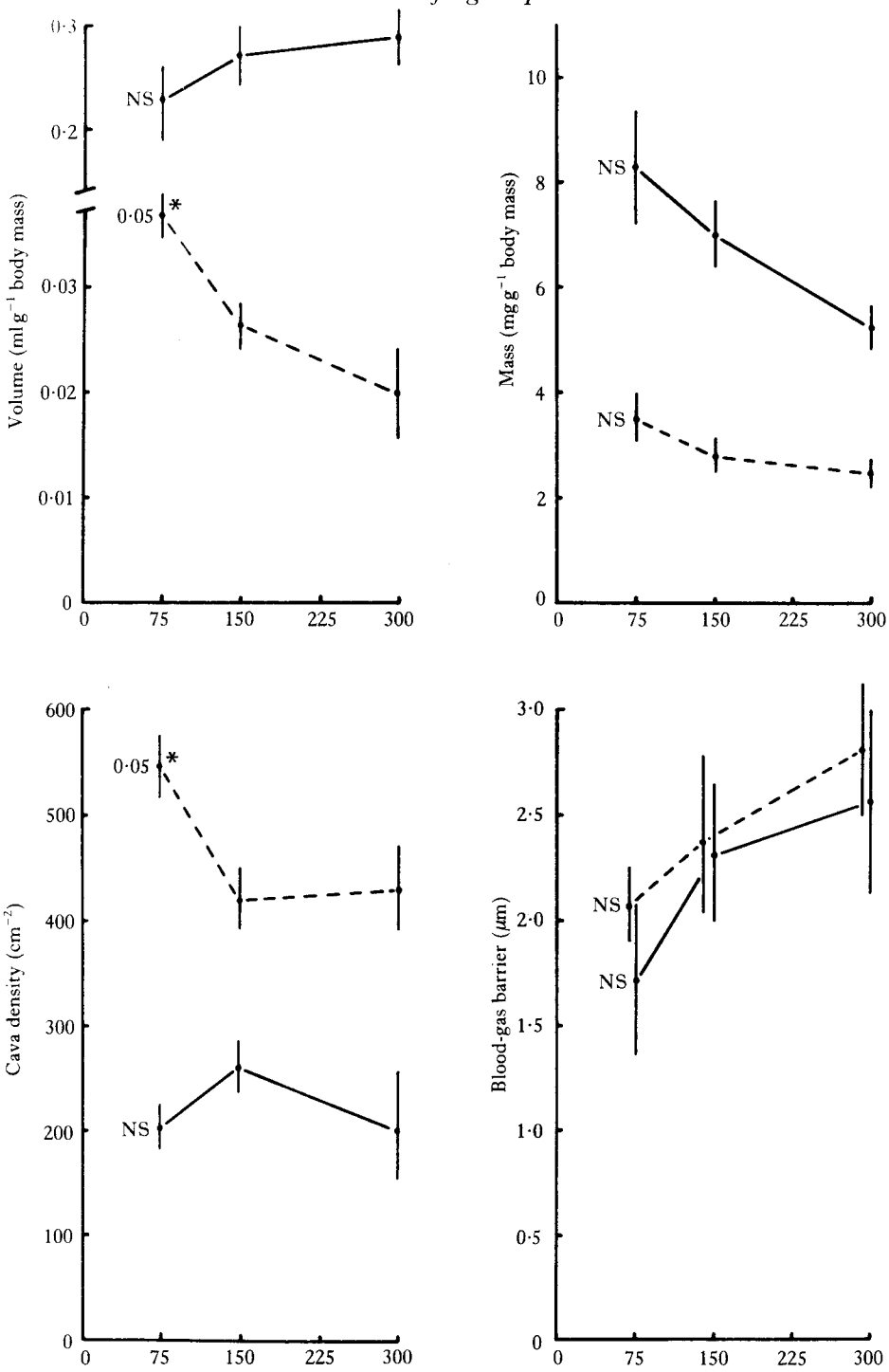

Volume (ml g^{-1} body mass)
0·3
0·2
0·03
0·02
0·01
0
NS
0·05
*
Mass (mg g^{-1} body mass)
10
8
6
4
2
0
NS
NS
0
75
150
225
300
Cava density (cm^{-2})
600
500
400
300
200
100
0
0·05
*
NS
Blood-gas barrier (μm)
3·0
2·5
2·0
1·5
1·0
0·5
0
NS
NS
Ambient P_{O_2} (mmHg)

Fig. 1

followed by Epon: Araldite resin embedding. Sections 1–4 μm thick were stained in toluidine blue and examined in a Bausch and Lomb Balplan phase contrast microscope.

Statistical analysis

Treatment effects (i.e. three oxygen levels) among larval populations and among adult populations were assessed initially by analysis of variance (ANOVA). Where significant ($P < 0{\cdot}05$) treatment effects existed, differences between specific means were subsequently assessed with Student's t-test for independent means.

RESULTS

In all experimental groups, including controls, 5–25 % of the animals died during the 3·5–4 week experimental period, most of them during the first week. The remaining animals all appeared to be in a healthy condition. Analyses were based on the following numbers of individuals surviving hypoxia, normoxia and hyperoxia: adults – 9, 7 and 6, respectively; tadpoles – 14, 7 and 7, respectively.

Lung morphometrics

Lung morphometric data for both adults and tadpoles are presented in Fig. 1, while photomicrographs of lung tissue are presented in Figs 9A, B and 10.

Environmental P_{O_2} had no significant treatment effect ($P > 0{\cdot}10$, ANOVA) on any measured lung variable in adult bullfrogs.

In sharp contrast to those of adults, the lungs of tadpoles exhibited significant ($P < 0{\cdot}05$ or lower, ANOVA) morphometric differences related to environmental P_{O_2}. Weight-specific lung volume and lung wall cavum density were significantly elevated by 30 % and 23 %, respectively, above control levels after 4 weeks of hypoxic exposure (Fig. 1). However, the lung measurements of hyperoxic populations were not significantly different ($P > 0{\cdot}1$) from those of normoxic populations after this time, nor was the minimum blood-gas barrier in the tadpole lung significantly affected by environmental P_{O_2}.

Skin morphometrics

Environmental P_{O_2} had no significant effect upon capillary mesh density or blood-water diffusion distance of flank or hindlimb skin in adult bullfrogs (Figs 2, 3).

Again in sharp contrast to the situation in adults, highly significant changes in skin morphology were correlated with environmental P_{O_2} in tadpoles. Tail skin thickness and weight were not greatly different between hypoxic and normoxic populations, but capillary mesh density was more than double, and the mean minimum diffusion pathway between blood and water fell by 58 % in the hypoxic populations (Figs 2, 8). Tail skin variables in hyperoxic tadpoles were not significantly different from those of the normoxic populations.

The flank skin of hypoxic tadpoles was also profoundly influenced by environmental P_{O_2}. Flank skin of tadpoles exposed to 4 weeks of hypoxia was approximately one-half as thick and as heavy as the skin of those in normoxic conditions. In addition, the

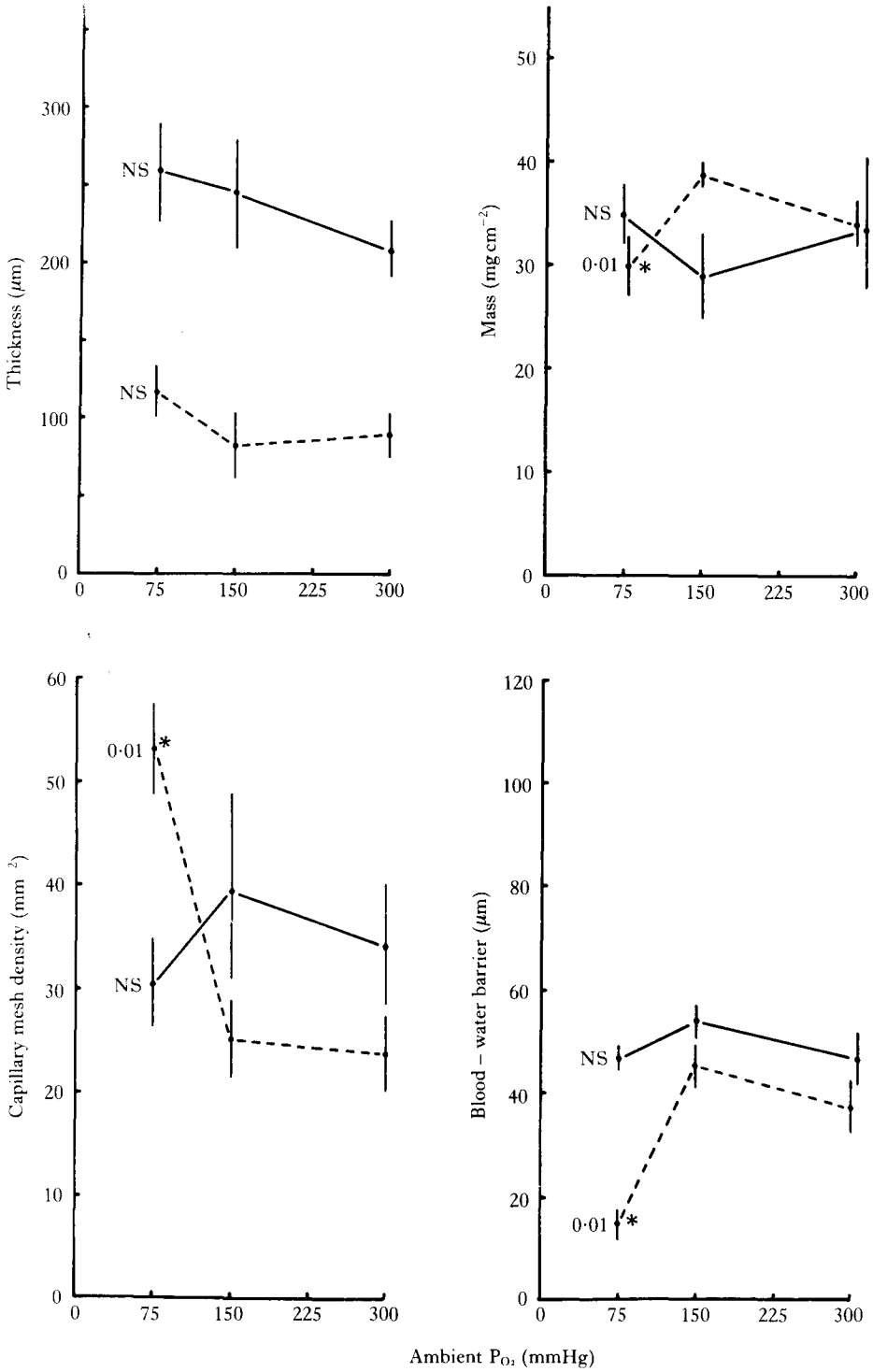


Fig. 2. Tail (larvae) or limb (adults) skin morphometrics in larval and adult bullfrogs as a function of chronic exposure to normoxia, hypoxia or hyperoxia. See legend to Fig. 1 for use of symbols and numbers of animals used.

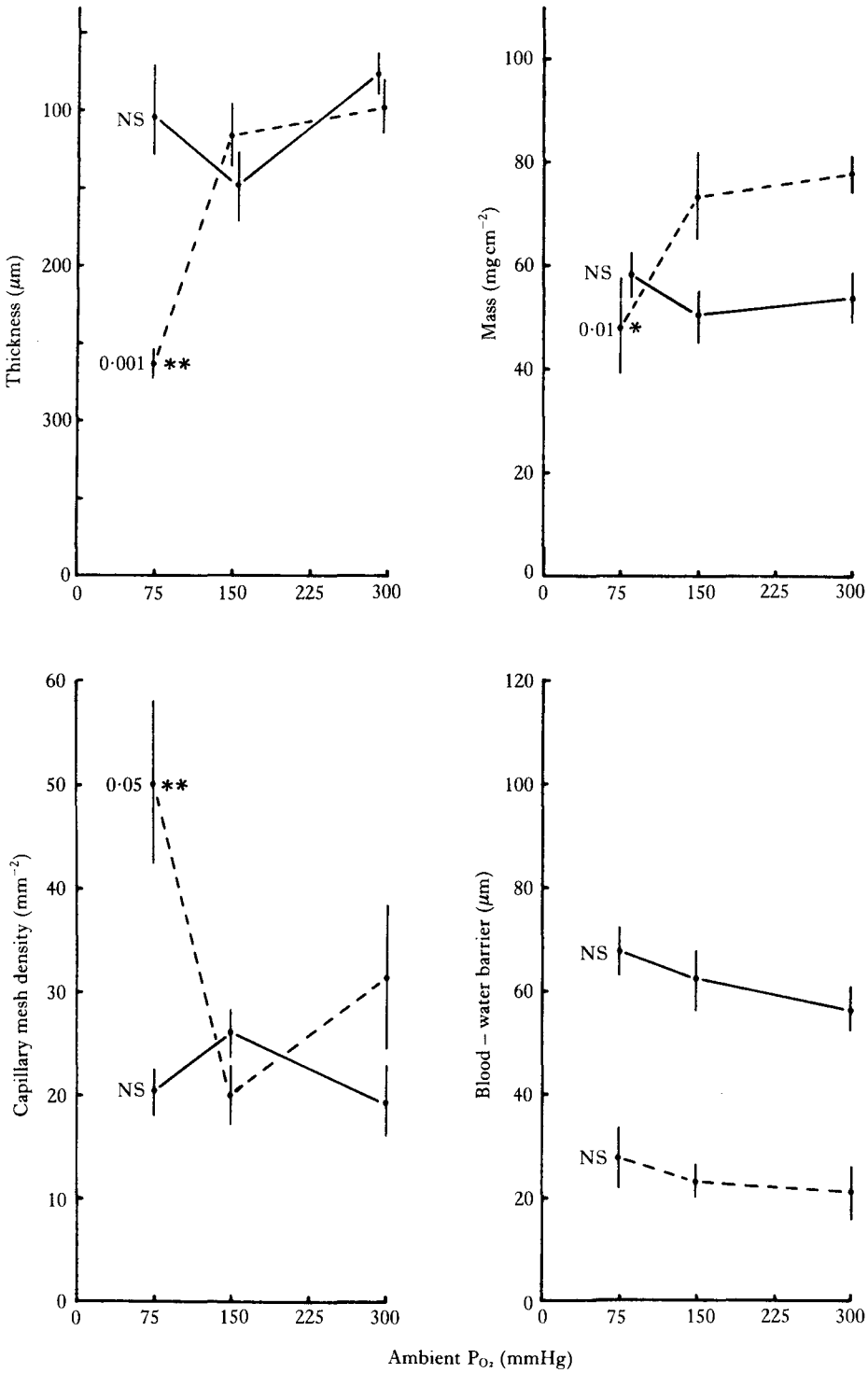


Fig. 3. Flank skin morphometrics in larval and adult bullfrogs as a function of chronic exposure to normoxia, hypoxia or hyperoxia. See legend to Fig. 1 for use of symbols and numbers of animals used.

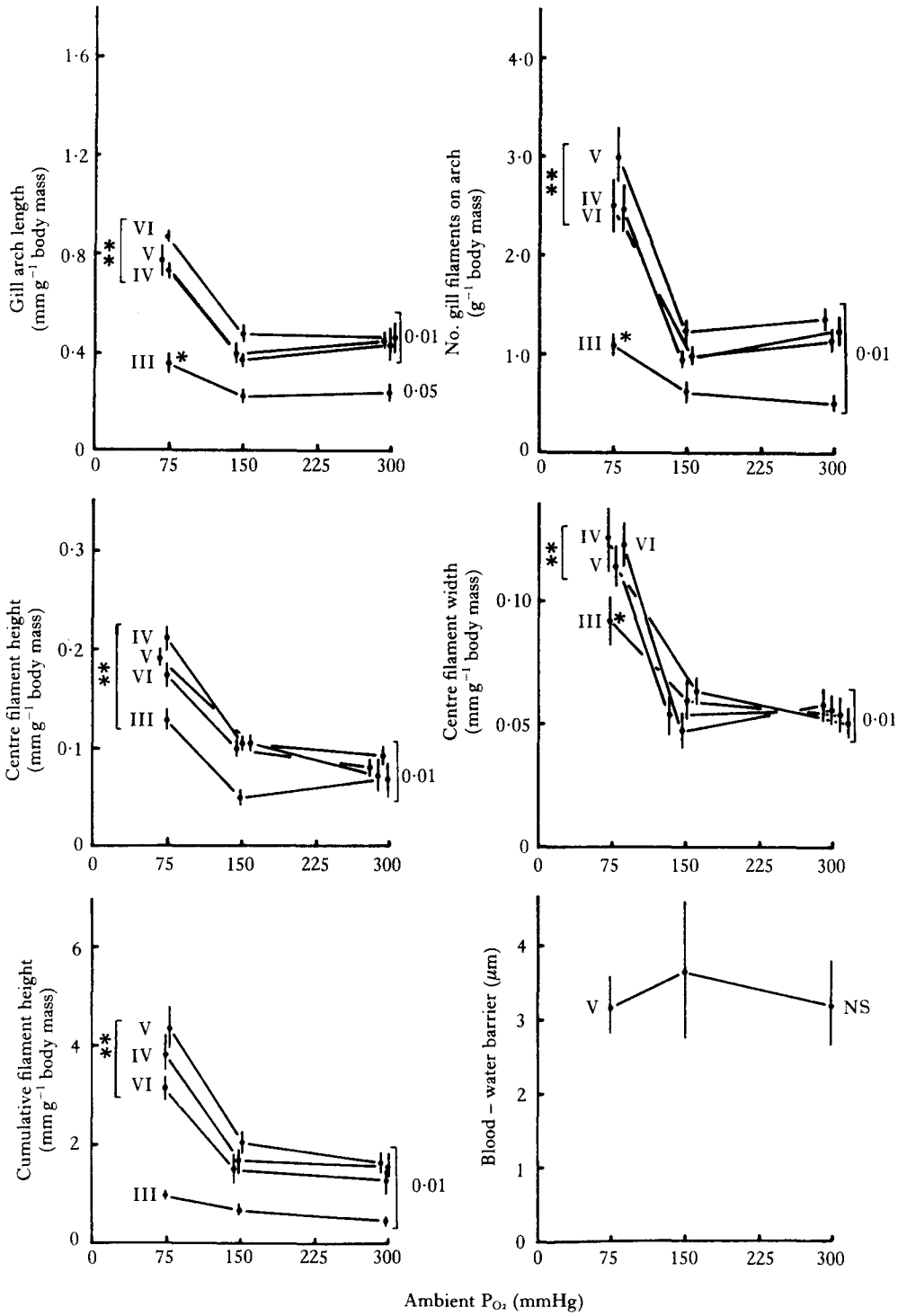


Fig. 4. Branchial morphometrics in larval bullfrogs as a function of chronic exposure to normoxia, hypoxia or hyperoxia. Roman numerals indicate embryonic arch number. See legend to Fig. 1 for use of symbols and numbers of animals used.

capillary density was elevated by 250% (Fig. 3). However, diffusion distance between blood and water remained unchanged. Hyperoxia produced no significant changes in flank skin from the normoxic condition.

Branchial morphometrics

Within any one of the three tadpole populations (i.e. independent of oxygen treatment effects), there was a significant correlation ($P < 0{\cdot}05$ for all correlation coefficients, r) between body mass and the following branchial variables; gill arch length, number of gill filaments/arch, filament width and height, and cumulative filament height/arch. These variables, therefore, were expressed on a mass-specific basis to facilitate comparison between tadpole populations.

Highly significant changes associated with different environmental P_{O_2} values occurred in every branchial variable (Fig. 4), with the exception of the mean minimum diffusion pathway between branchial blood and water. Considering gill arch V of hypoxic tadpoles, for example, the arch itself was 100% longer and bore 85% more gill filaments than gill arches of normoxic tadpoles. In addition, the centre gill filament was both 85% wider and taller than in normoxic tadpoles. The marked enlargement of individual gill filaments from the centre of arch V in hypoxic compared with normoxic or hyperoxic tadpoles is shown in Fig. 5. Cumulative filament height for gill arch V was more than doubled by chronic hypoxic exposure. Changes of similar magnitude were observed in gill arches IV and VI, but were attenuated in arch III, which in all three populations was a much smaller branchial structure. While respiratory surface area *per se* was not measured, these data clearly indicate a marked branchial hypertrophy associated with hypoxic exposure.

No significant branchial changes ($P > 0{\cdot}10$, ANOVA) from the control tadpole population were associated with chronic hyperoxic exposure.

DISCUSSION

Developmental changes in normoxic conditions

Important morphological differences emerge between the gills, skin and lungs of *R. catesbeiana* tadpoles which may affect oxygen transfer across these organs. In the gills and lungs, for example, minimum gas diffusion pathways range from 2 to 4 μm,

---

Fig. 5. The centre gill filaments removed from the left gill arch V of three, 15 g larval *Rana catesbeiana* exposed to chronic normoxia (*n*), hypoxia (*hyp*) and hyperoxia (*hypr*). Black bar indicates 1·0 mm.

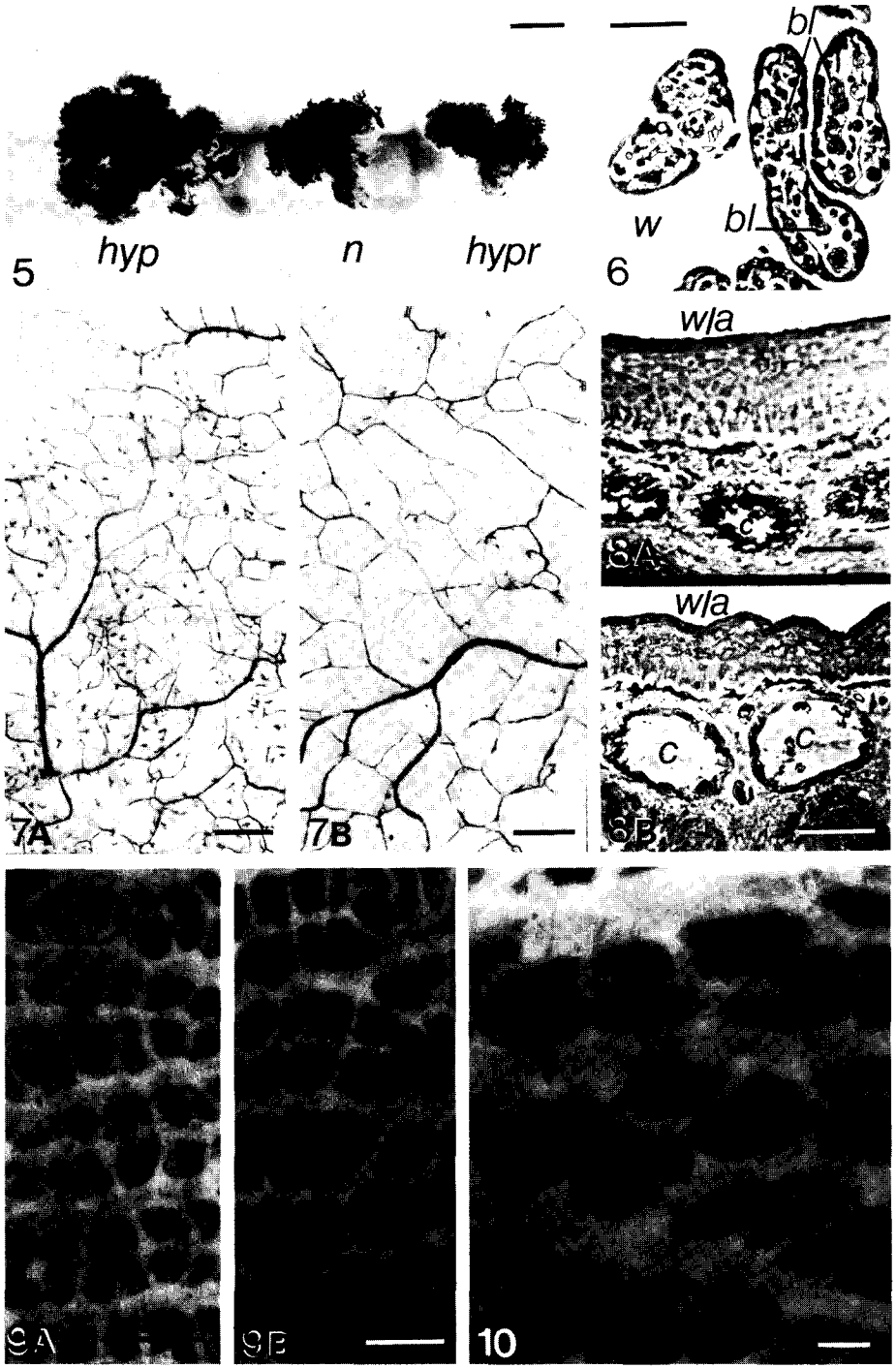
Fig. 6. Cross section through the gill filament of a larval *Rana catesbeiana* exposed to chronic normoxia. Black bar indicates 10 μm. *bl*, blood channel; *w*, branchial water.

Fig. 7. Tail skin perfused with India ink and removed from larval *Rana catesbeiana* exposed to chronic hypoxia (A) and normoxia (B). Black bars indicate 250 μm on both figures.

Fig. 8. Thin sections through the tail skin of larval *Rana catesbeiana* exposed to chronic normoxia (A) and hypoxia (B). Bars indicate 20 μm in both figures. *w/a*, water or air; *c*, capillary.

Fig. 9. Internal surface of the lung walls of larval *Rana catesbeiana* exposed to chronic hypoxia (A) and normoxia (B). Bars indicate 500 μm.

Fig. 10. Internal surface of the lung wall of an adult bullfrog exposed to chronic normoxia. Bar indicates 500 μm.


5
hyp
n
hypr
6
bl
w
bl
7A
7B
w/a
c
8A
w/a
c
c
8B
9A
9B
10

whereas in the skin covering the tail and trunk, the diffusion pathway is ten times greater (Figs 1–4). In the earlier developmental stages, however, the organs of oxygen uptake in decreasing order of importance are skin, gills and lungs (Burggren & West, 1982; Burggren, Feder & Pinder, 1983). Clearly, other factors such as respiratory surface area, the P_{O2} gradient between capillary blood and respiratory medium, and the convective supply of both water/air and blood, greatly influence the relative oxygen uptake performance of these respiratory structures.

Metamorphosis in *R. catesbeiana* tadpoles is accompanied by the total loss, reduction, or rearrangement of entire organ systems. The gills atrophy and disappear, as does the large tail, which in early developmental stages has a high surface area to mass ratio (Burggren & West, 1982), and probably makes a large contribution to cutaneous gas exchange. With respect to oxygen uptake, at least, there is thus a major shift of gas exchange from aquatic sources to aerial uptake *via* the enlarging lungs (see Burggren & West, 1982), which undergo a 10-fold increase in mass-specific lung volume (Fig. 1). Although the degree of septation of the adult lung is about one-half that in larvae (Fig. 1), the net effect of metamorphosis must be a large increase in mass-specific surface area. The minimum diffusion distance between pulmonary capillary blood and lung gas, however, was unaffected by metamorphosis. The lungs of lower vertebrates are generally perfused at much higher blood pressures with fluid of lower colloid osmotic pressure than in mammals, which may produce a large net plasma filtration into the lungs (Burggren, 1982). To prevent accumulation of excessive fluid in the alveolar spaces, the reductions in capillary and lung wall structure and thickness (which might alter gas diffusion path length) possible upon maturity may be restricted by a need to maintain a comparatively thick and 'leak-free' blood/gas barrier.

In *R. catesbeiana* tadpoles of developmental stages IV–XIX, about 60 % of oxygen uptake occurs *via* the skin, while this falls to only 20 % in the adult bullfrog (Burggren & West, 1982). This may result in part from the changing ventilation/perfusion relationships of the cutaneous and pulmonary vascular bed, but can also be partially explained by the three-fold increase in diffusion distance between the skin surface and cutaneous capillaries (Figs 2, 3).

Effects of environmental P_{O2} on respiratory morphology

In essence, the morphology of all the gas exchange organs of *R. catesbeiana* tadpoles was profoundly adjusted during the course of 4 weeks of low oxygen exposure, while the respiratory structures of adult bullfrogs were essentially unaltered. Within a single amphibian species, then, the morphological response to chronic hypoxic stress clearly differs with developmental state.

The magnitude of hypoxic-induced changes in the skin, gills and lungs of tadpoles was extraordinary. For example, in tadpole skin (Figs 2, 3, 7, 8), the blood-water gas diffusion pathway as well as capillary mesh density doubled, reflecting a large increase in both number and surface area of cutaneous capillaries. All of these changes may help to maintain cutaneous oxygen uptake in the face of decreasing ambient P_{O2}.

Mass-specific lung volume and the degree of lung septation in tadpoles were also significantly higher after chronic hypoxic exposure which, coupled with physiological responses of hyperventilation during hypoxic exposure (West & Burggren, 1982, 1983), would facilitate pulmonary oxygen uptake during aerial hypoxia.

A marked branchial hypertrophy also occurred, particularly in the largest gill arches (Figs 4, 5). A similar branchial hypertrophy after hypoxic exposure for 7–30 days has been reported in larval amphibians (Babák, 1907; Drastich, 1925; Bond, 1960; Guimond & Hutchinson, 1976) and larval fishes (see McDonald & McMahon, 1977). As with the urodele amphibians examined in these earlier studies, a large increase in the respiratory surface area of the internal gills of the bullfrog tadpole should facilitate aquatic oxygen uptake, particularly if any component of the profound branchial hyperventilation in response to acute hypoxia (West & Burggren, 1982) remains during chronic hypoxic exposure.

In contrast to the profound morphological adjustments in tadpoles, the respiratory structures (lungs, skin) of the adult bullfrog essentially remained unaltered after 4 weeks of hypoxic exposure (Figs 1–4).

What clearly emerges, then, are two quite different responses to longterm exposure to low oxygen levels by different developmental stages of *R. catesbeiana*. The tissues of larval amphibians obviously undergo tremendous differentiation and alteration during metamorphosis. This 'plasticity' of tissues and even whole organs, which usually manifests itself only during the normal metamorphic process, apparently can be stimulated by environmental factors unrelated to development, such as chronic hypoxic exposure, to produce morphological adjustments in respiratory structures which facilitate gas exchange under sub-optimum conditions.

Morphological changes to lungs and skin might be expected to aid gas exchange in adult bullfrogs, as well, but are completely absent (Figs 1–4). Changes in the convective delivery of lung gas and blood, along with large increases in blood oxygen affinity and oxygen capacity of adult bullfrogs in chronic hypoxia (Pinder & Burggren, 1983) – few of which occurs in tadpoles – may suffice to maintain oxygen uptake without morphological adjustments in the gas exchange organs. Whether the 'cost' of changes in the morphology of respiration structures *vs* changes in blood properties and physiological responses is much greater in the terminal adult stage compared to the tadpole stages, or whether the adult simply lacks regulatory mechanisms for tissue growth which are stimulated by environmental hypoxia, remains to be demonstrated.

We thank Lourdes Algarin for assistance with tissue fixation, Alan Pinder for preparation of the figures and Juan Markin for comments during preparation of the manuscript. This project was supported by NSF grant PCM80-03752 and a University of Massachusetts Faculty Research Grant.

REFERENCES

BABÁK, E. (1907). Über die funktionelle Anpassung der äusseren Kiemen bei Sauerstoffmangel. *Zentbl. Physiol.* **21**, 97.

BOND, A. N. (1960). An analysis of the response of salamander gills to changes in the oxygen concentration of the medium. *Devl Biol.* **2**, 1–20.

BOUTILIER, R. G. & TOEWS, D. P. (1977). The effect of progressive hypoxia on respiration in the toad *Bufo marinus*. *J. exp. Biol.* **68**, 99–108.

BURGGREN, W. W. (1982). Pulmonary blood plasma filtration in reptiles: a wet vertebrate lung? *Science, N.Y.* **215**, 77–78.

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

CZOPEK, J. (1965). Quantitative studies on the morphology of respiratory surfaces in amphibians. *Acta anat.* **62**, 296–323.

DEJOURS, P. (1981). *Principals of Comparative Respiratory Physiology*. New York: Elsevier/North Holland. 265 pp.

DRASTICH, L. (1925). Über das Leben der Salamandre-Larven bei hohem und neidrigem Sauerstoffpartialdruck. *Z. vergl. Physiol.* **2**, 632–657.

GUIMOND, R. W. & HUTCHINSON, V. H. (1976). Gas exchange of the giant salamanders of North America. In *Respiration of Amphibious Vertebrates*, (ed. G. M. Hughes), pp. 313–338. New York: Academic Press.

MCDONALD, D. G. & MCMAHON, B. R. (1977). Respiratory development in Arctic char *Salvelinus alpinus* under conditions of normoxia and chronic hypoxia. *Can. J. Zool.* **55**(9), 1461–1467.

PINDER, A. W. & BURGGREN, W. W. (1983). Respiration during chronic hypoxia and hyperoxia in larval and adult bullfrogs. II. Changes in blood respiratory properties and haematology. *J. exp. Biol.* **105**, 205–213.

SHELTON, G. (1970). The Regulation of Breathing. *Fish Physiology*, Vol. 4, (eds W. S. Hoar & D. J. Randall), pp. 293–359. New York: Academic Press.

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

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

WEST, N. H. & BURGGREN, W. W. (1983). Reflex interactions between aerial and aquatic gas exchange organs in the larval bullfrog. *Am. J. Physiol.* (in the press).

WEST, N. H. & JONES, D. R. (1975). Breathing movements in the frog *Rana pipiens*. II. The power output and efficiency of breathing. *Can. J. Zool.* **53**(3), 345–353.