

## Acclimation of CTM, LD<sub>50</sub>, and Rapid Loss of Acclimation of Thermal Preferendum in Tadpoles of *Limnodynastes peronii* (Anura, Myobatrachidae)

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### ABSTRACT

Groups of tadpoles of *Limnodynastes peronii* (Anura, Myobatrachidae) were kept at two temperatures (15° and 25°C) for 45-105 days. In a thermal gradient (6-36°C), initial thermal preferenda were significantly different in 15° and 25°C history groups, approximately 13° and 21°C respectively. However, after 2.5 hours these initial differences were no longer seen and the final thermal preferendum was approximately 18°C regardless of previous thermal history. Critical thermal maxima and LD<sub>50</sub> increased following warm thermal history. Considering these results and the lack of metabolic acclimation reported elsewhere, these tadpoles can be seen as ectotherms whose thermal strategies are behavioural rather than physiological or biochemical. The rapid loss of acclimation of preferred temperature is discussed in relation to Fry's "final preferendum" paradigm.

### INTRODUCTION

Tadpoles of *Limnodynastes peronii* show no evidence of thermal metabolic acclimation when kept at 15°C and 25°C for period of 45-75 days (Marshall and Grigg, 1979). This suggests that the major thermally significant strategies in these tadpoles may be behavioural rather than biochemical or physiological, and leads to questions about the ability of tadpoles of *L. peronii* to select a preferred temperature within the habitat.

Field observations on other species suggest that tadpoles seek out suitable thermal environments (Brattstrom, 1963). Lucas and Reynolds (1967) examined the behaviour of tadpoles of *Rana pipiens* and *Rana catesbeiana* in a thermal gradient. They found that tadpoles of both species aggregated at specific temperatures within the gradient and that the preferred temperature was influenced by previous thermal history. The main aims of this study were to determine the extent to which tadpoles of *L. peronii* aggregate within a thermal gradient and whether or not any thermal preference is affected by previous thermal history.

Many studies of Amphibia have emphasised the dependence of various physiological parameters upon previous thermal history. Brown (1969) showed that

heat resistance of four species of tadpoles could be increased by previous exposure to warm temperatures. Rapid thermal acclimation of CTM (critical thermal maximum), thermal LD<sub>50</sub> and OS (onset of spasms) temperatures have been demonstrated in many Amphibia (Brattstrom and Regal, 1965; Brattstrom, 1968, 1970; Holzman and McManus, 1973). Accordingly, further aims of the study were to determine the effects of thermal history on thermal LD<sub>50</sub> and CTM.

## MATERIAL AND METHODS

*L. peronii* is a large (to 65 mm total length) widespread myobatrachid frog which occurs on the coastal plains and in the ranges of eastern Australia. In suburban areas it frequently makes use of artificial ponds for breeding and seems tolerant of polluted water (Barker and Grigg, 1977). Its distribution from 18-42°S latitude suggests that it is eurythermal. The tadpoles are long-lived, often overwintering (Barker, pers. comm.).

Egg masses of *L. peronii* were collected from the Sydney suburb of Sylvania, transported to the laboratory in water from the site of collection, and kept at room temperature (ca. 20°C) until hatching. Groups of tadpoles were then subjected to short-term (45-60 days) and long-term (90-105 days) exposures to warm (25°C) and cool (15°C) temperatures. Tadpoles were fed frozen lettuce every second day and the water was replaced weekly with fresh water at the required temperature. Tadpoles were kept in constant temperature rooms under constant illumination. Measurements of PBT (preferred body temperature), LD<sub>50</sub> or CTM were made on 15° and 25°C tadpoles alternately to minimise any likelihood of a time-bias being introduced.

### (a) CRITICAL THERMAL MAXIMUM

CTM was determined for 30 tadpoles from each of the four experimental groups, using the method described by Hutchison (1961).

### (b) THERMAL LD<sub>50</sub> DETERMINATION

Only tadpoles exposed to constant temperatures for 90-105 days were used for this measurement.

Preliminary experiments indicated that LD<sub>50</sub> for cold-history and warm-history tadpoles would be within the ranges 34-36°C and 35-37°C respectively. Accordingly, water baths were set up at 34, 35, 36 and 37°C ( $\pm 0.1^\circ\text{C}$ ). Thirty, 60 and 30 cold-history tadpoles were placed in water baths at 34, 35 and 36°C ( $\pm 0.1^\circ\text{C}$ ) respectively and the number of tadpoles surviving 24 hours was recorded. Thirty, 60 and 30 warm-history tadpoles were tested at 35, 36 and 37°C respectively. LD<sub>50</sub> was determined by Probit analysis.



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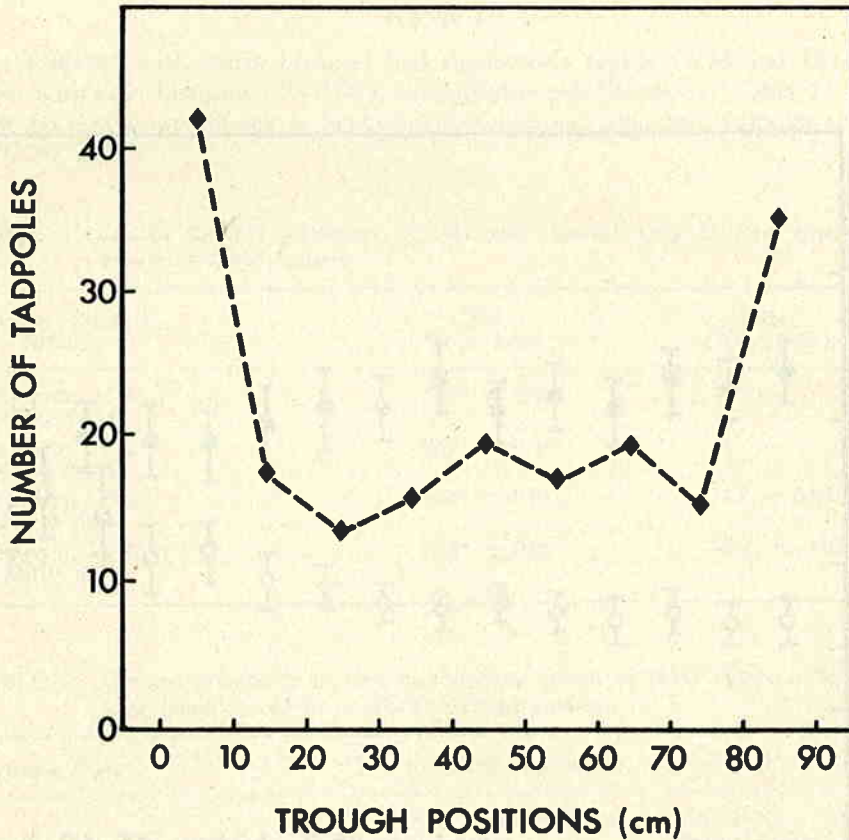


FIG. 1. Distribution of tadpoles in the trough at uniform temperature (20°C). Each point represents the cumulative number of tadpoles found at given positions over a succession of six 10-minute intervals.

(c) THERMAL PREFERENDA

Thermal preferendum was determined in a simple (6-36°C) thermal gradient, similar to that described by Lucas and Reynolds (1967). We found, as they did, that when no gradient was present tadpoles favoured the ends rather than the middle regions of the apparatus (Fig. 1). This suggests that any aggregation towards the centre of the thermal gradient, when established, indicates a response to temperature rather than to some other characteristic of the trough. Tadpoles of *L. peronii* are not known to school.

In each experiment, 30 tadpoles were introduced to the middle of the gradient (about 20°C) and their positions and corresponding temperatures were noted each 10 minutes for 2.5 hours.

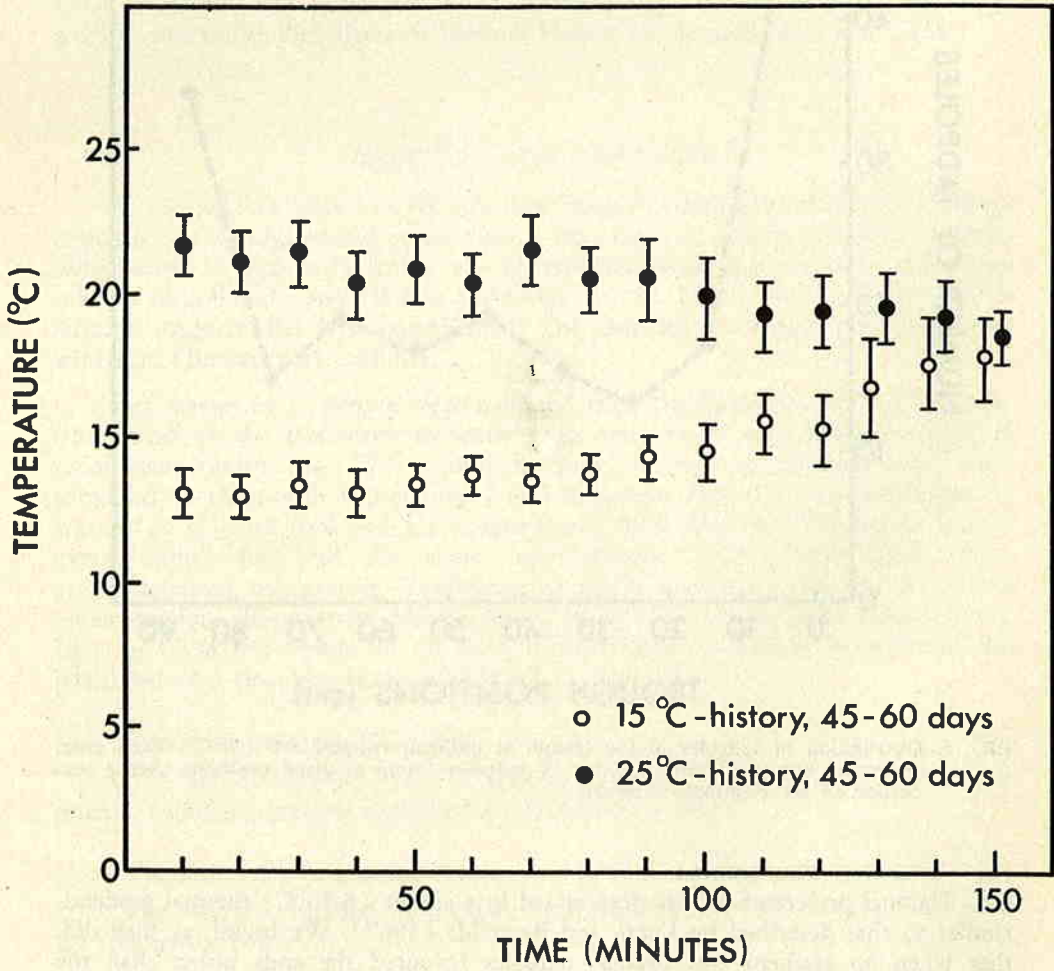


FIG. 2. Changes in thermal preferenda of two groups of tadpoles reared at 15°C and 25°C for 45-60 days. Vertical bars represent the 95% confidence limits about the mean. No measurements were made beyond 150 minutes.

## RESULTS

Tadpoles with warm histories had significantly higher CTM and LD<sub>50</sub> than those with cold histories ( $P < 0.005$ , by Analysis and Variance) (Table 1). There were no significant differences between short-term and long-term tadpoles at either 15°C or 25°C.

TABLE 1. Critical thermal maximum (CTM) and thermal LD<sub>50</sub> in four experimental groups of thirty tadpoles.

Previous Thermal History	CTM (°C ± S.E.)	LD <sub>50</sub> (°C ± S.E.)
Short-term, 15°C (45-60 days)	37.5° ± 0.06	—
Short-term, 25°C (45-60 days)	39.3° ± 0.04	—
Long-term, 15°C (90-105 days)	36.8° ± 0.05	34.9 ± 0.05
Long-term, 25°C (90-105 days)	39.3° ± 0.05	36.9° ± 0.07

TABLE 2. Thermal preferenda of four experimental groups of thirty tadpoles, 10 minutes after being placed in a 6-36°C thermal gradient.

Exposure Time	Preferred Temperature (°C ± S.E.)	
	15°C Thermal History	25°C Thermal History
Short-term (45-60 days)	13.2° ± 0.41 (range 8-17°)	21.5° ± 0.53 (range 17-27°)
Long-term (90-105 days)	12.5 ± 0.26 (range 10-15°)	21.8 ± 0.47 (range 18-26°)

In both short-term and long-term groups, thermal preferenda of 15°C history and 25°C history tadpoles were significantly different ( $P < 0.005$ ), warm history tadpoles having a higher preferred temperature (Table 2).

In every case, the thermal preferenda of tadpoles changed significantly between the first and last 10 minute period ( $P < 0.005$ ). (Figs. 2, 3).

No significant differences were found between final thermal preferenda of any of the groups (Table 3) and no seasonal trends were seen in either initial or final preferenda during the period over which experiments were carried out.



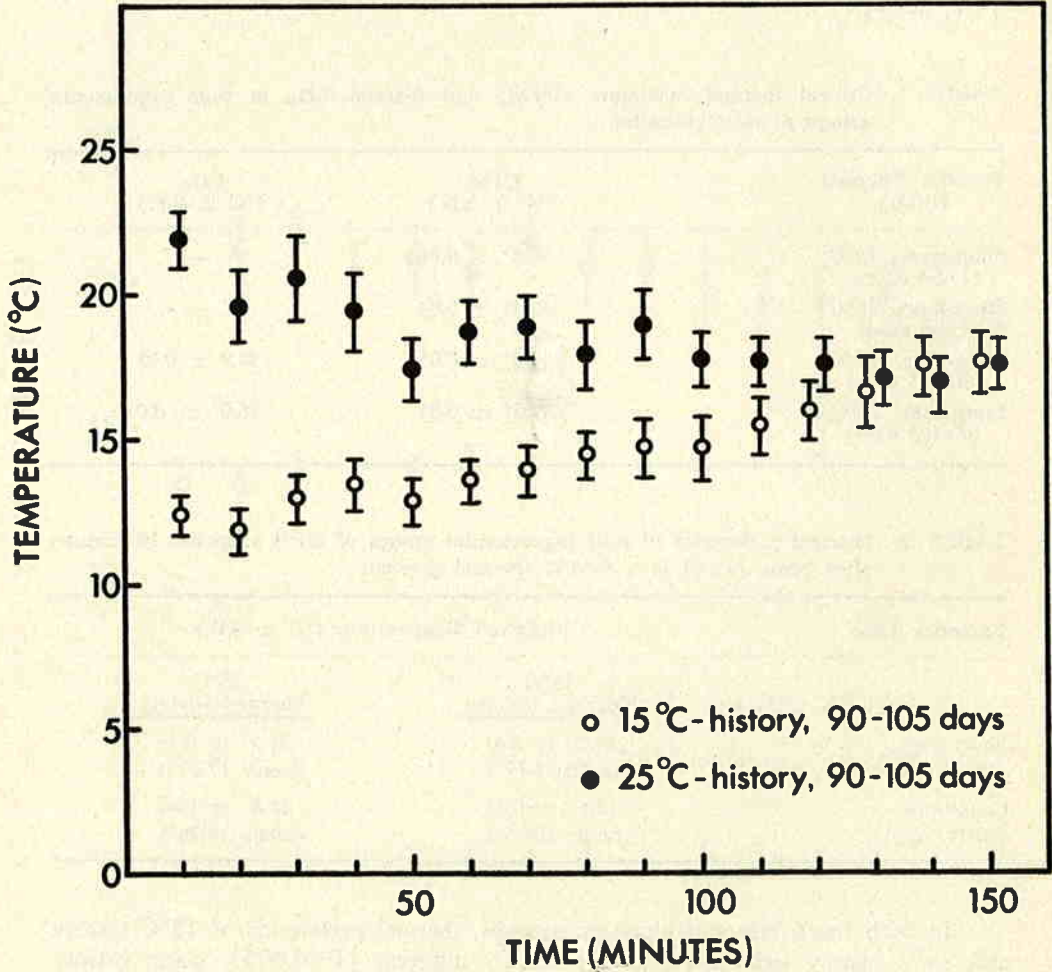


FIG. 3. Changes in thermal preferenda of two groups of tadpoles reared at 15°C and 25°C for 90-105 days. Vertical bars represent the 95% confidence limits about the mean. No measurements were made beyond 150 minutes.

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TABLE 3. Thermal preferenda of four groups of tadpoles, 2.5 hours after being placed in a 6-36°C thermal gradient.

Exposure time	Preferred Temperature (°C ± S.E.)	
	15°C Thermal History	25°C Thermal History
Short-term (45-60 days)	17.9° ± 0.74 (range 8-28°C)	18.4° ± 0.58 (range 13-23°)
Long-term (90-105 days)	17.7° ± 0.53 (range 11-22°)	17.8° ± 0.40 (range 14-21°)

DISCUSSION

Both CTM and thermal LD<sub>50</sub> of tadpoles of *L. peronii* are dependant upon previous thermal history, heat resistance being increased by warm thermal history. This seems to be typical for Amphibia (Brattstrom and Regal, 1965; Brattstrom, 1968, 1970; Holzman and McManus, 1973; Brown, 1969). The results for thermal preferenda are more interesting and warrant further comment.

At a uniform temperature, tadpoles showed a preference for the ends of the test-trough (Fig. 1), in striking contrast to their aggregation at specific temperatures when a thermal gradient was established within the trough (Tables 2, 3, Figs. 2, 3). This shows that the tadpoles of *L. peronii* have a well developed ability to adjust their body temperature by selecting an appropriate water temperature. This finding is in agreement with that of Lucas and Reynolds (1967) who found that tadpoles of *R. pipiens* and *R. catesbeiana* aggregated at specific temperatures within a thermal gradient.

The effect of previous thermal history on preferred temperature (Table 2) is so transient, however, being lost after only 2.5 hours in the gradient (Table 3) that it is very difficult to ascribe any functional significance. One is led to the conclusion that tadpoles of *L. peronii* at this latitude and for at least this part of the year have a preferred body temperature of about 18°C regardless of previous thermal history. In fact, 95% of all tadpoles tested in the gradient selected temperatures between 13 and 22°C within 2.5 hours. It is difficult to compare our results with those of Lucas and Reynolds (1967) because their method of data calculation and presentation neglected the possibility of thermal preferenda changing with time while in the gradient.

Licht and Brown (1967) found similar results in the Red Bellied Newt, *Taricha rivularis*, where initial differences in preferred temperatures of newts acclimated at 5°C compared with groups acclimated at 15°C and 23°C disappeared, suggesting a final thermal preferendum of 22-24°C.

Reynolds and Casterlin (1979) have called for wider attention to be given to Fry's (1947) "final preferendum" paradigm. Briefly the "final preferendum" is the temperature to which an ectotherm in a thermal gradient will gravitate regardless of its prior thermal experience. This concept distinguishes an "acute thermal preferendum", (measured shortly after introduction to the gradient), which may be influenced by previous thermal history, and a "final thermal preferendum" which is essentially independent of prior thermal history because re-acclimation occurs during the gravitation process. The time course for reaching a final thermal preferendum is normally some days in fishes (Fry, 1947) and presumably relates to the fish's state of metabolic acclimation. The lack of thermal metabolic acclimation in tadpoles of *L. peronii* (Marshall and Grigg, 1979) may account for the rapidity with which a final preferendum is reached.

A thermal preferendum of about 18°C, as found in *L. peronii* may seem surprisingly low at times of the year when temperatures of 25°C or more can easily be encountered. One becomes accustomed to the idea that behavioural strategies of many ectotherms are directed towards the maintenance of a relatively high body temperature (which in this case would result in decreased development time). The low preferendum of *L. peronii* tadpoles shows that such a view is simplistic. It would be very interesting to see whether or not the thermal preferendum of this tadpole varies throughout its wide latitudinal range and to examine the temperature-sensitivities of their enzyme systems. Also, the preferred temperature under field conditions is unknown.

In summary, tadpoles of *L. peronii* show only minimal physiological and biochemical acclimation to high or low temperature. Whereas they exhibit an increase in heat resistance (CTM and LD<sub>50</sub>) when kept at warmer temperatures, they are unable to undergo thermal metabolic acclimation (Marshall and Grigg, 1979) and show only a transient effect of acclimation temperature on thermal preferendum. Their behaviour in a thermal gradient coupled with only very limited physiological and biochemical flexibility supports the view that behaviour may be the main mechanism by which tadpoles of *L. peronii* could minimise the effects of fluctuations in environmental temperature.

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