The Effects of Acute and Developmental Temperature on Burst Swimming Speed and Myofibrillar ATPase Activity in Tadpoles of the Pacific Tree Frog, *Hyla regilla*

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ABSTRACT

The effects of acute and developmental temperature on maximum burst swimming speed, body size, and myofibrillar ATPase activity were assessed in tadpoles of the Pacific tree frog, *Hyla regilla*. Tadpoles from field-collected egg masses were reared in the laboratory at 15° (cool) and 25°C (warm). Body size, maximum burst swimming speed from 5° to 35°C, and tail myofibrillar ATPase activity at 15° and 25°C were measured at a single developmental stage. Burst speed of both groups of tadpoles was strongly affected by test temperature ($P < 0.001$). Performance maxima spanned test temperatures of 15°–25°C for the cool group and 15°–30°C for the warm group. Burst speed also depended on developmental temperature ($P < 0.001$), even after accounting for variation in body size. At most test temperatures, the cool-reared tadpoles swam faster than the warm-reared tadpoles. Myofibrillar ATPase activity was affected by test temperature ($P < 0.001$). Like swimming speed, enzyme activity was greater in the cool-reared tadpoles than in the warm-reared tadpoles, a difference that was significant when assayed at 15°C ($P < 0.01$). These results suggest a mechanism for developmental temperature effects on locomotor performance observed in other taxa.

Introduction

Environmental temperature can have profound consequences for whole-animal performance traits. Maximum locomotor performance of ectothermic vertebrates, for example, is highly sensitive to environmental temperature, typically peaking at intermediate temperatures and decreasing substantially above and below the "optimal" temperature or plateau (reviewed in Bennett 1990). In adult ectotherms, acclimation to different temperatures (usually on a timescale of weeks to months) can alter the relationship between environmental temperature and performance (Bennett 1990; Johnston et al. 1990). As a result of acclimation, two individuals within a single population may differ greatly in performance—and possibly in Darwinian fitness—even at the same environmental temperature if they have different thermal histories. The functional mechanisms underlying thermal acclimation of maximum burst speed have been examined extensively in several species of fish (e.g., Beddow and Johnston 1995; Johnson and Bennett 1995; Ball and Johnston 1996; Johnson et al. 1996). In general, acclimatory changes in performance are accompanied by parallel changes in muscle contractile kinetics, myofibrillar structure, and myofibrillar ATPase activity.

Locomotor performance of ectotherms can also be influenced by the temperature experienced during embryonic development (e.g., Burger 1991; Janzen 1993; Parichy and Kaplan 1995; Elphick and Shine 1998). Because of their occurrence early in ontogeny, when developmental "decisions" are made, such temperature effects may have long-lasting consequences throughout an individual's life span. Thus, two individuals may differ in performance and fitness even at a late stage if they were incubated at different temperatures. Such differences may be particularly important in species that reproduce and develop in thermally heterogeneous environments and those in which parents can choose incubation temperature. Furthermore, if such developmental temperature effects are indeed long lasting, they may confound comparisons involving adults even when an experimenter attempts to eliminate the effects of thermal history by acclimating animals to a common environment before measuring traits of interest. In contrast to thermal acclimation in adults, there has been no attempt to identify the mechanisms underlying the effects of developmental temperature on burst speed in any ectothermic animal. (Although developmental temperature has been shown to affect muscle structure and enzymatic activity in some fish [Nathanailides et al. 1995; Johnston et al. 1996, 1997], it is not known whether locomotor performance in those animals is similarly affected.) Elucidating such mechanisms is essential to understanding how variation in ecologically important performance traits arises in natural populations.

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The Pacific tree frog, *Hyla regilla*, experiences a highly variable thermal environment throughout its life cycle. In natural populations, temperatures commonly range at least 20°C between breeding sites, during a day, and over the course of a breeding season (Cunningham and Mullally 1959; Brown 1975; Schaub and Larsen 1978). Larvae of this species can be subject to heavy predation, and the maximum burst swimming speed of a tadpole is an important determinant of its survival in the presence of natural predators (Watkins 1996).

How might variation in burst speed among *H. regilla* tadpoles arise? The thermal dependence of locomotor performance in this species is unknown, but the results of previous work (Watkins 1997*b*) suggest that developmental temperature may influence speed (see also Parichy and Kaplan 1995). This study therefore examines the effects of acute and developmental temperature on burst swimming speed, body size and shape, and myofibrillar ATPase activity in a single population of *H. regilla* tadpoles. Body size and shape are examined because they can affect speed in tadpoles (Parichy and Kaplan 1995) and because they can influence survival in the presence of some predators (e.g., Van Buskirk and Relyea 1998). Myofibrillar ATPase activity is measured because it is correlated with maximum shortening velocity of unloaded muscles (Edman et al. 1988) and because it underlies variation in performance among acclimation temperature treatment groups (fish: Johnson and Bennett 1995) and among individuals (tadpoles: Watkins 1994).

A potentially confusing semantic issue arises when discussing environmental effects on tadpole traits. "Developmental temperature effects" often refers to the influence of embryonic or larval temperature on animals that have attained their adult gross morphology (i.e., after hatching in reptiles [e.g., Janzen 1993] and after metamorphosis in insects [e.g., Olvido and Mousseau 1995]). Tadpoles, of course, are larval forms that have not yet completed development. Consequently, the meaning of "developmental temperature effects" in our study may differ somewhat from that of other studies and may instead be equated with thermal acclimation that happens to occur during the larval period (e.g., in tadpoles of *Limnodynastes peronii*, a species with a very long larval period; Wilson and Franklin 1999). In any case, since *H. regilla* larvae develop rapidly, the potential effects of developmental temperature are considered here to be distinct from thermal acclimation seen in adult animals.

Material and Methods

Animal Collection and Maintenance

Approximately 12 distinct embryo masses were collected from a single pond in Susanville, California (Lassen Co.), on the night of April 14, 1998. Most of the embryos were in the early postneurulation stages (approximately Gosner [1960] stages 16–18) when collected. The embryos were transported to the laboratory in Irvine, California, the following day, and each mass was divided equally between two temperature treatments. One-half of each mass was placed into one of two plastic shoe boxes with 2 L of aerated dechlorinated water at 25°C (hereafter, the warm group) or 15°C (the cool group). Each shoe box therefore contained embryos from each mass. Splitting each mass between treatments ensured that variation among masses was not confounded with temperature treatment; because masses were pooled within treatments, variation among families (e.g., genetic variation) was not examined. The embryos were kept in these boxes under a 14L : 10D photoperiod until all hatched (3 d for the warm group, 6 d for the cool group). The choice of developmental temperatures was arbitrary, but both fall well within the range of temperatures experienced in the field (Cunningham and Mullally 1959; Schaub and Larsen 1978) and at which development is normal (Brown 1975).

After hatching, tadpoles were reared in 10 plastic tanks, each of which held 10 L of dechlorinated water that was continuously aerated. The tanks were arranged on shelves in a temperature-controlled room set to 15°C with a 14L : 10D photoperiod. The five warm tanks contained thermostatically controlled aquarium heaters set to 25°C and were arranged alternately with the cool tanks on the shelves. Tank temperatures were recorded every 1–4 d. Mean temperature ($\pm$SEM) was 15.3° $\pm$ 0.05°C for the cool group and 25.2° $\pm$ 0.1°C for the warm group.

Each rearing tank held 20 tadpoles that were selected at random from the appropriate hatching container. Tadpoles that died within the first 3 d were replaced with surplus hatchlings; thereafter, no replacements of dead tadpoles were made. The animals were fed a 3 : 1 (w : w) mixture of finely ground guinea pig food and fish flakes ad lib. Tank water was changed about twice a week as appeared necessary. While survival in each tank was not recorded, it was neither noticeably low nor were there obvious differences in survival between temperature treatments.

Locomotor Performance Trials

All measurements were made at Gosner (1960) stage 37, an easily identifiable stage at which the hind limbs are not used during locomotion (Steuhower and Farel 1984). Maximum burst speed was measured at test temperatures (T_{test}) of 5°, 15°, 25°, 30°, and 35°C, with the exception that the cool-reared tadpoles were not raced at 35°C, because the few individuals tested at this high temperature were unable to swim. Because of their rapid development rates, each tadpole could be raced at only one T_{test}, which was chosen haphazardly with an effort to distribute the tadpoles from each rearing tank evenly across test temperatures.

When a tadpole reached Gosner (1960) stage 37, it was placed into a beaker with about 500 mL of water from its rearing tank, and the beaker was placed in a water bath set to the chosen T_{test}. The tadpole was brought to temperature over a period of 10–55 min; rates of heating or cooling ranged from 0.16° to

0.50°C min^{-1}. Tadpoles then remained at their T_{test} undisturbed for an additional 30 min or more. Subsequently, a single tadpole was placed into a plastic racetrack (100 × 10 × 7 cm) filled with water at the T_{test} and immediately chased up and down the track four times without rest by tapping its tail with a metal wire. Each time the tadpole crossed a 15-cm mark, the event was recorded by pressing a key on a computer loaded with a timer program. Maximum burst swimming speed (U_{max}) was calculated from the single fastest 30-cm interval. This method produces repeatable differences among individuals (Watkins 1996) and compares favorably to high-speed video analysis (Watkins 1997*a*). Following its trial, each tadpole's total length and tail length were measured with a dial caliper; snout-vent length (SVL) was calculated as the difference between the two measures.

Myofibrillar ATPase Measurements

After their locomotor and body size measurements, a subsample of tadpoles (all at stage 37) was selected for measurement of tail myofibrillar ATPase activity. Each tadpole was chilled on ice and quickly killed by decapitation. The tail was cut from the body, the fins and as much overlying skin as possible were removed, and the tail was then stored in an empty microfuge tube at −80°C until use (2–3 mo later).

Myofibrillar ATPase activities were measured at 15° and 25°C following a modification of Johnson and Bennett (1995). Frozen tails were homogenized individually for 30 s in about 2 mL ice-cold extraction buffer (10mM Tris-HCl, 1% Triton-X, 100 mM KCl, 5 mM EDTA, pH 7.2 at 0°C). The crude homogenate was centrifuged for 10 min at 7,800 g and 4°C, and the pellet was rehomogenized in fresh extraction buffer. Each tail was subjected to three homogenization-centrifugation cycles. Following the final centrifugation, each pellet was suspended in 500 μL of suspension buffer (30 mM Tris-HCl, 150 mM KCl, pH 7.2 at 0°C) and kept on ice. Total protein content was determined with a commercially available kit (Bio-Rad DC Protein Assay) using bovine serum albumin as a standard. Subsequently, 100 μL of myofibril suspension (0.5–2.4 mg protein) were incubated for 2 min at the assay temperature in a reaction buffer (30 mM Tris-HCl, 1.17 mM EGTA, 1.17 mM $MgSO_4$, 2.8 mM $CaCl_2$, pH 7.4 at assay temperature). The reaction was then initiated by the addition of 200 μL of ATP (from fresh stock) and stopped by the addition of ice-cold trichloroacetic acid. The final concentration of inorganic phosphate was determined spectrophotometrically at 660 nm by the procedure of Rockstein and Herron (1955). All samples and phosphate standards were run in duplicate at each assay temperature.

Statistical Analysis

Myofibrillar ATPase activity values were log transformed because variance scaled with the mean. All other variables were not transformed, and assumptions of normality and equal variance were met for all analyses. Initial ANOVAs revealed no significant effects of rearing tank on any dependent variable within the developmental temperature groups. Because the cool-reared tadpoles did not swim at 35°C, comparisons of burst speed between rearing temperature groups were based on measurements made between 5° and 30°C only. All analyses were performed on SAS version 6.11 (SAS Institute 1989).

Results

Maximum burst swimming speed demonstrated a typical unimodal relationship with test temperature (Fig. 1). Both test temperature and developmental temperature had large and significant effects on U_{max} (T_{test}: $F_{3,99} = 25.54$, $P < 0.001$; T_{dev}: $F_{1,99} = 11.47$, $P < 0.001$), but there was no significant effect of their interaction ($F_{3,99} = 1.84$, $P > 0.10$). Plateaus in performance existed between 15° and 25°C for the cool group and between 15° and 30°C for the warm group (these plateaus were defined as regions in which means within a rearing group did not differ significantly from one another [Tukey pairwise comparisons, $P > 0.05$]).

Tadpoles in the cool group were significantly larger than those in the warm group (Table 1), and their tails were relatively longer (ANCOVA on tail length with SVL as a covariate: $F_{group} = 89.29$, df = 1, $P < 0.001$). However, two separate analyses show that the differences in U_{max} between rearing groups (Fig. 1) were not explained by differences in body size or shape. First, neither total length, tail length, nor relative tail length (calculated as residuals from the regression of tail length on

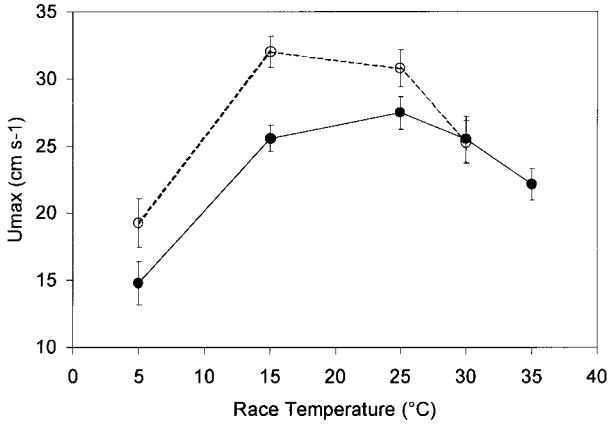

Figure 1. Maximum burst speed of tadpoles as a function of race temperature and developmental temperature. Tadpoles reared at cool temperature (15°C; *open circles*) swam faster than those reared at warm temperature (25°C; *filled circles*). Values are means ± 1 SEM. Sample sizes, reading left to right, are as follows: cool-reared tadpoles 9, 13, 16, 9; warm-reared tadpoles 8, 20, 20, 12, 12.

Table 1: Body sizes of tadpoles reared at two different temperatures

Developmental Temperature (°C)	N	Total Length (cm)	Tail Length (cm)
15	49	5.34 ± .047	3.81 ± .036
25	72	4.72 ± .037	3.29 ± .029

Note. Values are means ± 1 SEM. All tadpoles were at Gosner (1960) stage 37. The rearing groups differed significantly for both variables (t-tests, $P \ll 0.001$ in each case).

SVL) affected U_{max}, nor did any of their interactions with T_{dev} or T_{test} (ANCOVA, $P > 0.3$ in all cases). The differences in U_{max} between groups remained significant in all of these analyses. Second, at each of the four race temperatures, the slopes of U_{max} versus total length (Fig. 2), tail length, and relative tail length were never different from zero or from each other (ANCOVA, all $P > 0.20$). The difference in U_{max} between groups was significant at T_{test} of 15°C, even after removing any effect of size or shape (ANCOVA, all $P < 0.05$).

Because acclimation of adult ectotherms to a particular temperature often enhances performance at that temperature, it was of interest to test the hypothesis that development at a given temperature significantly improves performance at that temperature. To do so, orthogonal linear contrasts were conducted on speeds measured at T_{test} of 15° and 25°C only (i.e., on a subset of the data). As Figure 1 suggests, over this restricted range of T_{test}, there was a significant effect of T_{dev} on U_{max} ($F_{1,65} = 16.37$, $P < 0.001$) but no effect of either T_{test} ($F_{1,65} = 0.07$, $P > 0.70$; temperature coefficients [Q_{10} values] were 0.96 and 1.07) or the interaction ($F_{1,65} = 1.67$, $P > 0.20$).

Developmental temperature had a significant effect on myofibrillar ATPase activity (Fig. 3; $F_{1,24} = 8.94$, $P < 0.01$). The difference between groups was greater when assayed at 15°C (orthogonal contrast: $F_{1,24} = 11.37$, $P < 0.01$) than at 25°C ($F_{1,24} = 0.74$, $P > 0.30$); this pattern matches the effects of T_{dev} on U_{max} (cf. Figs. 1, 3). There was also a significant effect of T_{test} on ATPase activity ($F_{1,24} = 57.88$, $P < 0.001$; Q_{10} values were 2.97 and 4.85), which is in contrast to the thermal independence of U_{max} over the same range of T_{test}. The interaction of T_{dev} and T_{test} had no effect on myofibrillar ATPase activity ($F_{1,24} = 3.16$, $P = 0.088$).

Discussion

Effects of Test Temperature

The thermal dependence of larval *Hyla regilla* swimming speed is similar in form (unimodal curve with a plateau at intermediate temperatures) to that observed for various adult anurans (summarized in Fig. 3 of Whitehead et al. 1989), larval fish (Batty and Blaxter 1992), and many other ectothermic vertebrates (reviewed in Bennett 1990). Likewise, the thermal dependence of myofibrillar ATPase reported here is quite similar to that observed in fish white muscle (Johnston and Walesby 1977; Penney and Goldspink 1981; Johnson and Bennett 1995; Ball and Johnston 1996; Johnson et al. 1996).

At intermediate test temperatures, the U_{max} of both cool- and warm-reared tadpoles is relatively independent of T_{test}, but the activity of myofibrillar ATPase in both groups is highly dependent on temperature (Figs. 1, 3). Given that myofibrillar ATPase activity is an important determinant of a muscle's maximum shortening velocity (Edman et al. 1988) and is positively correlated with maximum burst speed in *H. regilla* tadpoles (Watkins 1994), one might predict concordant thermal sensitivities of ATPase activity and U_{max}. Clearly, the lack of concordance suggests that there are other determinants of U_{max} whose thermal dependence over intermediate temperatures negates or compensates for that of myofibrillar ATPase. Such determinants may include thermally insensitive elastic elements (Marsh and Bennett 1985), the activity of creatine kinase (Gatten et al.

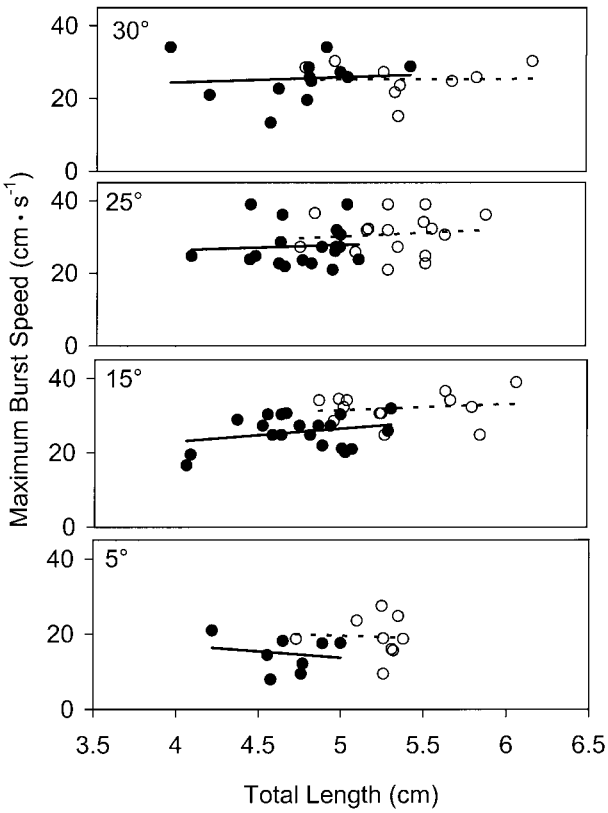

Figure 2. Maximum burst speed of tadpoles as a function of total length and developmental temperature at each of four race temperatures (indicated in upper left of each panel). The differences in speed between developmental temperature groups are not explained by differences in body size. Symbols are as in Figure 1.

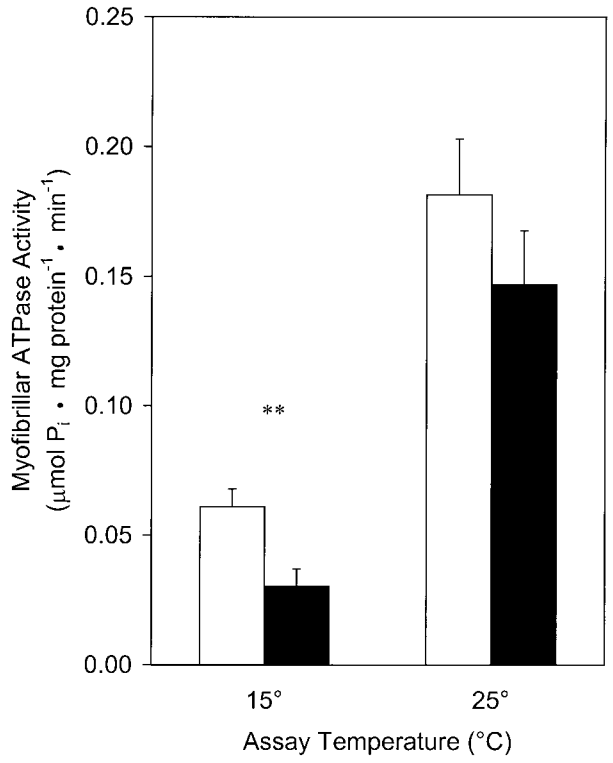

Figure 3. Myofibrillar ATPase activity as a function of temperature. Activity depends on both assay temperature and developmental temperature. Values are untransformed means ± 1 SEM. Dark bars are warm-reared tadpoles ($N = 7$); light bars are cool-reared tadpoles ($N = 7$). Two asterisks indicate $P < 0.01$.

1984), or kinematic parameters like tail-beat amplitude and stroke length (Else and Bennett 1987; Batty and Blaxter 1992). Another possibility is that the thermal dependence of the shortening velocity of an unloaded muscle (which is indexed by myofibrillar ATPase activity) may be greater than that of a partially loaded muscle; such, however, seems not to be the case in adult amphibians (Rome 1983; Else and Bennett 1987). In any case, the relative contributions to variation in tadpole swimming speed by elastic storage mechanisms, muscle contractile kinetics, and muscle enzyme activities are unknown. Functional analyses of tadpole locomotion similar to those completed on teleost fish (reviewed in Wardle et al. 1995) would reveal much about how tadpoles power swimming.

Effects of Developmental Temperature

Figure 1 indicates that tadpoles that develop at two different temperatures have different temperature-performance curves. One explanation for this pattern is that swimming speed is affected directly by developmental temperature. An alternative explanation is that swimming speed is influenced only by the magnitude of the difference between each developmental temperature and each test temperature and that the apparent group differences are the result of the groups being in different physiological states at a given test temperature. The former explanation is better supported by the results in at least two ways. First, the ANOVA on the entire data set revealed a large and significant overall effect of developmental temperature on U_{max} despite the fact that U_{max} was measured over a large range of deviations (−20° to +15°C) from the groups' respective developmental temperatures. Second, a 10°C decrease for the cool-reared tadpoles (i.e., T_{test} of 15°C vs. T_{test} of 5°C) substantially reduced speed, while an identical decrease in temperature for the warm-reared tadpoles (i.e., T_{test} of 25°C vs. T_{test} of 15°C) resulted in no significant change in speed. Therefore, rearing temperature directly affected burst swimming speed over a range of test temperatures.

The effect of developmental temperature on locomotor performance is independent of body size and at least one measure of shape. The independence of speed and size is common when tadpoles are reared at constant temperature and measured at the same developmental stage (Watkins 1994, 1996, 1997*b*; J. Richardson, unpublished data), owing to very little variation in size. The claim that the performance differences between groups is not due to differences in size or shape is based on the results of ANCOVA, in which group differences in size are removed statistically. Nonetheless, there is a long tradition of reporting specific swimming speeds (body lengths per second) in an attempt to remove the effects of size on speed. In the present case (data not presented), the specific swimming speeds of the cool-reared tadpoles are greater than the warm-reared tadpoles at low test temperatures, but the differences are not statistically significant. However, the use of specific speed does not remove the effects of size from these data, since the line relating U_{max} and size does not pass through the origin (e.g., Fig. 2). The analysis of covariance is thus the more appropriate method for analyzing differences between groups in size-independent speed (Packard and Boardman 1987, 1988, 1999; Hayes and Shonkwiler 1996). Furthermore, absolute rather than specific speed is more important for escaping predators: regardless of its size, a prey animal needs to sprint absolutely faster than a pursuing predator in order to escape. This is not to say that size is unimportant in predator-prey relations; in fact, size can be very important for a prey animal if it is captured by a gape-limited predator.

Over most of the test temperatures used in this study, tadpoles reared at 15°C swam faster than their counterparts reared at 25°C. Likewise, in the first study of thermal acclimation of locomotor performance in amphibian larvae, Wilson and Franklin (1999) reported that *Limnodynastes peronii* tadpoles acclimated to 14°C swam faster at all three test temperatures than those acclimated to 24°C. (Because Wilson and Franklin [1999] reared field-caught tadpoles rather than egg masses and

because *L. peronii* has a long larval period [>1 yr], the reported effect of long-term temperature exposure in that species is "acclimatory" rather than "developmental," as defined above in the "Introduction".) These effects of temperature are particularly interesting in light of the fact that thermal acclimation of locomotor performance in adult amphibians is weak or absent (Rome 1983; Else and Bennett 1987; Londos and Brooks 1988; Whitehead et al. 1989; Knowles and Weigl 1990). Larval and adult anuran amphibians thus appear to be distinct in their capacity for thermally induced locomotor plasticity. It is possible that rapid and widespread morphogenesis (even in species with long larval periods) confers greater plasticity on larvae relative to adults.

The effect of developmental temperature on locomotor performance has previously been examined for only one other larval amphibian, the toad *Bombina orientalis* (Parichy and Kaplan 1995); all other such studies have been conducted on reptiles (Burger 1990, 1991; Van Damme et al. 1992; Janzen 1993; Shine and Harlow 1996; Shine et al. 1997). These various studies report both positive and negative relationships between developmental temperature and speed among the various taxa. Evaluating the effects of developmental temperature in these previous studies is difficult, however, since they often failed to control for at least three important variables. First, some studies (e.g., Burger 1990) confound family effects with treatment effects by failing to distribute eggs from a given female evenly across temperatures. Second, experiments with low or differential survival between treatments (e.g., Van Damme et al. 1992) may erroneously imply phenotypic effects of temperature that are in fact the result of laboratory selection. Third, the authors of all the above studies measured locomotor performance at only one test temperature. Any unknown shift in the temperature-performance curves of treatment groups relative to one another can greatly affect conclusions concerning the magnitude and direction of developmental temperature effects. In this study, for example, three very different conclusions would be obtained had U_{max} been measured only at 15°, 30°, or 35°C.

Two recent studies (Elphick and Shine 1998; Downes and Shine 1999) have examined the effects of temperature on locomotor performance while controlling for all three of the above potential artifacts and have shown variation among species in the direction of developmental temperature effects. In one species of high-altitude skink (*Nannoscincus maccoyi*), incubation in a cool environment results in greater running speed across all test temperatures than does incubation in a warm environment. In other sympatric species of skinks (*Bassiana duperreyi*, *Lampropholis delicata*, *Saproscincus mestelina*), warm-incubated animals are faster than their cool-incubated siblings at all or some test temperatures (Elphick and Shine 1998; Downes and Shine 1999). As in this study, variation in body size and shape does not explain the differences in burst speed between incubation treatments. In the case of skinks, differences between species in the direction of the incubation-temperature effect may reflect alternative evolutionary adaptations to reproducing in cold climates (Shine 1999).

What are the proximate mechanisms by which differences in developmental temperature produce differences in size-independent burst speed? Change in myofibrillar ATPase activity is one important factor, as suggested by the concordant responses of U_{max} and ATPase activity to developmental temperature (i.e., both variables are higher in the cool-reared tadpoles, especially at a T_{test} of 15° C; Figs. 1, 3). Similar concordances between thermally induced changes in performance and myofibrillar ATPase have been found in recent studies of temperature acclimation in adult fish (Beddow and Johnston 1995; Beddow et al. 1995; Johnson and Bennett 1995; Ball and Johnston 1996; Johnson et al. 1996). Thus, thermal acclimation in mature fish and thermally induced plasticity in tadpoles appear to involve similar changes in muscle phenotype. The effects of developmental temperature on muscle phenotype may be common to taxa other than anurans, as similar temperature-induced changes have been reported for axial muscles of larval Atlantic herring (Johnston et al. 1996, 1997) and carp (Nathanailides et al. 1995).

Ecological Implications of Developmental Temperature

The consequences and adaptive value, if any, of the phenotypic response to developmental temperature are unknown. There is not sufficient information on patterns of selection at different temperatures to draw conclusions concerning the consequences of the temperature effects demonstrated in this study. Some careful speculation seems warranted, however, since predator-mediated selection on the phenotypes studied here has been demonstrated previously. For example, it is known that fast swimming speed confers a survival advantage to tadpoles in the presence of predators (Watkins 1996; Feder 1983). If such an advantage persists across all relevant environmental temperatures, then the results shown in Figure 1 would be consistent with the "colder is better" hypothesis of phenotypic plasticity (Huey et al. 1999), although the inability of the cool-reared tadpoles to swim at 35°C suggests that their fitness at very high environmental temperatures is reduced. The lack of a significant interaction between T_{test} and T_{dev} on U_{max} in the middle of the performance curve fails to support the hypothesis that development at one temperature improves performance (and possibly fitness) at that temperature.

The changes in body size and relative tail length induced by developmental temperature (Table 1) may also have a survival advantage. In other species of tadpoles, changes in size and shape can be induced by the presence of natural predators (e.g., Van Buskirk and Relyea 1998), and the resulting morphologies can be favored by selection in the same environments that induced them (Van Buskirk et al. 1997; Van Buskirk and Relyea 1998). Whether temperature-induced changes in body size and

shape in the present case support the hypothesis that plasticity is adaptive is unknown; again, such a possibility can be revealed only by studying selection across a range of temperatures.

Tadpoles of several species have been shown to prefer warm temperatures both in the field and the lab (reviewed in Ultsch et al. 1999). Choosing the warmest available temperature may maximize growth and development rates (Smith-Gill and Berven 1979) but may also reduce body size and maximum swimming speed (this study). The survival consequences of such behavioral thermoregulation depend on the selective environment. For a population preyed on by garter snakes, which eat both tadpoles and juvenile frogs (Arnold and Wassersug 1978), and that lives in a pond that dries only occasionally, there may not be an advantage to achieving rapid development by selecting warm temperatures; in fact, there may be a strong disadvantage of reduced locomotor performance (Watkins 1996). In populations not subject to predation by size-unlimited predators but subject to pond drying, achieving rapid development by selecting warm temperatures should confer a survival advantage. The adaptive value of behavioral thermoregulation has been suggested in comparisons of thermal physiology between species that occupy different microhabitats (e.g., *Bufo terrestris* and *Rana pipiens*; Noland and Ultsch 1981). However, comparisons between two species can lead to erroneous conclusions concerning adaptation (Garland and Adolph 1994).

The constant temperature regimes used in this study are undoubtedly artificial, as *H. regilla* eggs experience considerable thermal variation in the field (Cunningham and Mullally 1959). Variability in developmental temperature has been shown to influence offspring phenotype in a skink apart from the effects of mean temperature (Shine and Harlow 1996); whether such an effect exists for *H. regilla* is unknown. But while they are artificial, the constant temperature regimes used here may nonetheless be relevant to the field, as there can be large differences in average temperature among adjacent breeding ponds (Schaub and Larsen 1978). Likewise, the occurrence of eggs in both small and large bodies of water ensures that persistent microsite differences in mean temperature exist. Eggs (even, potentially, from the same female) will therefore be distributed across a spatially heterogeneous thermal environment. Furthermore, given that breeding at a particular location lasts for several weeks, eggs laid early in the season may experience very different temperatures than those laid late in the season. There are no available data on whether female *H. regilla* choose oviposition sites or times with regard to water temperature. If they do, and if there is genetic variation among females in oviposition choice, then locomotor performance and its functional determinants may evolve in response to selection acting on female breeding behavior. In any case, the effects of rearing temperature on locomotor performance and its functional determinants, coupled with parental breeding behavior, offer an excellent opportunity to examine the causes and evolutionary dynamics of plasticity in a complex functional system.

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