DEVELOPMENTAL CHANGES IN CARDIAC AND METABOLIC PHYSIOLOGY OF THE DIRECT-DEVELOPING TROPICAL FROG *ELEUTHERODACTYLUS COQUI*

BY WARREN W. BURGGREN, ROBERT L. INFANTINO, JR

Department of Zoology, University of Massachusetts, Amherst, MA 01003-0027, USA

AND DANIEL S. TOWNSEND

Department of Biology, University of Scranton, Scranton, PA 18510-2192, USA

Summary

The neotropical frog *Eleutherodactylus coqui* (Thomas) undergoes direct development within the egg, emerging at hatching as a juvenile frog. The aim of this study was to investigate cardiorespiratory changes that accompany development, as well as to determine how the developmental physiology of direct-developing anurans differs from anurans with free-living larval stages. Heart rate (f_H), oxygen uptake ($\dot{M}_{O_2}$), vitelline fluid pH, and the protein and lactate concentrations of vitelline fluid were measured as a function of development. At 24–25°C, f_H doubled from 50 to 100 beats min^{-1} during the early stages of heart development, then increased more slowly with later embryonic development to 120 beats min^{-1} at hatching. The act of hatching itself was accompanied by a transient increase in f_H to 155 beats min^{-1}, this mild tachycardia lasting for at least 2 h. In juveniles and adults, heart rate decreased with increasing body mass, falling to about 80 beats min^{-1} in 6 g frogs at 24–25°C. $\dot{M}_{O_2}$ at 24–25°C increased from about 2 μmol g egg^{-1} h^{-1} for the earliest embryos examined to about 12 μmol g egg^{-1} h^{-1} at hatching. $\dot{M}_{O_2}$ in adults decreased with increasing body size, ranging from 4 to 5 μmol g^{-1} h^{-1} in the largest adults examined (6 g). Both f_H and $\dot{M}_{O_2}$ were also measured at 18°C. The Q_{10} for f_H was between 1.5 and 2 throughout development. The Q_{10} for $\dot{M}_{O_2}$ in embryos ranged between 3.5 and 6.5 until hatching, falling to 1.6–2.0 in juveniles and small adults. Vitelline fluid pH (24–25°C) decreased from 5.2 in early embryonic stages to 4.7 at the time of hatching. Egg dehydration caused a significant decrease in the pH of vitelline fluid and was associated with a significant rise in f_H. Vitelline fluid protein concentration was about 5–6 mg dl^{-1}, and showed no consistent change with development. Whole-egg lactate concentration ranged from 4 to 11 μg g egg^{-1}, and also showed no consistent change during development. These findings indicate considerable changes in cardiac and metabolic physiology associated with development in *Eleutherodactylus coqui*. These changes appear to be associated with both organogenesis (primary influence before hatching) and body mass changes (primary influence after hatching).

Introduction

Amphibian development provides many opportunities for studying and understanding the ontogeny of physiological processes in vertebrates. Building upon earlier studies of the endocrinological regulation of development (for a review see Fox, 1984), recent work has expanded to include developmental changes in cardiorespiratory and metabolic physiology, especially during metamorphic climax (for reviews, see Burggren, 1984; Feder, 1984; Burggren and Just, 1991). Collectively, these studies have revealed ontogenetic changes in physiology that occur broadly in different amphibian families. For example, the larvae of both anurans (*Rana* spp.) and urodeles (*Ambystoma* spp.) show a decreasing dependence upon gills and skin for oxygen uptake as development of the lungs proceeds, but the skin remains important for elimination of CO_2 throughout the animal's larval and adult life (see Feder and Burggren, 1985, for a review). Similarly, a rise in blood P_{CO_2} and HCO_3^- concentration with development occurs in anurans (Erasmus *et al.* 1970/1971; Just *et al.* 1973) and urodeles (Burggren and Wood, 1981), and mechanisms for acid–base balance during metamorphosis change to reflect those of terrestrial air-breathing animals.

While some ontogenetic changes in physiology are quite clearly associated with the transition from an aquatic to a terrestrial habitat (e.g. the changing dependence upon the skin for oxygen uptake), others are less so. For example, heart rate falls precipitously following hatching in strictly water-breathing stages of larval *Rana catesbeiana*, remains constant during much of middle larval development (the period including the transition to air breathing), and falls sharply once again with metamorphosis to the adult (Burggren and Doyle, 1986). Vagal tone, which lowers heart rate in resting animals, is absent in young larvae, appears during middle larval development and disappears in the adult. Such changes are not obviously correlated with the transition from aquatic to terrestrial habitat, and could be accounted for in part by changes in body mass or other developmental changes (e.g. organogenesis).

One approach that can help to discern which changes are due to environmental transition and which might be due to unrelated developmental changes is to examine the cardiorespiratory physiology of species with modes and rates of development different from those of the most commonly studied amphibians (i.e. *Rana* spp., *Xenopus* spp., *Ambystoma* spp.). Members of these three genera all hatch from aquatic eggs as very immature larvae, all undergo extensive development as free-living, aquatic larvae, and all develop into obligate air breathers, and thus present relatively little variation in developmental life history. If we examine a species that begins breathing air only as an adult (or becomes terrestrial as a larva before metamorphosis), then the opportunity arises to test whether a given set of physiological patterns is associated with being a larva as opposed to being an adult, or is connected with being an aquatic rather than a terrestrial animal.

The present study was undertaken on the neotropical frog *Eleutherodactylus coqui* (Thomas), commonly called the 'coquí'. This endemic Puerto Rican species is of considerable importance to physiological studies of amphibian development

for three reasons. First, *E. coqui* lays large, terrestrial eggs that develop through to the adult body form before hatching (see Townsend and Stewart, 1985). Thus, all development culminating in the adult form occurs while the organism is within the aquatic environment of the egg and has yet to breathe air. Only then does hatching and an abrupt transition to air breathing occur. Second, the genus *Eleutherodactylus* is the largest single vertebrate genus, with more than 400 named species (Frost, 1985). At least on the basis of number of species, *Eleutherodactylus* is a far more 'representative' anuran genus than *Rana*. Third, while direct development in amphibians is relatively widespread, comparatively little is known about the physiology associated with this life history pattern (but see Bradford and Seymour, 1988).

The specific goals of this study were to determine if heart rate and oxygen consumption change during ontogeny in *E. coqui* and, if changes do occur, to determine whether they can be attributed to development *per se*, the transition in respiratory medium at hatching or other factors like changes in body mass.

Materials and methods

Animal collection and maintenance

E. coqui adults, juveniles and egg clutches were collected in second-growth rain forest in the Caribbean National Forest in the Luquillo mountains of northeastern Puerto Rico (elevation=350 m) during May and June, 1988. Adults and juveniles were held in the laboratory in small, vented plastic bags containing moist paper towels. Individual eggs were separated from the clutch and placed on moist paper towels in small plastic containers. Animals were initially held in the laboratory at $25\pm2°C$, which approximates the mean daily temperature on the forest floor during May and June (Townsend and Stewart, 1986). Measurements were carried out at $25\pm1°C$ and $18\pm1°C$, unless otherwise indicated.

Eggs were staged according to Townsend and Stewart (1985). Individual eggs were weighed, and embryos (with attached yolk sac) were then weighed after separation from the vitelline fluid and egg capsule. Body mass was recorded for hatchlings (frogs less than 0.1 g), while both body mass and snout–vent length (SVL) were recorded for juveniles and adults (all animals $\geqslant0.1$ g).

Measurement of heart rate

The egg capsules and body wall of the embryo of *E. coqui* are transparent throughout embryonic development, and the ventral surface of the thorax of adults and juveniles is translucent. Thus, in all developmental stages the beating heart was visible under a binocular dissecting microscope (magnification 10–20×) using substage fiber-optic illumination. Adults and juveniles, still within their small plastic bags, were moved with minimal disturbance to the microscope stage for determination of heart rate (f_H). Eggs were carefully transferred using forceps to a small transparent holding chamber on the microscope stage. Temperature was monitored on the microscope stage immediately adjacent to the animal (or egg) to

ensure that there was no temperature change from the pre-recording holding temperature. f_H was counted twice for each individual during a 30 s period, and the two readings were averaged. Measurements were made at 24–25 °C and at 18 °C. In each case animals or eggs were acclimated for at least 2 h at each temperature prior to measurement.

The effect of emergence from the egg on f_H was studied in eight stage 14–15 embryos, which are viable if hatching is induced and would normally hatch within 1–3 additional days at 25 °C if left undisturbed (Townsend and Stewart, 1986). Eggs were transferred to individual 20 ml glass vials maintained at 28±1 °C. Control f_H was counted every 15 min for 45 min, and the embryos were then gently hatched by rupturing the egg capsule with fine forceps. In every case, the hatchling emerged from the collapsed egg membranes and hopped at least 1 cm before becoming stationary again. Heart rate was measured within the first 2 min of hatching, and continued every 15–20 min for several hours thereafter.

Oxygen consumption

Oxygen consumption ($\dot{M}_{O_2}$) of embryos, hatchlings and adults was measured using closed-system respirometry in gas-tight glass syringes (Vleck et al. 1979; Bradford, 1984). Individual eggs or hatchlings were placed in 2 ml gas-tight glass syringes, and $\dot{M}_{O_2}$ of juveniles and adults was measured in 2–30 ml syringes, depending upon body size. All syringes were covered with aluminum foil to shield the animals from movements of the investigators. Every frog or egg was allowed an initial acclimation period of 6–12 h, during which the narrow ends of the syringes were left unsealed to allow fresh air into them. After the acclimation period, the syringes were flushed several times with humidified room air, filled to a known volume, and the syringe tips sealed with needles inserted into rubber bungs. The subsequent rate of O_2 depletion depended upon the mass of the animal, the volume of gas in the syringe and the $\dot{M}_{O_2}$ of the animal. Initial experiments allowed empirical determination of the approximate period for a given set of conditions that would produce a P_{O_2} drop of 0.7–1.3 kPa within the syringe. The P_{O_2} change at the end of this period (which varied between 3 and 12 h) was then carefully measured. This was achieved by removing the rubber bung (while maintaining a slight positive pressure on the syringe plunger to avoid aspirating room air), inserting the needle tip into the injection port of a IL 213 Micro Blood Gas Analyzer, and injecting at least 200 μl of gas. The syringe was resealed and the volume of gas injected and the volume remaining in the syringe were recorded. A replicate measurement was then made on each egg or frog. The two replicates were averaged to generate an $\dot{M}_{O_2}$ for that animal.

Measurements of $\dot{M}_{O_2}$ were made at 24–25 °C and 18 °C. Animals within their syringe were allowed to acclimate to a new temperature for 6–12 h before measurements were resumed.

Because we occasionally observed a slight discoloration of the egg capsule that could indicate bacterial growth, experiments were performed to determine the significance of microfaunal respiration in the eggs of E. coqui. $\dot{M}_{O_2}$ was measured

on a combined sample consisting of the egg capsules and vitelline fluid of 10 stage 9B eggs from which the embryos (with yolk intact) had been removed. This experiment revealed no measurable $\dot{M}_{O_2}$ by the non-embryonic components of the eggs, and no further corrections were made to the $\dot{M}_{O_2}$ values calculated for intact eggs.

Oxygen consumption (μmol g^{-1} h^{-1}) was calculated from the volume of gas in the syringe (the syringe volume minus the animal/egg volume, the latter measured at the conclusion of gas sampling), the decline in gas P_{O_2} over the measurement period, the elapsed time and the body mass. In the case of eggs, the mass used was that of the embryo plus yolk sac after removal from the egg.

Characteristics of vitelline fluid

The pH of vitelline fluid in eggs of stages 5 and 9 was measured using a micro combination pH electrode housed in the tip of a 20 gauge needle (World Precision Instruments, New Haven, CT). The tip of the needle electrode was gently inserted through the egg capsule and into the vitelline fluid using a micromanipulator. Fluid pH was displayed on a Fisher Accumet pH meter. The pH electrode was calibrated frequently with buffers of pH 4.00 and 7.40.

The effect of the extent of egg hydration on vitelline fluid pH was assessed by separating eggs from the same clutch into two groups. The first group was fully hydrated by placing the eggs on moist paper towels. The second group was dehydrated by placement on dry paper towels in air with a relative humidity of 50–70%. After 10 h of either dehydration or hydration, egg pH was measured as described above.

The protein concentration of vitelline fluid was measured using a spectrophotometric assay for protein (Sigma diagnostics procedure no. 610). Fluid samples of 50–75 μl were drawn up using a Hamilton 100 μl syringe, transferred to microcentrifuge tubes and frozen. The frozen samples were transported from Puerto Rico to Amherst, Massachusetts, and analyzed 1 month later.

To measure lactate content, individual eggs, as well as new hatchlings, were homogenized in 2 vols of 8% $HClO_4$ in a glass tissue homogenizer. The homogenate was transferred to a microcentrifuge tube and spun for 15 min at 2000 g. The supernatant was decanted into a new microcentrifuge tube and frozen. Frozen samples were transported to Amherst, Massachusetts, and analyzed 1 month later using a spectrophotometric assay for lactate (Sigma diagnostics procedure no. 826-UV).

Data analysis

Data for embryos were grouped, plotted and analyzed as a function of development. For embryos, extent of development was defined by the staging scheme of Townsend and Stewart (1985), while data for post-hatch individuals were grouped, plotted and analyzed as a function of body mass. For general comparative purposes, data on body and yolk mass (embryos) and snout–vent length (adults) were collected from other, non-experimental groups reared at

ambient field temperatures (19–24°C) and plotted along with the measurements of embryonic stage and body mass actually measured in the experimental animals.

All data are presented as means ±1 standard error, unless otherwise indicated. The 'treatment' effect, essentially the extent of ontogeny (expressed either as developmental stage or body mass), was analyzed using a one-way analysis of variance (ANOVA). Differences between pairs of means were assessed with Student's t-test for independent means (two-tailed).

Results

Morphometric relationships

The relationship between embryonic development and changes in mass of the yolk/embryo complex and the embryo and yolk individually are shown in Fig. 1. Embryonic mass increased progressively and significantly with development ($F_{7,21}=208.2$, $P<0.001$). Yolk mass changed little until about stage 10, after which it decreased significantly to a hatching value of about 50% of its mass at stage 5 ($F_{7,21}=93.1$, $P<0.001$). The percentage of the yolk/embryo complex comprised of the yolk alone declined progressively from over 80% at stage 5 to about 25% at the time of hatching (stage 15).

The relationship between snout–vent length (SVL) and body mass is shown for 72 post-hatch juveniles and adults whose SVL ranged from 7 to 53 mm (Fig. 2). There was a highly significant relationship ($F_{3,74}=890$, $P<0.001$) between these two variables. The second-order polynomial equation describing the relationship, $y=0.005x^2-0.106x+0.826$, was used to calculate predicted SVL from measured body mass (both variables are indicated in Figs 3 and 5).

Heart rate

Heart rate and development

Heart rate (f_H) in *E. coqui* as a function of development (stage 5 embryos to 6 g adults) at two temperatures is presented in Fig. 3. There were highly significant variations of f_H with ontogeny, expressed by a combination of stage and body mass changes (18°C, $F_{19,211}=33.8$, $P<0.001$; 24–25°C, $F_{18,305}=70.9$, $P<0.001$). In stage 5 embryos, the earliest stage of development in which heart beat can be observed, f_H was approximately 50 beats min^{-1} at 24–25°C. Embryonic development to stage 6 was accompanied by a near doubling of f_H, but f_H showed much less change during the remainder of embryonic development.

Heart rate in hatchlings (spontaneously hatched) at 24–25°C was nearly 130 beats min^{-1}, which was the highest rate at any time during development. Heart rate after hatching declined substantially as body mass and snout–vent length increased, falling to about 80–85 beats min^{-1} in the largest adults measured.

Changes in f_H at 18°C showed a pattern similar to that at 24–25°C. f_H increased sharply in early developmental stages, stabilized until hatching, and then declined
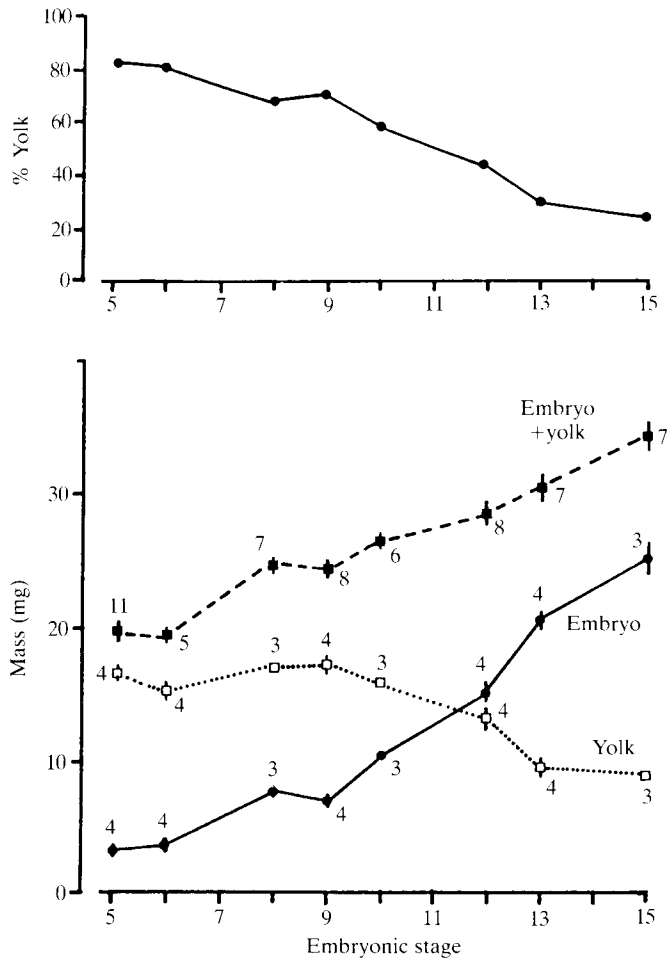
Fig. 1. Relationship between mass of embryo with and without yolk and embryonic stage in *Eleutherodactylus coqui*. Means ±1 standard error are presented (in many cases the standard error is smaller than the symbol). The number beside each mean value shows the number of different animals contributing to that point. Also indicated (top panel) is the percentage of the yolk–embryo complex occupied by the yolk.

with increasing body mass in adults. The Q_{10} for f_H was between 1.5 and 2 throughout development.

Heart rate and hatching

Artificial hatching at developmental stage 14 had a significant effect on f_H ($F_{9,70}=35.6$, $P<0.001$). Fifteen minutes after hatching f_H was significantly higher than when measured in the same animals prior to hatching ($P<0.01$, independent *t*-test) (Fig. 4). Within 1 min of hatching, f_H had increased from about 120 beats min^{-1} to nearly 160 beats min^{-1} at 27–29°C. f_H then began to fall slowly after
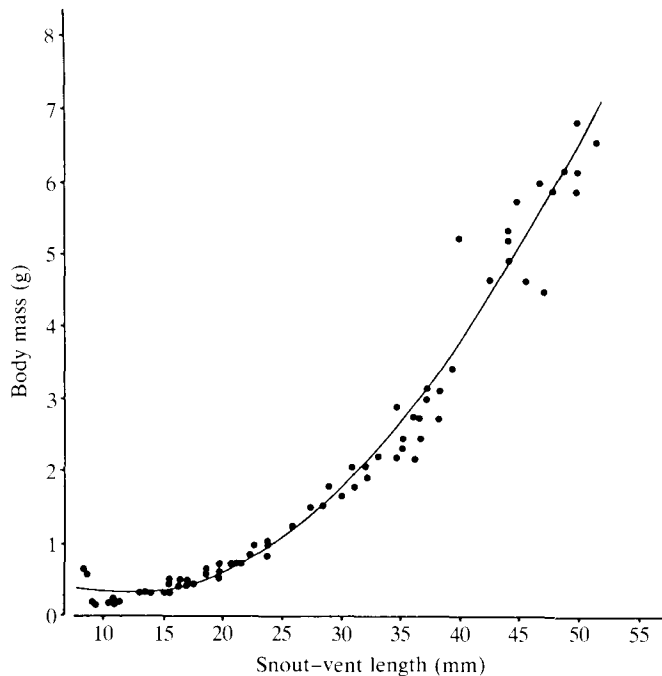
Fig. 2. Relationship between snout–vent length and body mass in 72 post-hatch, juvenile and adult *Eleutherodactylus coqui*. See text for statistical analysis and line equation.

hatching, and after 4 h had returned to values not significantly different from pre-hatch rates ($P>0.1$, independent t-test).

Heart rate and embryonic dehydration

Table 1 presents the effects of dehydration on egg mass, pH of vitelline fluid and f_H in stage 5 and stage 9 embryos. Dehydration to about 50% of the egg mass of the control (fully hydrated) group resulted in a significant fall in pH of vitelline fluid in both stages, with the largest decrease of almost 1 unit occurring in the younger embryonic stage. Dehydration also produced a significant fall in f_H, from 146 beats min^{-1} when hydrated to 130 beats min^{-1} after dehydration in stage 9 embryos.

Oxygen consumption and development

The effects of development on mass-specific $\dot{M}_{O_2}$ in *E. coqui* at 24–25 °C and 18 °C are shown in Fig. 5. Since no significant $\dot{M}_{O_2}$ was measured in egg capsules and vitelline fluid without embryos, all subsequent values are attributed to embryonic respiration. Development had a highly significant effect on mass-specific $\dot{M}_{O_2}$, with a pattern of change similar to that for f_H. Separate ANOVAs were performed for each temperature, with embryonic stage before hatching and
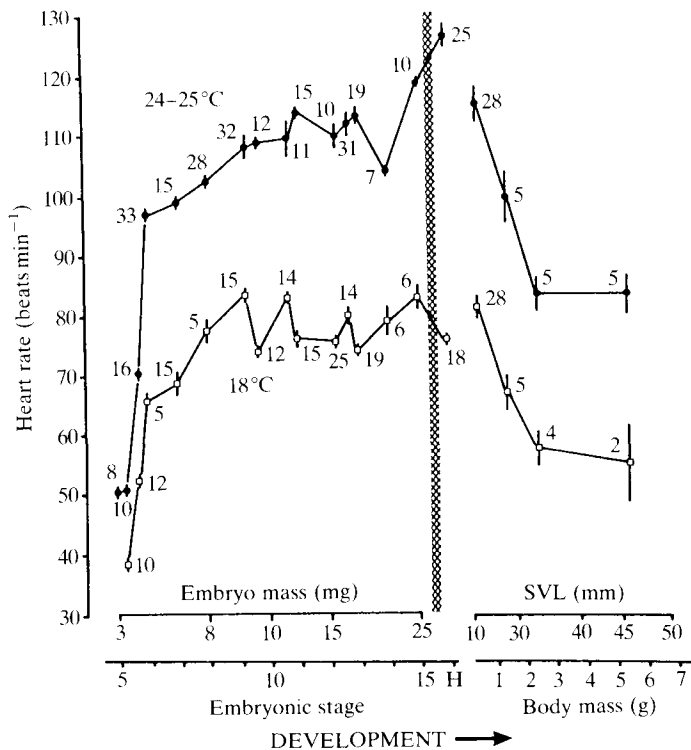
Fig. 3. Heart rate (mean ±1 standard error) at 24–25°C and 18°C as a function of development in *Eleutherodactylus coqui*. The vertical shaded area indicates the time of hatching. The number beside each mean value shows the number of different animals contributing to that point. See text for statistical analysis. The data for embryos are plotted according to the developmental stage at which they were measured. Hatchlings (H following stage 15) were defined as newly hatched frogs weighing less than 100 mg. The data for juveniles and adults are plotted according to the measured body mass. For comparative purposes, the embryonic body masses (minus egg capsule, fluid and yolk) at specific stages measured in separate groups of embryos (previously presented in Fig. 1) are also provided. Also indicated for juveniles and adults are the associated snout–vent lengths (SVL), as determined from the relationship between SVL and body mass depicted in Fig. 2. Note that there is neither a 1:1 nor a logarithmic correlation between embryonic body mass and embryonic stage nor between posthatch body mass and SVL.

body size after hatching as the treatment effects (18°C, $F_{11,82}$=13.0, $P<0.001$; 24–25°C, $F_{12,109}$=31.3, $P<0.001$). $\dot{M}_{O_2}$ at 25°C increased sharply from about $2\,\mu\text{mol}\,\text{g}^{-1}\,\text{h}^{-1}$ at stage 5 to about 6–$8\,\mu\text{mol}\,\text{g}^{-1}\,\text{h}^{-1}$ at stage 6. $\dot{M}_{O_2}$ then showed less change during the rest of embryonic development. Hatchlings exhibited the highest $\dot{M}_{O_2}$ of any developmental stage recorded. Post-hatchling growth was accompanied by a decline in $\dot{M}_{O_2}$, which in adults was between 4 and $6\,\mu\text{mol}\,\text{g}^{-1}\,\text{h}^{-1}$.

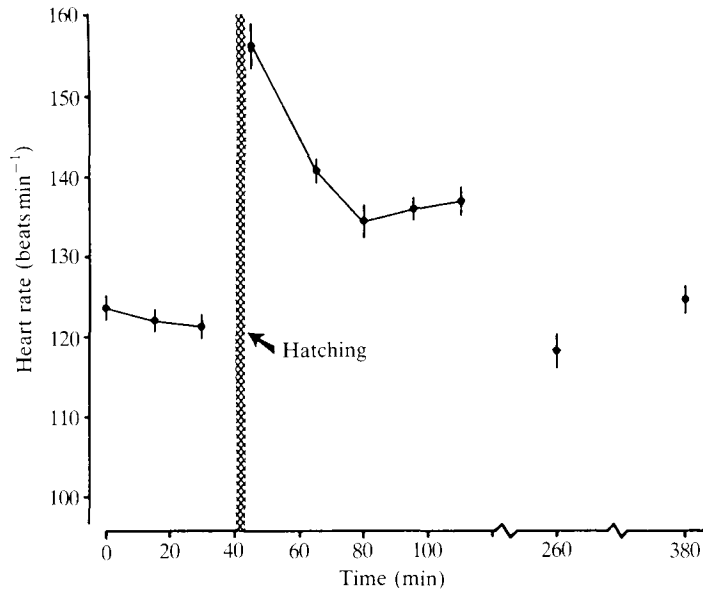$\dot{M}_{O_2}$ at 18°C showed relatively less change with development, though the effect

Fig. 4. The influence on heart rate of experimental hatching (27–29 °C) in eight stage 14 embryos. Means ±1 standard error are presented. See text for statistical analysis.

Table 1. *Effects of dehydration on egg mass, pH of vitelline fluid and heart rate in two stages of embryos of* Eleutherodactylus coqui

	Variable	Hydrated	Dehydrated	P
Stage 5	N	22	19	
	Egg mass (mg)	71±2	32±1	<0.001
	pH	5.13±0.06	4.13±0.03	<0.001
	f_H (beats min^{-1})	—	—	—
Stage 9	N	8	8	
	Egg mass (mg)	116±2	58±5	<0.001
	pH	4.97±0.04	4.64±0.07	<0.025
	f_H (beats min^{-1})	146±1	130±1	<0.001

All measurements were made at 25–27 °C.
Mean values ±1 standard error are presented.
Significance was assessed by an independent t-test between hydrated and dehydrated groups.
See text for additional details.

was still highly significant, as indicated above. At 18°C hatchlings showed essentially the same $\dot{M}_{O_2}$ as that of pre-hatching embryos and larger juveniles.

Temperature effects on f_H *and* $\dot{M}_{O_2}$

Fig. 6 shows the Q_{10} (the fractional rate change produced by a 10°C temperature change) for heart rate and oxygen consumption. The data points represent single
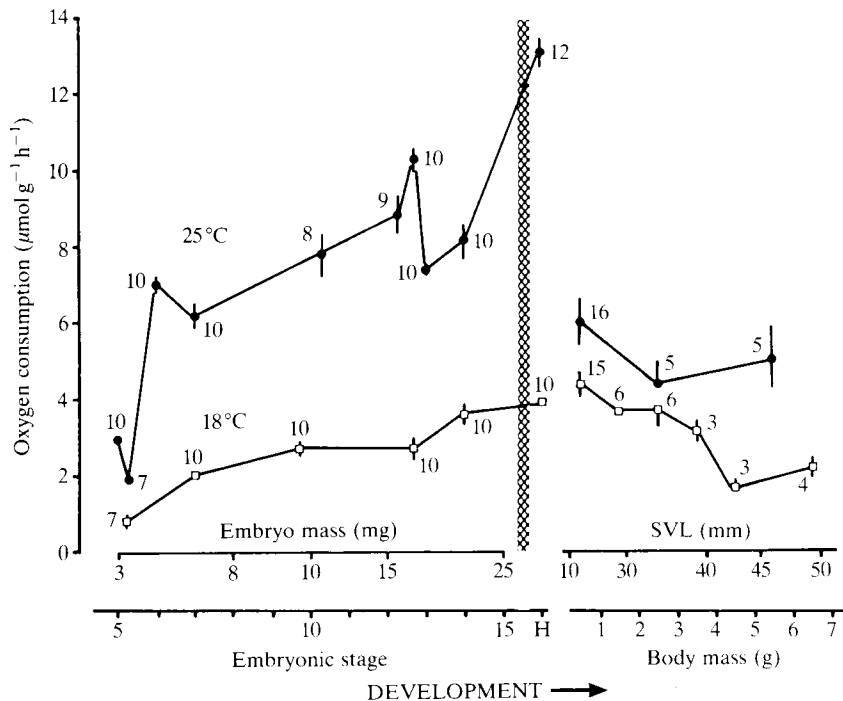
Fig. 5. $\dot{M}_{O_2}$ (mean ±1 standard error) at 25°C and 18°C as a function of development in *Eleutherodactylus coqui*. For stages 5–15, mass (g) for $\dot{M}_{O_2}$ refers to embryo plus yolk. The form of data presentation is as explained in the legend to Fig. 3. See text for statistical analysis.

values calculated from the mean values for f_H and $\dot{M}_{O_2}$ at 18°C and 24–25°C plotted in Figs 3 and 5. As is evident from Fig. 6, there are considerable differences between f_H and $\dot{M}_{O_2}$ in terms of the patterns of change in Q_{10} with development. Whereas the Q_{10} for f_H remained between 1.5 and 2 throughout embryonic and adult development, the Q_{10} for $\dot{M}_{O_2}$ ranged between 3.5 and 6.5 until hatching, falling to 1.6–2.0 in juveniles and small adults. Therefore, an identical temperature change had a two- to fourfold greater effect on $\dot{M}_{O_2}$ than on f_H throughout embryonic development.

Characteristics of vitelline fluid

The pH of vitelline fluid varied significantly ($F_{7,73}=13.0$, $P<0.001$) as embryonic development progressed (Fig. 7). In very early development pH increased significantly from about 5.0 in stage 5 to 5.3 in stage 6 (independent t-test, $P<0.001$), but then progressively declined to a low value of 4.65 immediately before hatching.

The concentration of lactate in whole eggs (embryo plus vitelline fluid and egg membranes) varied significantly with embryonic development (ANOVA, $F_{7,67}=3.4$, $P<0.01$). There was no discernible pattern of change during develop-
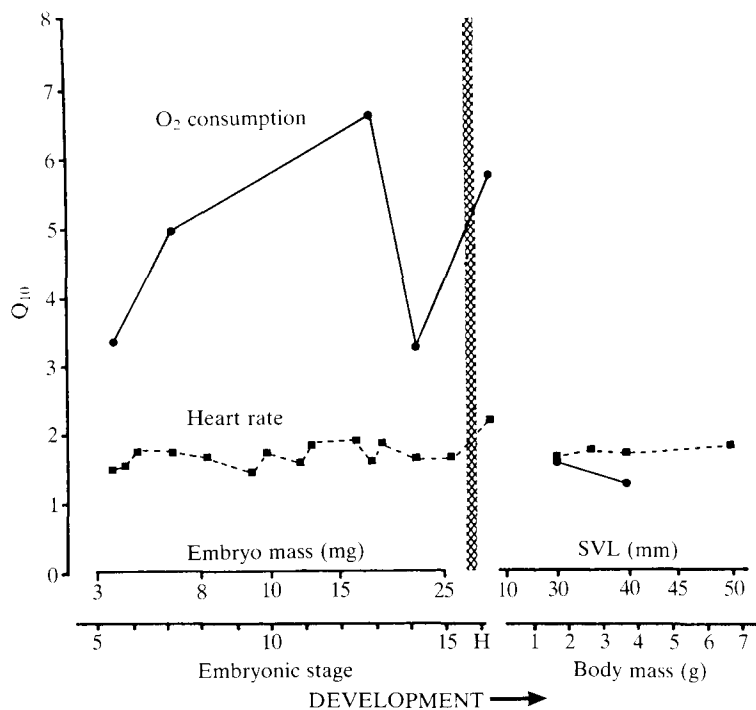
Fig. 6. The temperature quotient, Q_{10}, for heart rate (f_H, dashed line) and oxygen consumption ($\dot{M}_{O_2}$, solid line) as a function of development in *Eleutherodactylus coqui*. Each point represents the Q_{10} determined from the mean values of f_H and $\dot{M}_{O_2}$ at 24–25°C and 18°C, as presented in Figs 3 and 5.

ment, however, with means for individual developmental stages ranging from 4 to 11 μg lactate g egg^{-1}. Lactate concentration in hatchlings (37.5±4.2 μg lactate g tissue^{-1}) was 3–4 times higher than that of whole eggs, though this is not a direct comparison because of the lack of aqueous and egg membrane components in hatchlings.

The concentration of protein in vitelline fluid did not change significantly during embryonic development (ANOVA, $F_{4,47}=1.4$, $P>0.10$). Values were quite low and variable, with a grand mean and s.e. of 5.3±1.4 mg dl^{-1}.

Discussion

Developmental changes in heart rate

Large, predictable changes in f_H accompany development in *Eleutherodactylus coqui*. The pattern is both temperature independent and complex, with a sharp rise in f_H during early embryonic development, a steady but slower rise until hatching, and then a sharp fall after hatching with further development from juvenile to adult stages. 'Development' in *E. coqui* consists not only of the obvious processes of organogenesis, but also includes great changes in body mass and a
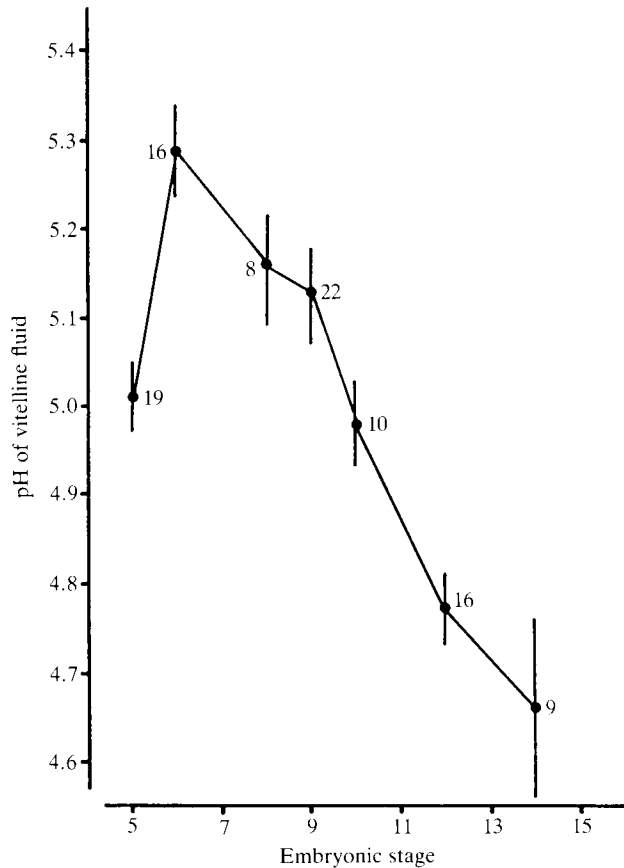
Fig. 7. Effects of development on the pH of vitelline fluid in the eggs of *Eleutherodac-tylus coqui*. Mean values ± 1 standard error are presented. Numbers beside each mean value represent the number of eggs sampled. See text for statistical analysis.

transition from an aquatic to a terrestrial environment. Several conclusions can be drawn from this study about the putative factors influencing f_H in developing *E. coqui*.

First, profound changes in f_H in embryos appear to be associated with organogenesis *per se*, or at least *not* with scaling effects or major environmental changes (i.e. from an aquatic to terrestrial habitat). The approximate doubling of f_H from embryonic stage 5 to stage 6 is accompanied by a less than 1 mg increase in body mass (approximately 3–4 mg), while only small additional increases in f_H occurred until hatching, even though body mass of the embryo increased seven- to eightfold. These changes are inconsistent with known allometric changes in physiological performance of juvenile and adult animals. Second, the transition from an aquatic to a terrestrial environment with the concomitant switch from fluid to air breathing does not seem to be correlated with a persistent change in f_H. Two lines of evidence support this contention. Although there is a significant

increase in resting f_H between stage 15 embryos and hatchlings (Fig. 3), the increase is less than 7 %, and probably has little biological significance. Moreover, after embryos that have been artificially hatched have recovered from the stress associated with that event (see below), the heart rate of new hatchlings returned within 4 h to values not significantly different from those of the embryo before hatching (Fig. 4).

The changes in body mass that occur after hatching in *E. coqui* doubtlessly influence f_H. There is a more than 100-fold increase in body mass from hatching to full maturity. Heart rate, like many other physiological processes, scales negatively with body mass in almost every vertebrate that has been examined (see Peters, 1983; Schmidt-Nielsen, 1984). With such a large increase in body mass during post-hatching development, a decrease in f_H related strictly to body mass change would certainly be anticipated. Unfortunately, to our knowledge there are no allometric data for amphibian f_H with which to make informed predictions of f_H changes associated with body mass changes.

The act of hatching in *E. coqui* had a stimulating effect on f_H that required several hours to disappear (Fig. 4). The natural process of explosive hatching from the egg capsule requires considerable activity on the part of the embryo, and the immediate response upon leaving the egg capsule, during either natural or artificial hatching, is to take several large jumps (unpublished observations of the authors). Locomotor activity is well known to result in increased f_H in amphibians (Boutilier *et al.* 1980; McDonald *et al.* 1980; Hillman *et al.* 1985, 1987). Although the effects of activity on f_H were not examined directly in the present study, there is no reason to suspect that *E. coqui* should differ from other amphibians in this respect. The slow time course (at least 2 h) of the return of f_H to pre-hatch levels after hatching and its associated activity in *E. coqui* is noteworthy, for it resembles the slow recovery of f_H in adult anurans and urodeles that have been exercised to the point of inducing a severe metabolic acidosis and oxygen debt. In the present study, after the initial burst of activity, hatchlings of *E. coqui* show little further ability to hop for at least several minutes, which is also highly suggestive of a recovery period from an exhaustive activity bout.

Developmental changes in f_H have been reported for very few amphibian species. In the bullfrog *Rana catesbeiana*, which hatches and then undergoes larval development in water, f_H falls progressively during larval development (Burggren and Doyle, 1986). However, meaningful comparison between f_H in early developmental stages of *E. coqui* and *R. catesbeiana* may be negated by the different developmental patterns exhibited by these species.

Developmental changes in oxygen consumption

Oxygen consumption, like f_H, shows a distinctive, complex pattern associated with ontogeny. Interestingly, the pattern of change in mass-specific $\dot{M}_{O_2}$ displayed the same general trends as for f_H, rising sharply early in development, rising more slowly until hatching, and then falling sharply with further post-hatch development. $\dot{M}_{O_2}$ also increases during development in the direct-developing plethodon-

tid salamander *Ensatina eschscholtzii*, but in this species the greatest rate occurs immediately prior to hatching (Bradford, 1984).

Heart rate has been used as an index of $\dot{M}_{O_2}$ in a study of the physiology of activity in *Bufo marinus* (McDonald *et al.* 1980). The relationship between f_H and $\dot{M}_{O_2}$, and in particular its utility in predicting one from the other, has been much debated for vertebrates generally (e.g. Fedak *et al.* 1988). It is not the purpose of the present paper to continue the debate over the precision of the relationship between f_H and $\dot{M}_{O_2}$. Nonetheless, given that the convective transport of blood provides the substrates for aerobic metabolism, it is not surprising to find that the general pattern of ontogenetic change in f_H and $\dot{M}_{O_2}$ resemble each other in *Eleutherodactylus coqui*.

Following the same reasoning for $\dot{M}_{O_2}$ as for f_H discussed above, it is clear that the patterns of change in $\dot{M}_{O_2}$ have components related to organogenesis, changes in body mass and the transition from fluid to air breathing. The transition from stage 14 embryos to hatchlings at 25 °C results in a 25 % rise in $\dot{M}_{O_2}$. It is tempting to assign *biological* (as opposed to statistical) significance to this rise at hatching, which of course represents a transition from fluid to air breathing. Yet, there is no rise when hatching occurs at 18 °C. Further experiments would be necessary to determine if the depression in $\dot{M}_{O_2}$ in stage 13 and 14 embryos at 25 °C is consistent in all populations of *E. coqui*.

Finally, it is worthwhile noting that the values of $\dot{M}_{O_2}$ we measured at 18 °C for approximately 4 g adult *E. coqui* are very similar to those measured in similar-sized adults of this species at 20 °C by Taigen *et al.* (1982) and Pough *et al.* (1983). Previous measurements of $\dot{M}_{O_2}$ in embryonic *E. coqui* have not been made. Although measurements of embryonic $\dot{M}_{O_2}$ have been made for many other amphibian species (e.g. see Bradford, 1984; Burggren, 1984, for reviews), different investigators have used variable methods for expressing $\dot{M}_{O_2}$ (per gram total egg mass, per gram embryo plus yolk, per gram total nitrogen, per gram protein, etc.), obviating direct comparison between studies.

Temperature effects

As indicated above, the pattern of developmental change in f_H was essentially the same at the two measurement temperatures. The Q_{10} for f_H, approximately 1.5–2, is a value that might be anticipated on the basis of temperature sensitivity of f_H in vertebrates generally (Schmidt-Nielsen, 1984; Prosser, 1986). The greater temperature sensitivity of $\dot{M}_{O_2}$ compared with f_H in embryos is apparent in the much higher Q_{10} for $\dot{M}_{O_2}$ up to the point of hatching (Fig. 6). The Q_{10} for development in *E. coqui* is also quite high at 3.92 (Townsend and Stewart, 1986), which indicates that general metabolic processes in this animal may be more temperature sensitive than in other vertebrates.

One of the most interesting aspects regarding temperature effects is the considerably different temperature sensitivities of f_H and $\dot{M}_{O_2}$ prior to hatching. As discussed above, f_H is commonly regarded as at least a rough index of $\dot{M}_{O_2}$. The present study for *E. coqui* would indicate that, as temperature changes, the

relationship between f_H and $\dot{M}_{O_2}$ changes fundamentally. If temperature in the natural environment increased from 20 to 30°C, the embryonic oxygen consumption would increase from three to as much as seven times (depending upon embryonic stage), while f_H would increase only 1.5–2 times. If we assume that cardiac output is matched to $\dot{M}_{O_2}$ over a range of temperature, then under these circumstances considerable changes in the stroke volume of the embryonic heart would have to occur to maintain oxygen and nutrient delivery to the metabolically active tissues. Yet, as hatchlings develop into juveniles and adults, the temperature sensitivity of $\dot{M}_{O_2}$ falls sharply to about that of the heart.

Why is there a dramatic change in Q_{10} of $\dot{M}_{O_2}$ as development proceeds in *E. coqui*? The answer to this question from a mechanistic viewpoint awaits an investigation that includes the kinetics of rate-limiting enzymes in oxidative metabolism. Certainly, the Q_{10} for $\dot{M}_{O_2}$ tends to be highest in the lower end of the normally encountered temperature range in amphibians (Hutchison and Dupre, 1991), and 18°C is near the lowest temperature encounterd by *E. coqui* (Townsend and Stewart, 1986). Thus, the Q_{10} for $\dot{M}_{O_2}$ reported in the present experiments may be higher than if the Q_{10} had been determined over the range 24–30°C, for example.

While it is tempting to assign an ecological importance to the effect of temperature on these physiological functions, the thermal biology of this species is quite complex and precludes simple adaptive explanations. For example, the eggs, typically laid within the protective confines of palm fronds, probably experience less temperature change than do free-ranging juveniles and adults. In addition, parental care by the male is known to affect egg water loss (Taigen *et al.* 1984; Townsend *et al.* 1984). Since water loss results in evaporative cooling, egg temperature is certain to be affected by the presence or absence of the male or by his position over the eggs. Finally, juveniles and adults, although living in a more eurythermal environment, are known to thermoregulate behaviorally (Pough *et al.* 1983).

Thus, the adaptive significance, if any, of the ontogenetic changes in temperature sensitivity, awaits further experiments that test the relative contributions of these factors. It is worth noting that embryos of both freshwater and marine teleost fishes similarly show a Q_{10} for $\dot{M}_{O_2}$ of up to 5 prior to hatching. After hatching, however, the Q_{10} for $\dot{M}_{O_2}$ falls to an average of 2 (see Rombough, 1988). This phenomenon may be a general characteristic of lower vertebrate embryos, rather than a specific trait of *E. coqui*.

The vitelline environment

The pH of vitelline fluid is quite acidic, ranging between 4.7 and 5.3 depending upon developmental stage. Although water lost by evaporation from the eggs is thought to be replaced by water transferred through the skin of the brooding male, the eggs certainly will absorb some environmental water. The pH of rain water dripping from the forest canopy into the leaf litter can be in the range 5–6, as in the temporary ground-water pools in the area in which eggs and animals were

collected (unpublished observations of the authors). Thus, the vitelline fluid is maintained slightly acidic to 'ambient' water. The vitelline fluid of the eggs of the salamander *Ambystoma maculatum* also remains about 0.5 pH units more acidic than ambient water with a pH of 6.0 (Robb and Toews, 1987). Generally speaking, amphibian larvae are able to survive and develop in water with a pH in the range 4–5 (Clark and Hall, 1985; Dunson and Connell, 1982).

After an initial increase, the pH of vitelline fluid fell progressively during development. An extensive chemical analysis of vitelline fluid was not made. However, the very low levels of protein compared with plasma, for example, would provide little buffering capacity. Thus, the addition of only small amounts of H^+ to the vitelline fluid would make it relatively acidotic. The acidosis could be of either respiratory or metabolic origin. A mild respiratory acidosis (caused by retention of CO_2) is possible, given the progressive rise in $\dot{M}_{O_2}$ (and presumably $\dot{M}_{CO_2}$) with development. Alternatively, the accumulation of acidic end-products of metabolism excreted into the vitelline fluid could produce a metabolic acidosis. Lactic acid would not seem to account for this increasing acidity, as there was no significant change in lactate concentration in vitelline fluid during embryonic development. The form of nitrogenous waste elimination could be indirectly involved in pH changes, but this deserves much further study, particularly with respect to its interactions with developmental transitions from ammoniotelism to ureotelism.

Dehydration is a real and common threat in the natural environment in which the eggs of *E. coqui* develop, and accounts for much of the embryonic mortality (Townsend *et al.* 1984). Dehydration had a profound effect on vitelline fluid pH, especially in earlier developmental stages (Table 1). A simple calculation can be made that assumes that dehydration results only in the loss of water molecules (retaining all pre-existing H^+ associated with metabolic acids). If half of the water in vitelline fluid were lost to evaporation, the resulting concentration of metabolically produced H^+ would result in a fall in pH of less than 0.2 units. Since the actual decrease is in the range 0.3–1.0 units, dehydration must result in the increase either in production of metabolic acids or the retention of CO_2. Further work is necessary to determine the source of the dehydration acidosis. Analysis is additionally complicated by the fact that, even in aquatic amphibian eggs, a decrease in pH of either vitelline fluid or the surrounding water results in shrinkage of the perivitelline space. Clearly, the causal relationship between dehydration and environmental pH has yet to be established.

In summary, this study has revealed highly significant changes in cardiac and metabolic physiology associated with development in *Eleutherodactylus coqui*. These changes appear to be associated with three factors: organogenesis, body mass changes and the transition from fluid to air breathing. Organogenesis is the overriding influence before hatching, while changes in body mass affect metabolism and f_H to the greatest extent in juveniles and adults. The shift from water to air breathing at hatching does not have a chronic effect on $\dot{M}_{O_2}$ or f_H. The relatively greater body mass changes during development in *Rana* spp., *Xenopus*

spp. and *Ambystoma* spp., together with the gradual rather than abrupt transition from fluid to air breathing during development in these species, add to the complexity of interpreting ontogenetic physiological change. Comparative studies involving amphibians with different developmental patterns (e.g. direct development, free-swimming larval intermediates, ovoviparity) will provide new insights into the ontogeny of cardiac and metabolic function.

References

Boutilier, R. G., McDonald, D. G. and Toews, D. P. (1980). The effects of enforced activity on ventilation, circulation and blood acid–base balance in the aquatic gill-less urodele, *Cryptobranchus alleganiensis*; a comparison with the semi-terrestrial anuran, *Bufo marinus*. *J. exp. Biol.* **84**, 289–302.

Bradford, D. F. (1984). Physiological features of embryonic development in terrestrially-breeding plethodontid salamanders. In *Respiration and Metabolism of Embryonic Vertebrates* (ed. R. S. Seymour), pp. 87–98. Dordrecht, Netherlands: Dr W. Junk Publishers.

Bradford, D. F. and Seymour, R. S. (1988). Influence of environmental P_{O_2} on embryonic oxygen consumption, rate of development, and hatching in the frog *Pseudophryne bibroni*. *Physiol. Zool.* **61**, 475–482.

Burggren, W. W. (1984). Transition of respiratory processes during amphibian metamorphosis: from egg to adult. In *Respiration and Metabolism of Embryonic Vertebrates* (ed. R. S. Seymour), pp. 31–53. Dordrecht, Netherlands: Dr W. Junk Publishers.

Burggren, W. W. and Doyle, M. (1986). Ontogeny of heart rate regulation in the bullfrog, *Rana catesbeiana*. *Am. J. Physiol.* **61**, 331–337.

Burggren, W. W. and Just, J. J. (1991). Ontogenetic change in physiological systems. In *Environmental Physiology of Amphibians* (ed. M. E. Feder and W. W. Burggren). Chicago: University of Chicago Press. (in press).

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

Clark, K. L. and Hall, R. J. (1985). Effects of hydrogen ion and aluminium concentrations on the survival of amphibian embryos and larvae. *Can. J. Zool.* **63**, 116–123.

Dunson, W. A. and Connell, S. (1982). Specific inhibition of hatching in amphibian embryos by low pH. *J. Herpetol.* **16**, 314–316.

Erasmus, B.-E. W., Howell, B. J. and Rahn, H. (1970/1971). Ontogeny of acid–base balance in the bullfrog and chicken. *Respir. Physiol.* **11**, 46–53.

Fedak, M. A., Pullen, M. R. and Kanwisher, J. (1988). Circulatory responses of seals to periodic breathing: heart rate and breathing during exercise and diving in the laboratory and open sea. *Can. J. Zool.* **66**, 53–60.

Feder, M. E. (1984). Consequences of aerial respiration for amphibian larvae. In *Respiration and Metabolism of Embryonic Vertebrates* (ed. R. S. Seymour), pp. 71–86. Dordrecht, Netherlands: Dr W. Junk Publishers.

Feder, M. E. and Burggren, W. (1985). Cutaneous gas exchange in vertebrates: Design, patterns, control and implications. *Biol. Rev.* **60**, 1–45.

Fox, H. (1984). *Amphibian Morphogenesis*. Clifton, New Jersey: Humana.

Frost, D. (1985). (ed.) *Amphibian Species of the World*. Lawrence, Kansas: Allen Press.

Hillman, S. S., Withers, P. C., Hedrick, M. S. and Kimmel, P. B. (1985). The effects of

erythrocythemia on blood viscosity, maximal systemic oxygen transport capacity and maximal rates of oxygen consumption in an amphibian. *J. comp. Physiol.* B **155**, 577–581.

HILLMAN, S. S., WITHERS, P. C., PALIOCA, W. P. AND RUBEN, J. A. (1987). Cardiovascular consequences of hypercalcemia during activity in two species of amphibians. *J. exp. Zool.* **242**, 303–308.

HUTCHISON, V. H. AND DUPRE, R. K. (1991). Thermoregulation. In *Environmental Physiology of Amphibians* (ed. M. E. Feder and W. W. Burggren). Chicago: University of Chicago Press.

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

McDONALD, D. G., BOUTILIER, R. G. AND TOEWS, D. P. (1980). The effects of enforced activity on ventilation, circulation and blood acid–base balance in the aquatic gill-less urodele, *Cryptobranchus alleganiensis*: a comparison with the semi-terrestrial anuran, *Bufo marinus. J. exp. Biol.* **84**, 289–302.

PETERS, R. H. (1983). *The Ecological Implications of Body Size.* Cambridge: Cambridge University Press.

POUGH, F. H., TAIGEN, T. L., STEWART, M. M. AND BRUSSARD, P. F. (1983). Behavioral modification of evaporative water loss by a Puerto Rican frog. *Ecology* **64**, 244–252.

PROSSER, C. L. (1986). *Adaptational Biology.* New York: Wiley.

ROBB, L. AND TOEWS, D. (1987). Effects of low ambient pH on perivitelline fluid of *Ambystoma maculatum* (Shaw) eggs. *Env. Pollution* **44**, 101–107.

ROMBOUGH, P. J. (1988). Respiratory gas exchange, aerobic metabolism, and effects of hypoxia during early life. In *Fish Physiology*, vol. XIA (ed. W. S. Hoar and D. J. Randall), pp. 59–161. New York: Academic Press.

SCHMIDT-NIELSEN, K. (1984). *Scaling: Why is Animal Size so Important?* Cambridge: Cambridge University Press.

TAIGEN, T. L., POUGH, F. H. AND STEWART, M. M. (1984). Water balance of terrestrial anuran (*Eleutherodactylus coqui*) eggs: importance of parental care. *Ecology* **65**, 248–255.

TOWNSEND, D. S. AND STEWART, M. M. (1985). Direct development in *Eleutherodactylus coqui* (Anura: Leptodactylidae): A staging table. *Copeia* **1985**, 423–436.

TOWNSEND, D. S. AND STEWART, M. M. (1986). The effect of temperature on direct development in a terrestrial-breeding, neotropical frog. *Copeia* **1986**, 520–523.

TOWNSEND, D. S., STEWART, M. M. AND POUGH, F. H. (1984). Male parental care and its adaptive significance in a neotropical frog. *Anim. Behav.* **32**, 421–431.

VLECK, C. M., HOYT, D. F. AND VLECK, D. (1979). Metabolism of avian embryos: Patterns in altricial and precocial birds. *Physiol. Zool.* **52**, 363–377.