Larval Cardiorespiratory Ontogeny and Allometry in *Xenopus laevis*

Kent Orlando
Alan W. Pinder*
Biology Department, Dalhousie University, Halifax, Nova Scotia B3H 4J1, Canada

Accepted 9/6/94

Abstract

Very little is known about the early development of cardiorespiratory regulatory mechanisms in newly hatched amphibian larvae. We tested whether early cardiovascular responses to hypoxia reflect local flow regulation in tissues and whether regulation of ventilation would improve during larval development. Cardiac output was calculated from heart rate and stroke volume, and buccal pumping rate was measured at 19°–21° C for Xenopus laevis *larvae between Nieuwkoop and Faber stages 44 (just after hatching) and 57 (4–1,102 mg) denied access to air at a range of ambient aquatic P_{O_2} from normoxia (150–155 mmHg) to severe hypoxia (27–45 mmHg). Cardiac output decreased in severe hypoxia in stage 44–49.5 larvae, but not in stage 51–54 larvae, because heart rate decreased significantly in the early larvae, probably a direct effect of O_2 limitation on cardiac metabolism. Stroke volume did not change significantly in hypoxia in either early- or late-stage larvae. Thus there was no evidence of a tissue-mediated increase in cardiac output in hypoxia. Buccal pumping increased by about 50% over normoxic rates in moderate hypoxia in all larvae but sharply decreased in severe hypoxia, decreasing more in younger larvae than older. Younger larvae show significantly more variability in buccal pumping than older larvae, which suggests that regulatory mechanisms are not yet fully developed in early larvae. Cardiac output scales to body mass with a allometric coefficient of 1.15 ± 0.15 (95% confidence limits), significantly higher than literature values for O_2 uptake (0.83), implying that cardiovascular gas transport may be less important (compared to direct diffusion) in very small early-stage larvae than in larger, late-stage larvae.*

Introduction

Xenopus larvae hatch just over 2 d after fertilization. Only rudimentary gill bars have formed, and the developing oropharyngeal cavity is overlaid by

* To whom correspondence should be addressed.

Physiological Zoology 68(1):63–75. 1995. 0031-935X/95/6801-9401$02.00

a cement gland (Nieuwkoop and Faber 1967). Although circulation of blood is established, heart chamber partitioning and valve formation is incomplete. Thus, the cardiorespiratory system of larvae is anatomically and functionally immature on hatching into an environment where the O_2 availability over the course of a day may fluctuate greatly. At hatching, *Xenopus* larvae are less than 1 mm wide. At this small size, respiratory demands are probably met primarily by diffusion through the body wall directly to tissues (Burggren and Pinder 1991). With growth, however, more complex cardiovascular and respiratory processes develop that compensate for the increasing diffusing distance to metabolizing tissues and the decreasing surface-area-to-volume ratio.

Little is known about the premetamorphic development of physiological regulatory mechanisms in amphibians. Much of the work on amphibian larvae has concentrated on metamorphosis to adult (Burggren and Pinder 1991; Burggren et al. 1992), with few studies on newly hatched larvae. Extrinsic neural and humoral regulation, especially of the heart, has been studied to the near exclusion of intrinsic mechanisms, although intrinsic mechanisms of peripheral blood vessel regulation and the Starling mechanism of cardiac output regulation can be argued to be more fundamental to cardiovascular regulation and may appear first in development.

The heart is not innervated at hatching in anuran larvae, but responds to acetylcholine and catecholamines by stage II or III (Taylor and Kollros [1946] staging) (Kimmel 1990; Protas and Leontieva 1992; Pelster et al. 1993). Cholinergic and adrenergic receptors appear before the heart is innervated (Protas and Leontieva 1992), however, and the route of agonist administration and whether the heart is studied in situ or in vitro appear to give quite different results (Burggren and Doyle 1986*a;* Protas and Leontieva 1992; Pelster et al. 1993); thus, the importance of these receptors for chronotropic and inotropic regulation is unclear. The heart becomes innervated midway through larval development (Bride [1975] for *Xenopus laevis;* Protas and Leontieva [1992] for *Rana temporaria*), but there seems to be little neural regulation of heart rate in response to hypoxia or exercise until late larval development (Burggren and Doyle 1986*a*). There may be humoral regulation of heart rate, but no measurements of catecholamine concentrations in larvae have been made.

Heart rate may not be a good indicator of cardiovascular response, however. Cardiac output is a more important measure of cardiac performance and can vary over a wide range even at a constant heart rate because stroke volume is variable. It is well known that the microcirculation responds to O_2 and tissue metabolism to regulate local blood flow through intrinsic mechanisms. The sum of tissue perfusion changes can affect cardiac output

by changing venous return to the heart, acting through Frank-Starling modulation of stroke volume. Cardiac output in lower vertebrates may be regulated more through intrinsic mechanisms than central neural cardiac regulation (Farrell 1992). Burggren and Doyle (1986*a*) noted an exercise tachycardia in bullfrog larvae that could not be blocked by cholinergic or adrenergic blockers; they speculated that these changes were caused by increased venous return secondary to local tissue blood flow regulation.

There are no intrinsic mechanisms regulating gill ventilation because ventilation is powered by skeletal muscles and must be controlled by the central nervous system. *Rana* and *Xenopus* larvae can ventilate the buccal cavity at hatching, and there appears to be a hypoxic drive for ventilation even before the operculum encloses the gills so that buccal ventilation is effective for irrigating the gills (Feder and Wassersug 1984; Burggren and Doyle 1986*b*). Regulation is unlikely to be fully mature at hatching, however; thus, gill ventilation might be expected to show greater variability in younger larvae than in older larvae. Glomus cells in the external carotid artery of *Rana catesbeiana* larvae appear to be nonfunctional; chemoreception by these receptors seems to start after metamorphosis (Kusakabe 1992).

It is unclear how cardiac output and cardiovascular transport functions scale with body mass in very small vertebrates. Cardiac output in adult mammals and birds scales with an allometric coefficient similar to that for O_2 uptake (Calder 1981), and heart mass in fish scales very close to unity (Farrell 1992). In the absence of data to the contrary, it has been assumed that cardiac output scales isometrically with body mass in early larvae (Pelster and Burggren 1991), but because of the very small body size and incompletely developed circulatory system this is highly speculative.

We investigated the ontogeny of cardiorespiratory reflexes in response to aquatic hypoxia and allometric changes of cardiac output in submerged *X. laevis* larvae. *Xenopus laevis* was chosen for experiments for several reasons: the adults can be artificially induced to spawn; the life stages from egg to adult are well documented (Nieuwkoop and Faber 1967); they develop to metamorphosis in only 8 wk; and the larvae are transparent, allowing in vivo analysis of cardiac function without surgery.

Material and Methods

Experimental Animals

All *Xenopus laevis* larvae were siblings obtained as embryos from an induced breeding of a single adult pair. The larvae were reared and all experiments were conducted at room temperature (19°–21°C). The hatched larvae were

fed brewer's yeast supplemented with nettle powder and ground alfalfa leaves every other day. Larvae were not fed 1 d before testing. Larvae were maintained at local natural light-dark cycles. Some larvae were placed in a refrigerator at 10°C to slow development and delay metamorphosis, which extended experimental testing. These larvae were given a minimum of 3 d to acclimate to room temperature and feeding schedules before being tested. Experiments were conducted on larvae from Nieuwkoop and Faber (NF) stages 44–57 (Nieuwkoop and Faber [1967] staging).

Apparatus and Experimental Protocol

Larvae were placed in chambers made from three microscope slides, 3 mm, 7 mm, and 12 mm wide to accommodate larvae of various developmental stages and sizes. Larvae were restrained with a piece of nylon screen that restricted movement of the tail, wedged with a length of rubber tubing above the larva preventing access to air but allowing unrestricted gill ventilation. Larvae that appeared not to have free buccal pumping movement because of the design of the chamber, or that struggled frequently during the experiment, were not included in the analysis. Larvae were allowed to acclimate to the chamber for 10 min after any initial struggles before testing began.

The chamber received water from a stripping column that regulated P_{O_2}. Larvae were oriented so they faced the flow of water into the chamber. Nitrogen gas and air were mixed with a Matheson dual flowmeter to control water P_{O_2} in the stripping column. Water exiting the bottom of the column was channeled through two tubes; one drained into the experimental chamber and the other flowed passed a Radiometer Copenhagen O_2 electrode (model E5046-0) housed in a D616 thermostated cell that maintained the temperature of the electrode at a constant 20°C by flowing water around it from a Haake refrigerated water bath. The P_{O_2} was read from the O_2 electrode by a custom-made O_2 meter (Max-Planck-Institut für Experimentelle Medizin, Göttingen).

Measurements were taken at successively decreasing P_{O_2}'s from normoxia (150–155 mmHg) to severe hypoxia (30–40 mmHg) in 10–15 mmHg steps at approximately 10-min intervals, so that the entire experiment took about 2 h. Testing was terminated when the larvae reached a critical activity point (CAP) at which the larvae greatly decreased buccal pumping rate and were unresponsive to physical stimuli (Feder and Wassersug 1984). The measurement prior to the CAP was the last one to be analyzed for all larvae. Larvae were revived following the procedure by turning off the N_2 gas and weighed. Each larva was tested only once.

Data Collection

Heart rate, buccal pumping rate, and cardiac stroke volume were measured from videotapes of the larvae. Video recordings of the hearts of the larvae were taken with a Panasonic video camera mounted on a Wild M650 surgical microscope focused horizontally on a small mirror mounted at a 45° angle below the experimental chamber, giving a view of the ventral side of the larva. Because the ventral side of *X. laevis* larvae is transparent, the ventral aspect of the heart can be seen. The heart rates and buccal pumping rates of all the larvae were counted for all P_{O_2} levels. Stroke volume measurements were taken by advancing the tape on the VCR by single frames and tracing the dimensions of the larva's ventricle at systole and diastole from the video monitor onto acetate sheets. Measurements of stroke volume and cardiac output were made for larvae only up to stage 54 because the body wall became opaque, obscuring the image and preventing precise ventricle tracings. After stage 49 the shape of the ventricle was estimated from tracings of the pericardium, which becomes opaque at this stage. Drawings were made in the following P_{O_2} ranges: 150–155, 95–110, 60–75, and 30–46 mmHg. A generalized prolate spheroid geometric shape was used to calculate the volume of the ventricle at systole and diastole. The volume of this "rugby ball" shape was calculated according to the formula $(4/3)\pi ab^2$, where a is one-half the length of the major axis and b is one-half the length of the minor axis. These two axes were measured from the tracings of the ventricle at systole and diastole. Stroke volume was calculated by subtracting the ventricle volume at systole from the volume at diastole. Cardiac output was calculated by multiplying the stroke volume by the heart rate. Because the calculation of cardiac output depends on an approximation of the ventricle shape, the accuracy of this calculation was checked by measuring the difference in surface area of the two-dimensional ventricle tracings at systole and diastole. Measurements of projected ventricular surface area were made with Stereometric Measurement Analysis revision 3.2 on an Apple II with a graphics tablet. Although this does not give a measure of absolute cardiac output, the patterns of change with development and hypoxia should be similar to those seen with the calculated cardiac output. The patterns observed with projected area were identical to those for calculated stroke volume; thus, only the stroke volume calculations are presented.

Statistical Analysis

Data from different developmental stages were pooled into two groups (stages 44–49.5 and 53–57) for analysis of developmental changes in buccal

pumping rate. Data were also pooled for heart rate, stroke volume, and cardiac output measurements but only for larvae in stages 44–49.5 and 51–54 because cardiac output could not be measured after the body wall became opaque at stage 54. Buccal pumping was also measured at stages 51–52, and heart rate was measured at stages 55–57; these data were used in the allometric analysis. Statistical significances of differences between developmental stages and treatment effects were determined with ANOVA followed by paired *t*-tests (Statview statistical package). Variances of stage 44–49.5 and stage 53–57 heart rate and buccal pumping rate responses to hypoxia were compared with an equality of variance *F*-test (Sokal and Rohlf 1981, pp. 189–190). Allometric relationships were analyzed with least squares linear regression analysis on log-transformed data (SYSTAT statistical package).

Results

Responses to the Chamber

Most larvae struggled occasionally in the chamber, which may have increased O_2 consumption or heart rate. However, larvae were no more likely to struggle at low P_{O_2} (until CAP was reached) than at normoxia. Larvae typically settled during the initial acclimation period. Struggling, when it occurred, would last for no more than 5 s. Younger larvae appeared to struggle more frequently than older larvae. Because of the restraints necessary to measure cardiac output visually the larvae cannot be considered to be resting or undisturbed.

Effects of Hypoxia

Changes in heart rate, stroke volume, and cardiac output are shown in figure 1. Heart rate did not increase at any level of hypoxia and decreased significantly ($P < 0.05$) both in young (NF stages 44–49.5) and older (NF stages 53–57) larvae in severe hypoxia. Heart rates were usually very constant from one measurement to the next in individual larvae. Stroke volume did not change significantly in hypoxia. Cardiac output decreased significantly only in severe hypoxia in younger larvae ($P < 0.05$; paired *t*-test after repeated-measures ANOVA).

Changes in buccal pumping rates are shown in figure 2. Individual buccal pumping rates and patterns of change with hypoxia are extremely variable in younger larvae (NF stages 44–49.5), while older larvae have a much more predictable rate and pattern of buccal pumping. Hypoxia significantly affects

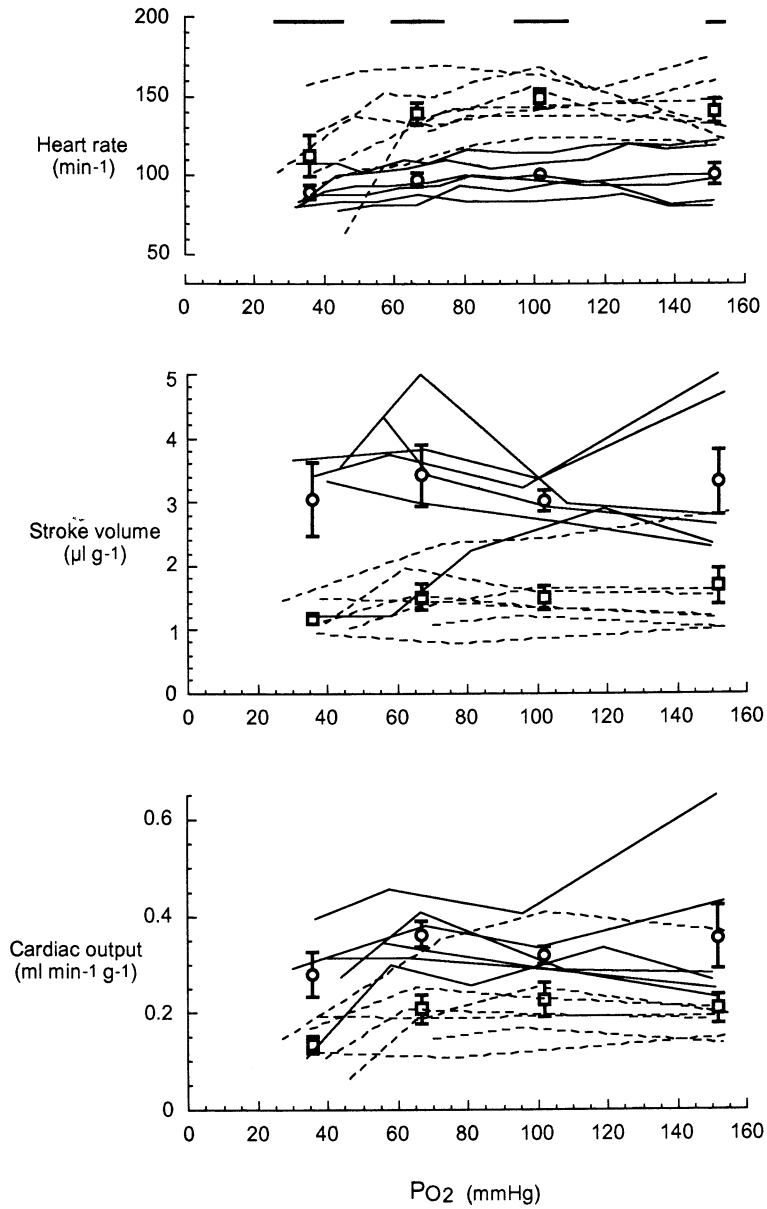

Fig. 1. Changes in heart rate, stroke volume, and cardiac output in response to hypoxia. Dashed lines represent responses in individual young larvae (NF stages 44–49.5), and solid lines represent individual older larvae (NF stages 51–54). Means and standard errors are also provided for the two groups, shown by squares for young larvae and by circles for older larvae. The horizontal bars at the top of figure show the P_{O_2} range over which data were pooled to calculate means and standard errors. Only one measurement from each animal fell within each P_{O_2} range.

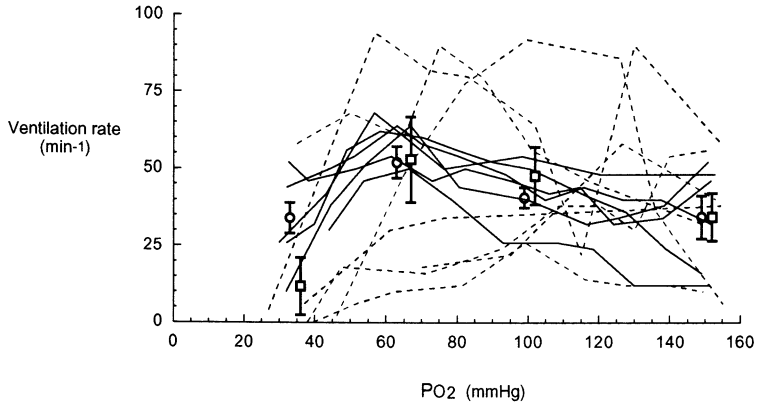

Fig. 2. Changes in buccal ventilation in response to hypoxia. Data presentation is as in fig. 1 except that the older larvae were stages 53–57.

buccal pumping rates both in stage 44–49.5 larvae ($P < 0.01$) and stage 53–57 larvae ($P < 0.05$) (repeated-measures ANOVA). In older larvae, buccal pumping rate increased significantly ($P < 0.05$) with decreasing O_2 to Po_2's of 60–75 mmHg, then decreased back to normoxic rates in severe hypoxia (Po_2's of 27–46 mmHg). In younger larvae, the apparent increase in buccal pumping rate is not significant because of the much greater variability. Buccal pumping rate in severe hypoxia decreased significantly ($P < 0.05$) below rates in normoxia. A test for equality of variance shows that the variability in buccal pumping rates of stage 44–49.5 larvae is significantly greater than in older stage 53–57 larvae ($P < 0.05$) at Po_2 ranges of 95–110 mmHg and 60–75 mmHg.

Allometry

The larvae grew by two orders of magnitude in body mass during the experiments (4–5 mg wet wt at stage 44 to 550–650 mg at stage 57). Figure 3 shows linear regressions for normoxic heart rate, stroke volume, and cardiac output against wet body mass for log-transformed data. Heart rate decreased slightly but significantly with increasing body mass (allometric coefficient −0.089) while stroke volume increased much more quickly than body mass (allometric coefficient 1.24 ± 0.15 [95% confidence interval]), so that mass-specific cardiac output also increased with positive allometry (allometric coefficient 1.15 ± 0.15). Buccal pumping rate was not significantly correlated with body mass ($r^2 = 0.045$; $P > 0.1$).

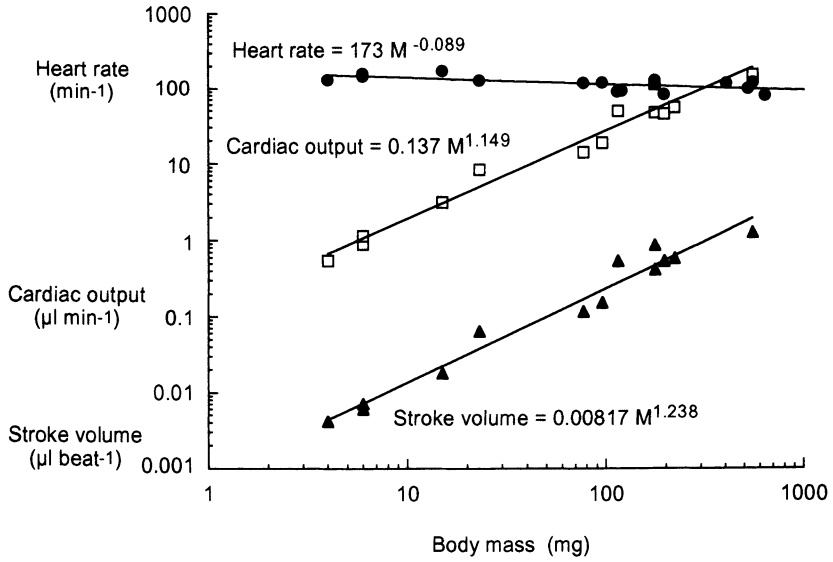

Fig. 3. Allometry of cardiovascular variables. All regressions are statistically significant ($P < 0.05$ for heart rate; $P < 0.0001$ for cardiac output and stroke volume).

Discussion

Cardiac Output

Our estimates of cardiac output, 0.21 ± 0.029 mL g^{-1} min^{-1} in NF stage 44–49 larvae and 0.35 ± 0.066 mL g^{-1} min^{-1} in NF stage 51–54 larvae, are similar to estimates made with similar image analysis techniques in anesthetized normoxic *Xenopus* larvae, in which cardiac output increased from 0.078 mL g^{-1} min^{-1} at NF stage 40 to 0.284 mL g^{-1} min^{-1} at NF stage 51 (Hou 1992). Cardiac output in the neotenic salamander *Ambystoma tigrinum* is about 0.10 mL g^{-1} min^{-1} (measured with pulsed Doppler flow probes; Hoyt, Eldridge, and Wood 1984).

The development of cardiovascular reflexes in larval amphibians has received little attention to date. Most studies have focused on the change in heart rate over development—for example, in *Eleutherodactylus coqui* (Burggren, Infantino, and Townsend 1990), *Rana catesbeiana* (Burggren and Doyle 1986*a*), and *Xenopus laevis* (Feder and Wassersug 1984). Newly hatched *R. catesbeiana* do not regulate heart rate. There is little vagal tone before Taylor and Kollros (TK) stage X (approximately equivalent to NF stage 55; Burggren and Just 1992) and little sympathetic adrenergic tone until after metamorphosis (Burggren and Doyle 1986*a*). In our study there was little change in heart rate with hypoxia except at the lowest P_{O_2} measured

(26–47 mmHg) in the youngest larvae and even less response in the older larvae. These changes were similar to responses of larval *R. catesbeiana* (Burggren and Doyle 1986*a*). There was no indication of a hypoxic bradycardia, which is present in adult anurans (Pinder and Smits 1993). The decrease in heart rate at the lowest P_{O_2} was probably the result of direct inhibition of cardiac metabolism by low O_2 (Burggren and Just 1992).

Changes of cardiac output in response to hypoxia had not previously been measured in larval anurans, but, like heart rate, cardiac output changed little with decreasing P_{O_2} in larvae between NK stages 44 and 54 (TK stages I–IX). An increase in cardiac output in hypoxia was expected to compensate for decreases in the difference between arterial and venous O_2 content caused by low ambient P_{O_2}. The lack of response suggests that there was no hypoxemic hyperemia, which would have increased venous return to the heart, stroke volume, and cardiac output even without central regulation. Increased gill ventilation could not have prevented hypoxemia in these larvae (see below). Hypoxia was sufficiently severe that larvae became unresponsive.

Two possible explanations for a lack of response are that (1) cardiac output was already maximal or (2) the vasculature was not responsive to tissue hypoxia. The larvae struggled infrequently and briefly during experiments, but this exertion may have been enough to fully recruit blood flow to the skeletal musculature, maximizing cardiac output. This seems unlikely, especially since our measurements of cardiac output in conscious larvae are very similar to those for anesthetized larvae (Hou 1992), but the possibility cannot be ruled out. Nonetheless, our larvae were restrained and cannot be considered to be resting or undisturbed. Metabolic regulation of local blood flow is thought to be intrinsic to the microcirculation (Granger et al. 1983); thus, its apparent absence in these larvae is surprising, especially in the absence of hypoxic bradycardia and accompanying redistribution of blood flow. It is possible that there was a small hypoxic bradycardia that was only large enough to obscure peripheral responses, but more detailed observations of the distribution of blood flow are needed before we can investigate local blood flow regulation. An exercise tachycardia in larval *R. catesbeiana* that is not blocked by atropine or adrenergic blockers suggests that there are intrinsic mechanisms of blood flow regulation even in early larvae (Burggren and Doyle 1986*a*).

Ventilation

Although early and late larvae show a superficially similar pattern of changes in buccal ventilation with hypoxia, increasing ventilation by about 50% in

moderate hypoxia and decreasing ventilation in severe hypoxia, individual responses are very different between the two age-groups (fig. 3). Ventilation frequencies of individual early larvae are extremely variable from one measurement to the next, showing little overall pattern except for a sharp decrease in ventilation frequency in severe hypoxia, presumably due to a direct inhibition by low O_2. In contrast, older larvae have a much more stereotyped response, with significantly less variability than the younger larvae. Previous studies of submerged *X. laevis* and *R. catesbeiana* larvae show similar patterns of response to hypoxia (West and Burggren 1982; Feder and Wassersug 1984), although these studies did not note differences in individual variability between younger and older larvae. Older *R. catesbeiana* larvae (Burggren and Doyle 1986*b*), *Rana berlandieri* larvae (Feder 1983*a*), and the exclusively aquatic *Bufo woodhousei* (Feder 1983*b*) increased gill ventilation even in severe hypoxia, which suggests a higher tolerance to hypoxia in these *Bufo* and *Rana* species than in *Xenopus* when prevented from air breathing.

Clearly the neuromuscular control mechanisms for ventilation are in place by hatching, but the high variability in young larvae suggests a lack of effective feedback regulation while the stereotyped response of older larvae suggests that the regulatory feedback mechanism is much better developed. In *R. catesbeiana,* glomus cells in the external carotid artery are not innervated and thus not functional until late larval stages or after metamorphosis (Kusakabe 1992). Other O_2 chemosensors must be functional for larvae to have any response at all to hypoxia, but some inputs appear to be incomplete.

Xenopus larvae are normally bimodal breathers, but air breathing in normoxia is infrequent, providing only about 17% of total O_2 uptake, and larvae can survive for weeks without breathing air (Feder and Wassersug 1984). Air breathing provides a greater proportion of total O_2 uptake during hypoxia, prevents the increase in gill ventilation seen in submerged larvae (Feder and Wassersug 1984), and presumably mitigates hypoxemia. We prevented our larvae from air breathing both to simplify making the measurements and because we wanted to observe cardiovascular responses to hypoxemia. Gill ventilatory reflexes change throughout larval development in association with lung development and ventilation (West and Burggren 1982; Burggren and Doyle 1986*b*).

Allometry

The allometric coefficients for stroke volume (1.23) and cardiac output (1.15) are significantly greater than allometric coefficients for O_2 consumption in *X. laevis* larvae (0.83; Feder 1982) and other anuran larvae (0.59,

interspecific comparison; Gatten, Miller, and Full 1992). Because the allometric coefficients for stroke volume and cardiac output are above 1.0, the older and larger larvae have greater mass-specific stroke volume and cardiac output than younger and smaller larvae. The high exponent for cardiac output suggests that older larvae are more dependent on cardiovascular convection for delivering O_2 than younger larvae. Newly hatched *Xenopus* larvae (NF stages 44–46) were only 4–5 mm long, 1 mm wide, and 4–6 mg in body mass. Small size, and thus short diffusion distances to most tissues, may allow direct diffusion of O_2 from the body surface to most tissues, decreasing the importance of blood flow for O_2 delivery. We found no relationship between buccal pumping rate and body mass, although Feder and Wassersug (1984) found that differences in body mass accounted for 30%–40% of the variation in buccal pumping rate in *Xenopus* larvae.

Acknowledgments

This research was supported by a National Engineering and Science Research Council research grant and a University Research Fellowship to A.W.P.

Literature Cited

Bride, M. 1975. Établissement de l'innervation dans le coeur du têtard de Xènope et ses repercussions sur le fonctionnement de l'organe. C. R. Soc. Biol. Paris 169: 1265–1271.

Burggren, W. W., J. E. Bicudo, M. L. Glass, and A. S. Abe. 1992. Development of blood pressure and cardiac reflexes in the frog *Pseudis paradoxsus*. Am. J. Physiol. 263:R602–R608.

Burggren, W. W., and M. E. Doyle. 1986*a*. Ontogeny of heart regulation in the bullfrog, *Rana catesbeiana*. Am. J. Physiol. 251:R231–239.

———. 1986*b*. Ontogeny of regulation of gill and lung ventilation in the bullfrog, *Rana catesbeiana*. Respir. Physiol. 66:279–291.

Burggren, W. W., R. L. Infantino, and D. P. Townsend. 1990. Developmental changes in cardiac and metabolic physiology of the direct-developing frog *Eleutherodactylus coqui*. J. Exp. Biol. 152:129–148.

Burggren, W. W., and J. J. Just. 1992. Developmental changes in physiological systems. Pages 467–530 in M. E. Feder and W. W. Burggren, eds. Environmental physiology of the amphibians. University of Chicago Press, Chicago. 646 pp.

Burggren, W. W., and A. W. Pinder. 1991. Ontogeny of cardiovascular and respiratory physiology in lower vertebrates. Annu. Rev. Physiol. 53:107–135.

Calder, W. A. 1981. Scaling of physiological processes in homeothermic animals. Annu. Rev. Physiol. 43:301–322.

FARRELL, A. P. 1992. The heart. Pages 1–72 in W. S. HOAR, D. J. RANDALL, and A. P. FARRELL, eds. Fish physiology. Vol. 12A. Academic Press, New York.

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.

———. 1983*a*. Responses to acute hypoxia in larvae of the frog *Rana berlandieri*. J. Exp. Biol. 104:79–95.

———. 1983*b*. Effect of hypoxia on the energy metabolism of lungless tadpoles, *Bufo woodhousei*, and air-breathing anuran larvae. J. Exp. Biol. 228:11–19.

FEDER, M. E., and R. J. WASSERSUG. 1984. Aerial versus aquatic oxygen consumption in larvae of the clawed frog, *Xenopus laevis*. J. Exp. Zool. 108:231–245.

GATTEN, R. E., K. MILLER, and R. J. FULL. 1992. Energetics at rest and during locomotion. Pages 314–377 in M. E. FEDER and W. W. BURGGREN, eds. Environmental physiology of the amphibians. University of Chicago Press, Chicago. 646 pp.

GRANGER, H. J., J. L. BORDERS, G. A. MEININGER, A. H. GOODMAN, and G. E. BARNES. 1983. Microcirculatory control systems. Pages 209–236 in N. A. MORTILLARO, ed. The physiology and pharmacology of the microcirculation. Vol. 1. Academic Press, New York.

HOU, P.-C. L. 1992. Development of hemodynamic regulation in larvae of the African clawed toad, *Xenopus laevis*. Ph.D. diss. University of Massachusetts, Amherst.

HOYT, R. W., M. ELDRIDGE, and S. C. WOOD. 1984. Noninvasive pulsed Doppler determination of cardiac output in an unanesthetized neotenic salamander, *Ambystoma tigrinum*. J. Exp. Zool. 230:491–493.

KIMMEL, P. B. 1990. Ontogeny of cardiovascular control mechanisms in the bullfrog, *Rana catesbeiana*. Ph.D. diss. University of Massachusetts, Amherst.

KUSAKABE, T. 1992. Ultrastructural characteristics of glomus cells in the external carotid artery during larval development and metamorphosis in bullfrogs, *Rana catesbeiana*. Anat. Rec. 233:461–466.

NIEUWKOOP, P. D., and J. FABER. 1967. Normal table of *Xenopus laevis*. North-Holland, Amsterdam.

PELSTER, B., and W. W. BURGGREN. 1991. Central arterial hemodynamics in larval bullfrogs (*Rana catesbeiana*): developmental and seasonal influences. Am. J. Physiol. 260:240–246.

PELSTER, B., W. W. BURGGREN, S. PETROU, and I. WAHLQVIST. 1993. Developmental changes in the acetylcholine influence on heart muscle of *Rana catesbeiana: in situ* and *in vitro* effects. J. Exp. Zool. 267:1–8.

PINDER, A. W., and A. W. SMITS. 1993. Mechanisms of acute hemoconcentration in bullfrogs in response to hypoxemia. Am. J. Physiol. 264:R687–R695.

PROTAS, L. L., and G. R. LEONTIEVA. 1992. Ontogeny of cholinergic and adrenergic mechanisms in the frog (*Rana temporaria*) heart. Am. J. Physiol. 262:R150–R161.

SOKAL, R. R., and J. F. ROHLF. 1981. Biometry. W. H. Freeman, San Francisco. 776 pp.

TAYLOR, A. C., and J. KOLLROS. 1946. Stages in the normal development of *Rana pipiens* larvae. Anat. Rev. 94:7–23.

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