

# THE EFFECT OF METAMORPHOSIS ON THE REPEATABILITY OF MAXIMAL LOCOMOTOR PERFORMANCE IN THE PACIFIC TREE FROG *Hyla regilla*

TIMOTHY B. WATKINS\*

*Department of Ecology and Evolutionary Biology, University of California, Irvine, CA 92697, USA*

*Accepted 24 July 1997*

## Summary

Measuring the repeatability of inter-individual differences in locomotor performance is an important first step in elucidating both the functional causes and the ecological consequences of performance variation. Thus, repeatability of whole-animal performance traits provides a crucial link between functional and evolutionary biology. In the present study, repeatability of maximal burst locomotor performance was estimated for a single population of the Pacific tree frog *Hyla regilla*.

Animals were reared individually from eggs through metamorphosis in the laboratory. Maximum burst swimming speed of tadpoles was measured before metamorphosis (Gosner stage 37) and again at the onset of the metamorphic climax (stage 42). Maximum jump distance was measured on the same individuals as juvenile frogs.

Locomotor performance was repeatable over a 24 h period for both premetamorphic tadpoles and juvenile frogs. Performance was not repeatable across metamorphosis or between any two of the three developmental stages investigated. A high-performance individual at one developmental stage does not necessarily retain that performance advantage at another stage. This lack of repeatability contrasts sharply with several previous studies on non-metamorphosing vertebrates, but concurs with a single previous study on a metamorphosing salamander. Metamorphosis appears to place strict temporal constraints on individual consistency in locomotor ability.

Key words: Pacific tree frog, *Hyla regilla*, metamorphosis, locomotion, locomotor performance, repeatability.

## Introduction

The temporal repeatability of whole-animal performance traits has a number of important implications in organismal and evolutionary biology. First, the presence of consistent differences in performance among individuals within a species suggests functional explanations which may be revealed without confounding phylogenetic effects inherent to traditional species comparisons. Studies of individual temporal repeatability of performance traits thus represent an important first step for a functional analysis (Bennett, 1987). Second, repeatability defines the time period over which accurate predictions of a trait's future value can be made on the basis of the current value. For instance, maximum burst running speed in the lizard *Sceloporus occidentalis* is repeatable over a 2 month period in the summer but is not repeatable between summers (van Berkum *et al.* 1989). Identifying such temporal limits to predictability is an important first step in the study of natural selection on performance traits. Because selection acts on differences among individuals, the selective value of a performance trait may only be estimated over a time frame short enough such that individual differences are persistent (Huey and Dunham, 1987; Boake, 1989; van Berkum *et al.* 1989). Third, repeatability measured over the pre-reproductive

portion of the lifespan provides information on the genetic transmission of traits across generations by placing an upper limit on heritability (Falconer, 1989). For all these reasons, repeatability of individual differences in performance traits plays a central role in linking functional and evolutionary investigations of animal performance (Bennett, 1997).

The repeatability of locomotor speed and endurance has been estimated in several studies of terrestrial vertebrates, and typically manifests as correlation coefficients of 0.2–0.7 (see Table 1 in Austin and Shaffer, 1992). Locomotor performance in terrestrial vertebrates is almost always significantly repeatable over time periods ranging from seconds (Austin and Shaffer, 1992) to a year (Huey and Dunham, 1987; Jayne and Bennett, 1990). Many of these studies have spawned further investigations of the functional determinants and evolutionary consequences of variation in locomotor performance (e.g. Bennett and Huey, 1990).

Most previous studies of locomotor repeatability have been conducted on animals that do not metamorphose, yet metamorphosis can have profound effects on locomotor performance. For example, in anurans, metamorphosis is associated with sharp decreases in mean swimming endurance

\*e-mail: twatkins@uci.edu

(Wassersug and Sperry, 1977) and maximum burst speed (Huey, 1980; Brown and Taylor, 1995) and an increase in evasiveness (Brown and Taylor, 1995). However, the consequences of metamorphosis for the repeatability of individual differences in locomotor performance are completely unknown for anurans. Only one previous study, on the salamander *Ambystoma californiense*, addressed whether locomotor speed and endurance were repeatable across the metamorphic transition (Shaffer *et al.* 1991). The present study reports on the repeatability of maximum burst locomotor performance (measured as tadpole burst speed and frog jump distance) during and across metamorphosis in the Pacific tree frog *Hyla regilla*. Previous work (Watkins, 1996) showed a selective advantage for high maximum burst speed among tadpoles during encounters with a natural predator. The principal objective of the present study was to estimate the temporal and ontogenetic limit to a such a selective advantage.

Given that metamorphosis produces large and very rapid changes in the morphology, behaviour, physiology and ecology of anurans (Wilbur, 1980; Wassersug, 1989; Burggren and Just, 1992), tadpole swimming speed and frog jump distance may not represent the same 'trait' from a functional perspective, and thus consideration of repeatability may seem contrived (Boake, 1989). But the definition of 'trait' is arbitrary and subject to personal bias. Furthermore, regardless of whether swimming and jumping represent different 'traits,' the interesting question that nevertheless remains is whether an individual's performance in one stage of a complex life cycle is correlated with its performance in the other stage. A lack of correlation is predicted by theoretical models for the evolution of metamorphosis (e.g. Ebenman, 1992), while the presence of a correlation is required for Carrier's (1996) claim that locomotor performance in adults reflects the action of selection on performance in juveniles.

## Materials and methods

### *Animal collection and maintenance*

Eggs of *Hyla regilla* (Baird & Girard, 1852) were collected in early February 1995 from three populations in Irvine and Laguna Beach, California, USA. The eggs were brought into the laboratory and, after hatching, tadpoles were transferred to a single 391 aquarium filled with aerated dechlorinated tap water. Ten days after the eggs hatched, 29 tadpoles were selected at random from the aquarium and placed in individually labeled plastic cups each containing approximately 350 ml of water. This procedure ensured that these tadpoles represented a random sample that was fully mixed with regard to the source population. The positions of the cups on the laboratory bench were randomized every other day. The tadpoles were fed frozen romaine lettuce *ad libitum* and the water was changed every 2–4 days. Laboratory temperature was  $20.1 \pm 1.4^\circ\text{C}$  (mean  $\pm$  S.D.), with a 12h:12h L:D photoperiod.

During metamorphosis, each animal was placed in a separate plastic shoebox with water. The date of completion of

metamorphosis (disappearance of the tail stub) was recorded for each animal. Juvenile frogs were kept in shoeboxes with water dishes and fed crickets dusted with vitamin powder.

### *Tadpole locomotion trials*

Burst swimming speed was measured on each individual at developmental stages 37 and 42 (Gosner, 1960). Stage 37 was chosen as a premetamorphic stage in which the hindlimbs do not augment locomotion (Stehouwer and Farel, 1984). Stage 42 was chosen because it is considered to be the start of metamorphosis, when the forelimbs erupt and impair swimming performance (Wassersug and Sperry, 1977; Huey, 1980). At this stage, tadpoles kick with their hindlimbs, and this behaviour presumably provides some locomotor power. Each tadpole was used at stage 42 only upon eruption of the second forelimb so as not to introduce variation among individuals in gross morphology and drag.

Measurements were made in a 100 cm  $\times$  10 cm  $\times$  7 cm plastic trough marked off in 15 cm intervals and filled with water to a depth of approximately 4 cm. All measurements were made at a water temperature of 30  $^\circ\text{C}$  in a temperature-controlled room. This temperature was chosen because it is experienced naturally during the day (Cunningham and Mullally, 1956), it is within the thermal tolerance limits of this species (Brattstrom, 1963; T. B. Watkins, unpublished data) and because previous studies of selective predation were performed at 30  $^\circ\text{C}$  (Watkins, 1996).

When a tadpole reached the appropriate developmental stage, it was placed in a cup with approximately 50 ml of water and left undisturbed for at least 45 min to equilibrate to the new temperature. It was then transferred quickly to the trough and immediately chased with a fine wire at least four times without rest down the length of the track. Each time the tadpole crossed a 15 cm mark, the time was recorded by striking a key on a laptop computer (Toshiba T1000) loaded with a custom-designed timer program. Maximum burst speed ( $V_{\text{max}}$ ) was taken as the fastest 30 cm interval. After  $V_{\text{max}}$  had been determined, the tadpole was placed on its side and total length and tail length (distance from the tip of the tail to the junction of the tail musculature and the body wall, slightly anterior to the vent) were measured to the nearest 0.05 mm using a dial caliper.

To determine whether maximum locomotor performance is repeatable within a developmental stage, 17 tadpoles from a separate natural population in Malibu, CA, USA, were raced as described above at Gosner stage 37 on two consecutive days. Tadpoles grew out of stage 42 in less than 24 h, so similar repeatability measurements could not be made on metamorphosing animals.

The accuracy of these speed measurements depends on the investigator's reaction time as a tadpole crosses a line. To estimate this accuracy, six tadpoles were raced as described above and simultaneously filmed using a high-speed video system (HSV500, NAC Inc., Tokyo, Japan) at 500 frames  $\text{s}^{-1}$ . This system has a theoretical maximal error of  $\pm 2$  ms, although in practice it is somewhat greater owing to some ambiguity in

determining from the video image when a tadpole crosses a line. The hand-timed method resulted in tadpole speeds between 3.39 and 36.59 cm s<sup>-1</sup>. Over this range, the percentage error (i.e. the difference between the video and hand-estimated values divided by the video value) was quite small (mean 3.3 %, range 0.41–11.81 %,  $N=6$ ; all but one of these error values was below 4 %).

#### Frog locomotion trials

Jump distance was recorded 38±7 days (mean ± s.d.,  $N=29$ ) after completion of metamorphosis. Survival to this age suggests that the animals remained healthy throughout the study. Jump distance was measured on the paper-covered floor of a temperature-controlled room at 30.0±0.50 °C (mean ± s.d.). An open container of water maintained high humidity in the room and ensured that the frogs did not cool through evaporation. Juvenile frogs in their shoeboxes were placed in the room for 45 min and allowed to equilibrate to the new temperature, then transferred to separate covered plastic cups containing moist paper towel. At the start of a trial, a frog was removed from its cup and quickly placed on a covered jumping platform resting on the floor. This platform consisted of a piece of cotton gauze glued to a thin sheet of plastic and soaked in either water-soluble black ink or food coloring. The transfer was made quickly to avoid any exertion by the animal; frogs showed little sign of struggle during this transfer. After 3 min, the plastic cover was lifted and the frog was stimulated to jump by prodding it gently with a wire. A trial consisted of 3–8 consecutive jumps, after which the frog was returned to its cup for 15–20 min before the next trial. Each frog was used in four trials, then weighed to the nearest milligram.

The distances between the vent marks of consecutive frog prints were measured to the nearest millimeter. Maximum jump performance was taken as the single longest jump from all four trials.

#### Statistics

All statistical tests were computed in SigmaStat 2.0, with significance levels set at  $P<0.05$ . Repeatabilities were estimated using the Pearson product moment correlation coefficient rather than the intraclass correlation coefficient,

since the latter is sensitive to changes in mean values between trials (van Berkum *et al.* 1989). As noted above, performance was estimated from single maximum values for each individual. With one exception (see below), there were no qualitative differences in results or conclusions when performance was instead estimated as the mean of the two highest values for each individual.

## Results

### Tadpole performance

Table 1 reports the mean maximum swimming speed and size measurements for the tadpoles at stages 37 and 42, with probabilities from paired comparisons between stages. On average, there were 15±3 days (±s.d.) between the two stages. Development was associated with a slight decrease in  $V_{\max}$ , which was nearly significant ( $P=0.07$ ). This decrease was highly significant ( $P<0.01$ ) when performance was scored as the mean of the two fastest 30 cm intervals from each individual (Table 1). Maximum burst speed was more variable at stage 37 (coefficient of variation 0.33) than at stage 42 (coefficient of variation 0.21). Total length and body length both decreased slightly; tail length did not decrease significantly between the stages. The change in maximum burst speed between these two tadpole stages was positively correlated with the change in relative tail length (tail length/total length) (Spearman correlation,  $r_s=0.39$ ,  $P<0.04$ , d.f.=27), but not with changes in any of the other body size variables.

At neither stage did  $V_{\max}$  depend on body size. Linear regressions of speed on body size variables (total length, tail length and relative tail length, measured as residuals calculated from the regression of tail length on total length) at both stages all revealed non-significant slopes ( $P>0.50$  in all cases). Coefficients of determination ( $r^2$ ) were below 2 % in all cases.

### Juvenile frog performance

Mean maximum jump distance was 30.9±8.5 cm (mean ± s.d.,  $N=29$ ). Maximum jump distance was independent of body mass and age (linear regressions, distances were squared to improve normality:  $F=0.093$ ,  $r^2=0.003$ ,  $P=0.762$  for body

Table 1. Swimming speed and body size measurements of tadpoles at two developmental stages

	$V_{\max}$ (cm s <sup>-1</sup> )	Total length (cm)	Tail length (cm)	Body length (cm)	Tail length/ total length
Stage 37	35.93±11.71	4.04±0.19	2.86±0.16	1.19±0.07	0.71±0.01
Stage 42	31.13±6.46	3.95±0.24	2.82±0.25	1.13±0.08	0.71±0.03
$P$	0.07 <sup>a</sup>	0.02	0.33	<0.001 <sup>b</sup>	0.03 <sup>b</sup>

Values are means ± s.d.,  $N=29$ .

$V_{\max}$ , maximum swimming speed.

$P$  values are calculated using paired  $t$ -tests.

<sup>a</sup>When  $V_{\max}$  was estimated as the mean of the two fastest 30 cm intervals, tadpoles were significantly faster at stage 37 than at stage 42 (34.52±10.18 versus 28.40±5.95 cm s<sup>-1</sup>; paired  $t=2.93$ ,  $P<0.01$ ).

<sup>b</sup>Wilcoxon signed-ranks test.

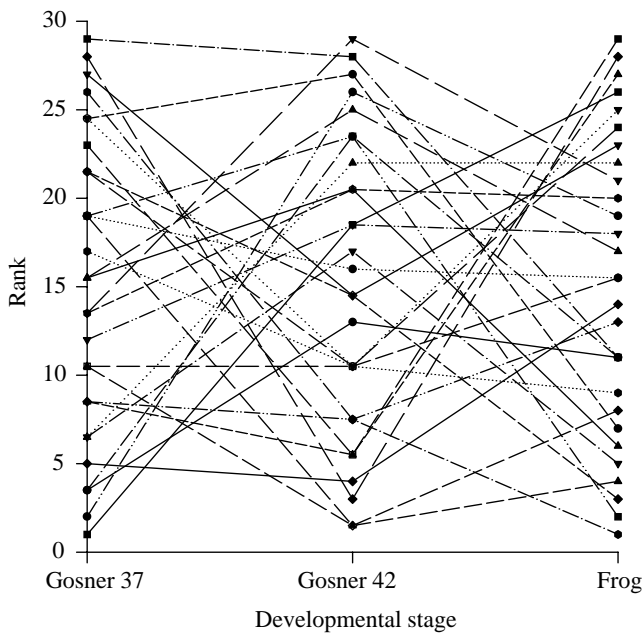


Fig. 1. Relative maximum burst locomotor performance across metamorphosis for 29 individuals at 30 °C. The crossing lines indicate a lack of repeatability.

mass, and  $F=2.81$ ,  $r^2=0.06$ ,  $P=0.106$  for age). Among all jumps, there was no significant effect of trial number, indicating that the frogs were not becoming fatigued during the measurements (Kruskal–Wallis analysis of variance on ranks:  $H=1.08$ , d.f.=3,  $P=0.783$ ).

#### Repeatability of performance

Because speed and jump distance were independent of all size, age and trial variables, repeatabilities were calculated using raw performance values (analysis of size-corrected performance gave identical results). Maximum burst speed on one day was correlated with speed on the following day for the Malibu tadpoles at stage 37 ( $r=0.65$ ,  $P<0.01$ , d.f.=15), indicating that speed is repeatable over a short period within this developmental stage. Among the juvenile frogs, maximum jump distance was consistent among individuals across trials (Kendall's coefficient of concordance:  $W=0.791$ ,  $\chi^2=88.55$ ,  $P<0.001$ ). Furthermore, maximum distance in the first trial was positively correlated with maximum distance in the fourth trial ( $r=0.83$ ,  $P<0.001$ , d.f.=27), indicating that maximum distance was highly repeatable over a period of approximately 1 h. In an independent sample of juvenile frogs, maximum jump distance was highly repeatable over a 24 h period ( $r=0.97$ ,  $P\leq 0.001$ , d.f.=22). However, in the present sample relative locomotor performance across metamorphosis was not repeatable (Fig. 1; Kendall's coefficient of concordance,  $W=0.277$ ,  $\chi^2=23.25$ ,  $P>0.50$ ), nor was relative performance correlated between any two stages (Table 2).

#### Discussion

In this sample of *Hyla regilla*, both maximum burst speed

Table 2. Correlation matrix for maximum burst locomotor performance across three developmental stages

		$V_{\max}$		Maximum jump distance, juvenile frog
		Stage 37	Stage 42	
$V_{\max}$ , stage 37	$r$	–	–0.029	–0.008
	$P$		0.88	0.97
$V_{\max}$ , stage 42	$r$	–	–	–0.157
	$P$			0.42

The top row of each cell is the Pearson correlation coefficient  $r$  and the second row is its associated probability  $P$ .  
 $N=29$ .

of stage 37 tadpoles and maximum jump distance of juvenile frogs were significantly repeatable over two consecutive days. Thus, an individual's performance on one day was a good predictor of its performance at least 24 h later. The Pearson correlation coefficient of 0.65 for maximum burst speed matches closely the repeatabilities obtained previously for maximum burst speed from several amphibian and reptilian species over the same time span (Table 1 in Austin and Shaffer, 1992). Similarly, the correlation coefficients of 0.83 and 0.97 for maximum jump distance are similar to the repeatability of burst performance over 24 h in *Bufo woodhousei* (Walton, 1988). These high repeatabilities over a relatively short time span suggest that performance differences among individuals are caused by underlying functional properties (e.g. muscle contractile rates, oxygen delivery rates) that are amenable to investigation and that are themselves repeatable. Furthermore, the significant repeatability measured in the present study means that the fitness advantage of a fast tadpole during a 2 h encounter with garter snakes (Watkins, 1996) extends over at least 24 h, a significant fraction of the larval period. The similar repeatabilities measured in other vertebrates suggest that if selection were to act on locomotor performance in these other species, as is often assumed (Bennett and Huey, 1990), then the fitness advantages of high performance values may likewise apply over a similar time span.

Maximum burst speed was not repeatable between stages 37 and 42, a period of only 15 days. This result was surprising because during this time only relatively minor metamorphic changes occurred; at both stages, the animals were tadpoles, they did not change appreciably in size and they swam using tail oscillations in the same aquatic environment. The lack of repeatability between these two stages may reflect the effects of initial metamorphic change *per se*, the protracted erosion of consistency in individual differences with time that has been observed in previous studies, or both. However, the fact that nearly all previous studies (including one on rapidly growing juvenile lizards; (van Berkum *et al.* 1989) demonstrate repeatable maximum performance over periods much greater than 15 days implicates metamorphosis *per se* (as opposed to time or growth) in the lack of repeatability. A possible

mechanistic basis for this lack of repeatability may be variation among individuals in the proportion of locomotor power derived from the tail *versus* power derived from the legs. No data have been collected on the relative power output of the tail and hindlimb musculature in developing tadpoles, although such information would be useful in understanding how tadpoles swim.

Regardless of why performance is not repeatable between stages 37 and 42, a potentially important consequence is that the fitness benefit enjoyed by fast tadpoles at one stage may not persist to later tadpole stages. Thus, estimates of future speed and fitness as a function of current speed must be confined to a narrow developmental window. In fact, the present data suggest that the fitness advantage lasts for at least 24 h but less than 15 days.

Locomotor performance rank was similarly disrupted as metamorphosis progressed. Maximum jump distance was not correlated with maximum burst swimming speed at either of the two tadpole stages (Table 2). This lack of concordance among locomotor performance measurements before and after metamorphosis is not surprising because of the substantial metamorphic changes in locomotor mode, anatomy and environment. Furthermore, several weeks separated the tadpole speed measurements from the frog jump distance measurements. As noted above, the lack of repeatability may be affected in part by the time span separating these two measures, so some caution is warranted in interpreting these observations solely as a result of metamorphosis.

A similar effect of metamorphosis on aquatic and terrestrial maximum burst speed and endurance was found by Shaffer *et al.* (1991) for the salamander *Ambystoma californiense*, an animal that experiences considerably less metamorphic change than anurans, although it does shift from reliance on tail and body undulations to leg-stepping during metamorphosis (Duellman and Trueb, 1994). The present study and that of Shaffer *et al.* (1991) provide some phenotypic evidence in support of the hypothesis that metamorphosis eliminates genetic correlations between the same trait (e.g. maximal locomotor performance) expressed in two different life stages, thereby allowing independent adaptation in the two stages (Ebenman, 1992). Thus, locomotor performance during the two stages of this complex life cycle may represent discrete characters that can evolve independently of one another. Metamorphosis, then, may provide an exception to Carrier's (1996) hypothesis that selection on juvenile locomotor performance greatly influences adult locomotor performance.

I thank A. F. Bennett, A. J. Cullum, and J. McLister for guidance and comments on the manuscript. Three anonymous reviewers provided helpful comments on an earlier draft. This study was supported by NSF grant IBN 9420155 to A. F. Bennett.

## References

AUSTIN, C. C. AND SHAFFER, H. B. (1992). Short-, medium- and long-

- term repeatability of locomotor performance in the tiger salamander *Ambystoma californiense*. *Funct. Ecol.* **6**, 145–153.
- BENNETT, A. F. (1987). Interindividual variability, an underutilized resource. In *New Directions in Ecological Physiology* (ed. M. E. Feder, A. F. Bennett, W. W. Burggren and R. B. Huey), pp. 147–166. Cambridge: Cambridge University Press.
- BENNETT, A. F. (1997). Adaptation and the evolution of physiological characters. In *Handbook of Physiology*, section 13, *Comparative Physiology*, vol. 1 (ed. W. H. Dantzler), pp. 3–16. Oxford: Oxford University Press.
- BENNETT, A. F. AND HUEY, R. B. (1990). Studying the evolution of physiological performance. In *Oxford Surveys of Evolutionary Biology*, vol. 7 (ed. D. Futuyma and J. Antonovics), pp. 251–284. Oxford: Oxford University Press.
- BOAKE, C. R. B. (1989). Repeatability – its role in evolutionary studies of mating behavior. *Evol. Ecol.* **3**, 173–182.
- BRATTSTROM, B. H. (1963). A preliminary review of the thermal requirements of amphibians. *Ecology* **44**, 238–255.
- BROWN, R. M. AND TAYLOR, D. H. (1995). Compensatory escape mode trade-offs between swimming performance and maneuvering behaviour through larval ontogeny of the wood frog, *Rana sylvatica*. *Copeia* **1995**, 1–7.
- BURGGREN, W. W. AND JUST, J. J. (1992). Developmental changes in physiological systems. In *Environmental Physiology of the Amphibians* (ed. M. E. Feder and W. W. Burggren), pp. 467–530. Chicago: University of Chicago Press.
- CARRIER, D. R. (1996). Ontogenetic limits on locomotor performance. *Physiol. Zool.* **69**, 467–488.
- CUNNINGHAM, J. D. AND MULLALLY, D. P. (1956). Thermal factors in the ecology of the Pacific tree frog. *Herpetologica* **12**, 68–79.
- DUELLMAN, W. E. AND TRUEB, L. (1994). *Biology of Amphibians*. Baltimore: Johns Hopkins University Press.
- EBENMAN, B. (1992). Evolution in organisms that change their niches during the life cycle. *Am. Nat.* **139**, 990–1021.
- FALCONER, D. S. (1989). *Introduction to Quantitative Genetics*, 3rd edn. New York: John Wiley and Sons.
- GOSNER, K. L. (1960). A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica* **16**, 183–190.
- HUEY, R. B. (1980). Sprint velocity of tadpoles (*Bufo boreas*) through metamorphosis. *Copeia* **1980**, 537–540.
- HUEY, R. B. AND DUNHAM, A. E. (1987). Repeatability of locomotor performance in natural populations of the lizard *Sceloporus merriami*. *Evolution* **41**, 1116–1120.
- JAYNE, B. C. AND BENNETT, A. F. (1990). Selection on locomotor performance capacity in a natural population of garter snakes. *Evolution* **44**, 1204–1229.
- SHAFFER, H. B., AUSTIN, C. C. AND HUEY, R. B. (1991). The consequences of metamorphosis on salamander (*Ambystoma*) locomotor performance. *Physiol. Zool.* **64**, 212–231.
- STEHOUWER, D. J. AND FAREL, P. B. (1984). Development of locomotor behaviour in the frog. *Devl. Psychobiol.* **17**, 217–232.
- VAN BERKUM, F. H., HUEY, R. B., TSUIJ, J. S. AND GARLAND, T., JR (1989). Repeatability of individual differences in locomotor performance and body size during early ontogeny of the lizard *Sceloporus occidentalis* (Baird & Girard). *Funct. Ecol.* **3**, 97–105.
- WALTON, M. (1988). Relationships among metabolic, locomotory and field measures of organismal performance in the Fowler's toad (*Bufo woodhousei fowleri*). *Physiol. Zool.* **61**, 107–118.
- WASSERSUG, R. J. (1989). Locomotion in amphibian larvae (or 'Why aren't tadpoles built like fishes?'). *Am. Zool.* **29**, 65–84.

2668 T. B. WATKINS

WASSERSUG, R. J. AND SPERRY, D. G. (1977). The relationship of locomotion to differential predation on *Pseudacris triseriata* (Anura: Hylidae). *Ecology* **58**, 830–839.

WATKINS, T. B. (1996). Predator-mediated selection on burst

swimming performance in tadpoles of the Pacific tree frog, *Pseudacris regilla*. *Physiol. Zool.* **69**, 154–167.

WILBUR, H. M. (1980). Complex life cycles. *A. Rev. ecol. Syst.* **11**, 67–93.