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The effect of temperature on the growth and development of the endangered green and golden bell frog (*Litoria aurea*)

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Abstract

1. The affects of temperatures of 15°C, 22°C and 28°C on the growth and development of juvenile green and golden bell frogs (*Litoria aurea*) were investigated over 42 days.
2. Snout-vent length increased only at 28°C.
3. Sexual maturation of males occurred only at 28°C.
4. Growth was not proportional to food intake.
5. Over the range of *L. aurea* ambient temperatures are seasonally lower than the optimum for growth and development.
6. Morning temperatures are 2°C colder, during the cooler months, in regions where *L. aurea* is extinct when compared with those still occupied.

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1. Introduction

The extinction of the endangered Australian green and golden bell frog (*Litoria aurea*) at cooler high altitudes (NSW TSCA, 1995; White and Pyke, 1996) suggests that temperature could affect the species survival. Torpor in adults during mid-winter (June–July), basking in air temperatures above 30°C (Pyke and White, 2001), and an optimum growth temperature above 22°C for tadpoles (Browne et al., 2003), suggest that in post metamorphic *L. aurea* low temperatures could adversely affect the physiological processes of growth and development or reduce foraging and food consumption. However, body temperatures above 22°C are not necessary for food consumption as nocturnal foraging occurs below 15°C (Browne pers. obs.).

The interaction of temperature with growth, development, and food intake and assimilation influences the survival of frog populations (Hutchison and Dupré, 1992). Exposure of anurans (frogs and toads) to higher temperatures generally increases growth rates and decreases maturation times, therefore affecting reproductive output (Hadfield, 1966; Smith, 1976; Lillywhite, 1970). Successful mating and high fecundity require both maturity and a high condition index. A high condition index is needed as the body mass of male anurans decreases during the reproductive period as a consequence of energetically expensive calling behaviour, and in female anurans a significant proportion of body mass is partitioned into oocytes during reproduction (Smith, 1976). Therefore, behavioural thermoregulation through basking could affect growth, development, and reproductive output if either growth or food assimilation is temperature dependent.

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Basking is widespread in anurans and increases body temperature from approximately 3–10°C above ambient air temperature (Lillywhite, 1970; Hutchison and Dupré, 1992). However, the advantages of increased temperature on growth and development through basking must be balanced against increased predation risk by diurnal predators (Duellman, 1978). Therefore, in conservation programs for anurans the provision of optimum basking microhabitats requires a sound knowledge of each species thermal physiology and their thermoregulatory behaviour.

Populations of *L. aurea* are subject to intense monitoring, including mark recapture growth studies. The effects of different ambient temperatures, between seasons or regions, on the growth of populations or individuals will influence the accuracy of the von Bertalanffy growth model. In this model the mean size difference between mark and recapture time is used to calculate growth curves of size against time for discrete populations. Thus the prediction of frog age and population demographics depends on individual growth being consistent with the previously calculated growth curve (Hota, 1994). Differences in growth rates due to altered body temperatures will affect the accuracy of these predictions.

Laboratory investigations using varying temperatures and solar irradiance can provide information on the optimum physiological temperature, the preferred temperature, and the lapse time to reach the preferred temperature (Hutchison and Dupré, 1992). To test the affect of temperature on the optimal physiological temperature of *L. aurea* we measured the length and mass, food intake, and the maturation of juveniles at three temperatures (15°C, 22°C and 28°C) over 42 days. Above 22°C both growth and development increased. Therefore, to test the need for basking the daily and seasonal temperature variations at weather stations over the historical range of *L. aurea* were compared between currently and previously occupied regions.

2. Materials and methods

Captive bred *L. aurea* originally derived from populations at Kooragang Island, New South Wales, Australia (32°51′S, 151°42′E) were raised as tadpoles at 22±1°C in a temperature controlled room until metamorphosis (Browne et al., 2003). Frogs were then kept in a temperature-controlled room at 28°C and fed mealworms (*Tenebrio molitor*) with vitamin supplements until developing into early juveniles. 120 frogs selected at random were divided equally between 12 polyethylene storage boxes (78 L × 44 W × 17 H cm). A 30 × 45 × 6 cm plastic tray filled to 1 cm with tap water was placed at one end of each box, and each box was provided with moistened, absorbent paper towelling to improve sanitation. Four boxes were kept in each of three temperature-controlled rooms at 15°C, 22°C and 28±1.5°C. The photoperiod was 12 h light and 12 h dark for all treatments.

At alternate 3 or 4 day intervals, we fed 200 mealworms per box (individual mean mealworm mass = 0.097 g). Live mealworms were confined by a plastic plate (30 cm diameter) with smooth sloping sides placed at the opposite end of the box to the water tray. Uneaten mealworms were removed and counted at the end of each interval, the trays and boxes were washed, and boxes relined with moistened absorbent paper towelling.

At days 0, 14, 28 and 42 snout-vent length (SVL) was measured with Vernier callipers to 0.1 mm, and frog mass was determined to 0.01 g. From the values obtained we calculated condition index CI = (frog mass (g)/SVL^3 (mm)) × 10^4). The mean food consumption per frog in each treatment was calculated as the accumulating mass of mealworms consumed for each 14 day interval and as a total (Ml) over 42 days. The mean food conversion ratio (FCR) was calculated as the total mass of mealworms consumed (Ml) divided by the initial (IM) minus the final (FM) frog mass (Ml/FM−IM(g)). The specific growth rate of mass SGR^{mass} (% day^{-1}) was calculated as: SGR^{mass} (% day^{-1}) = 100 × ($\log_e$ FM − $\log_e$ IM)/time (42 days) and the specific growth rate of SVL SGR^{SVL} (% day^{-1}) by a corresponding formula. Every 14 days the presence or absence on male frogs of a secondary sexual characteristic, the nuptial pad, was used to determine progress to sexual maturation. Frogs with a nuptial pad were considered mature and in breeding condition (Pyke and White, 2001).

The statistical ranges of mean monthly maximum, 9 am, and 3 pm temperatures in mid-summer (January) and mid-winter (July) were calculated from the individual means of all 149 weather stations of the Australian Bureau of Meteorology (ABM, 2002) logged over the previously inhabited range of *L. aurea*. The benefit of basking was also tested (*t*-tests) by comparison of the mean 9 am temperature between regions currently and previously occupied by *L. aurea* in eastern Australia.

Condition index data were arcsine transformed before testing for normality (Shapiro-Wilk W test) and homogeneity of variance (O'Brien's). All data sets were normal. Comparison of means within each treatment between day 1 and other times were made by student *t*-tests (Table 1), and between treatments by Tukey–Kramer least significant difference for multiple comparison of means. Means are presented ±SE (Tables 1 and 2). All analyses were performed using the JMP 3.2 software package (SAS Institute Inc.).

Table 1
The snout-vent length, mass and condition index of *L. aurea* kept at 15°C, 22°C and 28°C, for each 14 day period between day 1 and day 42 (means ± SE)

Response	Day	15°C	22°C	28°C
Snout-vent length (mm)	1	45.0 ± 0.6	44.7 ± 1.0	43.7 ± 0.9
	14	43.9 ± 0.8	44.9 ± 0.9	44.9 ± 0.5
	28	44.3 ± 0.6	46.6 ± 0.8	$47.3 \pm 0.5^{**}$
	42	44.3 ± 0.5^{b}	45.8 ± 0.8^{b}	$50.1 \pm 0.7^{*a}$
Mass (g)	1	6.3 ± 0.3	6.7 ± 0.6	5.6 ± 0.3
	14	$7.7 \pm 0.5^{**}$	8.1 ± 0.5	$7.4 \pm 0.3^{**}$
	28	$8.0 \pm 0.1^{**}$	$8.9 \pm 0.1^{**}$	$9.1 \pm 0.5^{*}$
	42	$8.0 \pm 0.4^{**b}$	$8.5 \pm 0.5^{**b}$	$10.5 \pm 0.4^{*a}$
Condition index	1	6.9 ± 0.2	7.4 ± 0.3	6.9 ± 0.2
	14	$9.3 \pm 0.1^{**}$	$8.9 \pm 0.5^{**}$	$8.1 \pm 0.4^{**}$
	28	$9.2 \pm 0.3^{**}$	$8.6 \pm 0.3^{**}$	$8.5 \pm 0.3^{**}$
	42	$8.9 \pm 0.2^{**}$	$8.4 \pm 0.1^{**}$	$8.2 \pm 0.3^{**}$

Comparison of means within treatments is between day 1 and each time (superscripts $^{**}P<0.05$, $^{*}P<0.01$). Comparison of means by ANOVA (superscripts ^{a,b}, $P<0.05$) is between treatments. Means with different superscripts are significantly different.

Table 2
The difference between the final (FM) and initial (IM) frog mass (FM−IM), the mass of mealworms consumed (MI), the food conversion ratio (FCR), specific growth rate weight (SGR^{mass}) and snout-vent length (SGR^{SVL}) (means ± SE)

Treatment	FM−IM (g)	MI (g)	FCR	SGR^{mass}	SGR^{SVL}
15°C	1.69 ± 0.4^{b}	6.3 ± 0.4^{c}	3.8 ± 0.4^{b}	0.56 ± 0.12^{b}	-0.04 ± 0.03^{b}
22°C	1.89 ± 0.3^{b}	10.6 ± 0.4^{b}	5.9 ± 0.7^{a}	0.61 ± 0.06^{b}	0.08 ± 0.04^{b}
28°C	4.8 ± 0.5^{a}	13.5 ± 0.9^{a}	2.8 ± 0.2^{b}	1.47 ± 0.04^{a}	0.34 ± 0.02^{a}
Probability	$P<0.001$	$P<0.001$	$P<0.005$	$P<0.001$	$P<0.001$

Within columns means with different superscripts are significantly different ($P<0.05$).

3. Results

3.1. Snout-vent length, mass and condition index

At day 1 the mean length of frogs was normally distributed with no significant difference between the treatment means. Table 1 shows that at day 42 there was a significantly greater ($P<0.05$) SVL at 28°C than the other treatments. The 28°C treatment showed a significant ($P<0.05$) increase in SVL by day 28 which increased in significance to ($P<0.01$) by day 42. The other treatments showed no significant increase in SVL to day 42. Increases in SVL between day 1 and day 42 were 6.62 ± 0.46 mm at 28°C, 1.43 ± 0.79 mm at 22°C and 0.74 ± 0.64 mm at 15°C.

At day 1 the mean mass of frogs was normally distributed with no significant difference between the treatment means. After day 1 the mean masses of frogs showed no significant differences between treatments except at day 42 when the 28°C treatment was significantly ($P<0.01$) greater than at 22° and 15°C. At 28°C frogs showed a significant ($P<0.05$) increase in mass at day 14, and to day 42 ($P<0.01$). At 22°C frogs showed a significant ($P<0.05$) increase in mass from day 28 which did not increase to 42. At 15°C frogs showed a significant ($P<0.05$) increase in mass after day 14 which did not increase to day 42 (Table 1). Increases in mass from day 1 to day 42 were 4.8 ± 0.23 g at 28°C, 1.89 ± 0.28 g at 22°C and 1.69 ± 0.21 g at 15°C.

Condition index increased significantly ($P<0.05$) for all treatments by day 14. From day 14 to day 42 there was no significant change in CI for any treatment. There was no significant difference in condition index between any treatments at any time periods.

3.2. Food intake, food conversion ratio and specific growth rate

Mean food consumption per 14 day period declined from days 1 to day 42 in all treatments, from 2.4 ± 0.05 to 0.4 ± 0.05 g at 15°C, from 3.0 ± 0.1 to 1.0 ± 0.01 g at 22°C, and from 3.4 ± 0.1 to 2.0 ± 0.2 g at 28°C. Food

intake per period was always significantly higher ($P<0.001$) at 28°C, than at 15°C and from day 14 ($P<0.05$) at 22°C. The total food consumption was significantly greater ($P<0.01$) at 28°C and 22°C than at 15°C at all times, and significantly greater ($P<0.001$) at 28°C than at 22°C from day 28 (data not shown). The FCR was significantly lower ($P<0.05$) at 15°C and 28°C when compared with 22°C. The specific growth rates as expressed by mass and SVL were significantly higher ($P<0.001$) at 28°C than at 15°C and 22°C (Table 2).

3.3. Maturation

Male frogs matured only in the 28°C treatment. At day 28 nine frogs from all replicates developed nuptial pads. The SVL (51.7 ± 0.8 mm; mean ± SE) was not significantly different to the 28°C treatment mean.

3.4. Climatic variables

The statistical ranges of mean monthly maximum, 9 am, and 3 pm temperatures for January were 21–34°C, 15.7–27.3°C, and 19.6–31.3°C, respectively, and for July were 7–24°C, 1.7–14.5°C, and 6–19.6°C, respectively. The mean 9 am temperature was lower than 22°C in both regions in all months, and was significantly warmer in the occupied areas by approximately 1.5–2°C from May (autumn; $P<0.01$; 14.3 ± 0.7°C, 12.4 ± 0.3°C) to September (spring; $P<0.04$; 15.1 ± 0.4°C, 13.7 ± 0.3°C), currently and previously occupied regions, respectively.

4. Discussion

In this study we report a coupling between temperature, growth and maturation in *Litoria aurea*. Growth in the SVL and maturation of males occurred only at 28°C. Both daily and seasonal temperatures over the current and previous range of *L. aurea* were frequently below 22°C showing the ability of basking to increase growth. The higher 9 am temperature, during the cooler months in areas currently occupied by *L. aurea* than those previously occupied, shows that basking coupled with air temperature could effect survival. These relationships between the temperature requirements for growth and development, and climate have implications in the conservation of *L. aurea,* particularly in the provision of suitable microhabitats for basking.

Food types can affect the growth of anurans, with some foods producing poor growth and development rates and even resulting in dietary deficiencies (Larsen, 1992). However, mealworms as used in this study appear to provide a suitable diet for *L. aurea*. The SGR^{mass} at 28°C of *L. aurea* in this study was approximately that of maturing juveniles of other anurans (Rodriguez-Serna et al., 1996; Hilken et al., 1995), with no pathology observed during the experimental period. In anurans the FCR increases and the specific growth rate (SGR^{mass}) lowers with maturation (Rodriguez-Serna et al., 1996). The FCR at 28°C of *L. aurea* corresponded with those in maturing *R. catesbeiana* (Rodriguez-Serna et al., 1996) and *Bufo terrestris* (Smith, 1976).

Temperature was shown to alter the relationship between food intake, assimilation and the growth of *L. aurea*. In anurans growth stimulation by hormones, or natural growth phases entrained by increased temperature or photoperiod, decrease lipid stores and increase the conversion of nutrients to other tissues. Conversely, low temperatures per se or anticipation of cool periods with associated torpor increase lipid storage (Pasanen and Koskela, 1974) . In *L. aurea* corresponding affects were shown when high temperature increased growth and reduced the storage of lipids. Duellman and Trueb (1986) concluded that basking increased digestive rates, which consequently maximised growth in juveniles, and allowed greater accumulation of lipids. Whether the low FCRs of *L. aurea* at increased temperature were due to lower assimilation, or greater energy demands from increased activity or metabolism is undetermined. However, at 15°C food intake had declined to a minimum and condition index had reached a maximum by day 14, with a similar but less pronounced pattern followed at 22°C. Our results show that with *L. aurea* temperatures above 22°C are not required for increased food assimilation but are necessary for growth.

High temperatures promote sexual maturation in frogs, with androgens stimulating the development of nuptial pads in males (Lofts et al., 1972). The pituitary releases gonadotrophins (Kanamadi and Jirankali, 1993) which promote luteinising hormone (LH) stimulated secretion of androgen from the testis (Licht et al., 1983). Conversely low temperatures decrease circulating androgens which coincides with lower LH sensitivity (Emerson et al., 1997). Therefore low temperatures increase the age of sexual maturity and consequently reduce reproductive output. Whether size or temperature was responsible for the maturation of males in the 28°C treatment is uncertain, as their SVL was the same as that shown in maturating males in nature (Pyke and White, 2001). Nevertheless, irrespective of whether maturation is determined by temperature or size, higher body temperatures through basking would allow *L. aurea* to shorten maturation time. However, basking may not completely compensate for low air temperatures. In cool climates this could result in reduced growth and development rates and lower reproductive output from populations.

Climate records over the historic range of *L. aurea* showed that all seasons had temperatures where basking would increase growth and development. The 9.00 am temperature range of 15.7–27.3°C in January

(mid-summer) showed at some locations and times basking would increase growth rate. In July (mid-winter) even the daily maximum and 3 pm temperatures were generally lower than those needed for growth. The 2°C lower temperature at 9 am from late autumn (May) and early spring (September), in regions where *L. aurea* is extinct compared to those occupied, could result in increased basking behaviour. Increased basking behaviour with increased predation, especially in habitats offering degraded basking sites, could have contributed to the species decline. Even with basking the high variability in temperature over the range of *L. aurea* could result in differences in growth rates between cohorts. Thus growth models derived for one location may be inapplicable to another. At the same location growth curves may also vary from one cohort to another depending on the season of metamorphosis. These differences would be ameliorated by basking in suitable microhabitats (Hutchison and Dupré, 1992).

Our study shows that over the historic range of *L. aurea* the daily and seasonal ambient temperature is generally lower than the optimum for growth and development. Early morning temperatures were also higher in occupied regions than where *L. aurea* is extinct from autumn to spring. The dependence of growth on higher temperatures suggests that thermoregulation through basking maximises growth and lowers maturation time. Therefore, behavioural thermoregulation coupled with ambient temperatures could influence the population demographics and reproductive output of *L. aurea* and consequently the survival of populations. Further laboratory studies between the thermoregulatory behaviour of *L. aurea* and their body temperature, growth and development, and field studies of the ecology of thermoregulation, could contribute to the species conservation.

References

ABM, 2002. Climatic averages. Australian Bureau of Meteorology, http://www.bom.gov.au.

Browne, R.K., Pomering, M., Hamer, A.J., 2003. High density effects on the growth, development and survival of *Litoria aurea* tadpoles. Aquaculture 215 (1–4), 109–121.

Duellman, W.E., 1978. The biology of an equatorial herpetofauna in Amazonoan Ecuador. Misc. Publ. Mus. Natl. Hist. Univ. Kansas 65, 1–352.

Duellman, W.E., Trueb, L., 1986. Biology of Amphibians. The Johns Hopkins University Press, Baltimore, MD, 217pp.

Emerson, S.B., Carroll, L., Hess, D., 1997. Hormonal induction of thumb pads and the evolution of secondary sexual characteristics of the South East Asian Fanged frog, *Rana blythii*. Exp. Zool. 279, 587–596.

Hadfield, S., 1966. Observations on body temperature and activity in the toad *Bufo woodhousei fowleri*. Copeia, 581–582.

Hilken, G., Dimigen, J., Iglauer, F., 1995. Growth of *Xenopus laevis* under different laboratory rearing conditions. Lab. Anim. 29, 152–162.

Hota, A.K., 1994. Growth in amphibians. Gerontology 40, 147–160.

Hutchison, V.H., Dupré, R.K., 1992. Thermoregulation. In: Feder, M.E., Burggren, W.W. (Eds.), Physiology of Amphibians. University of Chicago Press, Chicago, pp. 207–215.

Kanamadi, R.D., Jirankali, C.S., 1993. Role of temperature and light in regulation of ovarian cycle of tree frog *Polypedates maculatus*. Ind. J. Exp. Biol. 31, 732–737.

Larsen, L.O., 1992. Feeding and digestion. In: Feder, M.E., Burggren, W.W. (Eds.), Physiology of Amphibians. University of Chicago Press, Chicago, pp. 378–380.

Licht, P., Mc Creery, B.R., Barnes, R., Pang, R., 1983. Seasonal and stress related changes in plasma gonadotrophins, sex steroids, and corticosterone in the Bullfrog, *Rana catesbiana*. Gen. Comp. Endocrinol. 18, 344–364.

Lillywhite, H.B., 1970. Behavioural thermoregulation in the Bullfrog *Rana catesbiana*. Copeia, 158–168.

Lofts, B., Wellen, J.J., Benraad, T.S., 1972. Seasonal changes in endocrine organs of the male Common Frog, *Rana temporaria* III. The gonads and cholesterol cycle. Gen. Comp. Endocrinol. 18, 344–363.

NSW Threatened Species Conservation Act, 1995. New South Wales Consolidated Acts Government of New South Wales, Australia, 1995.

Pasanen, S., Koskela, P., 1974. Seasonal and age variation in the metabolism of the common frog, *Rana temporaria* L. in northern Finland. Comp. Biochem. Physiol. 47, 635–654.

Pyke, H.G., White, W.W., 2001. A review of the biology of the green and golden bell frog *Litoria aurea*. Aust. Zool. 31 (4), 563–598.

Rodriguez-Serna, M., Flores-Nava, A., Olvera-Novoa, M.A., Carmona-Osalde, C., 1996. Growth and production of bullfrog *Rana catesbeiana* Shaw, 1802, at three stocking densities in a vertical intensive culture system. Aquacult. Eng. 15, 233–242.

Smith, G.C., 1976. Ecological energetics of three species of ectothermic vertebrates. Ecology 57, 252–264.

White, A.W., Pyke, G.H., 1996. Distribution and conservation status of the green and golden bell frog *Litoria aurea* in New South Wales. Aust. Zool. 30 (2), 177–189.