



---

Metabolic Cost of Development in Terrestrial Frog Eggs (*Pseudophryne bibronii*)

Author(s): Roger S. Seymour, Fritz Geiser, David F. Bradford

Reviewed work(s):

Source: *Physiological Zoology*, Vol. 64, No. 3 (May - Jun., 1991), pp. 688-696

Published by: [The University of Chicago Press](#)

Stable URL: <http://www.jstor.org/stable/30158201>

Accessed: 16/04/2012 20:08

---

Your use of the JSTOR archive indicates your acceptance of the Terms & Conditions of Use, available at

<http://www.jstor.org/page/info/about/policies/terms.jsp>

JSTOR is a not-for-profit service that helps scholars, researchers, and students discover, use, and build upon a wide range of content in a trusted digital archive. We use information technology and tools to increase productivity and facilitate new forms of scholarship. For more information about JSTOR, please contact [support@jstor.org](mailto:support@jstor.org).



The University of Chicago Press is collaborating with JSTOR to digitize, preserve and extend access to *Physiological Zoology*.

---

# Metabolic Cost of Development in Terrestrial Frog Eggs (*Pseudophryne bibronii*)

---

Roger S. Seymour

Fritz Geiser<sup>1</sup>

David F. Bradford<sup>2</sup>

Department of Zoology, University of Adelaide, G.P.O. Box 498, Adelaide, South Australia 5001 Australia; <sup>1</sup>Department of Zoology, University of New England, Armidale, New South Wales 2351, Australia; <sup>2</sup>Environmental Science and Engineering Program, School of Public Health, University of California, Los Angeles, California 90024-1772

Accepted 10/31/90

## Abstract

We incubated *Pseudophryne bibronii* eggs at selected  $T_a$  (7°, 12°, 17°, 22° C) and substrate water potentials (0 and -25 kPa) to determine their effects on rate of  $O_2$  consumption ( $\dot{V}O_2$ ) of the embryos, incubation time, growth rate, and differentiation rate. Incubation to median hatching stage (stage 27) increased from 17 d at 22° C to about 140 d at 7° C, and  $\dot{V}O_2$  increased in proportion to age at all temperatures. At 0 kPa water potential, the total  $O_2$  consumed until hatching stage decreased from about 1.2 mL at 7° C, to 0.81 mL at 12° C, to a low of 0.58 mL at 17° C, and increased slightly to 0.67 mL at 22° C. However, gut-free dry mass of 12° C hatchlings was significantly higher than those at 17° and 22° C, so the energy cost of producing 1 mg of dry, gut-free embryo was similar (0.47–0.55 mL/mg) between 12° and 22° C. Incubation at lower water potential (-25 kPa) reduced  $\dot{V}O_2$  by 19%–28% and retarded growth rate but did not affect differentiation rate or incubation time.

---

## Introduction

The energy for development in amphibian embryos originates in the yolk. Very little is known about the fate of this energy, such as how much is metabolized during incubation and how much ends up in the embryo and residual yolk at hatching. In the terrestrially breeding frog, *Pseudophryne bibronii*, the energy contained in the egg at laying must be sufficient not only to carry the embryo through to hatching but also to supply energy during the delayed-hatching period, during which the terrestrial embryos await flooding by winter rain that stimulates them to hatch (Bradford and

---

Seymour 1985). At 12°C, embryos use only about 40% of the yolk mass by hatching time and have considerable reserve that lasts an additional 96 d. However, natural incubation temperatures can vary, affecting incubation time, metabolic rate, and the amount of energy remaining when embryonic development is complete. By measuring the total amount of O<sub>2</sub> consumed during incubation at different temperatures, we were able to evaluate the energy used for development.

The growth rate and metabolic rates of reptilian eggs have been shown to decrease when eggs are incubated under conditions of water restriction (Packard and Packard 1987). It is not clear whether this is true for birds (cf. Simkiss 1980; Davis and Ackerman 1987), but it may be true for amphibians (Smith-Gill and Berven 1979). In *P. bibronii*, we determined that growth rate was 32% less when eggs are incubated on a substrate at -25 kPa than at 0 kPa (Bradford and Seymour 1988), but we wished to find out whether this was correlated with a difference in rate of O<sub>2</sub> consumption. Therefore, at each of the four temperatures, we incubated eggs on moist filter-paper substrates at 0 and -25 kPa water potential.

## Material and Methods

The source and treatment of the eggs have been described elsewhere (Seymour, Geiser, and Bradford 1991). Briefly, eggs at Gosner (1960) stages 8–12 were collected, washed, and incubated in air under eight combinations of temperature (7°, 12°, 17°, and 22°C) and water potential (0 and -25 kPa). The eggs from seven clutches were randomly distributed among the eight treatments. The eggs were incubated without touching each other on stacks of moist filter paper within eight covered plastic containers. The eggs were occasionally selected at random for measurements of stage and metabolic rate and then returned to the incubator.

Embryos were staged according to Gosner (1960) except for stages 21 and 23, which were based on Woodruff (1972), and stages 19, 20, 24, and 25, which were interpolated in time between definitive stages, because *Pseudophryne bibronii* lacks the obvious features for these stages.

The rate of oxygen consumption ( $\dot{V}_{O_2}$ ) was determined with a Gilson model IG-14 single-valve differential respirometer equipped with 5-mL chambers and 1% KOH CO<sub>2</sub> absorbent. On every day of measurement at a given temperature, 4–5 chambers were prepared for each water potential, and each chamber contained 3–7 randomly selected eggs. After equilibration for at least 1 h,  $\dot{V}_{O_2}$  was measured for a further 3–6 h, and then the eggs were returned to their incubation containers. The average  $\dot{V}_{O_2}$  per egg ( $\mu\text{L STPD/h}$ ) was calculated for each chamber, and this value was assumed to

be independent of previous measurements and concurrent measurements in other chambers. Although this assumption is technically invalid for statistical analysis, it was impractical to identify individual eggs and to replicate constant temperature cabinets and egg containers for each treatment.

The effect of temperature on the mass of the hatchlings was determined in eggs incubated in water (0 kPa) because hatching does not normally occur in air. Embryos from two clutches were randomly separated into three groups of 9–10 individuals. Each group was placed at 12°, 17°, or 22°C in 90 mL of dechlorinated tap water, 1 cm deep. The groups were checked daily for hatchlings, which were removed and frozen. Subsequently the digestive tract and the carcass of the hatchlings were separated, dried to constant mass over silica gel, and weighed to 0.01 mg. Gut-free dry mass is presented in milligrams and as a percentage of total dry mass to reduce the variance due to differences in egg size. Measurements were not made at 7°C.

For the purposes of analysis, incubation time is taken as the time for terrestrial embryos to reach stage 27, which is the median stage at which hatching naturally occurs (Bradford and Seymour 1985). Differentiation rate is the inverse of the time taken to develop from one stage to another (Smith-Gill and Berven 1979).

## Results

### *Effect of Temperature and Water Potential on Incubation Time, Differentiation Rate, and Hatchling Mass*

In embryos incubated in air, temperature influenced incubation time and differentiation rate as measured by stage of development (fig. 1). Differentiation rates on substrates of 0 and –25 kPa were indistinguishable. The relationship between stage and age was not linear. The incubation time from stage 8 (the day after laying) to stage 27 was about 140 d at 7°C, 47 d at 12°C, 21 d at 17°C, and 17 d at 22°C. The  $Q_{10}$  values calculated for these three temperature intervals are 8.8 (7°–12°C), 5.0 (12°–17°C), and 1.5 (17°–22°C), indicating that differentiation rate becomes less temperature sensitive at higher temperature.

Nearly all embryos incubated in water survived to hatching at 12° and 17°C (i.e., 19 of 19 and 18 of 19, respectively), but only 5 of 20 survived at 22°C. Embryos in the two clutches did not differ significantly in incubation time at each temperature (Mann-Whitney  $U$ -test,  $P > 0.05$ ; Zar 1984). Median incubation times (and ranges) for the combined clutches, including 9 d at 12°C prior to placement at experimental temperatures, were 40 (36.5–65.5) d at 12°C; 23 (21.5–31) d at 17°C; and 20.5 (17–24.5) d at 22°C. Incubation

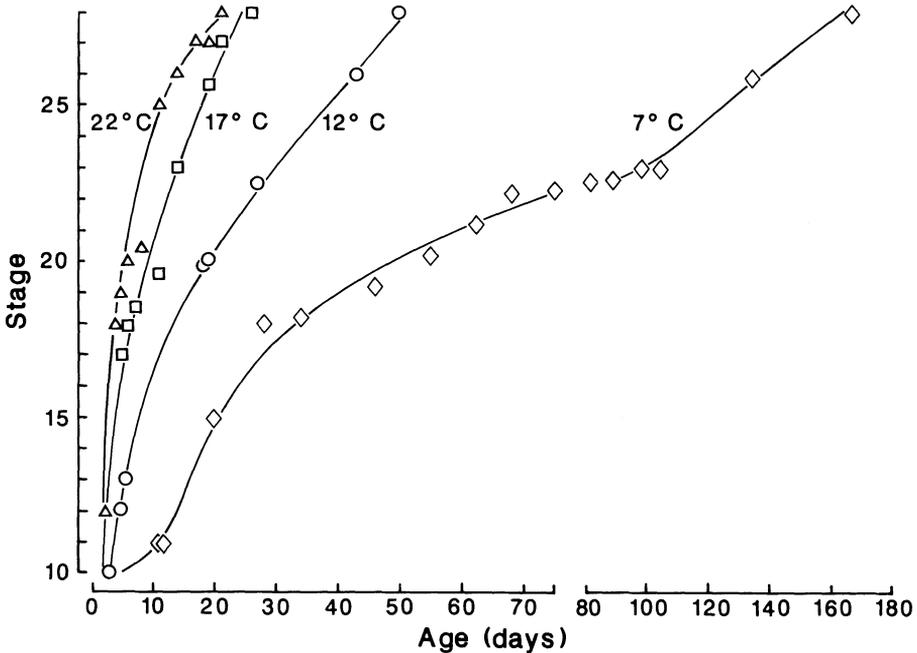


Fig. 1. Stage of development of *Pseudophryne bibronii* during terrestrial incubation on a saturated substrate (0 kPa) at four constant temperatures. Age represents time since fertilization. Each point is the mean of 4–5 embryos. Curves were fitted by eye.

times at 17° and 22°C were not significantly different but both were significantly shorter than at 12°C (*U*-test,  $P < 0.001$ ). Stage at hatching was not measured in aquatic embryos.

Embryos in water hatched at smaller dry mass at warmer temperatures (fig. 2). Similarly, embryos at warmer temperatures hatched when gut-free dry mass constituted a smaller proportion of total body mass (fig. 2). These differences were significant within each clutch between 12°C and the other temperatures (*t*-test,  $P < 0.05$ ; Zar 1984) but not between 17° and 22°C.

#### *Effects of Temperature and Water Potential on $\dot{V}O_2$*

Oxygen consumption increased during development in a fairly linear fashion, more quickly at higher temperature (ANCOVA:  $F = 32$ ,  $df = 3, 33$ ) (fig. 3). When the embryos reached hatching stage 27, the data tended to plateau. Oxygen consumption reached 1.30  $\mu\text{L}/\text{h}$  at 12°C, slightly higher than the value (1.05  $\mu\text{L}/\text{h}$ ) at 12°C in our earlier study (Bradford and Seymour 1985). The  $Q_{10}$  of  $\dot{V}O_2$  (stage 27, 0 kPa) was 2.49 between 12° and 17°C and 1.61 between 17° and 22°C. Embryos at 7°C did not reach stage 27, but  $Q_{10}$  at

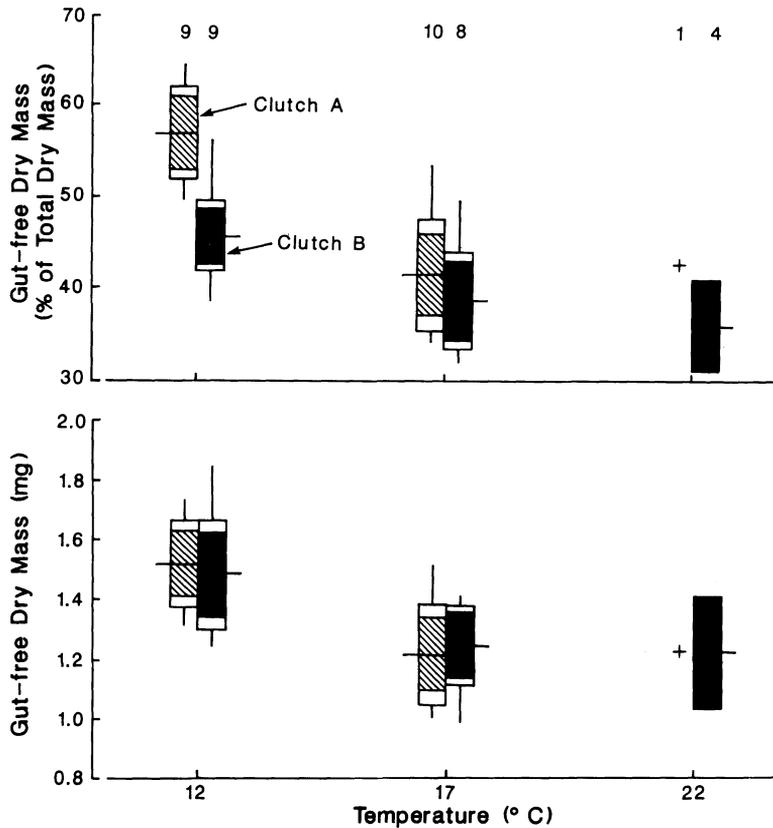


Fig. 2. Gut-free dry mass of *Pseudophryne bibronii* hatchlings in two clutches reared in water at three temperatures. Symbols represent means, 95% confidence limits of the mean, standard deviation, and range. Sample sizes are given at top. The "+" represents the single individual in clutch A that hatched at 22°C.

stage 26 was 2.51 between 7° and 12°C. Oxygen consumption was lower at -25 kPa water potential than at 0 kPa at all stages of development and at all temperatures except 7°C ( $P < 0.05$ ; Wilcoxon paired-sample tests; Zar 1984).

The integrated  $\dot{V}O_2$  up to hatching-stage 27 is given in table 1. At 12°, 17°, and 22°C, but not at 7°C, eggs at -25 kPa consumed less total  $O_2$  than those at 0 kPa. Total  $\dot{V}O_2$  was lowest at 17°C at both water potentials. The groups of eggs used for respirometry at 7°C died after they reached stage 26 at 0 kPa and stage 23 at -25 kPa. Because embryos can hatch at stage 26 (Bradford and Seymour 1985), these data are included in table 1. We attribute the late mortality of the 7°C eggs to fungus infections exacerbated by repeated handling. Other eggs at 7°C that were not removed from their incubation containers developed to stage 28 in about 156 d.

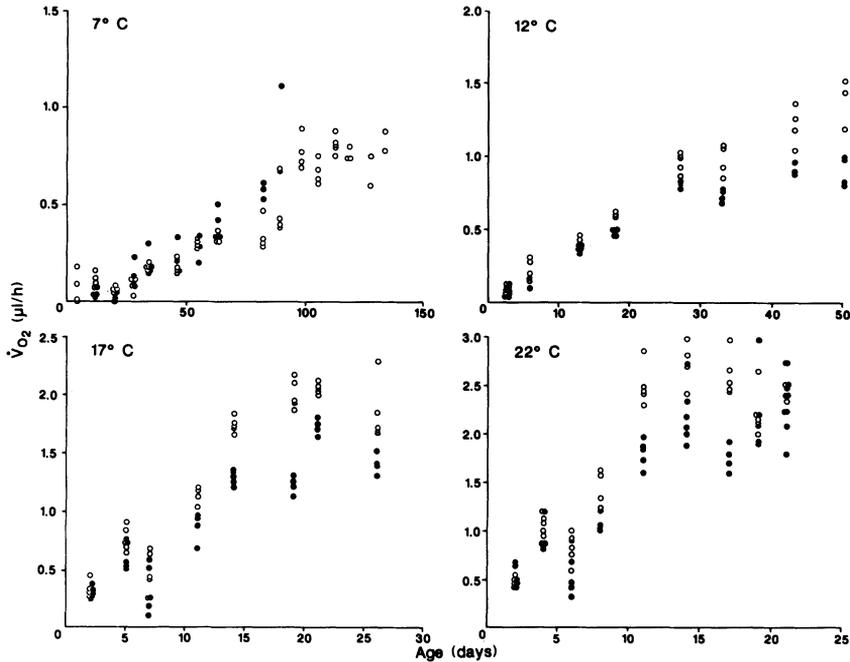


Fig. 3. Rate of  $O_2$  consumption per egg ( $\dot{V}O_2$ ) in *Pseudophryne bibronii* at four temperatures. Eggs were measured at 0 kPa (○) or -25 kPa (●) water potential. Each point is a mean of 4–5 embryos.

## Discussion

The energy used by an embryo can be estimated by measuring the total amount of  $O_2$  consumed during development. When an embryo of *Pseudophryne bibronii* reaches hatching stage after about 39–47 d at 12°C, it has consumed about 0.7 mL of  $O_2$  (table 1). If kept on land during the delayed-hatching period, it exhausts its yolk supply after 96 d, having consumed a total of about 1.7 mL, and dies at about 140 d, having consumed about 2.5 mL (Bradford and Seymour 1985). This viable limit of available energy is about  $2.5 \text{ mL} \times 20 \text{ J/mL} = 50 \text{ J}$ . Therefore development to hatching stage requires only 14 J, or 28% of the total viable limit. Considerable energy remains for survival during the delayed-hatching period, when the unhatched embryos wait for the nest to be flooded. Considerable reserve energy exists in other hatchling amphibians. For example, salamander (*Ambystoma*) embryos use about 0.3 mL of  $O_2$  to reach hatching and then about 1.0 mL as prefeeding larvae (Kaplan 1980). It would be valuable to compare embryonic energy budgets in other amphibians with different reproductive modes.

As is the case for other amphibians (Smith-Gill and Berven 1979; Kaplan 1980), incubation at lower temperatures increases hatchling size in *P. bi-*

TABLE 1

Total amount (mL) of O<sub>2</sub> consumed during development to stage 27 (median hatching stage), dry gut-free hatchling mass, and cost of development in *Pseudophryne bibronii*

Variable	Temperature (°C)			
	7	12	17	22
Total VO <sub>2</sub> :				
0 kPa (mL) . . . . .	1.21 <sup>a</sup>	.81	.58	.67
-25 kPa (mL) . . . . .	. . . <sup>b</sup>	.66	.42	.50
Dry hatchling:				
0 kPa (mg) . . . . .	. . .	1.50	1.23	1.22
Cost of development (mL/mg) . . . . .	. . .	.54	.47	.55

<sup>a</sup> Stage 26.

<sup>b</sup> Died after stage 23.

*bronii* (fig. 2). Larger hatchlings may be better able to survive larval life, but incubation at lower temperature carries disadvantages, especially for terrestrial eggs. For example, the total amount of O<sub>2</sub> consumed by embryos when they reach hatching stage increases as temperature decreases between 17° and 7°C (table 1). At colder temperatures, therefore, less energy remains in the egg to support metabolism in the delayed-hatching period. Incubation at 7°C is particularly disadvantageous. The total O<sub>2</sub> consumed to stage 26 (1.21 mL O<sub>2</sub>) is more than twice the value at 17°C and represents about half of the viable limit. Further development to stage 27 at 7°C would practically exhaust the yolk and leave the hatchling with little energy reserves except for those in its own body. This temperature is also disadvantageous because the exceptionally long incubation time (ca. 5 mo) increases the dangers of predation, infection, and desiccation and may require longer attendance by the male adult frog. Incubation at 12° and 17°C reduces total O<sub>2</sub> consumption and leaves the hatchling with progressively more energy. Interestingly, the trend does not continue to 22°C, and the total consumption begins to rise inexplicably. This temperature may be close to the tolerable limit; survivorship of aquatic embryos is only 25% at this temperature, and there is no survival at 27°C (D. F. Bradford, personal observation). High temperature possibly interferes with proper differentiation and makes development less energetically efficient.

This study allows us to examine the effect of temperature on the energy used to produce a unit of hatchling body mass in *P. bibronii*. This so-called

energy cost of development is obtained by dividing the total O<sub>2</sub> consumption by the gut-free, dry, hatchling mass (table 1). The values suggest that the cost of development is lowest at the intermediate temperature of 17°C, but any differences cannot be tested statistically until data become available for  $\dot{V}O_2$  and hatchling mass in the same eggs. Nevertheless, the differences are small, and the cost of building a unit of dry embryo is practically independent of temperature. The mass-specific energy cost of development in reptiles is also rather independent of temperature within the normal incubation range (Thompson 1983; Leshem et al. 1986; Whitehead and Seymour 1990).

The average energy cost of development is 0.52 mL/mg in *P. bibronii* (table 1). The values for 39 species of birds average about 0.78 mL/mg (Vleck and Vleck 1987). Assuming a hatchling water content of 78%, the energy cost of development at 30°C in 11 species of reptiles averages 0.49 mL/mg (Whitehead and Seymour 1990). Ackerman (1981) suggested that the difference in the energy cost of development between birds and reptiles rested in the difference in incubation temperature, but the present data from *P. bibronii* eggs incubated at much lower temperatures do not extend this idea. Not only are the costs similar in the frog, incubated at 12°–22°C, and the reptiles, incubated at 30°C, but also there is no consistent effect of temperature in the frog (table 1).

Embryos reared at –25 kPa consumed 72%–81% less O<sub>2</sub> during development to stage 27 than embryos at 0 kPa (table 1). This reduction is associated with a diminished growth rate at lower water potential; embryos at –25 kPa grew 71% slower than embryos at 0 kPa (Bradford and Seymour 1988). Surprisingly, the similar reductions in  $\dot{V}O_2$  and growth rate at lower water potential are not associated with retarded differentiation. Embryos at the two water potentials in our study did not noticeably differ in stage. A similar observation has been made in reptilian eggs (Packard and Packard 1987), but the physiological explanation is still unclear. Certainly the reductions in  $\dot{V}O_2$  and growth rate at lower water potential do not result from differences in O<sub>2</sub> availability. Although water potential greatly affects the size and thickness of the jelly capsule, its O<sub>2</sub> conductance is unaffected (Seymour and Bradford 1987).

## Acknowledgments

This research was supported by the Australian Research Grants Scheme. Veronica Ward and Sandi Poland offered excellent technical assistance.

## Literature Cited

- ACKERMAN, R. A. 1981. Oxygen consumption by sea turtle (*Chelonia, Caretta*) eggs during development. *Physiol. Zool.* 54:316–324.
- BRADFORD, D. F., and R. S. SEYMOUR. 1985. Energy conservation during the delayed-hatching period in the frog *Pseudophryne bibronii*. *Physiol. Zool.* 58:491–496.
- . 1988. Influence of water potential on growth and survival of the embryo, and gas conductance of the egg, in a terrestrial breeding frog, *Pseudophryne bibronii*. *Physiol. Zool.* 61:470–474.
- DAVIS, T. A., and R. A. ACKERMAN. 1987. Effects of increased water loss on growth and water content of the chick embryo. *J. Exp. Zool., suppl.*, 1:357–364.
- GOSNER, K. L. 1960. A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica* 16:183–190.
- KAPLAN, R. H. 1980. Ontogenetic energetics in *Ambystoma*. *Physiol. Zool.* 53:43–56.
- LESHEM, A., A. AR, R. DMI'EL, and R. A. ACKERMAN. 1986. Energy transformation during development of turtle (*Trionyx triunguis*) embryos. *Fed. Proc.* 45:642.
- PACKARD, G. C., and M. J. PACKARD. 1987. Physiological ecology of reptilian eggs and embryos. Pages 517–600 in C. GANS and R. B. HUEY, eds. *Biology of the Reptilia*. Vol. 16. Liss, New York.
- SEYMOUR, R. S., and D. F. BRADFORD. 1987. Gas exchange through the jelly capsule of the terrestrial eggs of the frog, *Pseudophryne bibronii*. *J. Comp. Physiol.* 157B:477–481.
- SEYMOUR, R. S., F. GEISER, and D. F. BRADFORD. 1991. Gas conductance of the jelly capsule of terrestrial frog eggs correlates with embryonic stage, not metabolic demand or ambient  $PO_2$ . *Physiol. Zool.* 64:673–687.
- SIMKISS, K. 1980. Water and ionic fluxes inside the egg. *Am. Zool.* 20:385–393.
- SMITH-GILL, S. J., and K. A. BERVEN. 1979. Predicting amphibian metamorphosis. *Am. Nat.* 113:563–585.
- THOMPSON, M. B. 1983. The physiology and ecology of the eggs of the pleurodiran tortoise *Emydura macquarii* (Gray), 1831. Ph.D. diss. University of Adelaide.
- VLECK, C. M., and D. VLECK. 1987. Metabolism and energetics of avian embryos. *J. Exp. Zool., suppl.*, 1:111–125.
- WHITEHEAD, P., and R. S. SEYMOUR. 1990. Patterns of metabolic rate in embryonic crocodylians, *Crocodylus johnstoni* and *C. porosus*. *Physiol. Zool.* 63:334–352.
- WOODRUFF, D. S. 1972. The evolutionary significance of hybrid zones in *Pseudophryne* (Anura: Leptodactylidae). Ph.D. diss. University of Melbourne.
- ZAR, J. H. 1984. *Biostatistical analysis*. Prentice-Hall, Englewood Cliffs, N.J. 718 pp.