Altitudinal Variation in Growth and Development Rates of Tadpoles of *Litoria chloris* and *Litoria pearsoniana* in Southeast Queensland, Australia

CLARE MORRISON¹ AND JEAN-MARC HERO

School of Environmental and Applied Sciences, Griffith University, PMB 50 Gold Coast Mail Centre,
Bundall Queensland 9726, Australia

ABSTRACT.—Altitudinal variation in larval growth and development rates in low- and high-altitude populations of two subtropical species of frog, *Litoria chloris* and *Litoria pearsoniana* was examined in the field using reciprocal transplant experiments. The larvae of both species raised at high altitudes (regardless of tadpole origin) had slower development rates than larvae raised at low altitudes. Slower growth rates were found in larvae from high altitudes in *L. chloris*, whereas a similar (but not significant) trend was recorded in *L. pearsoniana*. Despite slower growth rates overall, tadpoles raised at high altitudes tended to be larger at each Gosner Development Stage than those raised at low altitudes independent of tadpole origin. These results suggest that most of the variation in growth and development rates in the two species was caused by environmental factors (water temperature) rather than genetic or maternal factors. Tadpole survival in either species did not appear to be significantly affected by environmental or genetic factors in this study.

Intraspecific variation in life-history characteristics is common in species with broad geographical ranges. Amphibian traits that vary with altitude include embryonic development and growth rates (Berven, 1982b; Gollmann and Gollmann, 1996), size at metamorphosis (Pettus and Angleton, 1967; Berven et al. 1979), age and size at first reproduction (Berven, 1981; Berven, 1982a; Ryser, 1996) and longevity (Hemelaar, 1988).

In temperate regions, amphibian larval periods at high elevations are usually prolonged, growth rates are slower, and metamorphosis normally occurs at a larger size (Bizer, 1978; Berven et al., 1979; Berven, 1982b). This pattern is attributed to variation in temperature, which controls developmental and physiological processes (Smith-Gill and Berven, 1979; Blouin, 1992; Gollmann and Gollmann, 1996). Environmental variables influence life-history patterns in montane and lowland populations directly. The length of the larval period and the size at metamorphosis can be used as predictors of survival and reproductive success (Smith-Gill and Berven, 1979). Either early maturation, large body size, or both may have a significant selective advantage in certain environments (Pianka, 1972).

Differences in local environmental conditions may favor the evolution of either phenotypic plasticity or genuine genetic adaptation to the local environment. However, the relative influence of these factors in explaining geographic

variation in traits is poorly resolved (Berven, 1982b; Sorci et al., 1996).

Although the trend for longer larval periods and larger larval body sizes at high elevations occurs in temperate amphibians across great altitudinal ranges (Bizer, 1978; Berven et al., 1979; Berven, 1982b; Howard and Wallace, 1985), the relationship between altitude and tropical and subtropical amphibian ecology has not been investigated. Herein we examine the effects of altitude on growth and development rates of tadpoles of two subtropical species, *Litoria chloris* and *Litoria pearsoniana* in southeast Queensland, Australia.

MATERIALS AND METHODS

Study Species.—*Litoria chloris* and *L. pearsoniana* were chosen for the study not only because they are found along altitudinal gradients (< 100–900 m) but also for the predictable availability of numerous clutches and their relatively short larval periods (approximately 3–4 months). Calling, amplexus, and oviposition in both species occur in shallow, isolated pools beside streams in coastal rain forest or wet sclerophyll forest (Barker et al., 1995).

Eggs were collected from the field (between Gosner stages 13 and 17) and maintained in the laboratory for 4–5 days at 21°C until they had reached Gosner stage 21 (Gosner, 1960). This ensured that all tadpoles were at the same development stage at the start of the experiment and maximized survival rates at the initial transplant stage. Fourteen clutches of *L. chloris* eggs (eight from low altitude sites, six from high altitude sites) and 13 clutches of *L. pearsoniana*

¹ Corresponding Author. E-mail: morrison_c@usp.ac.fj

TABLE 1. Study site location details for tadpole growth and development experiments.

Site	Lat/Long	Altitude (m)
Tallebudgera	28°13'05"S/153°19'05"E	100
Austinvile	28°10'46"S/153°18'16"E	120
Curumbin	28°14'36"S/153°21'06"E	160
Natural Bridge (NB)	28°13'58"S/153°14'36"E	250
Purling Brook (PB)	28°11'30"S/153°16'12"E	600
Rush Ck	28°13'30"S/153°16'00"E	650
Eejung Ck	28°13'40"S/153°16'50"E	780
Mundora	28°13'28"S/153°17'02"E	800

eggs (seven from low altitude sites, six from high altitude sites) were used to minimize phylogenetic pseudoreplication (maximize genetic variation from each treatment/altitude). Individual tadpoles were randomly selected from different clutches (depending on the treatment) and allocated to each site. To avoid potential effects of local adaptation, no tadpole was allocated to its natal stream.

Experimental Design.—The relative importance of environmental factors and genetic origin on variability in larval growth and development rates were examined using a cross-transplant experiment. Four high-altitude and four low-altitude sites in southeast Queensland were selected for the experiment (Table 1). Each site had two treatments—tadpoles collected from high altitudes (> 600 m, treatment A) and those collected from low altitudes (< 250 m, treatment B)—for each species.

White, plastic, UV-proof containers (43 × 34 × 16) were used to house tadpoles in the field. Holes were cut in the sides and the lid and the plastic replaced with aluminium flymesh to facilitate water flow through the containers. The interiors of containers were divided into four compartments with aluminium flymesh so tadpoles could be raised individually within each container. Four containers were placed at each site (two species × two treatments = 16 tadpoles in total), and each set of four containers was placed in a wooden frame. The frame with floats was placed in a quiet, protected section of stream.

Species were kept in separate containers and the two origin treatments mixed, that is, two tadpoles originating from high altitudes and two originating from low altitudes were placed in each container to minimize container effects. Because the tadpoles are detritivores, 30 g (wet weight) of leaves and other detritus from each site were added to each compartment (after removal of predators) as food for the tadpoles. De-

tritrus was replaced as required (i.e., tadpoles were fed ad libitum).

Growth and Development Rates.—Tadpole sizes and development stages were measured weekly until the end of the experiment. Size was measured by volumetric displacement using a 10-ml cylinder, and the Gosner development stage was observed using a field microscope. Tadpoles were left in the field for nine weeks, at which time the experiment was terminated because of the loss of several enclosures damaged by heavy floods. Three water samples from each site were analyzed weekly using a TPS Model FLMV Microprocessor Analyzer to determine dissolved oxygen, pH, and water temperature.

Individual growth rates were calculated as the total change in body volume (cm³)/number of days, whereas development rates were calculated as (final Gosner development stage, GDS – initial GDS)/number of days.

Statistical Methods.—Multiple linear regression was used to examine the effects of altitude and tadpole origin on tadpole growth and development rates for each species. Because tadpoles used in this study were selected from multiple clutches, the growth and development rates of tadpoles from different clutches were compared to see whether maternity influenced larval development in this study (indirect test for phylogenetic pseudoreplication) using a one-way analysis of variance. A paired *t*-test was used to compare body size at each Gosner development stage between tadpoles raised at high and low altitudes and between tadpoles originating from high and low altitudes.

A significant relationship between altitude and water temperature was found ($r^2 = 0.875$, slope = -0.0032 , intercept = 18.187 , $P < 0.0001$). To correct for water temperature, residuals from linear regressions between water temperature and growth or development rates were used as the dependent variable in multiple regression models to examine the effects of altitude and tadpole origin on larval growth and development.

Several tadpoles died during the study before the floods and subsequently, a multiple logistic regression (used because the dependent variable was binomial—survived or died), was conducted to determine whether altitude, tadpole origin, or the interaction between the two factors had an effect on tadpole survival.

RESULTS

Tadpoles raised at higher altitudes developed significantly slower than those raised at lower altitudes in both *L. chloris* (Table 2, Fig. 1A) and *L. pearsoniana* (Table 2, Fig. 1B). Tadpoles of *L. chloris* raised at higher altitudes also grew slower than those raised at lower altitudes (Table 2,

TABLE 2. Multiple linear regressions of the two independent variables altitude and tadpole origin on tadpole development (DR) and growth rates (GR) in *Litoria chloris* and *Litoria pearsoniana*.

Species	Dependent variable	Independent variable	<i>t</i>	<i>P</i> (model)	<i>P</i> (variable)	<i>R</i> ²	df total
<i>L. chloris</i>	DR	Altitude	-12.160	<0.001	<0.001	0.764	48
		Tadpole origin	0.751				
	GR	Altitude	-8.856	<0.001	<0.001	0.632	48
		Tadpole origin	0.625				
<i>L. pearsoniana</i>	DR	Altitude	-12.835	-0.001	<0.001	0.800	44
		Tadpole origin	-1.127				
	GR	Altitude	-1.939	0.122	0.059	0.095	44
		Tadpole origin	-0.705

FIG. 1. Partial plots of mean ($\pm$ 1 SE) tadpole development rate (minus effects of tadpole origin) versus altitude in (A) *Litoria chloris* and (B) *Litoria pearsoniana* and partial plots of mean ($\pm$ 1 SE) tadpole growth rate (minus effects of tadpole origin) versus altitude in (C) *L. chloris* and (D) *L. pearsoniana*.

FIG. 2. Comparison of the relationship between mean body size and Gosner development stage for tadpoles raised at high (open squares) and low (closed triangles) altitudes in (A) *Litoria chloris* and (B) *Litoria pearsoniana*. Error bars represent one standard error. * denotes pairs that are significantly different.

FIG. 3. Comparison of the relationship between mean body size and Gosner development stage for tadpoles collected (tadpole origin) from high (open squares) and low (closed triangles) altitudes in (A) *Litoria chloris* and (B) *Litoria pearsoniana*. Error bars represent one standard error.

Fig. 1C). Altitude did not affect the growth rate of tadpoles of *L. pearsoniana* (Table 2, Fig. 1D). Tadpole origin had no effect on development or growth rates in either of the species (Table 2).

Maternity had no effect on development rates (*L. chloris* one-way ANOVA: $F_{13,35} = 0.458$, $P = 0.934$; *L. pearsoniana*: $F_{12,32} = 0.443$, $P = 0.933$) or growth rates (*L. chloris*: $F_{13,48} = 0.711$, $P = 0.740$; *L. pearsoniana*: $F_{12,44} = 0.936$, $P = 0.525$). Tadpoles from one clutch did not grow or develop faster or slower than those from any other clutch in either of the species.

There was a trend toward higher stage-specific growth in tadpoles of both species raised at high altitudes and in tadpoles collected from high altitudes (Figs. 2–3). However, none of these trends were significant (paired t -tests: *L. chloris*: altitude $t_4 = 1.500$, $P = 0.735$; origin $t_8 = -0.351$, $P = 0.208$; *L. pearsoniana*: altitude $t_9 = 1.500$, $P = 0.168$; origin $t_6 = 0.002$, $P = 0.998$).

There was no significant intersite or temporal difference in pH ($F_{71} = 1.293$, $p_{\text{model}} = 0.098$, $p_{\text{site}} = 0.119$, $p_{\text{week}} = 0.081$, $p_{\text{site} \times \text{week}} = 0.312$) or dissolved oxygen levels ($F_{71} = 1.190$, $p_{\text{model}} = 0.191$, $p_{\text{site}} = 0.574$, $p_{\text{week}} = 0.407$, $p_{\text{site} \times \text{week}} = 0.142$); therefore their influence on growth and development rates was not examined further.

Removing the effect of water temperature on development rates by using residual development rates of tadpoles of *L. pearsoniana* resulted in a decrease in the explanatory power of the multiple regression model ($R^2 = 0.145$ vs. $R^2 = 0.800$) and the overall significance ($P = 0.037$ vs. $P < 0.0001$). Using the residual development and growth rates of tadpoles of *L. chloris* resulted in large decreases in the explanatory power of both models and a shift from significance to nonsignificance ($R^2 = 0.764$, $P < 0.0001$ vs. $R^2 = 0.052$, $P = 0.292$; and $R^2 = 0.654$, $P < 0.0001$ vs. $R^2 = 0.048$, $P = 0.320$ for development and

growth rates, respectively). These results suggest that most of the altitudinal variation in growth and development rates can be explained by differences in water temperature.

No relationships were found between tadpole survival and either altitude or origin in both *L. chloris* ($N = 65$, $P = 0.711$) or *L. pearsoniana* ($N = 64$, $P = 0.581$). There was also no significant interaction between altitude and tadpole origin in determining survival in either of the species.

DISCUSSION

Altitude had a strong negative effect on the development rates of both species and the growth rates of *L. chloris*, results that are similar to most previous studies on temperate species that report slower larval development and growth at high altitudes (Berven et al., 1979; Berven, 1982b). Previous studies usually report larger body sizes at each development stage in high-altitude larvae caused by greater stage-specific growth (Bizer, 1978; Berven et al., 1979; Smith-Gill and Berven, 1979). That is, despite growth rates being slower at high altitudes, individuals spend more time growing at each development stage (caused by slower development rates) and also continue to grow when temperatures become too low for development (Smith-Gill and Berven, 1979). This results in larger body sizes at each development stage, including metamorphic climax. Although not significant, the same pattern appeared for both species in this study: tadpoles raised at high altitudes generally were larger at each development stage.

Comparison of the relationship between mean body size and Gosner development stage for tadpoles collected at high altitudes and low altitudes at each development stage in either of the species (Fig. 1) shows, however, that these factors did not play a significant role in determining growth and development rates (Berven 1982b), because previous studies have shown that genetic factors are often important in determining growth rates of tadpoles (Table 2, Fig. 1D). Tadpole origin had no effect on development or growth rates in either of the species (Table 2). Maternity had no effect on development rates and development rates were higher in low altitude *L. chloris* one-way ANOVA: $F_{1,33} = 0.458$, $P = 0.934$; *L. pearsoniana*: $F_{1,33} = 0.443$, $P = 0.933$ or garden conditions (Berven et al., 1979; Berven, 1982b; Gollmann and Gollmann, 1996). This suggests that natural selection has acted to produce faster intrinsic growth and development rates in these tadpoles to compensate for the colder temperatures at high altitudes (Berven et al., 1979; Berven, 1982b). These higher intrinsic rates are usually not reflected in the field because the effects of environmental variables (especially temperature) on larval development characteristics are usually stronger (Berven et al., 1979; Gollmann and Gollmann, 1996). Any effect of genetic factors (tadpole origin and year, $F_{1,33} = 0.351$, $P = 0.708$; *L. pearsoniana*: altitude $F_{1,33} = 1.500$, $P = 0.168$; origin $F_{1,33} = 0.002$, $P = 0.998$).

temperatures) on larval growth and development in this study may be masked by the effects of the environmental factors involved.

Altitudinal variation in growth and development rates in this study could be explained by differences in water temperature. The colder temperatures found at high altitudes restricted development rates in both species and growth rates in *L. chloris*. Water temperature has been shown to affect the larval development of other amphibian species with low temperatures reducing growth and development rates and in some cases, inhibiting metamorphosis altogether (Caley, 1973; Smith-Gill and Berven, 1979; Berven, 1982b).

Although pH and DO can influence larval development characteristics (Nie et al., 1999), they were not considered in this study because they did not vary significantly between sites. Other environmental factors such as density (Tejedo and Reques, 1992; Martinez et al., 1996), interspecific competition (Murray, 1990), and food levels (Berven and Chadra, 1988) also affect larval developmental patterns. However, these factors were not examined in this study because the two species were kept in separate containers, individual tadpoles were housed in separate compartments within a container, and equal amounts of food detritus were added to each compartment.

Delayed metamorphosis results in a larger body size that, in turn, can result in increased survival (Wilbur, 1980). Despite larger body sizes at low altitudes for *L. chloris* and no difference in body size in *L. pearsoniana*, there was no difference in survival between tadpoles collected at high altitudes and low altitudes at each development stage in either of the species (Table 2). This indicates that body size did not influence survival, and therefore, no interaction between altitude and tadpole origin. For example, tadpoles collected from high altitudes and raised at low altitudes did not experience higher mortality rates than tadpoles collected from low altitudes and raised at low altitudes. Although dissolved oxygen levels ($P = 0.191$, $F_{1,33} = 0.119$, $P = 0.731$) were higher at low altitudes, these results may be an artifact of the shortened duration of the experiment, they provide an initial insight into the role of altitude on growth and development and survival in an important life-history stage of these species.

Removal of the effect of water temperature on development rates by using residual development rates of tadpoles of *L. pearsoniana* resulted in a decrease in the explanatory power of the size at maturity (Smith, 1982). Delayed metamorphosis can result in overwintering by larvae, which adds a minimum of one year to the attainment of sexual maturity (Berven, 1982b). Although tadpoles of *L. chloris* and *L. pearsoniana* did not overwinter, delayed metamorphosis was evident at high altitude sites. Delayed metamorphosis can result in a shift from significance to nonsignificance ($R^2 = 0.764$, $P < 0.0001$ vs. $R^2 = 0.052$, $P = 0.651$) and a decrease in the explanatory power of the size at maturity (Smith, 1982). Delayed metamorphosis can result in a shift from significance to nonsignificance ($R^2 = 0.764$, $P < 0.0001$ vs. $R^2 = 0.052$, $P = 0.651$) and a decrease in the explanatory power of the size at maturity (Smith, 1982).

activity season and subsequently experiencing shortened periods to feed and prepare for colder seasons (Berven, 1982b). This, in conjunction with slower postmetamorphic growth and development rates, in turn can result in limited growth after emergence and a consequent delay in reaching the minimum size needed to attain maturity (Hemelaar, 1988; Goater, 1994). For example, a metamorph emerging around the middle of the activity period would have more time to feed and prepare for the coming winter than a later emerging individual (Berven, 1982b). The following year, the precocious individual would theoretically be in better condition and, if an early maturing species, may be able to achieve the minimum size needed for maturity the year after it emerges (Wilbur, 1980; Howard and Wallace, 1985). The year after the late-metamorphosing individual leaves the water, growth and development are delayed, and it may not be able to reach the minimum size needed to mature before the end of activity period therefore having to delay maturity till the following year (two years after emergence).

Acknowledgments.—We thank R. Schleich, C. McKay, and B. Purdon for help with the construction of the tadpole containers and enclosures and establishing them in the field. L. Shoo and two anonymous reviewers provided valuable comments that greatly improved the manuscript. Funding was provided by Griffith University Gold Coast as a Postgraduate Scholarship to CM. The microprocessor analyzer and other equipment used in the field were also provided by Griffith University Gold Coast. This study was carried out with the permission of Queensland Department of Environment, permit E3/001365/97/SAA.

LITERATURE CITED

- BARKER, J., G. GRIGG, AND M. J. TYLER. 1995. A Field Guide to Australian Frogs. Surrey Beatty and Sons, Chipping Norton, New South Wales, Australia.
- BERVEN, K. A. 1981. Mate choice in the wood frog, *Rana sylvatica*. *Evolution* 35:707–722.
- . 1982a. The genetic basis of altitudinal variation in the wood frog *Rana sylvatica*. I. An experimental analysis of life history traits. *Evolution* 36:962–983.
- . 1982b. The genetic basis of altitudinal variation in the wood frog *Rana sylvatica*. II. An experimental analysis of larval development. *Oecologia* 52:360–369.
- BERVEN, K. A., AND B. G. CHADRA. 1988. The relationship among egg size, density and food level on larval development in the wood frog (*Rana sylvatica*). *Oecologia* 75:67–72.
- BERVEN, K. A., D. E. GILL, AND S. J. SMITH-GILL. 1979. Countergradient selection in the green frog, *Rana clamitans*. *Evolution* 33:609–623.
- BIZER, J. R. 1978. Growth rates and size at metamorphosis of high elevation populations of *Ambystoma tigrinum*. *Oecologia* 34:175–184.
- BLOUIN, M. S. 1992. Comparing bivariate reaction norms among three species: time and size at metamorphosis in three species of *Hyla* (Anura: Hylidae). *Oecologia* 90:288–293.
- CALEF, G. W. 1973. Natural mortality of tadpoles in a natural population of *Rana aurora*. *Ecology* 54:741–758.
- GOATER, C. P. 1994. Growth and survival of post-metamorphic toads: interactions among larval history, density and parasitism. *Ecology* 75:2264–2274.
- GOLLMAN, B., AND G. GOLLMAN. 1996. Geographic variation of larval traits in the Australian frog *Geocrinia victoriana*. *Herpetologica* 52:181–187.
- GOSNER, K. L. 1960. A simplified table for staging anuran embryos and larvae with notes on their identification. *Herpetologica* 16:183–190.
- HEMELAAR, A. 1988. Age, growth and other population characteristics of *Bufo bufo* from different latitudes and altitudes. *Journal of Herpetology* 22:369–388.
- HOWARD, J. H., AND R. L. WALLACE. 1985. Life history characteristics of populations of the long-toed salamander (*Ambystoma macrodactylum*) from different altitudes. *American Midland Naturalist* 113:361–372.
- MARTINEZ, I. P., R. ALVAREZ, AND M. P. HERRAEZ. 1996. Growth and metamorphosis of *Rana perezi* larvae in culture: effects of larval density. *Aquaculture* 142:163–170.
- MURRAY, D. L. 1990. The effects of food and density on growth and metamorphosis in larval wood frogs (*Rana sylvatica*) from central Labrador. *Canadian Journal of Zoology* 68:1221–1226.
- NIE, M., J. D. CRIM, AND G. R. ULTSCH. 1999. Dissolved oxygen, temperature, and habitat selection by bullfrog (*Rana catesbeiana*) tadpoles. *Copeia* 1999:153–162.
- PETTUS, D., AND G. M. ANGLETON. 1967. Comparative reproductive biology of montane and piedmont chorus frogs. *Evolution* 21:500–507.
- PLANKA, E. R. 1972. *r* and *k* selection or *b* and *d* selection? *American Naturalist* 106:581–588.
- RYSER, J. 1996. Comparative life histories of a low- and high-elevation population of common frog from *Rana temporaria*. *Amphibia-Reptilia* 17:183–195.
- SMITH, D. C. 1987. Adult recruitment in chorus frogs: effects of size and date at metamorphosis. *Ecology* 68:344–350.
- SMITH-GILL, S. J., AND K. A. BERVEN. 1979. Predicting amphibian metamorphosis. *American Naturalist* 113:563–585.
- SORCI, G., J. CLOBERT, AND S. BELICHON. 1996. Phenotypic plasticity of growth and survival in the common lizard *Lacerta vivipara*. *Journal of Animal Ecology* 65:781–790.
- TEJEDO, M., AND R. REQUES. 1992. Effects of egg size and density on metamorphic traits in tadpoles of the natterjack toad (*Bufo calamita*). *Journal of Herpetology* 26:146–152.
- WILBUR, H. M. 1980. Complex life cycles. *Annual Review of Ecological Systematics* 11:67–93.

Accepted: 23 April 2002.