

THE ADAPTIVE SIGNIFICANCE OF TEMPERATURE-DEPENDENT SEX DETERMINATION: EXPERIMENTAL TESTS WITH A SHORT-LIVED LIZARD

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Abstract.—Why is the sex of many reptiles determined by the temperatures that these animals experience during embryogenesis, rather than by their genes? The Charnov-Bull model suggests that temperature-dependent sex determination (TSD) can enhance maternal fitness relative to genotypic sex determination (GSD) if offspring traits affect fitness differently for sons versus daughters and nest temperatures either determine or predict those offspring traits. Although potential pathways for such effects have attracted much speculation, empirical tests largely have been precluded by logistical constraints (i.e., long life spans and late maturation of most TSD reptiles). We experimentally tested four differential fitness models within the Charnov-Bull framework, using a short-lived, early-maturing Australian lizard (*Amphibolurus muricatus*) with TSD. Eggs from wild-caught females were incubated at a range of thermal regimes, and the resultant hatchlings raised in large outdoor enclosures. We applied an aromatase inhibitor to half the eggs to override thermal effects on sex determination, thus decoupling sex and incubation temperature. Based on relationships between incubation temperatures, hatching dates, morphology, growth, and survival of hatchlings in their first season, we were able to reject three of the four differential fitness models. First, matching offspring sex to egg size was not plausible because the relationship between egg (offspring) size and fitness was similar in the two sexes. Second, sex differences in optimal incubation temperatures were not evident, because (1) although incubation temperature influenced offspring phenotypes and growth, it did so in similar ways in sons versus daughters, and (2) the relationship between phenotypic traits and fitness was similar in the two sexes, at least during preadult life. We were unable to reject a fourth model, in which TSD enhances offspring fitness by generating seasonal shifts in offspring sex ratio: that is, TSD allows overproduction of daughters (the sex likely to benefit most from early hatching) early in the nesting season. In keeping with this model, hatching early in the season massively enhanced body size at the beginning of the first winter, albeit with a significant decline in probability of survival. Thus, the timing of hatching is likely to influence reproductive success in this short-lived, early maturing species; and this effect may well differ between the sexes.

Key words.—*Amphibolurus muricatus*, aromatase inhibitor, Charnov-Bull model, environmental sex determination, jacky dragon, phenotypes, survival, timing of hatching.

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Theory predicts that primary sex ratios evolve toward unity, whereby parents expend equal effort to produce sons and to produce daughters (Fisher 1930). Thus, sex-determining mechanisms that produce a balanced sex ratio should be favored by selection. Indeed, most organisms possess some form of genetically based sex-determining system that yields a 1:1 offspring sex ratio due to random segregation of sex chromosomes. However, there are numerous exceptions to this generalization, with many organisms possessing sex-determining mechanisms that do not yield an even sex ratio (Janzen and Paukstis 1991; McCabe and Dunn 1997; Voor-douw and Anhold 2002; Cook 2002). For example, species with environmental sex determination (ESD) often exhibit highly skewed sex ratios depending upon the environmental conditions that prevail during larval or embryonic development (Bull 1983). Because such sex-ratio biases among early life-history stages can strongly influence adult sex ratios, ESD can have significant consequences on the demographics of populations (Bulmer and Bull 1982). For example, highly biased sex ratios may be detrimental to some populations (Bulmer and Bull 1982). Why, then, does ESD occur in such a wide diversity of organisms?

Theoretical answers to this paradox abound, but few have been subject to rigorous experimental tests. The most widely-accepted model for the adaptive significance of ESD is that of Charnov and Bull (1977), who proposed that selection should favor a linkage between sex determination and some

environmental factor if that factor has a differential impact on the fitness of sons versus daughters. For example, suppose that incubation at high temperatures produces an offspring phenotype that is optimal for males but suboptimal for females, whereas low-temperature incubation generates “good” daughters but “poor” sons. Then selection should favor ESD whereby embryos that experience high incubation temperatures develop into sons, whereas those exposed to cooler conditions develop into daughters. Genes that had this effect would confer higher fitness than the alternative genetic sex-determination (GSD) system.

Many reptiles exhibit temperature-dependent sex determination (TSD) (Valenzuela and Lance 2004), with sex determined by the temperature that embryos experience during development. Intriguingly, TSD and GSD can co-occur among closely related species (Ewert and Nelson 1991; Harlow 2004). However, the adaptive significance of TSD remains unclear for reptiles, as it does for most phylogenetic lineages of organisms. The Charnov-Bull model is by far the most widely supported in published literature, but this acceptance reflects its overall plausibility—especially, consistency of its assumptions with known aspects of reptile biology—rather than specific experimental evidence (Gutzke and Crews 1988; Janzen 1995; Freedberg et al. 2004). Attempts to empirically test the Charnov-Bull model have been impeded by at least three factors:

(1) A diverse array of potential mechanisms: incubation

TABLE 1. Summary of the four differential fitness hypotheses tested for the adaptive significance of temperature-dependent sex determination (TSD).

Hypothesis	Role of incubation temperature	Prediction if adaptive	Examples
A. Different optimal egg size for sons vs. daughters	Enables mother to adjust clutch sex ratios via nest-site selection.	Sons and daughters produced from different-sized eggs, and sex-specific relationship between egg size and fitness.	Roosenburg 1996
B. Different phenotypic optima for sons vs. daughters	Induces changes to phenotype independent of sex.	Incubation temperature affects phenotypes, and the phenotypic determinants of fitness are sex specific.	Rhen and Lang 1995
C. Different norms of reaction for sons vs. daughters	Influences phenotypes and/or survival of hatchlings, but differently in sons and daughters.	Significant interaction between incubation temperature and sex on fitness-related phenotypes.	Shine et al. 1995
D. Different optimal hatching times for sons vs. daughters	Induces variation in time of hatching and seasonal variation in sex ratio.	Seasonal variation in offspring sex ratio production, and sex-specific relationship between time of hatching and fitness.	Conover 1984

temperature may differentially affect fitness in males and females via multiple, complex pathways (reviewed by Shine 1999), posing a substantial challenge to comprehensive empirical analysis. For example, TSD may enhance maternal fitness if (A) egg size (i.e., offspring size) has sex-specific fitness consequences (Roosenburg 1996); (B) incubation temperature influences phenotypes of offspring independent of sex, but the phenotypic determinants of fitness differ between males and females; (C) incubation temperature differentially modifies fitness-related phenotypes (and hence fitness) of male and female offspring (Shine et al. 1995; Elphick and Shine 1999); or (D) incubation temperature influences the timing of hatching, allowing offspring sex to be matched with the optimal seasonal time for hatching (Conover 1984). Any attempt to test the Charnov-Bull approach thus necessarily must examine a wide range of variables.

(2) Experimental design: To identify the sex-specific effects of incubation temperature, we need to produce offspring of both sexes at all incubation temperatures; and if incubation temperature determines sex, this is obviously a problem. Fortunately, the problem can be overcome by applying estradiol or aromatase inhibitors to eggs. With these manipulations, female offspring can be produced at male-producing temperatures, and males can be produced at female-producing temperatures (Crews et al. 1994; Rhen and Lang 1995), thus decoupling the otherwise confounded effects of sex and incubation temperature on phenotypic traits of hatchlings.

(3) Most TSD reptile species are poorly suited to fitness studies: Most reptile species known to have TSD are crocodylians and turtles, and measuring reproductive success (fitness) of these late-maturing, long-lived reptiles is extremely difficult, if not impossible. Furthermore, the sex of hatchling crocodylians and turtles is difficult to determine without sacrificing the animal, thereby precluding subsequent measurements of reproductive success. Again, however, a solution is available: recent studies by Harlow and co-workers have described TSD in many short-lived, early maturing Australian lizard species (Harlow and Shine 1999; Harlow and Taylor 2000; Harlow 2000, 2004).

We used an Australian agamid lizard, the jacky dragon

(*Amphibolurus muricatus*), to experimentally test predictions from the Charnov-Bull model described above. Jacky dragons are well suited for studies of the adaptive significance of TSD because: (1) they are abundant in coastal heathlands of south-eastern Australia; (2) they are relatively short lived and can mature within 12 months after hatching, facilitating the measurement of lifetime reproductive success; (3) females are highly fecund (up to four clutches of 3–9 eggs per season), facilitating experimental design; (4) the sex of hatchlings is easily and nondestructively identified by manually everting hemipenes on males (Harlow 1996); (5) adult jacky dragons are sexually dimorphic in traits such as adult head size and behavior, suggesting that the relationship between phenotype and fitness differs between the sexes (at least in adult life); (6) they lack sex chromosomes (Witten 1983) and have temperature-dependent sex determination, whereby relatively low and high incubation temperatures produce females and intermediate temperatures produce varying proportions of both sexes (Harlow and Taylor 2000); and (7) their sister taxon (*A. norrisi*) has genotypic sex determination (GSD) (Harlow 2004): The evolutionary lability in the mode of sex determination within this clade thus encourages the search for an adaptive significance.

Hypotheses to Be Tested

Table 1 summarizes the four hypotheses that we attempt to test in the present study. Two of these four hypotheses assume that the relationship between incubation temperature and morphology (egg size in one case, hatchling traits in another) is the same for sons versus daughters, but that the optimum values of these traits for fitness differ between the sexes. In the first hypothesis (different optimal egg sizes for sons vs. daughters), TSD is favored because it enables the mother to adjust the sex of her offspring to the egg size she has produced (in reptiles, among-clutch variance in egg size is much greater than within-clutch variance in this trait; Sinnero 1990). In the second hypothesis (different phenotypic optima for sons vs. daughters), incubation temperature directly affects phenotypic traits of the hatchlings, and does so

in the same way for sons and daughters. However, if the optimal values for these phenotypic traits differ between the sexes, then TSD can enhance maternal fitness by producing whichever sex is best advantaged by the phenotypic traits arising from those incubation conditions. Under the third hypothesis (different norms of reaction for sons vs. daughters), incubation temperature modifies fitness-related phenotypes (and hence fitness) differentially in male and female offspring. Thus, TSD enhances maternal fitness by ensuring that the embryo develops as the sex best suited to those incubation conditions. Lastly, the fourth hypothesis (different optimal hatching times for sons vs. daughters) relies on a covariation between incubation temperature and time of hatching. This covariation can arise in two (not mutually exclusive) ways. First, higher temperatures accelerate embryogenesis and thus hasten hatching (Andrews et al. 2000). Second, soil (nest) temperatures vary seasonally in many habitats, so that TSD might enable a seasonal shift in offspring sex ratios. Such a shift might enhance maternal fitness if, for example, one sex benefited more than the other from hatching early in the season (Conover 1984).

In all of these hypotheses, the putative differential fitness effects could be manifested at any age, from survival rates of embryos through to mating success or egg viability of adults. In the present paper, we focus on fitness-related traits (body size, growth, survival) measured during the early (embryo, hatchling, juvenile) stages of life history. Our experiments are continuing, and data on adult fitness will be presented in later papers.

MATERIALS AND METHODS

Collection and Maintenance of Gravid Females

Gravid jacky dragons were collected in the Sydney area from 27 September to 29 December 2003. After capture, females were housed in semi-natural outdoor enclosures (2 × 2 m) at Macquarie University (in Sydney, and thus within the natural range of *A. muricatus*). Lizards were fed (crickets and roaches dusted in vitamin/mineral mix) three times per week with ad libitum water.

Females were checked daily for signs of oviposition (loose folds of lateral and ventral skin). Oviposition occurred from 21 October 2003 to 9 February 2004. Eggs were brought back to the laboratory, weighed, and randomly assigned to six incubation treatments in a 2 × 3 factorial experimental design (see below).

Experimental Design

All eggs ($N = 221$, from 41 clutches) were incubated individually in glass jars (125 ml) and buried completely under vermiculite at a water potential of -200 kPa. To determine the effects of incubation temperature on hatchling sex and fitness-related phenotypes, eggs were placed in incubators set at three different thermal regimes: (1) a low female-producing temperature (23°C), (2) an intermediate temperature that produces an even sex ratio (27°C), and (3) a high female-producing temperature (33°C) (Harlow and Taylor 2000). All incubators were programmed to fluctuate $\pm 5^\circ\text{C}$ throughout a given day to simulate daily temperature fluctuations within

natural nests (Harlow and Taylor 2000). Jars were rotated within the incubators twice per week to minimize potential effects of thermal gradients within each incubator.

To examine the sex-specific effects of incubation temperature on offspring, we produced male offspring at each incubation temperature by applying Fadrozole (Ciba-Geigy CGS016949A, kindly provided by Novartis Pharmaceuticals AG, Basel, Switzerland) to half the eggs within each thermal treatment. Fadrozole is an aromatase inhibitor that blocks the conversion of androgens testosterone to estrogens estradiol, thus resulting in male offspring even at female-producing temperatures (Crews et al. 1994). We sex-reversed hatchlings in this way to decouple the effects of incubation temperature and sex (Rhen and Lang 1995). Thirty micrograms of Fadrozole (dissolved in 2.5 μl of 100% ethanol) was applied to each egg. The Fadrozole/ethanol solution was applied topically (directly on the vascularized surface) with a pipette 15% through the total incubation period (the critical sex-determining period; Bull 1987). Because incubation length decreases with temperature, the day at which Fadrozole was applied differed among treatments (day 6 at 33°C, day 8 at 27°C, and day 15 at 23°C). The remaining eggs within each temperature treatment were used as controls by applying 100% ethanol (2.5 μl to each egg) at the same time Fadrozole was applied to the experimental groups.

Hatchling Husbandry and Measurements

Immediately after eggs hatched, we measured several morphological traits of the hatchlings. We recorded hatching date and identified the sex of each individual by manual eversion of hemipenes (Harlow 1996). Hatchlings were weighed, measured, and marked uniquely for individual identification. Measurements of each individual included snout-vent length (SVL), tail length (TL), jaw length, head width, head depth, and the total lengths of all four limbs. Head sizes (jaw length, jaw width, and head depth) were scored as described by Harlow and Taylor (2000). Limb measurements were taken by restraining lizards and fully extending their limbs, using digital calipers to measure from the junction of the thigh (or upper arm) and body to the junction of the fourth and fifth toes.

Hatchlings were briefly housed in the laboratory in enclosures (30 cm tall × 36 cm wide × 50 cm long) with sand substrate, small branches for perching and basking, and a shelter for hiding. Enclosures were illuminated by 40-watt fluorescent reptile bulbs (Exo-Terra, Hagen Inc., Montreal, Canada), with 60-watt incandescent bulbs as a heat source. Room temperature was maintained at 25°C, and lights turned on at 0800 and turned off at 1800 h. Hatchlings were grouped together as they hatched so that each group contained five or fewer individuals. Hatchlings were provided with water and food (crickets dusted with vitamin/mineral mix) daily.

We measured locomotor performance of four- to five-day-old hatchlings on an electronically timed racetrack, in a room kept at 30°C. Hatchlings were given 30 min to acclimate to room temperature prior to each trial. The racetrack (1 m long) contained five infrared photocells at 25-cm intervals, connected to an electronic stopwatch that recorded times for each interval. Hatchlings were placed at one end of the racetrack

TABLE 2. The effect of Fadrozole on hatchling traits and survival. Naturally produced males were compared with sex-reversed males from the 27°C incubation treatment (analysis of covariance). Interaction terms between the main effect and the covariate were removed when these interactions were not significant. Variables were log-transformed when necessary. The effect of Fadrozole on hatchling survival was determined with a chi-squared test. SVL, snout-vent length.

Trait	Covariate	Fadrozole effect
Snout-vent length (mm)	egg mass	$F_{1,42} = 1.31, P = 0.258$
Body mass (g)	egg mass	$F_{1,42} = 0.23, P = 0.632$
Body mass (g)	SVL	$F_{1,43} = 1.39, P = 0.225$
Tail length (mm)	SVL	$F_{1,43} = 1.34, P = 0.254$
Running speed over 1 m (m/sec)		
at 0 degrees	SVL	$F_{1,41} = 0.00, P = 0.966$
at 15 degrees	SVL	$F_{1,41} = 0.32, P = 0.574$
at 30 degrees	SVL	$F_{1,40} = 0.16, P = 0.695$
at 45 degrees	SVL	$F_{1,40} = 0.53, P = 0.471$
Running speed over 25 cm (m/sec)		
at 0 degrees	SVL	$F_{1,41} = 2.26, P = 0.141$
at 15 degrees	SVL	$F_{1,41} = 0.95, P = 0.336$
at 30 degrees	SVL	$F_{1,41} = 0.00, P = 0.964$
at 45 degrees	SVL	$F_{1,41} = 0.62, P = 0.436$
Growth in SVL (in enclosures)	SVL	$F_{1,28} = 0.00, P = 0.986$
Growth in mass (in enclosures)	mass	$F_{1,28} = 0.35, P = 0.561$
Survival	—	$\chi^2 = 0.15, P = 0.699$

and encouraged to run by gently prodding them with a paintbrush. During each trial, we recorded times over each 25-cm interval, and the number of times hatchlings paused over the 1-m distance.

Because jacky dragons are semiariboreal, we also measured their locomotor performance at four slopes of 0-, 15-, 30-, and 45-degree angles. Each individual was tested four times at each slope (total of 16 trials each), with 2–3 min rest between trials within slopes, and a 1-h rest between trials at different slopes. The order in which lizards were run at each slope was randomized, within the restriction that all possible sequences (total of 24 orders) were represented within each incubation treatment. Locomotor performance for each slope was evaluated as the fastest speed (m/sec) over a 25-cm interval and fastest speed over the entire 1-m distance.

At an average age of six days ($SD = 2.3$), hatchlings were remeasured (mass, SVL, TL) and released into large outdoor enclosures (4×8 m, with metal walls 90 cm high) at Macquarie University, all within a mesh-covered aviary to eliminate bird predation. Each treatment and each clutch were equally represented in six replicate enclosures (30–32 hatchlings per enclosure). Each enclosure was subdivided into eight 2×2 -m sections, with the interior walls perforated by holes (58 mm diameter) such that all lizards had access to the entire 4×8 -m area, but had the option of remaining out of visual contact with other lizards.

All enclosures mimicked the natural habitat of jacky dragons, with sand substrate and branches for perching and basking. Dense vegetation, rocks, and logs provided shelter. Water was available ad libitum, and crickets (dusted in vitamin/mineral mix) were provided three times weekly to each enclosure. Hatchlings also took natural prey items that fell into the enclosures.

We attempted to recapture hatchlings at monthly intervals from January to June, to obtain data on growth and survival. Recaptured lizards were weighed and measured (SVL, TL, jaw length, head width, and head depth). Dead hatchlings

were identified (when possible) and preserved. We conducted a thorough search for hatchlings in June and August 2004 (winter) by overturning all logs, rocks, and vegetation within the enclosures. Hatchlings not found during these final recapture efforts were presumed dead.

Statistical Analyses

All data were checked for normality and homogeneity of variances. When needed, data were log-transformed to meet the assumptions of parametric analyses. Data were analyzed using SAS software (SAS Institute 1997) or JMP (ver. 5; SAS Institute 2002).

Chi-squared tests were used to evaluate the effect of incubation temperature on hatching success. A small sample of eggs ($n = 13$) that were laid on the soil surface (and thus, desiccated prior to discovery) were removed from our analyses of hatching success. Chi-squared tests were also used to evaluate the effects of incubation temperature and Fadrozole on hatchling sex ratios; sex ratios were compared among treatments and also tested against the null expectation of a 1:1 sex ratio.

To determine whether the application of Fadrozole on eggs affected hatchling phenotypic traits other than sex, we compared traits of naturally produced males from the 27°C control treatment to traits of sex-reversed males from the 27°C-Fadrozole treatment using analysis of covariance (ANCOVA). Based on results from these tests, we could not reject the null model that Fadrozole had no effect on hatchlings other than sex-reversal (Table 2). Therefore, independent variables for further analyses comprised sex, incubation temperature, and their interaction.

To evaluate sex-specific effects of incubation temperature on hatchling phenotypes, we used mixed-model ANOVAs or ANCOVAs using sex, temperature, and their interaction as fixed effects and clutch as a random effect. Incubation length was defined as days between oviposition and hatching. We

included initial egg mass as a covariate in comparisons of body size (mass and SVL), and included hatchling SVL as a covariate in analyses of tail, head, and limb sizes. The absolute value of the difference between the lengths of right and left limbs was used as an index of fluctuating asymmetry in limb length. To quantify body condition of hatchlings, we used residual scores from the general linear regression of \ln mass on \ln SVL. We analyzed locomotor performance in two ways; (1) fastest speed over a 1-m distance and (2) fastest speed over a 25-cm interval; in both cases, we included hatchling SVL as a covariate. The number of stops a hatchling made over a 1-m distance was evaluated by Kruskal-Wallis tests because these data did not meet the assumptions of parametric testing, even after transformation. We tested the effects of sex, incubation temperature, and their interaction on locomotor performance separately at each slope. Growth rate in the laboratory was evaluated as the size increment from hatching to release, divided by the number of days between measurements.

Growth rate in the outdoor enclosures was analyzed only for individuals that were recaptured at least once prior to winter. Because offspring growth rates were influenced by hatchling size at release, the interval between release and recapture (days), and date of hatching, we included all three of these factors in our analyses of growth rate. All body size measurements were natural-log-transformed to linearize relationships. Hatchling size at release (SVL and mass) and the interval (days) between release and final recapture were used as independent variables in a multiple regression with body size at final capture (SVL or mass) as the dependent variable. Residual scores from this multiple regression provided an index of growth rate. We used ANCOVA to determine the effects of enclosure, incubation temperature, sex, and their interactions (clutch was included as a random effect) on offspring growth rate (residual scores) with the date of hatching (Julian day) as a covariate.

The effects of enclosure, incubation temperature, and sex on hatchling survival (up to winter) were analyzed with chi-squared tests. We used multiple logistic regression to evaluate relationships between hatchling phenotypes and survival, including interactions among incubation temperature, sex, egg mass, and oviposition date. These analyses allowed us to compare logistic curves among incubation treatments and between sexes while adjusting for variation in egg size and oviposition date. We did not analyze rates of overwinter survival because only one individual died during this time period.

RESULTS

Egg Survival, Incubation Period, and Offspring Sex Ratios

Overall hatching success was 96% and did not differ among incubation treatments ($\chi^2 = 5.7$, $df = 5$, $P = 0.337$). The duration of incubation was influenced by incubation temperature but not by sex or their interaction (Table 3). Eggs that experienced cool incubation temperatures hatched much later (5–7 weeks) than did eggs from the intermediate and warm incubation temperatures (Fig. 1A). Incubation periods at the warm and intermediate temperatures also differed, but only by about 15 days. These thermal effects on incubation

duration thus influenced the date of hatching, even after adjusting for variation in oviposition date (Fig. 1B).

Incubation temperature also strongly influenced sex ratios ($\chi^2 = 36.1$, $df = 2$, $P < 0.001$; Fig. 2A). Eggs kept at the cool and warm temperatures produced 100% females, whereas those incubated at an intermediate temperature (27°C) produced an even sex ratio ($\chi^2 = 0.1$, $df = 1$, $P = 0.793$). The Fadrozole-treated eggs produced nearly 100% male offspring at all three incubation temperatures (Fig. 2B).

Hatchling Phenotypes

Incubation temperature influenced hatchling morphology, but did not differentially affect males and females (Table 3). Hatchlings from the warm incubation treatment were smaller (in mass and SVL), and had lower body condition than did hatchlings from the other treatments (Fig. 3A, B, C). Cool-incubated hatchlings had shorter tails relative to SVL than did hatchlings from the other treatments (Fig. 3D). In addition, the date of oviposition explained a small, but significant, amount of the variation in egg size ($r^2 = 0.02$, $P = 0.039$) and hatchling size ($r^2 = 0.02$, $P = 0.028$); eggs (and the resultant hatchlings) produced late in the season were larger than those produced early in the season.

Incubation temperature did not affect hatchling head measures, nor did it differentially affect male and female hatchlings in this respect (Table 3). However, males had deeper heads than females (Table 3). Limb length was generally not affected by incubation temperature, sex, or their interactions. The degree of asymmetry in hind limb length was greater in hatchlings from the warm incubation treatment than in hatchlings from cool incubation (Table 3); fluctuating asymmetry in limb length was not influenced by sex or the sex-by-temperature interaction.

Locomotor speeds of hatchlings were not influenced by incubation temperature and did not differ between males and females (Table 3). This pattern was consistent at all four slopes, as running speeds at each slope were tightly correlated (all $P < 0.001$). As expected, steeper slopes reduced running speeds. Hatchlings from the cool incubation treatment stopped less often (mean = 2.7 stops) over the 1-m distance than did hatchlings from the intermediate (mean = 3.2 stops) and warm (mean = 3.4 stops) incubation treatments (Kruskal-Wallis: $\chi^2 = 7.8$, $df = 2$, $P = 0.020$). Males and females did not differ in the number of stops made during the running trials (Kruskal-Wallis: $\chi^2 = 0.1$, $df = 1$, $P = 0.743$), with no significant interactions between sex and incubation temperature for any locomotor analyses.

Hatchling Growth

Over an average of six days (SD = 2.3) in the laboratory, hatchling growth rate was influenced by prior incubation temperature but did not differ between males and females (Table 3). Hatchlings from cool incubation grew more slowly than did individuals from intermediate or warm incubation, although this pattern was no longer significant if date of hatching was included as a covariate ($F_{2,137} = 2.0$, $P = 0.138$). Incubation temperature differentially affected SVL growth of male and female offspring (incubation temperature-by-sex interaction); sons and daughters from the 23°C and 27°C

TABLE 3. The effects of incubation temperature, sex, and their interaction on hatchling phenotypes. Mixed model ANOVAs or ANCOVAs were used with temperature and sex as fixed effects, and clutch as a random effect. When necessary, dependent variables were log-transformed to meet parametric assumptions. SVL, snout-vent length. The last four columns indicate the four differential fitness hypotheses that are supported (✓), rejected (×), or not impacted (—) by each statistical test. The four hypotheses state that there is a (A) different optimal egg size for sons versus daughters, (B) different phenotypic optima for sons versus daughters, (C) different norm of reaction for sons versus daughters, and (D) different optimal hatching time for sons versus daughters. Details of each hypothesis are in Table 1. Bold type indicates significance.

Trait	Covariate	Incubation effect	Sex effect	Interaction				Statistical test supports, rejects, or has no impact on hypothesis:					
				A	B	C	D	A	B	C	D		
Egg mass (g)	—	$F_{2,147} = 0.7, P = 0.521$	$F_{1,147} = 0.3, P = 0.613$	$F_{2,147} = 0.1, P = 0.868$	×	—	—	—	—	—	—	—	—
Incubation duration (days)	—	$F_{2,145} = 10.94, P < 0.001$	$F_{1,145} = 0.3, P = 0.589$	$F_{2,145} = 0.5, P = 0.595$	—	—	—	—	—	—	—	—	✓
Snout-vent length (mm)	egg mass	$F_{2,141} = 14.9, P < 0.001$	$F_{1,141} = 2.8, P = 0.097$	$F_{2,141} = 2.0, P = 0.140$	—	—	×	—	—	—	—	—	—
Body mass (g)	egg mass	$F_{2,141} = 7.5, P = 0.001$	$F_{1,141} = 0.4, P = 0.530$	$F_{2,141} = 0.2, P = 0.749$	—	—	×	—	—	—	—	—	—
Tail length (mm)	SVL	$F_{2,143} = 13.3, P < 0.001$	$F_{1,143} = 0.2, P = 0.667$	$F_{2,143} = 2.0, P = 0.140$	—	—	×	—	—	—	—	—	—
Body condition (residuals)	—	$F_{2,145} = 7.7, P = 0.001$	$F_{1,145} = 3.0, P = 0.086$	$F_{2,145} = 2.4, P = 0.093$	—	—	×	—	—	—	—	—	—
Jaw length (mm)	SVL	$F_{2,144} = 1.1, P = 0.324$	$F_{1,144} = 0.3, P = 0.562$	$F_{2,144} = 1.2, P = 0.307$	—	—	×	—	—	—	—	—	—
Head width (mm)	SVL	$F_{2,144} = 0.6, P = 0.561$	$F_{1,144} = 0.1, P = 0.724$	$F_{2,144} = 1.4, P = 0.240$	—	—	×	—	—	—	—	—	—
Head depth (mm)	SVL	$F_{2,144} = 1.9, P = 0.161$	$F_{1,144} = 0.5, P = 0.025$	$F_{2,144} = 1.0, P = 0.376$	—	—	×	—	—	—	—	—	—
Fluctuating asymmetry	—	—	—	—	—	—	—	—	—	—	—	—	—
Fore limbs (mm)	—	$F_{2,126} = 1.0, P = 0.383$	$F_{1,126} = 0.4, P = 0.543$	$F_{2,126} = 1.5, P = 0.218$	—	—	×	—	—	—	—	—	—
Hind limbs (mm)	—	$F_{2,125} = 3.4, P = 0.037$	$F_{1,125} = 0.2, P = 0.658$	$F_{2,125} = 0.3, P = 0.737$	—	—	✓	—	—	—	—	—	—
Running speed, 1 m (m/sec)	—	—	—	—	—	—	—	—	—	—	—	—	—
at 0 degrees	SVL	$F_{2,139} = 2.0, P = 0.136$	$F_{1,139} = 0.1, P = 0.709$	$F_{2,139} = 0.2, P = 0.807$	—	—	×	—	—	—	—	—	—
at 15 degrees	SVL	$F_{2,138} = 0.2, P = 0.851$	$F_{1,138} = 0.7, P = 0.391$	$F_{2,138} = 1.7, P = 0.188$	—	—	×	—	—	—	—	—	—
at 30 degrees	SVL	$F_{2,133} = 1.6, P = 0.201$	$F_{1,133} = 0.2, P = 0.660$	$F_{2,133} = 2.1, P = 0.133$	—	—	×	—	—	—	—	—	—
at 45 degrees	SVL	$F_{2,136} = 2.6, P = 0.081$	$F_{1,136} = 0.0, P = 0.950$	$F_{2,136} = 0.7, P = 0.488$	—	—	×	—	—	—	—	—	—
Running speed, 25 cm (m/sec)	—	—	—	—	—	—	—	—	—	—	—	—	—
at 0 degrees	SVL	$F_{2,139} = 1.3, P = 0.265$	$F_{1,139} = 3.7, P = 0.055$	$F_{2,139} = 1.3, P = 0.285$	—	—	×	—	—	—	—	—	—
at 15 degrees	SVL	$F_{2,138} = 0.6, P = 0.545$	$F_{1,139} = 0.5, P = 0.502$	$F_{2,138} = 1.4, P = 0.254$	—	—	×	—	—	—	—	—	—
at 30 degrees	SVL	$F_{2,135} = 1.6, P = 0.209$	$F_{1,135} = 0.2, P = 0.701$	$F_{2,135} = 2.5, P = 0.087$	—	—	×	—	—	—	—	—	—
at 45 degrees	SVL	$F_{2,139} = 0.4, P = 0.677$	$F_{1,139} = 0.1, P = 0.801$	$F_{2,139} = 1.3, P = 0.293$	—	—	×	—	—	—	—	—	—
Growth in laboratory	—	—	—	—	—	—	—	—	—	—	—	—	—
in SVL (Δ SVL/days)	SVL	$F_{2,137} = 3.1, P = 0.047$	$F_{1,137} = 0.3, P = 0.586$	$F_{2,137} = 3.4, P = 0.036$	—	—	✓	—	—	—	—	—	—
in mass (Δ mass/days)	mass	$F_{2,137} = 7.0, P = 0.001$	$F_{1,137} = 2.3, P = 0.133$	$F_{2,137} = 0.6, P = 0.560$	—	—	✓	—	—	—	—	—	—

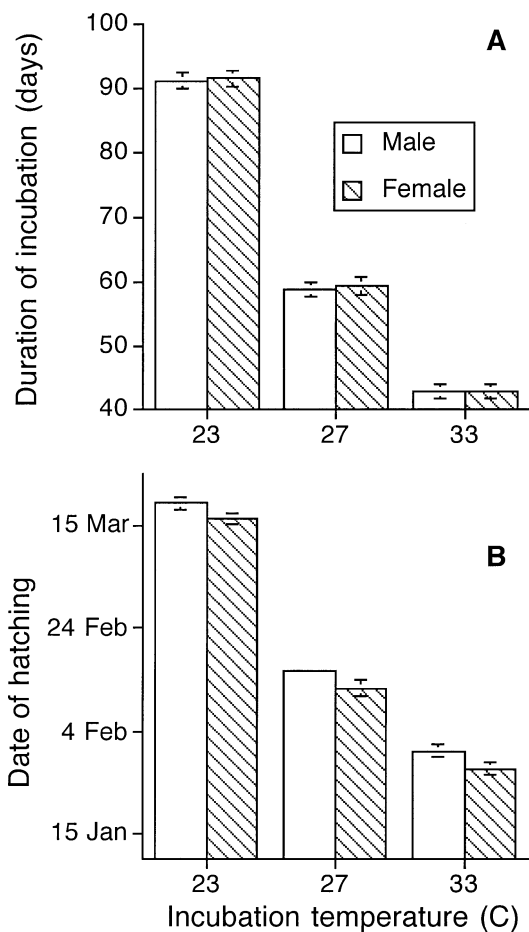


FIG. 1. The effect of incubation temperature on the timing of hatching. (A) Duration of incubation for male and female offspring. All three incubation treatments differed from each other ($F_{2,145} = 10942.8$, $P < 0.001$). (B) Date of hatching for male and female offspring. All three treatments differed from each other ($F_{2,145} = 8074.2$, $P < 0.001$). Date of hatching is adjusted for oviposition date (least-squares means are reported). Error bars represent 3 SE.

incubation treatments grew at similar rates, but daughters from the 33°C incubation treatment grew more slowly than their brothers. Because growth was measured over a short time period (about six days) and this interaction was not significant for growth rate in mass, the biological significance of this interaction is questionable. Nevertheless, the effect of incubation temperature on growth mimicked that found in the outdoor enclosures (see below).

In the outdoor enclosures, individuals that hatched relatively late in the season grew more slowly than did earlier-hatching conspecifics (Fig. 4A). Because temperature influenced incubation duration (and subsequently hatching date), this relationship between hatching date and hatchling growth was strongly driven by incubation temperature (growth in SVL: $F_{2,76} = 20.8$, $P < 0.001$; growth in mass: $F_{2,75} = 9.3$, $P < 0.001$; Fig. 4B); that is, relatively cool incubation temperatures produced slow-growing individuals via an effect on the timing of hatching. In support of this interpretation, incubation temperature had no apparent direct effect on hatchling growth rate when date of hatching was included as a covariate in the analysis ($F_{2,74} = 0.2$, $P = 0.813$). Hatchling

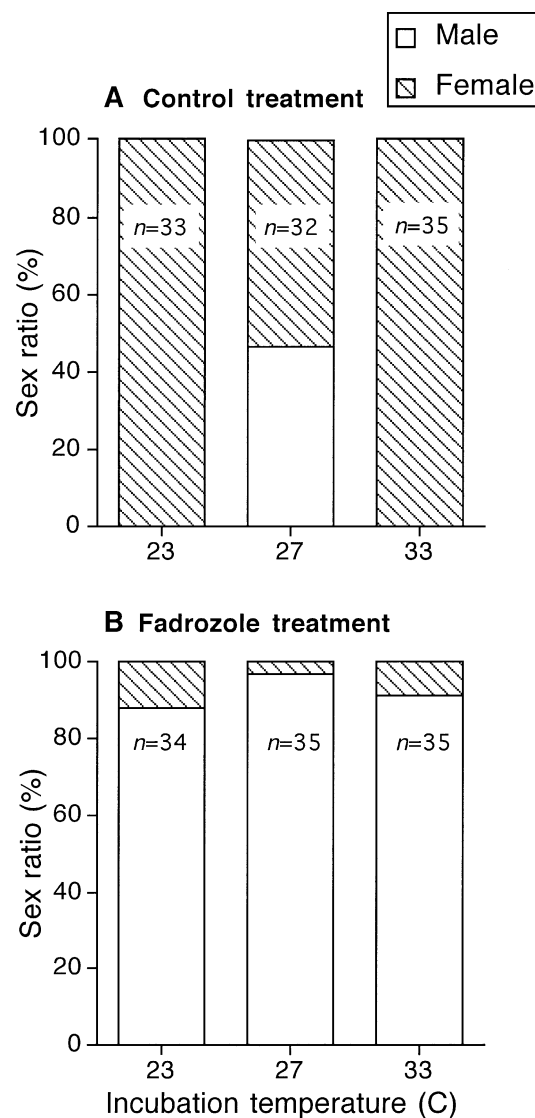


FIG. 2. The effect of incubation treatment on offspring sex ratios. (A) Sex ratios for control treatments at each incubation temperature. (B) Sex ratios for Fadrozole-treated offspring at each incubation temperature.

sex, the enclosures in which hatchlings were released, and their interaction had no significant effects on growth rate (all $P > 0.106$).

Incubation temperature indirectly influenced hatchling body size prior to winter via its effect on growth and the timing of hatching. Because incubation temperature influenced hatching date and growth rate, the size of offspring prior to winter was also strongly affected by incubation temperature (Fig. 4C). Thus, by the end of their first growing season, individuals from the warm and intermediate incubation treatments were much larger than their siblings from the cool incubation treatment ($F_{2,132} = 30.0$, $P < 0.001$; Fig. 4D).

Hatchling Survival

Hatchling survival in the laboratory was high (96%), and did not differ among treatments ($\chi^2 = 5.6$, $df = 5$, $P = 0.345$).

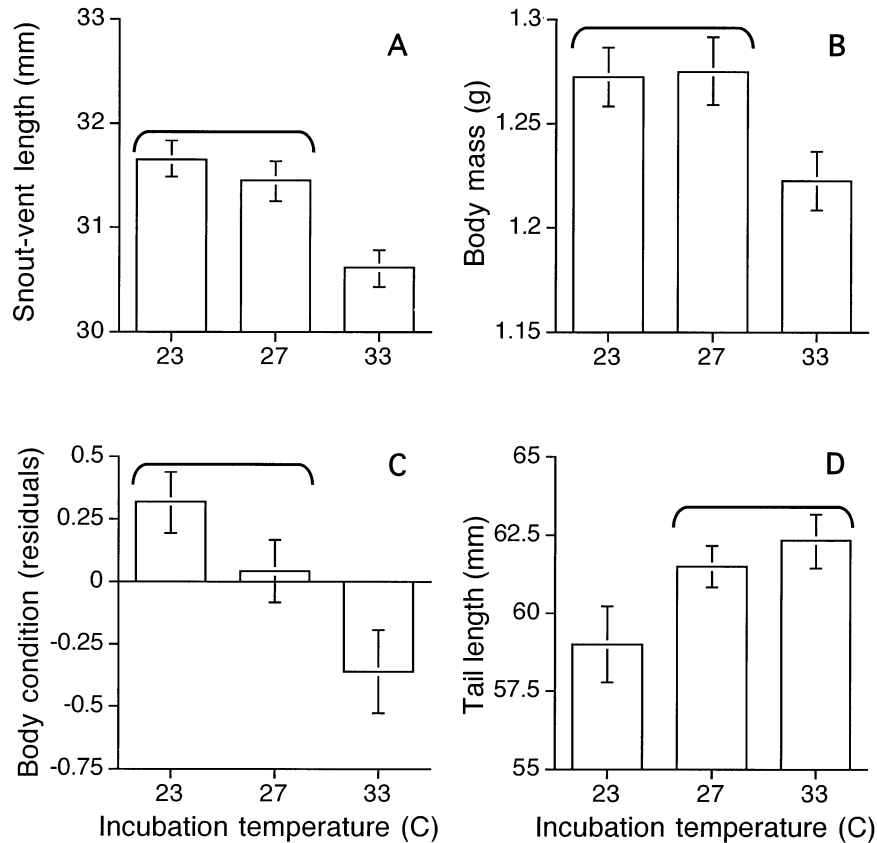


FIG. 3. Contrasts of offspring phenotypes from three incubation temperature regimes. (A) Snout-vent length versus incubation temperature. (B) Body mass versus incubation temperature. (C) Body condition versus incubation temperature. (D) Tail length versus incubation temperature. Least-square means are reported for snout-vent length, mass, and tail length. Brackets above bars group incubation treatments that are not significantly different from one another. Error bars represent 1 SE.

However, overall survival (in the laboratory and in outdoor enclosures combined) was influenced by incubation temperature ($\chi^2 = 6.7$, $df = 2$, $P = 0.036$) and differed marginally between male and female hatchlings ($\chi^2 = 3.3$, $df = 1$, $P =$

0.070; Fig. 5); analyses based on logistic regression provided similar results (see Table 4). For both sexes, individuals from the warm incubation treatment had lower prewinter survival than did their siblings from the cool and intermediate incu-

TABLE 4. Associations of incubation temperature, offspring sex, oviposition date, egg size, snout-vent length, running speed, and their interactions with hatchling survival prior to winter. Analyses were carried out with multiple logistic regression. The last four columns indicate the four differential fitness hypotheses that are supported (\checkmark), rejected (\times), or not impacted ($-$) by each statistical test. The four hypotheses state that there is a (A) different optimal egg size for sons versus daughters, (B) different phenotypic optima for sons versus daughters, (C) different norm of reaction for sons versus daughters, and (D) different optimal hatching time for sons versus daughters. Details of each hypothesis are in Table 1. We found no statistically significant patterns.

Dependent variables	Effect on hatchling survival	Statistical test supports, rejects, or has no impact on hypothesis:			
		A	B	C	D
Incubation temperature	$\chi^2 = 5.37$, $P = 0.068$	—	—	—	?
Oviposition date	$\chi^2 = 3.44$, $P = 0.063$	—	—	—	\checkmark
Egg mass (g)	$\chi^2 = 0.00$, $P = 0.980$	\times	—	—	—
Hatchling sex	$\chi^2 = 3.42$, $P = 0.064$	—	\checkmark	—	—
Snout-vent length (mm)	$\chi^2 = 0.32$, $P = 0.571$	—	—	—	—
Running speed (m/sec over 1 m)	$\chi^2 = 0.38$, $P = 0.537$	—	—	—	—
Incubation temperature \times hatchling sex	$\chi^2 = 0.01$, $P = 0.996$	—	—	\times	—
Oviposition date \times incubation temperature	$\chi^2 = 0.64$, $P = 0.726$	—	?	—	—
Oviposition date \times hatchling sex	$\chi^2 = 0.15$, $P = 0.696$	—	\times	—	—
Egg mass \times incubation temperature	$\chi^2 = 1.86$, $P = 0.394$	\times	—	—	—
Egg mass \times hatchling sex	$\chi^2 = 0.05$, $P = 0.830$	\times	—	—	—
Snout-vent length \times sex	$\chi^2 = 2.47$, $P = 0.116$	—	\times	—	—
Running speed \times sex	$\chi^2 = 1.05$, $P = 0.305$	—	\times	—	—

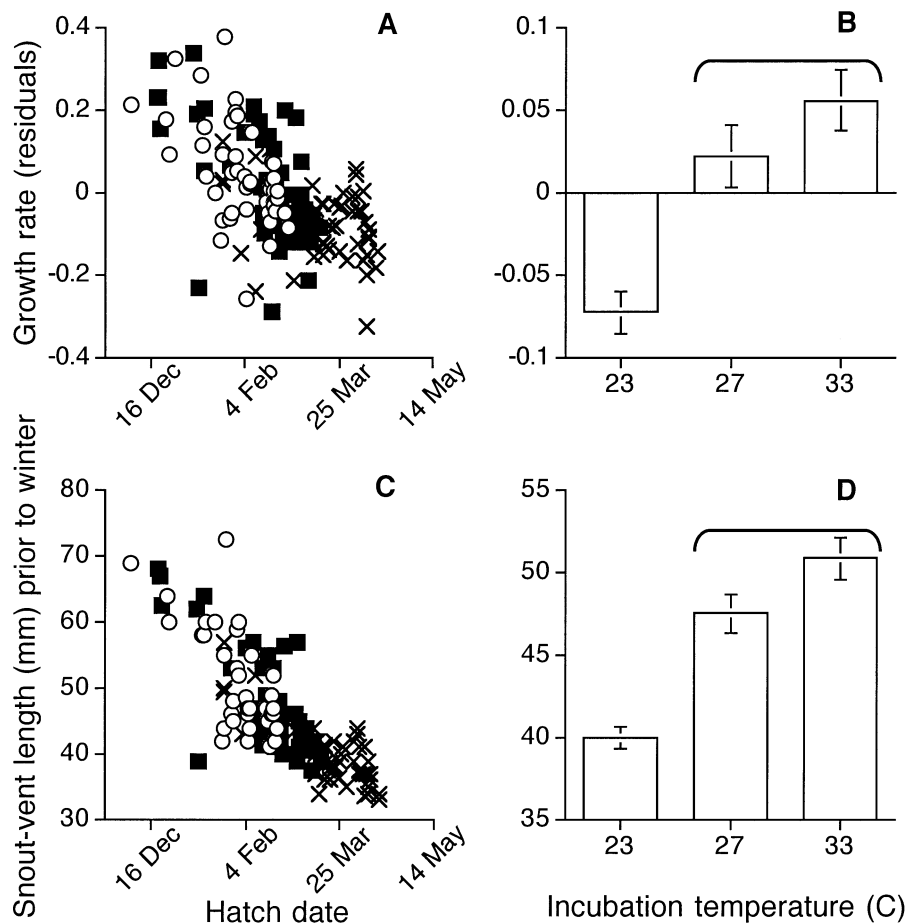


FIG. 4. Effect of hatching date and incubation temperature on hatchling growth rate and body size prior to winter in outdoor enclosures. (A) Relationship between hatching date and growth rate in snout-vent length ($r^2 = 0.381$, $P < 0.001$). (B) Effect of incubation temperature on growth rate ($F_{2,75} = 9.3$, $P < 0.001$). (C) Relationship between hatching date and snout-vent length prior to winter ($r^2 = 0.659$, $P < 0.001$). (D) Effect of incubation temperature on snout-vent length prior to winter ($F_{2,132} = 30.0$, $P < 0.001$). Symbols for left panel: ○, warm incubation treatment (33°C); ■, intermediate incubation treatment (27°C); ×, cool incubation treatment (23°C). Brackets above bars group incubation treatments that are not significantly different from one another. Error bars represent 1 SE.

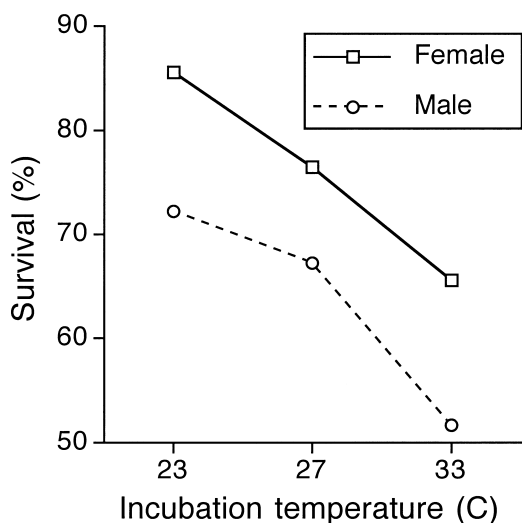


FIG. 5. Effect of incubation temperature and sex on hatchling survival up to winter.

Incubation temperature did not differentially influence male and female survival ($\chi^2 = 0.2$, $df = 2$, $P = 0.916$). Incubation temperature did not influence survival after adjusting for variation in oviposition date and egg size (incubation temperature \times oviposition date interaction; Table 4). The effect of egg mass on hatchling survival did not differ between males and females (egg mass \times sex interaction; Table 4).

The phenotypic traits of hatchlings that we measured were not related to the subsequent probability of survival of these animals (overall multiple logistic regression: $\chi^2 = 11.2$, $df = 8$, $P = 0.191$; Table 5), nor did the relationship between phenotypic traits and survival differ between male and female hatchlings (interactive effects of phenotypes and sex on survival: overall logistic model, $\chi^2 = 22.8$, $df = 17$, $P = 0.157$).

DISCUSSION

Ideally, a test of the Charnov-Bull model should be conducted in the field rather than in outdoor enclosures, and should incorporate fitness variation in all stages of life history (indeed, should be based on direct measurement of lifetime

TABLE 5. Sex-specific relationships between phenotypes and survival prior to winter. Analyses were carried out with a multiple logistic regression for each sex separately. The last four columns indicate the four differential fitness hypotheses that are supported (\surd), rejected (\times), or not impacted ($-$) by each statistical test. The four hypotheses state that there is a (A) different optimal egg size for sons versus daughters, (B) different phenotypic optima for sons versus daughters, (C) different norm of reaction for sons versus daughters, and (D) different optimal hatching time for sons versus daughters. Details of each hypothesis are in Table 1.

Phenotype	Male		Female		Statistical test supports, rejects, or has no impact on hypothesis:			
	χ^2	<i>P</i>	χ^2	<i>P</i>	A	B	C	D
Oviposition date	0.06	0.810	0.02	0.880	—	—	—	\times
Egg mass (g)	0.04	0.840	0.87	0.350	\times	—	—	—
Hatch date	0.97	0.325	1.16	0.281	—	—	—	\times
Snout-vent length (mm)	0.49	0.482	0.20	0.662	—	\times	—	—
Body mass (g)	0.02	0.876	2.24	0.135	—	\times	—	—
Tail length (mm)	0.30	0.582	0.80	0.373	—	\times	—	—
Body condition (residuals)	0.00	0.996	2.80	0.092	—	\times	—	—
Running speed (m/sec over 1 m)	1.88	0.171	0.51	0.475	—	\times	—	—
Growth in lab (Δ SVL/days)	0.20	0.653	3.42	0.065	—	\times	—	—

reproductive success). In practice, the logistical obstacles to such a study are so great that we were forced to rely on simulation of natural habitats using large field enclosures, and in the present study we have examined only one subset of fitness differentials: those that are evident prior to sexual maturation. This is an important limitation, because some sex-specific effects of incubation temperature might become apparent only after maturation (Gutzke and Crews 1988). Nonetheless, juvenile life is the phase of life history closest to incubation, and thus we might expect that any effects of incubation temperature would be most obvious at this time. Despite its limitations, our study enables an unusually extensive and detailed examination of the effects of incubation temperature and sex, and the interaction between these two variables, on fitness-relevant traits of individuals of a TSD species through most of their prereproductive life. In this respect, it is the first such study to be conducted in semi-natural conditions on any TSD reptile species. With the inevitable caveat that some components of fitness variation may not be expressed until the adult stage, we can nonetheless draw a variety of conclusions about the validity of alternative hypotheses that have been proposed to explain the adaptive significance of TSD in reptiles. In particular, we can evaluate the plausibility of the four hypotheses outlined in Table 1, because they each make specific predictions about relationships between the variables we have measured.

Hypothesis A: Different Optimal Egg Sizes for Sons versus Daughters

Variation in egg size can generate variation in offspring size and subsequent fitness (Sinervo et al. 1992), and the relationship between offspring size and fitness may differ between the sexes (Roosenburg 1996). Thus, a female parent may benefit by producing offspring of the sex that benefits most from a given egg size (Roosenburg 1996; but see Morjan and Janzen 2003). Temperature-dependent sex determination can enhance maternal fitness by allowing a female to select a nest site with a thermal regime that will produce the sex of offspring that is best suited to the mean egg size in her clutch.

Our results provide no support for this hypothesis. Hatching size was highly correlated with egg size, and the ex-

perimental design allowed male and female offspring to be produced from a wide range of egg sizes and incubation temperatures. We found no sex-specific optimal egg size, nor any incubation-temperature-specific optimal egg size (Table 4). The lack of interactive effects of egg size with sex and incubation temperature on offspring phenotypes and survival provides little support for the egg size/sex-matching hypothesis. A caveat is, however, that our hatchlings experienced a predator-free environment. Relatively large hatchlings (from large eggs) may have had greater survival than small individuals if predators were present (Janzen 1993; Sorci and Clobert 1999; Janzen et al. 2000), and this size-versus-survival relationship might differ between the sexes under more natural conditions.

Egg size (and subsequent hatchling size) also might play a role in the adaptive significance of TSD via an association between egg size and season. Indeed, our data show that egg size increased slightly but significantly over the course of the season. A previous study of *A. muricatus* suggests that male-biased clutches might be produced earlier in the season than female-biased clutches (Harlow and Taylor 2000), due to seasonal changes in nest temperatures. This putative seasonal shift in sex ratio coupled with the increase in egg size for later clutches, suggests that small eggs (early in the season) might tend to produce male offspring whereas large eggs (late in the season) tend to produce female offspring. However, our data do not support this scenario. We did not find any association between egg size and offspring sex: sons and daughters both were produced from a wide range of egg sizes, and we found no significant sex-specific or temperature-specific benefits of egg size at any time during the hatching season (Table 4). Additionally, the evidence for a seasonal shift in sex ratio is weak, based on only three nests (Harlow and Taylor 2000). Overall, we see no evidence within our data for any significant covariation between egg size and either offspring sex or incubation temperature.

Hypotheses B and C: Different Phenotypic Optima for Sons versus Daughters, and Different Norms of Reaction for Sons versus Daughters

Two different, but related, hypotheses attribute the adaptive significance of TSD to the effects of incubation tem-

perature on offspring phenotypes. Hypothesis B (different phenotypic optima for sons vs. daughters) assumes that incubation temperature modifies phenotypic traits of hatchlings, and does so in the same way for sons as for daughters. Despite this similarity in norms of reaction, a difference in the phenotypic determinants of fitness between males and females can generate a difference in optimal incubation temperatures between the two sexes, and thus favor TSD. Hypothesis C (different norms of reaction for sons vs. daughters) also posits that incubation temperature influences fitness-related phenotypic traits, but does so differently in male and female offspring (i.e., sex by incubation temperature interaction). For example, incubation temperatures that produce daughters induce phenotypic traits that are best suited for female fitness, and temperatures that produce sons induce phenotypic traits that are best suited for male fitness. Numerous studies demonstrate that incubation temperature influences phenotypes of hatchling reptiles in ways that may affect individual fitness (e.g., Van Damme et al. 1992; Downes and Shine 1999; Freedberg et al. 2004), and this effect can differ for male and female offspring (Shine et al. 1995; Elphick and Shine 1999).

Based on the phenotypes measured, our study does not support either of these models. Consistent with hypothesis B, but in direct falsification of hypothesis C, hatchling morphology was shaped by incubation temperature independent of sex. Hatchlings from the warm incubation treatment were smaller and had lower body condition than did hatchlings from the cool and intermediate treatments (Fig. 3). Although these effects of incubation temperature on the body size of hatchlings may influence offspring survival under field conditions (as proposed by hypothesis B), the magnitude of these effects in our study was so small (3–4% difference in body size) that we doubt they would have a significant impact on survival. For example, previous experiments on iguanid lizards where body size was greatly reduced (up to 20%) did not always influence rates of survival in the field (Sinervo et al. 1992; Warner and Andrews 2002). Moreover, the effects of incubation temperature on hatchling morphology in our dragons were short lived and rapidly overwhelmed by differentials induced by environmental conditions post-hatching. For example, although warm incubation temperatures produced small hatchlings, these individuals were among the largest by the onset of winter. As previously shown by Qualls and Shine (2000) for a scincid lizard, the phenotypic effects of incubation temperature may be trivial compared to effects of the post-hatching environment and especially the seasonal timing of hatching. Overall, the fitness consequences of phenotypic variation among hatchling *A. muricatus* did not differ between males and females, and no phenotypic traits influenced probability of survival (Tables 4 and 5). However, predation pressures have been shown to be sex specific in a turtle with TSD (Janzen 1995), and it is possible that seasonal variation in predator densities may induce sex-specific mortality due to temporal variation in the sex ratios produced.

Another consideration involves the measurement of appropriate phenotypes. For example, phenotypes other than those that we chose to measure may play an important role in determining offspring fitness. Indeed, sex-specific temperature effects may become apparent when offspring reach

sexual maturity. Thus, based on our prereproductive data, our results show no support for hypotheses B and C, but we still cannot completely rule out these hypotheses.

Hypothesis D: Different Optimal Hatching Times for Sons versus Daughters

In reptiles, incubation temperature strongly affects the duration of incubation and thus, the seasonal timing of hatching (Van Damme et al. 1992; Andrews et al. 2000). Thus, TSD may confer an adaptive advantage by allowing reproducing females to shift their clutch sex ratios seasonally, and hence to match the sex of their offspring to the seasonal conditions most suitable for that sex. This hypothesis has been supported in studies of fish and invertebrates with environmental sex determination (Conover 1984; McCabe and Dunn 1997), and finds support from our own study as well.

In jacks dragons, incubation temperature profoundly affects the duration of incubation: on average, eggs from the warm incubation treatment hatched about two months earlier than those from the cool treatment (Fig. 1B). A two-month “head start” may have dramatic effects on future reproductive success, particularly in a short-lived, early-maturing species such as *A. muricatus*. Indeed, early-hatched individuals (from the warm treatment) were substantially larger by the onset of winter than were individuals from cool incubation (Fig. 4). Moreover, by the onset of winter, some individuals from the warm incubation treatment were virtually adult sized (to 71 mm, with maturation occurring at about 72 mm, from Harlow and Taylor 2000). The much larger size of these warm-incubated animals than their cold-incubated counterparts reflects two advantages: (1) they had more time for growth prior to winter; and (2) they hatched into warmer conditions with few competitors for food.

Hatching early in the season, however, may have associated mortality costs; early-hatched lizards were less likely to survive until winter ($\chi^2 = 6.1$, $P = 0.014$). Thus, hatchlings from the warm incubation treatment had higher mortality than did those from the cool and intermediate incubation treatments (Fig. 5). This pattern was similar for males and females, although females had marginally higher rates of survival than did males for all treatments. The actual source of mortality in this study was unknown, but the most obvious reason for higher mortality rates of early-hatching lizards is that they had a greater time span prior to winter than individuals that hatched late, and thus they had more opportunities to die. However, most hatchlings that died did so within five days ($SD = 5.8$ days) after they were released in the enclosures; of 27 hatchlings for which the date of death was known, only two died more than a month after their release. Even late-hatched individuals spent at least a month in the outdoor enclosures prior to winter, and thus went through this phase of high risk. Another consideration involves nest predation; cool nests that hatch late have longer time of exposure to predators than warm nests. Currently, our data do not allow us to determine whether or not the benefits of hatching early (head-start on growth) outweigh its associated costs (low survival). Experimental work on the long-term effects of the seasonal timing of hatching on reproductive success are needed to address this question.

Our data establish that the seasonal timing of hatching (related to incubation temperature both as a direct effect, and as a correlate of high soil temperatures in early-season nests) is likely to have a major impact on fitness. By the end of the first growing season, hatchlings from early-season, relatively warm nests will be much larger than their conspecifics from later, cooler nests, and thus much more likely to attain adult body sizes early in their second summer of life (i.e., at about 11 months of age, vs. about 20 months for hatchlings emerging from later, cooler nests). Harlow and Taylor (2000) suggested that early-season jacky dragon nests might produce mainly males, because this sex would benefit more than their sisters from early maturation. However, our data point to the opposite scenario. Small, but sexually mature male dragons are unlikely to be able to compete successfully with the much larger territorial residents; thus maturation at one rather than two years of age may not enhance lifetime reproductive success for male jacky dragons. On the other hand, an early-hatched female would face less competition from conspecifics and likely be able to reproduce in her first year. Thus, high soil temperatures early in the season may produce mostly daughters, many of whom (despite high mortality rates) grow rapidly enough to mature and reproduce within a year of their own hatching. In contrast, later cooler nests produce mixed sexes, with both males and females requiring an additional year of growth prior to maturation. This hypothesis predicts that gravid female jacky dragons will tend to select nest sites with high temperatures early in the nesting season, and that such nests will produce daughters that reach sexual maturity in the following summer. Field studies of nest-site selection, nest thermal regimes, hatchling sex ratios, and growth rates under natural conditions are needed to test this hypothesis.

Conclusions

The adaptive significance of ESD, particularly TSD in reptiles, has been a problematic area of research for nearly three decades. To date, research in this field has focused on extremely long-lived reptiles (i.e., turtles and crocodylians), and hence has dealt only with very early life-history stages. Furthermore, few studies have appropriately decoupled the confounded effects of sex and incubation temperature on offspring characteristics via hormonal manipulations (Rhen and Lang 1995). Our experiment is unique in that it takes into account the above problems and simultaneously tests four differential fitness hypotheses underlying the Charnov-Bull model during most of the prereproductive life of a short-lived TSD reptile.

Our data, coupled with previously published work on the reproductive biology of jacky dragons, suggest that there is likely to be an adaptive value of TSD in this species. Specifically, our results suggest that TSD may be maintained because there are likely different optimal hatching times (which are driven by incubation temperature) for sons versus daughters in our study species. The generality of this hypothesis for the evolutionary significance of TSD in other reptiles is difficult to evaluate because TSD exists in a wide variety of taxa with extremely variable life histories, suggesting that TSD may have evolved for a variety of different reasons. Nonetheless, at least for short-lived, early-maturing

taxa such as our study species, experimental studies on the sex-specific effects of incubation temperature can provide insight into the selective forces involved in the evolution and maintenance of TSD.

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