

# Maternal investment and developmental plasticity: functional consequences for locomotor performance of hatchling frog larvae

D. M. PARICHY\* and R. H. KAPLAN†

\*Section of Evolution and Ecology and The Center for Population Biology, University of California Davis, Davis, CA 95616 and †Biology Department, Reed College, Portland, OR 97202, USA

## Summary

1. We examined the consequences of maternal investment and developmental plasticity for morphology and locomotor performance of early stage larvae of the frog *Bombina orientalis*.

2. We quantified the stages at which *B. orientalis* hatch in a natural population in the Republic of Korea. Greater than 95% of embryos hatched with prominent yolk masses and had not yet acquired circulation in the caudal fin (stages 19–21). Stage at hatching did not depend on egg size or the temperature experienced during development.

3. To investigate relationships between egg size, temperature during embryonic development, hatchling morphology and locomotor performance, we reared embryos at either a high or low temperature in the laboratory. Among individuals at the modal stage of hatching in the field (stage 21), egg size had positive effects on both snout–vent length (SVL) and tail length. In contrast, the temperature experienced prior to hatching influenced only tail length, with development at high temperature resulting in shorter tails. These effects of egg size and developmental temperature resulted in a range of hatchling morphologies that in turn influenced maximum sprint speed. Specifically, longer tails but not greater SVLs increased sprint speed. Moreover, development from larger eggs resulted in slower speeds, after controlling for the effects of total length.

4. We also examined morphology–performance relationships after larvae developed for one additional stage (stage 22). Among these individuals, egg size again had positive effects on both SVL and tail length, and a high developmental temperature resulted in shorter tails. But in contrast to individuals at the modal stage of hatching, both longer tails and greater SVLs increased sprint speeds and there were no residual effects of egg size after controlling for total length.

5. These results concur with previously demonstrated relationships between egg size, hatchling morphology and susceptibility to predation in the field (Kaplan 1992). This suggests that locomotor performance may be a functional link between maternal investment in individual offspring and subsequent offspring fitness. Finally, we discuss the rapid ontogenetic changes demonstrated in this study in relation to the causes and consequences of performance variability, as well as the evolution of optimal levels of maternal investment.

*Key-words:* *Bombina orientalis*, development, egg size, sprint speed, temperature

*Functional Ecology* (1995) **9**, 606–617

## Introduction

Differences in morphology have often been shown to influence fitness. Yet, the functional bases of such morphology–fitness relationships have been less frequently evaluated (Endler 1986; Jayne & Bennett 1990; Swain 1992a). One approach to this problem is to study aspects of individual performance, such as locomotor ability. Indeed, there is a growing realiza-

tion that studies of the causes and consequences of performance variability can provide a fuller understanding of natural selection and adaptation (Arnold 1983; Pough 1989).

The relationships between morphology and performance, and performance and fitness, may be understandable only if we consider multiple stages of an organism's life cycle. This is because morphological and performance characters, as well as selective

regimes, can change ontogenetically. Such changes could be especially important in developmentally plastic organisms, in which environmental factors can have lasting effects on an individual's phenotype (West-Eberhard 1989; Newman 1992; Sultan 1992; Scheiner 1993). We are using early amphibian development as a model in which to test the role of plasticity in producing phenotypic variability, and the consequences of plasticity for the fitness of individuals. In this study, we examine how plasticity can impact functional relationships between morphology and performance at the hatching stage, when larvae are especially susceptible to predation (e.g. Petranksa *et al.* 1987; Kaplan 1992; Sih & Moore 1993). Specifically, we focus on how the locomotor performance of hatchling larvae is affected by two environmental factors: (1) the level of maternal investment they received (a maternal effect: Falconer 1989; Bernardo 1991; Cowley & Atchley 1992); and (2) the temperature they experienced during embryonic development.

Maternal investment in individual offspring is often reflected in egg size, and egg sizes can vary considerably within species (e.g. Marsh 1986; Meffe 1987; Tessier & Consolatti 1989; Amundsen & Stokland 1990; Selcer 1990; Rossiter 1991; Sinervo *et al.* 1992). In amphibians, the environment experienced by a female during vitellogenesis can predictably influence the sizes of eggs she produces (Kaplan 1987). This plasticity in maternal provisioning, in conjunction with the female's genotype and the environment she experienced during her development, results in extensive variability in egg size both within and among clutches (e.g. Crump 1984; Kaplan 1985; Williamson & Bull 1989). Such differences in egg size are important because they represent variability in the earliest phenotypes of individual offspring. Moreover, the size of the egg influences later size and morphology, and the magnitude of these effects can vary among environments because of plasticity during later stages of the life cycle (Berven & Chadra 1988; Parichy & Kaplan 1992a,b). Thus, we might expect a particular level of maternal investment to have different consequences for the performance and fitness of offspring, depending on the environment they experience.

Temperature during embryonic development is one such factor that can potentially influence morphology, performance and fitness. The consequences of both egg size and developmental temperature have been studied in the Fire-Bellied Toad, *Bombina orientalis* Boulenger (Discoglossidae) (see Kaplan 1989, 1992). In this species, there is a positive relationship between egg size and hatchling snout-vent length. In contrast, developmental temperature acts predominantly on the length of the tail, and it does so in two opposing and unequal ways: tail growth rates are somewhat accelerated at high temperatures, but times to hatching are truncated. Overall, this yields a net decrease in tail length as developmental temperatures increase.

Because egg-size variation and temperature heterogeneity are ubiquitous at breeding sites used by *B. orientalis*, hatchling larvae exhibit a wide range of naturally occurring total lengths and body proportions. These differences in morphology are important because they can influence the likelihood of surviving predation.

During the time immediately after hatching (*c.* 1 day), larval *B. orientalis* are highly susceptible to predation by heterospecific tadpoles (*Rana amurensis coreana*). Although mortality can sometimes reach 100%, hatchlings with greater total lengths are less vulnerable to these predators (Kaplan 1992). To the extent that egg size and developmental temperature affect total length, they can thus impact survivorship as well. But the morphology-fitness relationship is complex. This is because for a given total length, hatchlings that develop from larger eggs can have *higher* probabilities of succumbing to predation than hatchlings that develop from smaller eggs. This effect is most accentuated when embryos experience higher temperatures during development and consequently have shorter tails at hatching.

One way that morphology could influence susceptibility to predation is through effects on hatchling locomotor performance (Kaplan 1992; Sih & Moore 1993; K. Nishikawa personal communication). In the present study, we investigate the possibility that susceptibility to predation in *B. orientalis* is mediated through effects of egg size and developmental temperature on larval morphology and sprint speed. We first quantify the developmental stages at which *B. orientalis* hatch naturally in the field. We then examine the consequences of egg size, developmental temperature, morphology and sibship identity for maximum sprint speed at these critical hatching stages, and we relate these findings to previous results for survivorship (Kaplan 1992). To better understand the ontogeny of these characters, we also investigate the relationship between these same variables and locomotor performance after larvae develop for one additional stage. We show that locomotor performance is influenced by interactions of these variables with each other and that the interactions change even during this brief period of post-hatching development.

## Materials and methods

### DEVELOPMENTAL STAGES AT HATCHING IN THE FIELD

The developmental stages at which individual *B. orientalis* hatch were determined at a field site near the city of Tonghae in the province of Kangwon-do, in the Republic of Korea. At this site the breeding season extends from the middle of May (middle to late Spring) through early July (beginning of the hot rainy season), and breeding takes place in small granite depressions adjacent to a mountain stream (see Kaplan 1989, 1992 for more detail).

the distal tip of the tail and we videotaped each trial (30 frames  $s^{-1}$ ) with a Panasonic VHS video camera. Larvae generally swam in a straight line towards the edge of the dish. Each larva was sprinted three times in rapid succession ( $<30$  s between trials) and was then immediately staged under the microscope and photographed. Using a mm rule in each photograph for calibration, we later measured ( $\pm 0.05$  mm) the snout to posterior margin of the vent length (SVL) and tail length of each hatchling from the transparencies and we calculated total length as the sum of these two measurements. We defined sprint speed as the mean velocity of an individual measured over 3 cm of travel and we included only the fastest sprint of each larva in our analyses. Because of differences in the resting posture of larvae and their initial direction of movement, we estimated sprint speeds from only the second to fourth cm of swimming.

#### STATISTICAL METHODS

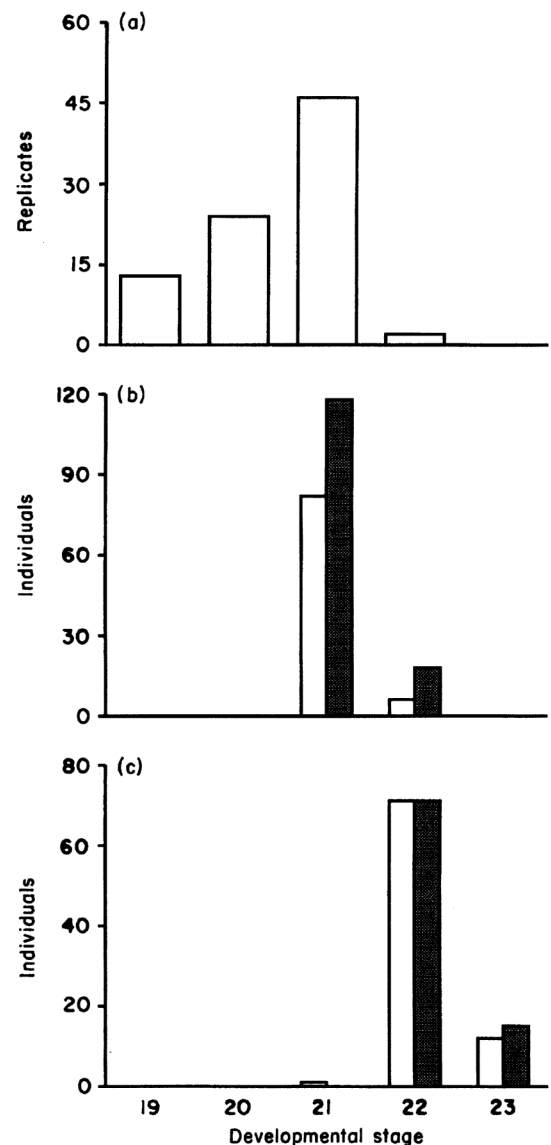
For analyses of hatching stage in the field we treated stages as ordinal and used the non-parametric Spearman rank correlation to test for correlations of hatching stages across egg-size treatments and association between hatching stage and mean developmental temperature. For morphological and performance variables in the laboratory, we analysed the two experiments separately after examining residuals for normality and homoscedasticity (Sokal & Rohlf 1981). Residual variances increased with sprint speeds in experiments I and II so we used logarithmic ( $\ln$ ) transformations of sprint speed in these analyses. We treated the covariates egg size, SVL, tail length and total length as fixed effects. Developmental temperature (two levels: high and low) was also treated as a fixed effect. We treated sibships and all interactions with sibships as random effects. Because of the presence of these random effects and unequal cell sizes, we analysed morphological and performance variables using restricted maximum likelihood estimation in SAS Proc MIXED (SAS Institute 1992; Searle, Casella & McCulloch 1992). Regression slopes for morphological and performance variables are partial regression coefficients from the final analyses and were obtained using least-squares estimation with SAS Proc MIXED. By controlling for sibship-specific effects on egg size, larval morphology and locomotor performance, we restricted our analyses to residual differences that represent variability found within clutches. For analyses of embryo survivorship to decapsulation we used multiple logistic regression and maximum likelihood estimation in SAS Proc CATMOD (SAS Institute 1989), with sibships treated as fixed effects owing to software limitations. We tested hypotheses using likelihood ratio tests at the  $P < 0.05$  level and we present only the significant effects from these analyses. For all dependent variables we performed preliminary analyses to test for homogeneity

of slopes of the covariates across sibships and developmental temperatures.

## Results

### DEVELOPMENTAL STAGES AT HATCHING IN THE FIELD

In the field, larvae hatched in most replicates at stage 21 although some hatched as early as stage 19 (Fig. 1a). Within each replicate, 10–20 individuals out of 25 survived to hatching. *Bombina orientalis* larvae at the modal stage of hatching (stage 21) have transparent corneas, prominent yolk masses and well-devel-



**Fig. 1.** Developmental stages at hatching in the field and laboratory. (a) Stages at hatching for embryos that developed in enclosures in the field. One replicate represents 10 to 20 individuals. (b) Stages at which embryos were decapsulated in experiment I. (c) Stages at which embryos were decapsulated in experiment II. (b,c) Open bars, individuals that developed at high temperature. Shaded bars, individuals that developed at low temperature.

oped oral suckers. One stage later (stage 22), tail-fin circulation begins and the gut starts to coil. Stages at hatching were identical for 10 of 11 matched pairs of replicates representing large eggs and small eggs developing in identical conditions (grand mean egg diameters = 2.19 mm, 1.94 mm; SD = 0.035, 0.039 respectively; correlation between large and small egg size treatments:  $r_s=0.94$ ,  $P<0.005$ ;  $n=11$  matched replicates). Stage at hatching was not related to the mean developmental temperature experienced by embryos ( $r_s=0.01$ ,  $P>0.9$ ;  $n=85$  replicates).

#### LABORATORY EXPERIMENT I: MODAL STAGE OF HATCHING

In this experiment we examined morphology and locomotor performance when most individuals were at the modal stage of hatching in the field (Fig. 1b). The proportions of hatchlings at different developmental stages did not differ significantly between the high and low developmental temperatures ( $\chi^2=2.30$ ,  $df=1$ ,  $P>0.1$ ), indicating that embryos had been decapsulated at comparable stages across treatments. Hatchlings developed from eggs ranging in diameter from 1.76 to 2.20 mm (mean = 2.00 mm, SD = 0.073,  $n=224$ ). Multiple logistic regression showed that survivorship did not vary significantly with egg size ( $P>0.9$ ) but did vary across sibships ( $\chi^2=51.40$ ,  $df=6$ ,  $P<0.0001$ ; Table 1) and temperature treatments ( $\chi^2=21.27$ ,  $df=1$ ,  $P<0.0001$ ).

We examined influences on hatchling snout-vent length and tail length by analysing simultaneously the effects of developmental temperature (fixed effect: two levels), egg size (covariate) and sibship identity (random effect), as well as interactions among these factors (Table 2). These analyses show that neither developmental temperature nor sibship identity had significant main effects on snout-vent length ( $P>0.07$  and  $P>0.9$ , respectively). But the temperature at which embryos developed did have a large main effect on tail length (Tables 1, 2; Fig. 2a). Overall, individuals that developed at high tempera-

ture had tails that were 0.41 mm shorter than individuals that developed at low temperature. These analyses also revealed significant developmental temperature  $\times$  sibship interactions for both snout-vent length and tail length, indicating that sibships responded differently across the two levels of developmental temperature (Fig. 3). Finally, egg size had significant positive effects on both snout-vent length (partial regression coefficient = 0.56, SE = 0.190,  $df=209$ ) and tail length (partial regression coefficient = 0.71, SE = 0.290,  $df=209$ ), after controlling for the effects of developmental temperature and sibship. The relationships between egg size and hatchling snout-vent length and tail length did not differ significantly among sibships or temperature treatments, and there were no significant three-way interactions (all  $P>0.09$ ).

Maximum sprint speeds ranged from 1.70  $\text{cm s}^{-1}$  to 10.00  $\text{cm s}^{-1}$  (mean = 6.27  $\text{cm s}^{-1}$ , SD = 1.643,  $n=224$ ). To evaluate the proximate determinants of hatchling sprint speed, we analysed simultaneously the effects of the covariates egg size, snout-vent length, tail length, and temperature at which larvae sprinted, as well as developmental temperature (a fixed effect), sibship identity (a random effect), and interactions among these variables. This analysis showed that longer tails significantly increased sprint speed (partial regression coefficient = 0.49, SE = 0.035,  $df=222$ ; Table 2). Snout-vent length was positively related to speed (partial regression coefficient = 0.12, SE = 0.071,  $df=221$ ) but this effect was not significant ( $\chi^2=0.78$ ,  $df=1$ ,  $P>0.4$ ). Developmental temperature did not have a significant direct effect on locomotor performance after controlling for the effects of tail length and there were no other significant main effects or interactions (all  $P>0.1$ ).

In this experiment, tail length was influenced by developmental temperature, and both snout-vent length and tail length were influenced by egg size. In the field, the probability of hatchlings surviving predation was shown to depend on total length, the size of the egg from which individuals develop, and developmental temperature (Kaplan 1992). To see if locomotor performance could be responsible for differential

**Table 1.** Sibship and developmental temperature means  $\pm$  1 SD for experiment I

	<i>n</i>	Egg size (mm)	SVL (mm)	Tail length (mm)	Sprint speed ( $\text{cm s}^{-1}$ )
Developmental temperatures					
High	88	2.01 $\pm$ 0.074	4.79 $\pm$ 0.180	3.36 $\pm$ 0.362	5.62 $\pm$ 1.497
Low	136	1.99 $\pm$ 0.071	4.78 $\pm$ 0.216	3.77 $\pm$ 0.348	6.69 $\pm$ 1.600
Sibships					
1	29	1.97 $\pm$ 0.047	4.89 $\pm$ 0.180	3.76 $\pm$ 0.355	6.83 $\pm$ 1.595
2	38	2.05 $\pm$ 0.044	4.87 $\pm$ 0.165	3.69 $\pm$ 0.345	6.30 $\pm$ 1.405
3	23	1.91 $\pm$ 0.073	4.61 $\pm$ 0.239	3.28 $\pm$ 0.459	4.90 $\pm$ 1.356
4	33	1.98 $\pm$ 0.037	4.94 $\pm$ 0.111	3.29 $\pm$ 0.277	5.48 $\pm$ 1.680
5	52	2.01 $\pm$ 0.005	4.66 $\pm$ 0.166	3.90 $\pm$ 0.237	7.21 $\pm$ 1.393
6	38	2.05 $\pm$ 0.069	4.81 $\pm$ 0.145	3.53 $\pm$ 0.395	6.30 $\pm$ 1.371
7	11	1.92 $\pm$ 0.036	4.59 $\pm$ 0.136	3.41 $\pm$ 0.295	5.40 $\pm$ 1.763

survivorship in the field, we also analysed the sprint speed data from experiment I using total length and egg size as the only measures of hatchling morphology, in combination with the other fixed and random effects and their interactions from the previous model. This second analysis showed that greater total lengths resulted in significantly faster sprint speeds (partial regression coefficient=0.42, SE=0.039, df=215;  $\chi^2=85.49$ , df=1,  $P<0.0001$ ). But among hatchlings of a given total length, those individuals that developed from larger eggs had significantly slower maximum sprint speeds (partial regression coefficient=-0.57, SE=0.242, df=215;  $\chi^2=4.17$ , df=1,  $P<0.05$ ; Fig. 4). Finally, this analysis also revealed sibship-specific effects on locomotor performance ( $\chi^2=8.04$ , df=1,  $P<0.01$ ). These differences in speed may reflect differences in body proportions because mean ratios of snout-vent length to tail length ranged from 1.31 to 1.51 among sibships and these ratios were negatively correlated with mean sprint speed (univariate analysis:  $r=-0.80$ ,  $P<0.05$ ;  $n=7$ ).

#### LABORATORY EXPERIMENT II: ONE STAGE PAST MODAL STAGE OF HATCHING

Here we examined morphology and locomotor performance when most individuals were at stage 22, which is one developmental stage past the modal stage of hatching in the field (Fig. 1c). The proportions of embryos at different developmental stages did not differ between the high- and low-temperature treatments ( $\chi^2=1.31$ , df=2,  $P>0.5$ ). Larvae developed from eggs ranging in diameter from 1.74 to 2.19 mm (mean=1.97 mm, SD=0.088,  $n=170$ ), and survivorship did not vary significantly with egg size or developmental temperature (both  $P>0.2$ ), but did vary across sibships ( $\chi^2=38.48$ , df=3,  $P<0.0001$ ; Table 3).

At these later stages, developmental temperature once again failed to influence snout-vent length, but it did affect tail length, with individuals that developed at high temperature having tails 0.27 mm shorter on average than individuals that developed at low temperature (Tables 3, 4; Fig. 2b). Sibships also differed

significantly in mean snout-vent lengths and tail lengths but developmental temperature  $\times$  sibship interactions were not found for either component of larval length. Finally, egg size continued to exert a significant positive effect on both snout-vent length (partial regression coefficient=1.53, SE=0.184, df=165) and tail length (partial regression coefficient=1.15, SE=0.399, df=164).

Larval sprint speed in experiment II ranged from 3.91 cm s<sup>-1</sup> to 10.00 cm s<sup>-1</sup> (mean=7.31 cm s<sup>-1</sup>, SD=1.483,  $n=170$ ). Tail length again had the greatest overall effect on sprint speed (partial regression coefficient=0.13, SE=0.036, df=166; Table 4). But in contrast to experiment I, greater snout-vent lengths also resulted in significantly faster sprint speeds (partial regression coefficient=0.19, SE=0.063, df=166). Moreover, developmental temperature in experiment II exerted an additional, direct effect on locomotor performance. Individuals that developed at low temperature were faster than individuals that developed at high temperature, even after controlling for the effects of developmental temperature on hatchling morphology (high temperature: least-squares mean = 1.92 ln(cm s<sup>-1</sup>), SD = 0.195,  $n=84$ , cm s<sup>-1</sup> = 6.82; low temperature: least squares mean = 2.02 ln(cm s<sup>-1</sup>), SD = 0.195,  $n=86$ , cm s<sup>-1</sup> = 7.54). No other factors had significant effects on sprint speed in experiment II (all  $P>0.1$ ).

We again analysed the sprint-speed data using total length and egg size as the only morphological variables. This analysis showed that larvae with greater total lengths had faster sprint speeds (partial regression coefficient = 0.15, SE = 0.028, df = 167;  $\chi^2 = 20.27$ , df = 1,  $P<0.0001$ ), as did larvae that developed at low temperature ( $\chi^2 = 4.53$ , df = 1,  $P<0.05$ ), but among hatchlings of a given total length, there was no significant effect of egg size on sprint speed ( $P>0.9$ ) and there were no other main effects or interactions (all  $P>0.1$ ).

## Discussion

This study shows that locomotor performance of early larval *B. orientalis* depends on egg size, larval morphology, developmental temperature and sibship-specific effects (Fig. 5). In each experiment, egg size had positive effects on both snout-vent length and tail length. In contrast, the temperature experienced during embryonic development had main effects only on tail length, with individuals that developed at high temperature having shorter tails than individuals that developed at low temperature. These effects of egg size and developmental temperature combined to generate a range of larval morphologies that influenced sprint speed. In experiment I (c. stage 21; Fig. 5a), longer tails and greater total lengths increased maximum sprint speed, but development from larger eggs reduced speed after controlling for total length (Fig. 4).

**Table 2.** Results of restricted maximum-likelihood analyses (see Statistical methods) for experiment I

	$\chi^2$	$P$
Snout-vent length:		
Egg size	6.888	<0.01
Developmental temperature $\times$ sibship	4.634	<0.05
Tail length:		
Developmental temperature	10.880	<0.001
Sibship	4.835	<0.05
Egg size	5.146	<0.05
Developmental temperature $\times$ sibship	10.762	<0.005
Sprint speed:		
Tail length	132.258	<0.0001

Thus, individuals that developed from large eggs could be *slower* than individuals that developed from small eggs. In experiment II (c. stage 22; Fig. 5b), greater tail lengths, snout-vent lengths and total lengths all increased sprint speed, and there were no residual effects of egg size on speed. Thus, individuals that developed from large eggs were *faster* than individuals that developed from small eggs. Taken together, these findings have implications for at least two active areas of enquiry: (1) the causes and consequences of variation in locomotor performance, and (2) the evolution of optimal levels of maternal investment.

#### LOCOMOTOR PERFORMANCE VARIABILITY

The effects on locomotor performance demonstrated in this study can be understood by considering the relationships between egg size and the different components of axial body length, and how these relationships change ontogenetically. At a stage when most embryos hatch in the field (c. stage 21), development from larger eggs significantly *reduced* locomotor performance among individuals of a given total length (Fig. 4). This residual influence of egg size could reflect: (1) reduced flexibility in the trunk, if greater quantities of yolk resist contraction of the myomeres (cf. Wassersug 1989); (2) increased drag if larger yolk masses project ventrally and laterally; (3) increased mass and slower acceleration; or (4) a combination of these factors. Such effects would be manifested primarily in the anterior trunk of hatchling larvae and could thus oppose any positive effects of this region on locomotor performance. This may explain the absence

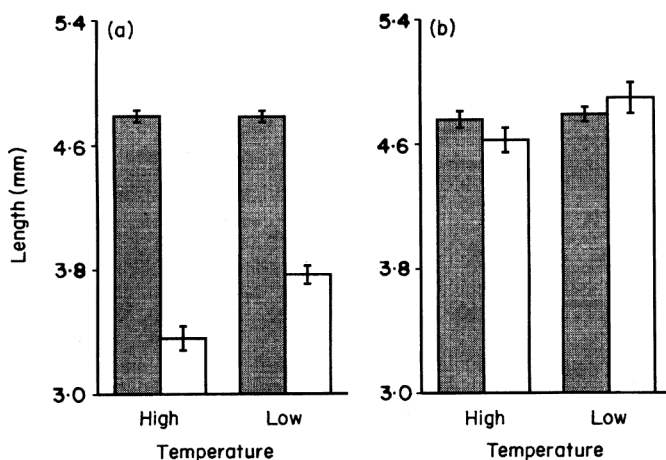


Fig. 2. Mean snout-vent lengths and tail lengths for experiments I (a) and II (b). (a,b) Shaded bars, snout-vent length. Open bars, tail length. (a) In experiment I, larval snout-vent length did not depend on the temperature experienced during embryonic development, but development at high temperature resulted in significantly shorter tails. (b) Similarly in experiment II, snout-vent lengths were independent of temperature treatment, but tail lengths were shorter among individuals that developed at high temperature. Error bars, two-tailed 95% confidence intervals.

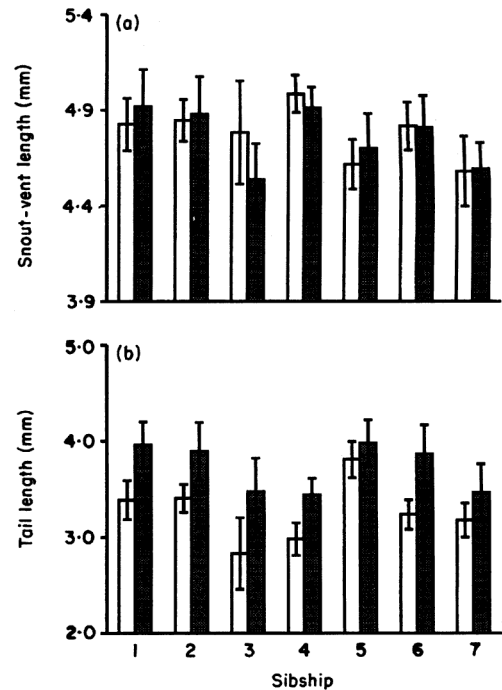


Fig. 3. Sibships responded differently across developmental temperatures in experiment I. (a) Mean snout-vent lengths. (b) Mean tail lengths. (a,b) Open bars, individuals that developed at high temperature. Shaded bars, individuals that developed at low temperature. Error bars, two-tailed 95% confidence intervals.

of significant snout-vent length effects on speed, despite the presence of large, positive effects of tail length at these early larval stages. When embryos developed to a slightly more advanced stage (c. stage 22), larvae had longer tails and greater total lengths, and consequently were faster than hatching stage larvae. Moreover, egg size no longer influenced speed after controlling for total length. This could indicate a reduction in the amount of inert yolk (as the gut begins to differentiate) or a change in the shape of the yolk mass or the larva as whole. Either of these possibilities is consistent with the observed positive effects of both snout-vent length and tail length on locomotor performance. During later stages, *B. orientalis* that develop from large eggs can attain larger sizes (Kaplan 1989; Parichy & Kaplan 1992a) and presumably faster sprint speeds (Wassersug & Sperry 1977; Huey 1980; Richards & Bull 1990) than individuals from small eggs. Any potential handicap on sprint speed among individuals developing from large eggs is therefore transitory. These results concur with other studies in which differences in axial proportions and morphology have been shown to influence locomotion (Arnold & Bennett 1988; Jayne & Bennett 1989; Wassersug 1989; Weihs 1989; Swain 1992a), as well as studies in which morphology-performance relationships have been shown to vary ontogenetically (Garland 1985; Shaffer, Austin & Huey 1991; Brown & Taylor 1995).

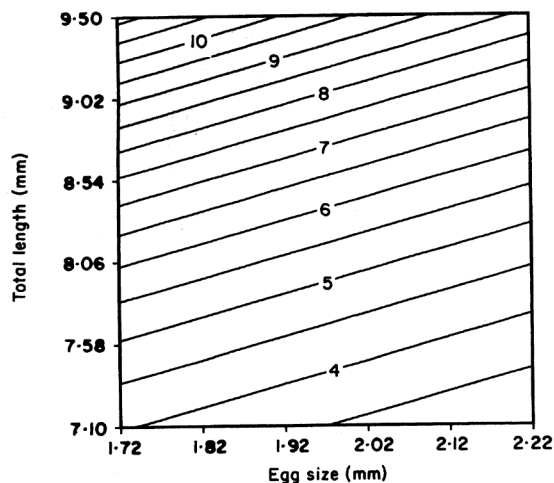


Fig. 4. Relationships among egg size, total length and maximum sprint speed in experiment I. Contour lines represent predicted sprint speeds ( $\text{cm s}^{-1}$ ) based on partial regression coefficients for covariates after controlling for variation among sibships (see text). Overall, hatchlings with greater total lengths had faster maximum sprint speeds. But for a given total length, hatchlings that developed from larger eggs had slower sprint speeds than hatchlings that developed from smaller eggs.

Several investigations have related variability in locomotor performance to differential survivorship (Christian & Tracy 1981; Snell *et al.* 1988; Jayne & Bennett 1990; Swain 1992b). Our results are also consistent with the hypothesis that differences in sprint speed impact fitness. In the field, *B. orientalis* hatchlings that developed from large eggs and at high temperatures suffered greater predation than hatchlings that developed from small eggs (Kaplan 1992). Similarly in the laboratory, hatchlings (*c.* stage 21) that developed from large eggs and at high temperature were slower than hatchlings that developed from small eggs (Fig. 5a). This concordance between studies suggests that egg-size effects on susceptibility to predation may be mediated through differential locomotor performance. Although the negative effect of egg size on speed was subtle, several lines of evidence indicate that our laboratory experiments may underestimate the

consequences of egg-size variation and temperature heterogeneity for locomotor performance and survivorship in the field. For instance, in the present study we controlled for the effects of sibships and thereby restricted our analyses to residual differences in egg size that represent variation within clutches. This intraclutch variability is less extreme than that found between repeated breedings of a single female or among females in general (Kaplan 1985, 1987; Cummins 1986; Berven 1988; R.H. Kaplan & E. King, in preparation). Moreover, we reared embryos at *c.* 21 °C and 28 °C, whereas in the field development can take place at 12.7–34.5 °C (see Materials and methods). The more restricted temperatures in the laboratory may have limited the range of hatchling morphologies and sprint speeds we were able to observe. Finally, we examined locomotor performance at stages 21–23, whereas in the field embryos hatch as early as stage 19. These precocious hatchlings have even shorter tails and proportionately larger yolk masses (R. H. Kaplan, unpublished data) and could suffer impaired locomotor performance relative to later-staged hatchlings (e.g. stage 21).

The rapid changes in morphology and performance demonstrated here are especially interesting because the hatching stages are a critical transition between embryonic and larval life. As detailed above, hatchling *B. orientalis* are highly susceptible to predation by heterospecific tadpoles and both hatchlings and older tadpoles are eaten by co-occurring salamander larvae (*Hynobius leechi* Boulenger, Hynobiidae; D. M. Parichy, unpublished data). Yet *B. orientalis* larvae that are slightly more advanced (*c.* stage 22) are larger, faster and less vulnerable to these predators. Such apparent advantages suggest that hatching at later stages could enhance survivorship. Indeed, studies of other amphibians have shown that delayed hatching can defend against predatory flatworms in the salamander *Ambystoma barbouri* (Sih & Moore 1993; K. Nishikawa, personal communication) and against predatory fish and shrimp in the frog *Agalychnis callidryas* (Warkentin 1995).

Table 3. Sibship and developmental temperature means  $\pm$  1 SD for experiment II

	<i>n</i>	Egg size (mm)	SVL (mm)	Tail length (mm)	Sprint speed ( $\text{cm s}^{-1}$ )
Developmental temperatures					
High	84	1.97 $\pm$ 0.089	4.76 $\pm$ 0.250	4.63 $\pm$ 0.363	6.82 $\pm$ 1.470
Low	86	1.96 $\pm$ 0.088	4.79 $\pm$ 0.222	4.90 $\pm$ 0.464	7.78 $\pm$ 1.340
Sibships					
8	21	1.85 $\pm$ 0.034	4.53 $\pm$ 0.174	4.22 $\pm$ 0.339	6.49 $\pm$ 1.276
9	48	2.04 $\pm$ 0.083	4.94 $\pm$ 0.222	4.92 $\pm$ 0.418	7.88 $\pm$ 1.501
10	64	1.95 $\pm$ 0.064	4.80 $\pm$ 0.185	4.79 $\pm$ 0.392	7.35 $\pm$ 1.382
11	37	1.96 $\pm$ 0.063	4.66 $\pm$ 0.184	4.81 $\pm$ 0.358	6.94 $\pm$ 1.470



Nevertheless, greater than 95% of *B. orientalis* embryos hatched at early stages in the field (stages 19–21). Why should individuals hatch at early stages as opposed to later ones? Since feeding does not commence for 2–3 days (stage 25; personal observation), resource availability and opportunities for rapid growth cannot themselves explain hatching at early stages. This suggests that there may be other trade-offs associated with the fine timing of this event. For example, egg masses are vulnerable to conspecific oophagy (Marshall, Doyle & Kaplan 1990), so it could be advantageous for individuals to escape from their egg capsules after a limited period of time. Alternatively there could be developmental or physiological limits on the duration of the embryonic period, even before embryos reach the feeding stage. We agree with Sih & Moore (1993) that further investigations are needed into both proximate influences on hatching and adaptive plasticity in hatching behaviour.

#### MATERNAL INVESTMENT IN OFFSPRING

In *B. orientalis* and many other ectothermic organisms that lack parental care, egg size can be considered a direct measure of maternal investment in individual offspring. Studies in a wide variety of taxa have found that larger propagule sizes and greater levels of investment per offspring can have positive effects on offspring growth, development and survivorship (e.g. McGinley, Temme & Geber 1987; Petranka *et al.* 1987; Tessier & Consolatti 1989; Amundsen & Stokland 1990; Rossiter 1991; Parichy & Kaplan 1992a; Janzen 1993). Recently, attention has focused on maternal effects on offspring performance. For example, the consequences of maternal investment for lizard morphology and locomotor performance have been examined by experimentally manipulating egg sizes (Sinervo 1990; Sinervo & Huey 1990). These studies have

shown that larger hatchlings (that developed from larger eggs) have faster sprint speeds.

The results reported here and from the field differ from these earlier studies because larger egg sizes can increase or decrease sprint speed (this study) and survival (Kaplan 1992). The particular effect depends on developmental stage as well as the temperature experienced prior to hatching. These findings add to a growing body of empirical evidence indicating that the consequences of particular levels of maternal investment can vary significantly with the environment that offspring experience. In plants, for example, successful establishment can be independent of seed size in good conditions but positively related to seed size when conditions are less favourable (Venable & Brown 1988). Similarly in amphibians, individuals that initially receive lower levels of maternal investment can sometimes equal (Crump 1984; Parichy & Kaplan 1992a,b) or exceed (Kaplan 1985; Berven & Chadra 1988) the growth and developmental rates of individuals that receive greater levels of investment, depending on the quality of the larval environment. The consequences of egg size for offspring survivorship can also depend on offspring sex and seasonal influences in lizards (Sinervo *et al.* 1992), available food level in fish (Hutchings 1991) and pond drying regime in salamanders (Semlitsch & Gibbons 1990). Finally, offspring receiving greater levels of investment can be more susceptible to predation if they are more conspicuous, or if predators prefer large over small individuals (Capinera 1979; Crump & Vaira 1991).

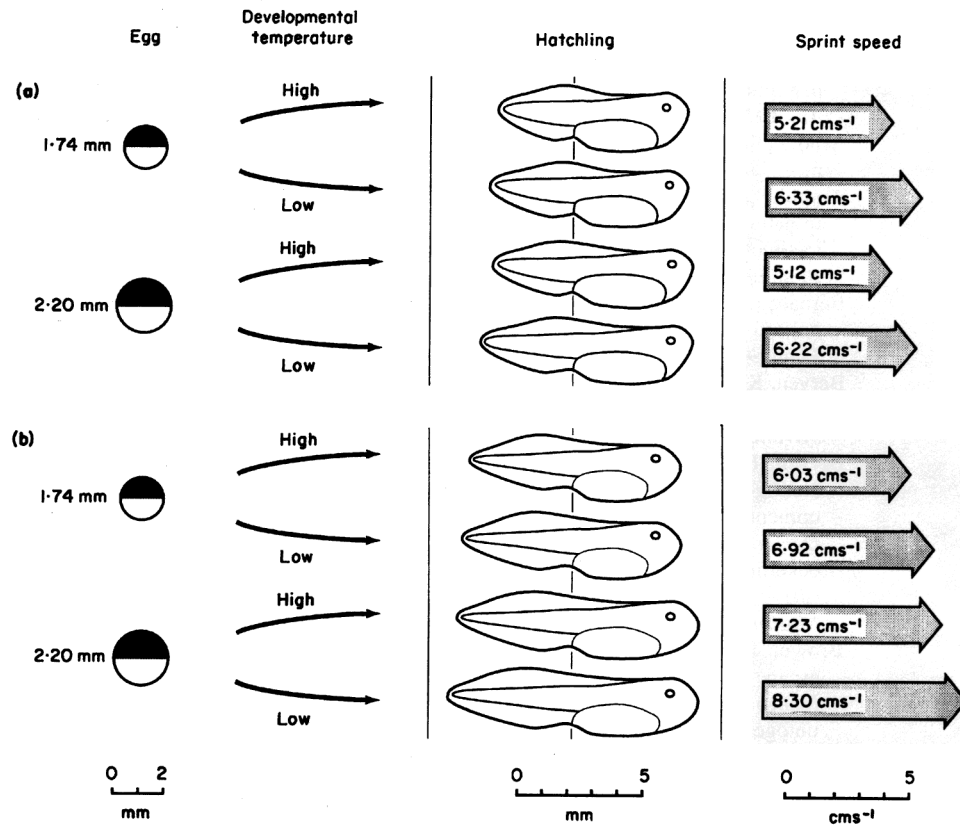
Given the existence of interactions between levels of investment per offspring and the offspring environment, different combinations of egg size and clutch size could be favoured in different environments. The situation is particularly complex when considering organisms that occupy two or more distinct, spatially heterogeneous and temporally unpredictable environments during different stages of the life cycle (Wilbur 1980; Ebenman 1992). In *B. orientalis*, for example, vitellogenesis occurs in a terrestrial environment prior to breeding (Kaplan 1987). Yet offspring develop in temporary pools that differ in temperature, predator type and abundance, conspecific density and persistence. Moreover, even if females selected oviposition sites (Crump 1991) favourable for the particular sizes and numbers of eggs to be deposited, breeding ponds are ephemeral and conditions can change rapidly prior to metamorphosis (Newman 1987, 1992).

Most theoretical investigations of optimal levels of maternal investment have assumed a monotonic, increasing relationship between levels of investment per offspring and offspring fitness (e.g. Smith & Fretwell 1974), and some have considered differences in the magnitude or convexity of this rela-

**Table 4.** Results of restricted maximum likelihood analyses (see Statistical methods) for experiment II

	$\chi^2$	P
Snout-vent length:		
Sibship	18.596	<0.0001
Egg size	53.739	<0.0001
Tail length:		
Developmental temperature	21.999	<0.0001
Sibship	13.462	<0.0005
Egg size	7.543	<0.01
Sprint speed:		
Tail length	8.329	<0.005
Snout-vent length	4.979	<0.05
Developmental temperature	4.930	<0.05





**Fig. 5.** Schematic representation of the relationships among egg size, developmental temperature, hatchling morphology and sprint speed. Figures are drawn to scale and based on the smallest and largest egg sizes used in this study (1.74 mm, 2.20 mm) and partial regression coefficients for covariates and fixed effects (see text) after controlling for variation among sibships in experiment I (a) and experiment II (b). In both experiments, egg size had positive effects on SVL and tail length and development at high temperature resulted in shorter tails (a,b). (a) At the modal stage of hatching in the field (experiment I, c. stage 21), greater tail lengths and total lengths resulted in faster sprint speeds. But among individuals that experienced the same temperature during development, hatchlings from larger eggs were slower than hatchlings from smaller eggs. (b) One stage past the modal stage of hatching (experiment II, c. stage 22), tail lengths and total lengths were greater overall. Among these later-staged larvae, greater SVLs and tail lengths (and hence total lengths) each increased sprint speed. But egg size did not influence speed after controlling for total length. Thus, larger eggs produced larger larvae with faster sprint speeds, relative to smaller eggs.

tionship (e.g. Brockelman 1975; Lloyd 1987; Winkler & Wallin 1987; Niewiarowski & Dunham 1994). Necessary conditions for the evolution of within- and among-clutch variance in maternal investment have also been explored (Kaplan & Cooper 1984; McGinley *et al.* 1987; Philippi & Seger 1989; Schultz 1991). The empirical study reported here and those reviewed above, suggest that maternal effects can depend on a variety of factors in the offspring environment, and that the magnitude and occasionally even the sign of these effects can change ontogenetically. Clearly the evolutionary significance of such interactions will depend on their strength and timing during development (Cowley & Atchley 1992), though the outcome of selection is especially difficult to predict when both genetic and maternal environmental effects are considered (Kirkpatrick & Lande 1989). Nevertheless, observations such as these indicate that greater emphasis should be placed on evaluating the functional consequences of different levels

of maternal investment per offspring across environments and ontogenetic stages.

### Acknowledgements

We thank F. J. Janzen, M. Mangel, P. C. Phillips, T. Price, D. Reznick, H. B. Shaffer, J. A. Stamps and R. J. Wassersug for helpful comments on various drafts of the manuscript. A. Jones provided valuable statistical advice, C. Smith assisted with videotaping and helped maintain the frogs, and L. S. Doyle, J. Kaplan, I. Kasman, S. Maley and P. Rajaraman provided assistance in the field. This research was supported by an M. J. Murdock Charitable Trust Grant of the Research Corporation, grants from the Explorers Club and Howard Hughes Medical Institute, and the Reed College Summer Research Fund. D.M.P. was supported by a NSF Predoctoral Fellowship and The Center for Population Biology (UC Davis) during later stages of this investigation.

## References

- Amundsen, T. & Stokland, J.N. (1990) Egg size and parental quality influence nestling growth in the shag. *Auk* **107**, 410–413.
- Arnold, S.J. (1983) Morphology, performance and fitness. *American Zoologist* **23**, 347–361.
- Arnold, S.J. & Bennett, A.F. (1988) Behavioural variation in natural populations. V. Morphological correlates of locomotion in the garter snake, (*Thamnophis radix*). *Biological Journal of the Linnean Society* **34**, 175–190.
- Bernardo, J. (1991) Manipulating egg size to study maternal effects on offspring traits. *Trends in Ecology and Evolution* **6**, 1–2.
- Berven, K.A. (1988) Factors affecting variation in reproductive traits within a population of wood frogs (*Rana sylvatica*). *Copeia* **1988**, 605–615.
- Berven, K.A. & Chadra, B.G. (1988) The relationship among egg size, density and food level on larval development in the wood frog (*Rana sylvatica*). *Oecologia* **75**, 67–72.
- Brockelman, W.Y. (1975) Competition, the fitness of offspring, and optimal clutch size. *American Naturalist* **109**, 677–699.
- Brown, R.M. & Taylor, D.H. (1994) Compensatory escape mode trade-offs between swimming performance and maneuvering behavior through larval ontogeny of the wood frog, *Rana sylvatica*. *Copeia* **1994**, 1–7.
- Capinera, J.L. (1979) Qualitative variation in plants and insects: effect of propagule size on ecological plasticity. *American Naturalist* **114**, 350–361.
- Christian, K.A. & Tracy, C.R. (1981) The effect of the thermal environment on the ability of hatchling Galapagos land iguanas to avoid predation during dispersal. *Oecologia* **49**, 218–223.
- Cowley, D.E. & Atchley, W.R. (1992) Quantitative genetic models for development, epigenetic selection, and phenotypic evolution. *Evolution* **46**, 495–518.
- Crump, M.L. (1984) Intraclutch egg size variability in *Hyla crucifer* (Anura: Hylidae). *Copeia* **1984**, 302–308.
- Crump, M.L. (1991) Choice of oviposition site and egg load assessment by a treefrog. *Herpetologica* **47**, 308–315.
- Crump, M.L. & Vaira, M. (1991) Vulnerability of *Pleurodema borelli* tadpoles to an avian predator: effect of body size and density. *Herpetologica* **47**, 316–321.
- Cummins, C.P. (1986) Temporal and spatial variation in eggs size and fecundity in *Rana temporaria*. *Journal of Animal Ecology* **55**, 303–316.
- Ebenman, B. (1992) Evolution in organisms that change their niches during the life cycle. *American Naturalist* **139**, 990–1021.
- Endler, J.A. (1986) *Natural Selection in the Wild*. Princeton University Press, Princeton, NJ.
- Falconer, D.S. (1989) *Introduction to Quantitative Genetics*, 3rd edn. John Wiley & Sons, Inc., New York.
- Garland, T., Jr (1985) Ontogenetic and individual variation in size, shape and speed in the Australian agamid lizard *Amphibolurus nuchalis*. *Journal of Zoology, London (A)* **297**, 425–439.
- 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.
- Hutchings, J.A. (1991) Fitness consequences of variation in egg size and food abundance in brook trout *Salvelinus fontinalis*. *Evolution* **45**, 1162–1168.
- Janzen, F.J. (1993) An experimental analysis of natural selection on body size of hatchling turtles. *Ecology* **74**, 332–341.
- Jayne, B.C. & Bennett, A.F. (1989) The effect of tail morphology on locomotor performance of snakes: a comparison of experimental and correlative investigations. *Journal of Experimental Zoology* **252**, 126–133.
- Jayne, B.C. & Bennett, A.F. (1990) Selection on locomotor performance capacity in a natural population of garter snakes. *Evolution* **44**, 1204–1229.
- Kaplan, R.H. (1979) Ontogenetic variation in 'ovum' size in two species of *Ambystoma*. *Copeia* **1979**, 348–350.
- Kaplan, R.H. (1985) Maternal influences on offspring development in the California newt, *Taricha torosa*. *Copeia* **1985**, 1028–1035.
- Kaplan, R.H. (1987) Developmental plasticity and maternal effects of reproductive characteristics in the frog, *Bombina orientalis*. *Oecologia* **71**, 273–279.
- Kaplan, R.H. (1989) Ovum size plasticity and maternal effects on the early development of the frog, *Bombina orientalis* Boulenger, in a field population in Korea. *Functional Ecology* **3**, 597–604.
- Kaplan, R.H. (1992) Greater maternal investment can decrease offspring survival in the frog *Bombina orientalis*. *Ecology* **73**, 280–288.
- Kaplan, R.H. & Cooper, W.S. (1984) The evolution of developmental plasticity in reproductive characteristics: an application of the 'adaptive coin-flipping' principle. *American Naturalist* **123**, 393–410.
- Kirkpatrick, M. & Lande, R. (1989) The evolution of maternal characters. *Evolution* **43**, 485–503.
- Lloyd, D.G. (1987) Selection of offspring size at independence and other size-versus-number strategies. *American Naturalist* **129**, 800–817.
- McGinley, M.A., Temme, D.H. & Geber, M.A. (1987) Parental investment in offspring in variable environments: theoretical and empirical considerations. *American Naturalist* **130**, 370–398.
- Marsh, E. (1986) Effects of egg size on offspring fitness and maternal fecundity in the orangethroat darter, *Etheostoma spectabile* (Pisces: Percidae). *Copeia* **1986**, 18–30.
- Marshall, C.J., Doyle, L.S. & Kaplan, R.H. (1990) Intraspecific and sex-specific oophagy in a salamander and a frog: reproductive convergence of *Taricha torosa* and *Bombina orientalis*. *Herpetologica* **46**, 395–399.
- Meffe, G.K. (1987) Embryo size variation in mosquitofish: optimality vs. plasticity in propagule size. *Copeia* **1987**, 762–768.
- Newman, R.A. (1987) Effects of density and predation on *Scaphiopus couchi* tadpoles in desert ponds. *Oecologia* **71**, 301–307.
- Newman, R.A. (1992) Adaptive plasticity in amphibian metamorphosis. *BioScience* **42**, 671–678.
- Niewiarowski, P.H. & Dunham, A.E. (1994) The evolution of reproductive effort in squamate reptiles: costs, trade-offs, and assumptions reconsidered. *Evolution* **48**, 137–145.
- Parichy, D.M. & Kaplan, R.H. (1992a) Maternal effects on offspring growth and development depend on environmental quality in the frog *Bombina orientalis*. *Oecologia* **91**, 579–586.
- Parichy, D.M. & Kaplan, R.H. (1992b) Developmental consequences of tail injury on larvae of the oriental fire-bellied toad, *Bombina orientalis*. *Copeia* **1992**, 129–137.
- Petranka, J.W., Sih, A., Kats, L.B. & Holomuzki, J.R. (1987) Stream drift, size-specific predation, and the evolution of ovum size in an amphibian. *Oecologia* **71**, 624–630.

- Philippi, T. & Seger, J. (1989) Hedging one's evolutionary bets, revisited. *Trends in Ecology and Evolution* **4**, 41–44.
- Phillips, P.C. & Kaplan, R.H. (1987) A personal computer-microscope interface for the analysis of size and shape. *Herpetologica* **43**, 384–385.
- Pough, F.H. (1989) Organismal performance and Darwinian fitness: approaches and interpretations. *Physiological Zoology* **62**, 199–236.
- Richards, S.J. & Bull, C.M. (1990) Size-limited predation on tadpoles of three Australian frogs. *Copeia* **1990**, 1041–1046.
- Rossiter, M.C. (1991) Maternal effects generate variation in life history: consequences of egg weight plasticity in the gypsy moth. *Functional Ecology* **5**, 386–393.
- SAS Institute Inc. (1989) *SAS/STAT User's Guide, Version 6*, 4th edn, vol. 1. SAS Institute, Inc., Cary, NC.
- SAS Institute Inc. (1992) *SAS Technical Report P-229, SAS/STAT Software: changes and enhancements*, Release 6.07. SAS Institute, Inc., Cary, NC.
- Scheiner, S.M. (1993) Genetics and evolution of phenotypic plasticity. *Annual Review of Ecology and Systematics* **24**, 35–68.
- Schultz, D.L. (1991) Parental investment in temporally varying environments. *Evolutionary Ecology* **5**, 415–427.
- Searle, S.R., Casella, G. & McCulloch, C.E. (1992) *Variance Components*. John Wiley & Sons, Inc., New York.
- Selcer, K.W. (1990) Egg-size relationships in a lizard with fixed clutch size: variation in a population of the Mediterranean gecko. *Herpetologica* **46**, 15–21.
- Semlitsch, R.D. & Gibbons, J.W. (1990) Effects of egg size on success of larval salamanders in complex aquatic environments. *Ecology* **71**, 1789–1795.
- Shaffer, H.B., Austin, C.C. & Huey, R.B. (1991) The consequences of metamorphosis on salamander (*Ambystoma*) locomotor performance. *Physiological Zoology* **64**, 212–231.
- Sih, A. & Moore, R.D. (1993) Delayed hatching of salamander eggs in response to enhanced larval predation risk. *American Naturalist* **142**, 947–960.
- Sinervo, B. (1990) The evolution of maternal investment in lizards: an experimental and comparative analysis of egg size and its effects on offspring performance. *Evolution* **44**, 279–294.
- Sinervo, B. & Huey, R.B. (1990) Allometric engineering: an experimental test of the causes of interpopulational differences in performance. *Science* **248**, 1106–1109.
- Sinervo, B., Doughty, P., Huey, R.B. & Zamudio, K. (1992) Allometric engineering: a causal analysis of natural selection on offspring size. *Science* **258**, 1927–1930.
- Smith, C.C. & Fretwell, S.D. (1974) The optimal balance between size and number of offspring. *American Naturalist* **108**, 499–506.
- Snell, H.L., Jennings, R.D., Snell, H.M. & Harcourt, S. (1988) Intrapopulation variation in predator-avoidance performance of Galápagos lava lizards: the interaction of sexual and natural selection. *Evolutionary Ecology* **2**, 353–369.
- Sokal, R.R. & Rohlf, F.J. (1981) *Biometry*, 2nd edn. W. H. Freeman & Company, New York.
- Sultan, S.E. (1992) Phenotypic plasticity and the Neo-Darwinian legacy. *Evolutionary Trends in Plants* **6**, 61–71.
- Swain, D.P. (1992a) The functional basis of natural selection for vertebral traits of larvae in the stickleback *Gasterosteus aculeatus*. *Evolution* **46**, 987–997.
- Swain, D.P. (1992b) Selective predation for vertebral phenotype in *Gasterosteus aculeatus*: reversal in the direction of selection at different larval sizes. *Evolution* **46**, 998–1013.
- Tessier, A.J. & Consolatti, N.L. (1989) Variation in offspring size in *Daphnia* and consequences for individual fitness. *Oikos* **56**, 269–276.
- Venable, D.L. & Brown, J.S. (1988) The selective interactions of dispersal, dormancy, and seed size as adaptations for reducing risk in variable environments. *American Naturalist* **131**, 360–384.
- Warkentin, K.M. (1995) Adaptive plasticity in hatching age: a response to predation risk trade-offs. *Proceedings of the National Academy of Sciences, USA* **92**, 3507–3510.
- Wassersug, R.J. (1989) Locomotion in amphibian larvae (or 'Why aren't tadpoles built like fishes?'). *American Zoologist* **29**, 65–84.
- Wassersug, R.J. & Sperry, D.G. (1977) The relationship of locomotion to differential predation on *Pseudacris triseriata* (Anura: Hylidae). *Ecology* **58**, 830–839.
- Weihs, D. (1989) Design features and mechanics of axial locomotion in fish. *American Zoologist* **29**, 151–160.
- West-Eberhard, M.J. (1989) Phenotypic plasticity and the origins of diversity. *Annual Review of Ecology and Systematics* **20**, 249–278.
- Wilbur, H.M. (1980) Complex life cycles. *Annual Review of Ecology and Systematics* **11**, 67–93.
- Williamson, I. & Bull, C.M. (1989) Life history variation in a population of the Australian frog *Ranidella signifera*: egg size and early development. *Copeia* **1989**, 349–356.
- Winkler, D.W. & Wallin, K. (1987) Offspring size and number: a life history model linking effort per offspring and total effort. *American Naturalist* **129**, 708–720.

Received 30 August 1994; revised 4 January 1995;  
accepted 6 January 1995