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EFFECT OF INCUBATION TEMPERATURE ON MORPHOLOGY, GROWTH, AND SURVIVAL OF JUVENILE *SCELOPORUS UNDULATUS*

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ABSTRACT: Incubation temperature affects a wide range of phenotypic traits of hatchling reptiles. The main objective of this research was to determine if such phenotypic traits persist long enough in the field to have an effect on fitness. Eggs of *Sceloporus undulatus* lizards were incubated at six temperature regimes, five constant and one fluctuating, with means ranging from 23–33 C. Hatchlings were measured and their subsequent morphology, growth, and survival were monitored for 7–9 months, one to two months before individuals reached adult size. Phenotypic traits of lizards that hatched at the field site were used for comparative purposes. Morphological traits persisted for 7–9 mo. In contrast, growth rates did not differ among incubation temperature treatments after individuals were released in the field. Overall, 29 (27%) of 107 individuals that were released survived to the spring following hatching, and individuals from eggs incubated at the lowest temperature had higher survival than individuals from all other groups. The phenotypes of lizards incubated at intermediate temperatures tended to be most similar to those of field hatched lizards. We rejected two predictions about phenotypic responses to incubation temperature. The first prediction was that extreme incubation temperatures would be associated with the most deviant phenotypes. Observed phenotypic responses to temperature were either linear or, only one extreme temperature produced a deviant phenotype. The second prediction was that hatchlings incubated at warm temperatures and that hatched early in the season would have higher survival in general and higher overwinter survival in particular than hatchlings incubated at cool temperatures and that hatched later in the season. The reverse was true; observed survival was greatest for hatchlings from the coolest incubation treatment that hatched last.

Key words: *Sceloporus*; Incubation Temperature; Growth; Survival

THE THERMAL CONDITIONS under which reptilian eggs are incubated affect many phenotypic traits of hatchlings including body size and shape, sex, locomotory performance, growth, thermal preference, and ability to escape predators (e.g., Burger, 1991; Shine and Harlow, 1996; Elphick and Shine, 1998; Rhen and Lang, 1999; Qualls and Andrews, 1999). For those reptiles with temperature-dependent sex determination, incubation temperature fixes an individual's sex for its lifetime (Janzen and Paukstis, 1991). In general, however, the ecological relevance of phenotypes that are induced by incubation temperature is virtually unknown. Several difficulties are associated with the projection of phenotypic traits exhibited by hatchlings in the laboratory to those individuals later in life and under natural conditions. First, hatchling phenotypes may be so transient that they have negligible effects on fitness. Second, incubation temperature has an important indirect effect on hatchling phenotype that is independent of temperature *per se*. Because eggs incubated at warm temperatures hatch before eggs incubated at cool temperatures, the environments that hatchlings encounter may differ considerably simply as a function of when hatching occurs.

To investigate the ecological relevance of incubation temperature on hatchlings of a squamate reptile, we incubated the eggs of the lizard *Sceloporus undulatus* under six temperature regimes and recorded the size, subsequent growth, and survival of the hatchlings. Individuals were maintained in the laboratory for about two weeks and then released at a field site where they were monitored through the following spring. We also recorded the size, growth, and survival of individuals that hatched naturally at the field site and

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compared these individuals, which presumably exhibited "normal" phenotypes, with the phenotypes of individuals hatched in the laboratory.

We tested two predictions. The first concerns optimal temperatures for development. Embryonic development of reptiles is successful only over a limited range of temperatures (Shine, 1983; Packard and Packard, 1988). Therefore, we predicted that extreme incubation temperatures would be associated with the most deviant phenotypes. We reasoned that either very high or very low incubation temperatures would be stressful and thus disrupt embryonic development. We selected a series of incubation temperatures based on previous studies on *Sceloporus* (Sexton and Marion, 1974; Overall, 1994); we assumed that the incubation temperatures associated with high mortality in these studies would also be stressful to surviving embryos.

The second prediction concerns the effect of hatching order on survival. Because of their "head start", individuals that hatch early because of incubation at high temperature may have a competitive advantage with regards to access to food and other resources (Fox, 1978; Ferguson et al., 1982; Ferguson and Fox, 1984). As the largest hatchlings, this treatment group may also benefit from enhanced locomotory performance relative to later hatchlings (Sinervo and Huey, 1990). Large hatchlings may also have a survival advantage during hibernation because they are relatively large and have had more time to build up energy stores (Shine, 1983). We therefore predicted that hatchlings incubated at warm temperatures would have higher survival in general and higher overwinter survival in particular than hatchlings incubated at cool temperatures.

MATERIALS AND METHODS

Collection and Husbandry of Gravid Females

Gravid female *Scelporus undulatus* (n = 14) were collected between 28 May and 25 June 1998, at two sites located between 700 and 780 m elevation near Blacksburg, Virginia. Females were housed individually in plastic tubs (46 × 24 × 20 cm) until oviposition. Each tub contained a substrate of moist soil, with flat slabs of wood for cover and basking sites. Light from windows provided a natural photoperiod. Tubs were illuminated by fluorescent Vitalites (0700–1800 h) and a 100 watt spot light at one end of the tub (0900–1800 h). The spot light established a temperature gradient for basking. Females were fed (crickets and wax worm larvae dusted with a 50:50 mixture of Pervinal vitamin-mineral supplement and calcium carbonate) and given water daily. Tubs were checked at least three times each day for eggs. After oviposition, females were released at the site of capture.

Oviposition occurred between 31 May and 29 June. One egg from each clutch was sampled to determine the developmental stage of the embryo (staged according to Dufaure and Hubert 1961). Some eggs were assigned to treatments in which eggs were sampled during development; the results of these observations will be reported elsewhere (Andrews, in preparation). The remaining eggs were assigned to six incubation temperature treatments such that all clutches had at least one egg assigned to each treatment.

Incubation Treatments

Controlled temperature chambers were used to produce the six experimental temperature treatments. Eggs in five treatments were incubated at a constant temperature of 23, 25, 28, 30, or 33 C. The range of constant temperatures was picked to fall within the range of temperature that supports development. *Sceloporus undulatus* embryos do not develop at temperatures of 17 C or lower (Andrews et al., 1997). Development occurs at temperatures as high as 35 C, although hatching success is relatively low (Sexton and Marion, 1974). Eggs in the sixth treatment were incubated at a fluctuating temperature regime: 8 hours at 23 C, 4 h when the temperature ramped linearly to 33 C, 8 hours at 33, and 4 hours when the temperature ramped linearly back to 23 C (overall mean of 28 C, designated as 28F

C). This latter treatment was used to determine if constant and fluctuating incubation temperatures are developmentally equivalent.

Eggs were placed individually in 65 ml glass jars containing moistened vermiculite which were sealed with plastic kitchen wrap and secured with rubber bands. The initial ratio of distilled water to dry vermiculite in the jars was 0.7:1.0, with a water potential of −200kPa (determined by thermocouple psychrometry). Because of the long incubation period of eggs at the two lower temperatures, vermiculite was replaced at monthly intervals to compensate for slight losses of water through evaporation from the jars. Eggs in all treatments took up comparable amounts of water, tripling or quadrupling their initial mass by the time of hatching.

Incubation Period and Morphology

The chambers were checked at least twice daily for hatchlings. We recorded the date of hatching, sex, mass (g), snout–vent length (SVL, mm), and tail length (TL, mm) of each hatchling, and gave each a unique toe clip for identification. Mass and SVL were also recorded on the day they were released.

Husbandry of Hatchlings

After hatching, individuals were placed under conditions similar to those described above for gravid females. Ten hatchlings were assigned to each cage based on the order in which they hatched. Hatchlings were fed (crickets, wax moth larvae, and flour beetle larvae dusted with the vitamin-mineral mix) and provided with water three times a day. Hatchlings were maintained under these conditions until they were about 2 weeks old (mean 14.5 days, range 9–21 days), and were then released.

Release and Recapture of Juveniles in the Field

One of the sites where females were captured was also used as the release site for juveniles. The site was a forest clearing about 1500 m^2 in area. The clearing, initially used as a log-deck, included a number of large piles of woody debris, stumps, and scattered small shrubs. It was bounded on two sides by a gravel road and on the other sides by forest.

Juveniles (n = 107) were released at the same debris pile at one-two week intervals. Release dates for the 33, 30, 28 and 28F, 25, and 23 C treatments were 27 July–21 August, 27 July–28 August, 6 August–4 September (both 28 and 28F), 26 August–27 September, and 14 September–9 October, respectively. The entire clearing and the periphery of the surrounding forest and road were searched weekly for juveniles from 3 August until 1 November. Juveniles were captured throughout the clearing and at the forest edge but were not seen in the forest proper until late October. Recaptured juveniles were identified, weighed, and measured (SVL) and then released where they were captured. Juveniles that had hatched on the site were also captured, measured, toe-clipped, and released. These individuals (n = 24) served as natural controls for the laboratory hatched individuals. To estimate overwinter survival and also to assess the extent of dispersal, the study area, surrounding forest, and road edges were searched 4 times between 10 April and 2 May 1999. Individuals were weighed, measured (SVL and TL), and released.

Because *Sceloporus* females often aggregate their nests in areas of high insolation (Blair, 1960; Rose, 1993; Andrews, unpublished data), the density of juveniles during our observations was probably not abnormally high. Moreover, juveniles were released at different times and about one-half of the juveniles were not seen again after release (see results).

Mean monthly temperatures during field observations were 22.3, 21.8, 20.0, and 12.6 C for July, August, September, and October, respectively. Rainfall during these months was 56, 116, 23, and 70 mm (climatic data from the Blacksburg Airport located 11 km from the field site at an elevation of 700 m).

Data Manipulation and Analysis

Preliminary analyses indicated that 1) the sex of hatchlings was not related to

their morphology or to their subsequent growth and survival and 2) the probability of recapture was not related to the morphology or growth of juveniles in the laboratory. These variables were therefore not considered in further analyses.

The effect of incubation treatment and clutch on morphological attributes was assessed with two-way ANOVA's and ANCOVA's. The effects of these two class variables on tail length at hatching were evaluated using the residuals of the overall regression of tail length on SVL. These effects on body condition (mass relative to SVL) at hatching, at release, and the last time individuals were captured in the field were evaluated using the residuals of the overall regression of $Mass^{0.33}$ on SVL. Mass was raised to the 0.33 power to adjust for the non-linear relationship between mass and length (Andrews, 1982).

Growth in the laboratory was evaluated as relative or specific growth rates (Andrews, 1982) to avoid confounding growth with body size *per se*. Growth in the laboratory was assessed as the difference between an individual's natural log transformed SVL (or mass) at the time of release and at hatching. Growth in the field was evaluated as the difference between the natural log transformed SVL (or mass) of each lizard at its last capture and at the time that it was released. The intervals of time were used as covariates in two-factor ANCOVA's with treatment and clutch as class variables.

Survival was based on the number of juveniles known to be alive 3 and 6 weeks after their release in 1998 and on 2 May 1999. Because the first juveniles were released 7 weeks before the last, survival to 2 May 1999 was adjusted to a common 8 mo interval. Survival over 8 mo was calculated as $[S_0^{1/T_0}]^8$ where S_0 was the proportion of individuals that survived over the period between the mean date of release for each treatment group and 2 May and T_0 was the length of the period in months between the mean date of release and 2 May.

Because few lizards were recaptured in spring 1999, some treatments were combined to ensure adequate sample sizes for statistical analyses. The particular combinations were based on results of analyses of the large data sets of 1998.

All statistical analyses were conducted with SAS software (SAS Institute, Inc., Vers. 6.12, 1997). Analyses of data collected in the laboratory were based on mean values for each clutch in each treatment. Analyses of data collected in the field (survival, morphological attributes and growth) were based on individual juveniles because the number of juveniles was so attenuated after release. ANCOVA's were conducted only if interaction terms were not significant ($P > 0.05$). A posteriori comparisons among means were made with Ryan-Eliot-Gabriel-Welsch (REGWQ) multiple range tests (ANOVA's) and probabilities from the SAS Least Square Means Procedure (ANCOVA's).

RESULTS

Overall, the effects of both incubation temperature and clutch explained a significant amount of variation in the phenotypes of the lizards in our experiments (Tables 1, 2). Because our major objective, however, was to assess the effects of incubation temperature on phenotypes, and because we did not know the relationships of field hatched juveniles, we do not consider clutch (maternal) effects in any detail (but see Discussion).

Incubation Periods and Egg and Hatchling Survival in the Laboratory

Eggs in the 23, 25, 28, 28F, 30, and 33 C treatments hatched after mean incubation periods of 90, 72, 52, 52, 43, 39 d, respectively, and embryos were at stages 27–29 at the time of oviposition. Mortality during incubation was low; of 119 eggs incubated, only 10 did not hatch (8.4% mortality). Mortality was distributed as 4, 1, 4, and 1 deaths in the 23, 25, 28, and 33 C treatments, respectively. The number of survivors (= number released) is given in Table 3. Mortality of hatchlings was negligible; only one died before release and one escaped in the laboratory and was not recovered.

TABLE 1.—Statistical tests on the effects of incubation temperature and clutch on morphology and growth of *Sceloporus undulatus* juveniles in the laboratory. SVL, Mass, and Tail Length were measured at hatching. Values used in the Tail Length analyses were the residuals of the regression of Tail Length on SVL. Values used in the condition analyses were the residuals of the regression of $Mass^{0.33}$ on SVL. Morphological analyses were two-way ANOVA's and growth analyses were two-factor ANCOVA's.

	Statistical test		
Variable	Treatment	Clutch	Overall model (covariate)
SVL	$F_{5,59} = 3.3$, $P = 0.012$	$F_{13,59} = 12.5$, $P < 0.001$	$F_{18,59} = 9.9$, $P < 0.001$
Mass	$F_{5,59} = 0.9$, $P = 0.485$	$F_{13,59} = 37.0$, $P < 0.001$	$F_{18,59} = 27.0$, $P < 0.001$
Tail length	$F_{5,59} = 17.9$, $P < 0.001$	$F_{13,59} = 4.1$, $P < 0.001$	$F_{18,59} = 8.2$, $P < 0.001$
Condition, at hatching	$F_{5,59} = 3.6$, $P = 0.006$	$F_{13,59} = 5.4$, $P < 0.001$	$F_{18,59} = 4.9$, $P < 0.001$
Condition, at release	$F_{5,58} = 6.4$, $P < 0.001$	$F_{13,58} = 3.0$, $P = 0.002$	$F_{18,58} = 4.0$, $P < 0.001$
Growth, SVL	$F_{5,58} = 4.9$, $P < 0.001$	$F_{13,58} = 2.4$, $P = 0.010$	$F_{19,58} = 6.7$, $P < 0.001$ ($F_{1,58} = 76.2$, $P < 0.001$)
Growth, mass	$F_{5,57} = 6.1$, $P < 0.001$	$F_{13,57} = 4.3$, $P < 0.001$	$F_{19,57} = 8.3$, $P < 0.001$ ($F_{1,57} = 65.6$, $P < 0.001$)

Estimation of Incubation Temperature in the Field

The nest temperature of field hatched individuals can be estimated from their mean length of incubation relative to the mean length of incubation for individuals in the laboratory whose eggs were incubated at known temperatures. Given the mean SVL of 27 mm and the mean date of first capture (28 August) of field hatched juveniles, a mean growth rate of about 0.4 mm/d (from that of laboratory hatched juveniles, see below), and an overall mean SVL of hatchlings of 24 mm, the mean date of hatching in the field would have been 8 August. The median date of oviposition was 13 June, giving a mean incubation period in the field of 56 days. In the laboratory, eggs incubated at 28 and 25 C hatched in 52 and 72 days, respectively. By extrapolation, the mean temperature of nests in the field was 27.4 C. This value matches the mean temperature of 27 C (range = 15–35 C) observed for a *S. undulatus* nest that was monitored at one of the collection sites from oviposition on 29 May 1998 until hatching 66 days later. Three eggs from this nest hatched and three eggs were destroyed by invertebrate predators.

Effects of Incubation Temperature on Morphology

Hatchlings from the two coldest treatments had shorter tails than hatchlings from the warmer treatments independent

TABLE 2.—Statistical tests on the effects of incubation temperature and clutch on morphology and growth of *Sceloporus undulatus* juveniles in the field (field hatched juveniles were not included in analyses because their clutch of origin was not known). Analyses for 1999 recaptures were based on four groups: 23 C and 25 C treatments combined, 28 C, 28F C, and 30 C treatments combined, and 33, and did not include clutch effects because only 29 of the original 107 hatchlings released were recaptured. Other statistical conventions as in Table 1.

	Statistical test		
Variable	Treatment	Clutch	Overall model (covariate)
Condition, last capture '98	$F_{5,33} = 3.3$, $P = 0.015$	$F_{13,33} = 2.4$, $P = 0.020$	$F_{18,33} = 2.5$, $P = 0.011$
Growth, SVL '98	$F_{5,32} = 0.7$, $P = 0.621$	$F_{13,32} = 1.1$, $P = 0.369$	$F_{19,32} = 9.0$, $P < 0.001$ ($F_{1,32} = 89.7$, $P < 0.001$)
Growth, mass '98	$F_{5,32} = 2.0$, $P = 0.099$	$F_{13,32} = 2.8$, $P = 0.008$	$F_{19,32} = 12.2$, $P < 0.001$ ($F_{1,32} = 75.7$, $P < 0.001$)
Tail length, '99			$F_{3,23} = 4.9$, $P = 0.009$
SVL, '99			$F_{3,25} = 6.7$, $P = 0.002$
Mass, '99			$F_{3,25} = 7.5$, $P < 0.001$
Condition, '99			$F_{3,25} = 0.7$, $P = 0.537$

TABLE 3.—Survival of individuals released in 1998 indexed by the numbers of individuals that were known to be present 21 and 42 days after release, and the following spring (2 May 1999). Survival to 2 May, 1999 is first shown as the unadjusted value and then as the value adjusted for the varying intervals between release and 2 May for the different treatment groups.

Incubation Trt. (C)	Released (*n*)	Survival to 21 day % (*n*)	Survival to 42 day % (*n*)	Survival to 2 May, 1999 % (*n*)
23	14	71.4 (10)	64.3 (9)	64.3, 59.7 (9)
25	17	35.3 (6)	29.4 (5)	29.4, 26.0 (5)
Field hatched	24	37.5 (9)	33.3 (8)	16.7, 16.6 (4)
28	14	28.6 (4)	14.3 (2)	14.3, 15.5 (2)
28F	18	44.4 (8)	27.8 (5)	22.2, 23.7 (4)
30	22	54.5 (12)	27.3 (6)	9.1, 10.8 (2)
33	22	54.5 (12)	22.7 (5)	18.2, 21.2 (4)

of SVL (Fig. 1A, Table 1). In spring 1999, juveniles from the two coldest treatments (23 and 25 C combined) still had shorter tails than hatchlings from the warmer treatments independently of SVL (Fig. 1B, Table 2). Field hatched juveniles had tail lengths similar to those from the 23–25 C incubation treatment.

Hatchlings from the cooler treatments had shorter SVL's than those from the warmer treatments (Fig. 1C, Table 1). Mass at hatching, however, did not differ among treatments; mean hatchling mass was 0.55 ± 0.075 g (Table 1). Laboratory hatched juveniles were slightly larger in size when they were released than field hatched juveniles at their first capture (P's < 0.05 for mass and SVL, REGWQ a posteriori tests, ANOVA's). Respective mean SVL's and masses were 30 versus 27 mm and 0.95 versus 0.65 g, respectively. At the time of their last capture in 1998, juveniles from all laboratory treatment groups and the field hatched juveniles had statistically indistinguishable SVL's and masses, even though body sizes decreased in order in which treatment groups were released (warmest to coldest). In spring 1999, however, variation in the body sizes of juveniles was significant (Table 2), with respective SVL's and masses for the 23/25, field hatched, 28/28F/30, and 33 C treatments of 37, 42, 42, and 47 mm and 1.8, 2.8, 2.9, and 4.0 g. Only the differences between the 33 C and all other treatments ($P < 0.05$, a posteriori REGWQ tests) were significant, however, probably because so few individuals were recaptured.

Body condition (essentially the ratio of mass to SVL) was related to incubation temperature at hatching and at release (Fig. 2A,B, Table 1), and when lizards were last captured in the field in 1998 (Fig. 2C, Table 2). The observed decrease in body condition with increasing incubation temperature is in accord with the nonsignificant treatment effect for mass and the increase in SVL with increasing incubation temperature. In contrast, body condition did not vary among incubation treatments in spring 1999 (Table 2). Field hatched juveniles had a similar body condition as laboratory hatched juveniles at this time (Fig. 2C).

Effect of Incubation Temperature on Growth

Growth was related to incubation temperature in the laboratory, but not after lizards were released in the field. In the laboratory, juveniles from the cooler treatments grew faster than juveniles from the warmest treatment in terms of SVL and mass (Fig. 3A,B, Table 1). After release in the field, growth was not related to incubation temperature (Table 2). Field hatched juveniles grew significantly faster than laboratory hatched juveniles from the 23/25 C and from the 33 C treatments ($P < 0.05$), but not faster than juveniles from the 28/28F C and 30 C treatments ($P > 0.05$, REGWQ a posteriori tests).

Effect of Incubation Temperature on Survival

Because the clearing was surrounded by habitat judged unsuitable for juveniles, we assume that the majority of disappearances

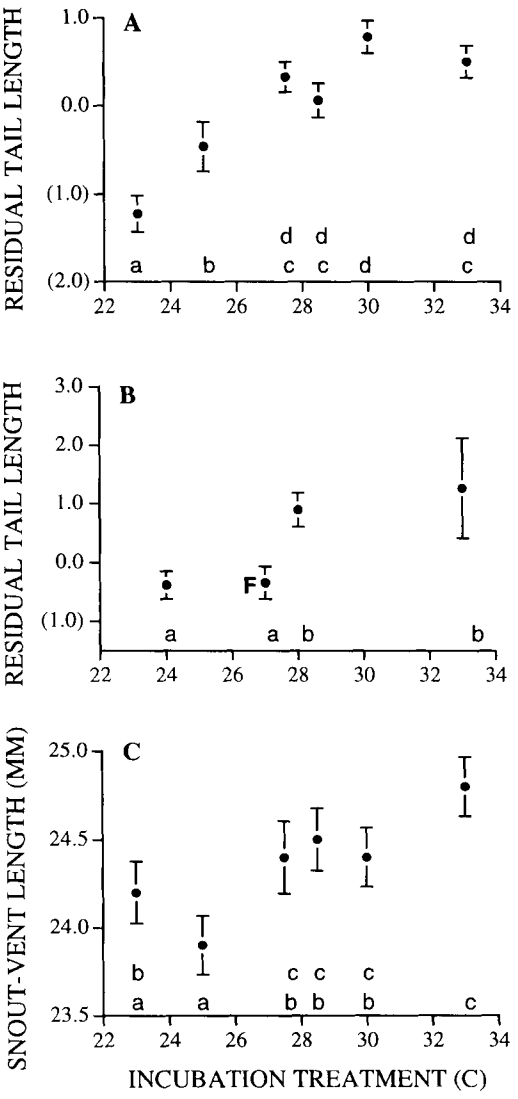

FIG. 1.—Effects of incubation treatment on tail and snout–vent length of hatchling and juvenile *Sceloporus undulatus*. Statistical results are presented in Tables 1 and 2. Means with the same letter do not differ ($P > 0.05$). Respective means for the 28 C treatment and the 28F C treatment are plotted at 27.5 and 28.5 C for clarity. **A.** Residual tail length at hatching. **B.** Residual tail length in April and May 1999 when juveniles were 6–9 mo old (23–25, 28–28F, and 30–33 treatments combined). The mean for field hatched juveniles (**F**) is plotted at 27 C. **C.** SVL at hatching.

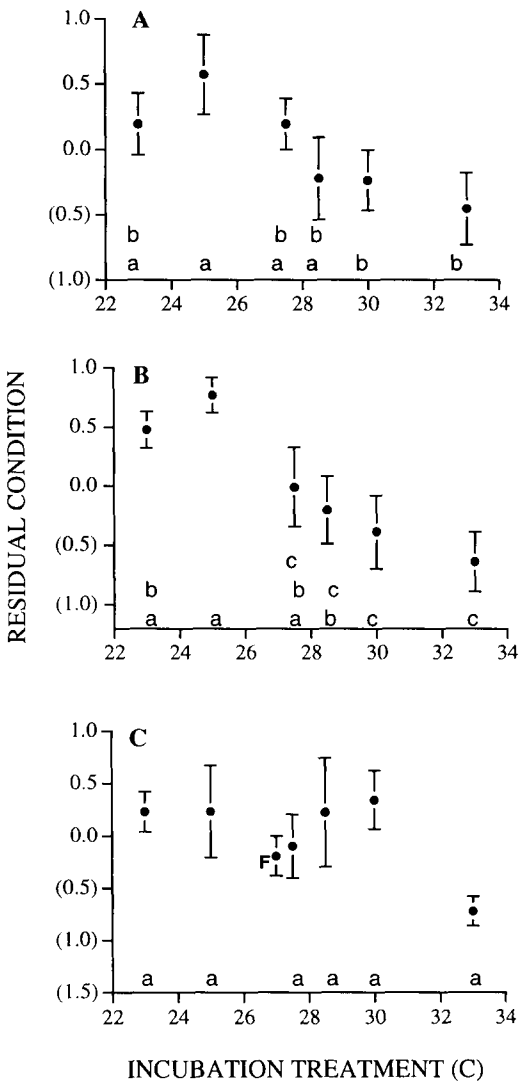

FIG. 2.—Effects of incubation treatment on body condition of hatchling and juvenile *Sceloporus undulatus*. **A.** Residual condition ($Mass^{0.33}$ relative to SVL) at hatching. **B.** Residual condition at the time of release (lizards were about 2 weeks old). **C.** Residual condition at last capture in 1998. The mean for field hatched juveniles (**F**) is plotted at 27 C for comparative purposes but was not included in the statistical analysis. Other conventions as in Fig. 1.

from the site represented deaths rather than emigration. High mortality of hatchling *Sceloporus* is typical (Blair, 1960; Jones and Ballinger, 1987; Niewiarowski and Roosenburg, 1993), and in our study, we can rule out mortality as an artifact of laboratory rearing; the survival of juveniles

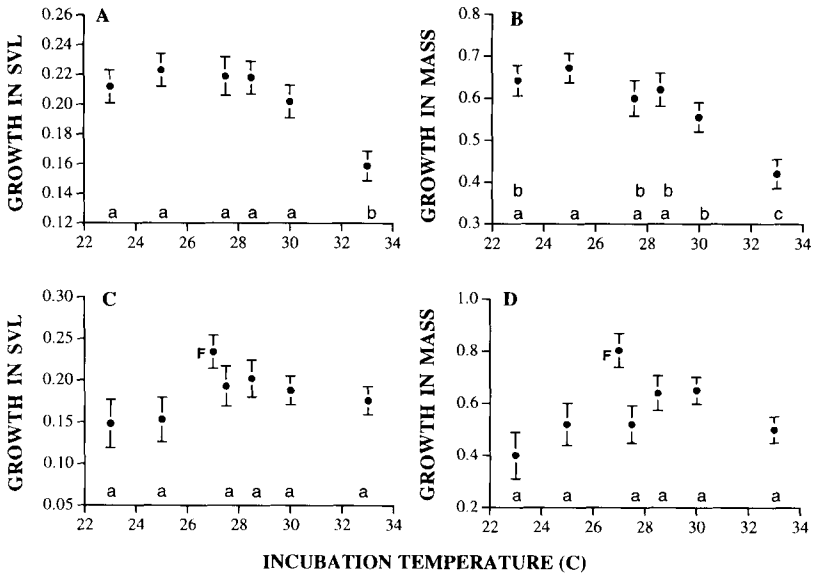

FIG. 3.—Effects of incubation treatment on growth of juvenile *Sceloporus undulatus*. **A.** Specific growth rate in SVL (mm) in the laboratory over a mean 15 days interval between hatching and release. **B.** Specific growth rate in Mass (g) in the laboratory over a mean 15 days interval between hatching and release. **C.** Specific growth rate in SVL (mm) in the field over a mean 36 days interval between release and last capture in 1998. **D.** Specific growth rate in Mass (g) in the field over a mean 36 days interval between release and last capture in 1998. Other conventions as in Fig. 2.

that were hatched in the laboratory was similar to that of juveniles that hatched on the study site. However, extensive searches in April and May 1999 outside of the area searched in 1998 included captures of four marked individuals. They were from the 23, 23, 25, and 33 C temperature treatments, with respective distances of 175, 60, 80, and 60 m from the release point. These individuals were included in the estimates of survival presented below.

The recapture rate of laboratory hatched juveniles was similar to that of field hatched juveniles. Of the 107 laboratory hatched juveniles released, 52 (49%), 32 (30%), and 26 (24%) were known to be alive 3 and 6 weeks after release, and the following spring, respectively (Table 3). Of the 24 juveniles hatched in the field, these respective numbers were 9 (38%), 8 (33%), and 4 (17%). The numbers of laboratory and field hatched juveniles that were survivors and non-survivors did not differ for any of the time periods (χ^2's < 0.5, P's > 0.05, df = 1, 2 $\times$ 2 chi-squared tests).

Survival of laboratory hatched juveniles did not differ among the six incubation treatments for the first 21 day after release ($\chi^2 = 7.6$, $P > 0.05$, df = 5). Survival did differ for the first 42 d after release and overwinter ($\chi^2 = 10.6$ and 17.3, respectively, df = 4, P's < 0.05, with the 28 and 28F C treatments combined to maintain minimum expected frequencies). Juveniles from the 23 C treatment had the highest survival for all time periods (Table 3). Survival during winter was high compared to the period when lizards were active. All juveniles from the 23, 25, and 28 C treatments that survived 42 days after release, survived overwinter, and survival of individuals from the remaining experimental groups was 63%. In general, recaptures appeared to be disproportionately represented among clutches. At the extremes, for example, two clutches from which six individuals each were released had no survivors in the spring, whereas, clutches from which 6 and 7 individuals each were released had 5 and 4 survivors.

Comparison Between the 28 C Constant and 28 C Fluctuating Treatments

The 28 C and the 28F C produced hatchlings that were similar for all the phenotypic attributes that were measured. Incubation periods for the two treatments were identical (52 days). Hatchlings from the two treatments had similar SVLs, masses, and TLs (P's > 0.05, REGWQ multiple range tests, Fig. 1A,C, Table 1). They did not differ in body condition at hatching nor at release (P's > 0.05, REGWQ multiple range tests, Fig. 2A,B), nor the spring after hatching ($P > 0.05$, REGWQ multiple range test, Fig. 2C). They did not differ in growth in the laboratory nor after release in the field (P's > 0.05, probabilities from SAS Least Squares means Procedures, Fig. 3). Finally, they did not differ in survival over 21 or 42 days, nor overwinter (2×2 χ^2 tests, P's $>>> 0.05$, Table 3).

Discussion

The major objective of this research was to determine if phenotypic traits induced by incubation temperature persist as juvenile reptiles grow towards adulthood under natural conditions. We found that some morphological features and the survival of juvenile *Sceloporus undulatus*, but not growth, were affected by incubation temperature for at least seven to nine months. Juveniles were within a month or two of sexual maturity at this time; two months later, we collected a gravid female from the 28F C treatment and a considerably larger female from the 30 C treatment. Long-term persistence of phenotypic traits induced by incubation temperature is in accord with laboratory observations on the Australian skink *Bassiana duperreyi* (Elphick and Shine, 1998). In Elphick and Shine's study, differences in several measures of locomotor performance for lizards that hatched from eggs incubated at a warm and at a cold temperature persisted for the entire 4.5 mo observation period. In contrast, initial differences in SVL, mass, mass relative to SVL, and TL of the skinks had disappeared by the time that the lizards were 2–3 mo old. These two relatively long-term studies indicate that temperature induced phenotypes can be persistent, and thus could affect fitness (see below).

We expected that phenotypes of laboratory hatched juveniles from the intermediate incubation temperatures would be most similar to those of field hatched juveniles. In general, this expectation was met; the phenotypes of laboratory hatched juveniles from the 28 C and 28F C treatments were most similar to those of field hatched juveniles. This result is in accord with our estimate of 27 C as the approximate mean incubation temperature for field hatched juveniles. The similarity of juvenile phenotypes from the 28 C and the 28F C treatments suggests that comparison between laboratory and field hatched juveniles is not confounded by effects of constant versus fluctuating temperature regimes. This particular result, however, may not be generalizable to other species or traits. For example, while Shine and Harlow (1996) found that the SVL and mass of *Bassiana duperreyi* skinks from eggs incubated at a constant temperature and a fluctuating temperature with the same mean did not differ, individuals from the constant temperature ran slower than individuals from eggs incubated at the fluctuating temperature.

We predicted that the phenotypic response to incubation temperature would be curvilinear, that is, the phenotypes of juveniles from the incubation temperature extremes would differ from the phenotypes of juveniles incubated at intermediate temperatures. This expectation was not met. Rather, the phenotypic response to temperature either appeared linear or only one extreme incubation temperature produced a distinctive phenotype. Relative tail length at hatching and at seven to nine months, and SVL at hatching increased and body condition decreased more or less linearly with increasing temperature. In contrast, juveniles from the highest incubation temperature were distinguished by relatively slow growth in the laboratory and juveniles from the lowest incubation temperature were distinguished by relatively high survival.

The lack of expected curvilinear response may be because eggs were not exposed to sufficiently extreme temperatures. If this is the case, higher or lower incubation temperatures would presumably produce more extreme phenotypes, and provide a better sense of how climatic change or range extension would affect fitness of hatchlings. On the other hand, the warmest and coolest mean temperatures we picked for laboratory incubation are unlikely to be more extreme than the mean temperatures that eggs of *Sceloporus undulatus* normally experience in the field. The length of incubation in the field suggests a mean nest temperature of about 27 C. Moreover, variation in mean temperature among nests of small reptile species tends to be low: *S. aeneus* (range = 16.2–16.9 C, n = 6 nests, Andrews, 1999); *Chrysemys picta* (range = 23.4–26.0 C, n = 12, Cagle et al., 1993). If eggs of *S. undulatus* are not normally exposed to mean temperatures as extreme as those used for the laboratory treatments, then even the range of phenotypic variation that we induced would not be commonly encountered in the field.

We also predicted that early hatchlings might have relatively high survival. This proved not to be the case. The juveniles from the 23 C incubation treatment that were released last had substantially higher survival than juveniles from all the other treatments during the first 21 days and the first 42 days after release. The high survival of juveniles from the 23 C incubation treatment may thus be related to the effects of incubation temperature *per se,* to relatively favorable physical or biological conditions late in the activity season, or both. For example, hatchlings were released from 27 July through 9 October, a sufficient span of time to encompass a decrease in the intensity of predation.

Given that incubation temperature has long-term effects on phenotypes, what are potential consequences of such phenotypic variation on individual fitness? Our results suggest that answering this question will not be easy. One difficulty is that the variation in phenotype associated with maternal effects is comparable to the variation associated with incubation temperature (this study; Shine et al., 1997; Packard, 1999). Observations on the long-term consequences of incubation temperature on fitness must thus additionally consider the direct effects of the maternal environment (genotype, nutrition status of female) as well as possible interactions between maternal effects and incubation temperature.

A more serious difficulty is that the association between a phenotype induced by incubation temperature and fitness is not necessarily direct or obvious (Travis et al., 1999). For example, hatching early in the season has many putative benefits (Elphick and Shine, 1998). In our study, however, lizards that hatched late in 1998, also had the highest survival from hatching to the following spring of all the treatment groups despite their relatively small size compared to earlier hatching individuals. Locomotor performance provides another example of the difficulty translating phenotypic traits into measures of fitness. Running speed is often related to incubation temperature, but speed may increase (Elphick and Shine, 1998) or decrease with incubation temperature (Van Damme et al., 1992; Qualls and Andrews, 1999). Moreover, speed *per se* has no necessary relationship to fitness. Escape behavior is complex, and the frequent stops and starts that reduce overall speed, exhibited by hatchling *Sceloporus* (Qualls and Andrews, 1999), could be more effective for the avoidance of predators than continuous high speed (Brodie, 1992; Schwarzkopf and Shine, 1992).

To be ecologically meaningful, an assessment of the phenotypic consequences of incubation conditions must be accompanied by an assessment of how phenotypes are related to fitness under natural conditions. Such an assessment has its own inherit problems. One of these is that physical and demographic environments vary from year to year (Dunham, 1980; Sinervo and DeNardo, 1996; Sinervo et al., 1992) and the fitness or lack thereof of particular phenotypes in one year therefore may not reflect fitness the following year or years. In view of these considerations, the ecological and evolutionary rel-

evance of the phenotypic variation associated with incubation conditions presents a complex and challenging problem for future research.

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