EFFECTS OF INCUBATION TEMPERATURE ON EMBRYONIC DEVELOPMENT AND SEX DETERMINATION IN THE NORTH AFRICAN AGAMID LIZARD, *AGAMA IMPALEARIS*

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The effects of temperature on incubation time, embryo survival, sex ratio, embryo growth and size at hatching were investigated in the north African Agamid lizard, *Agama impalearis*. Seven constant temperature treatments (spanning 20-36°C) were employed and a split clutch design was used to assign eggs from the same clutch to the different treatments. Incubation time varied significantly with temperature treatments. Embryos incubated at 32°C, 34°C and 36°C hatched between 41 and 46 days, whereas embryos incubated at 26°C and 28°C hatched at 83 and 67 days respectively. Hatching success was higher at 28°C, 30°C, 32°C and 34°C, but much lower at 26°C and 36°C; hatching did not occur at 20°C. Eggs incubated at 26°C and 36°C produced only females. At 28°C, 30°C, 32°C and 34°C, the percentages of males were 9%, 53.5%, 32%, and 58% respectively. These sex ratios can be explained by a temperature-dependent mechanism of sex determination. The relative growth rates are highest early in incubation and lower for several days prior to hatching. The relationship between snout-to-vent length and age of embryos seems to be best described by a polynomial fitted regression. Growth rates at 26°C were much lower than those at 34°C. Constant incubation temperatures affected both snout-to-vent length and body mass at hatching, with maximum body size occurring at intermediate constant incubation temperatures (30°C, 32°C and 28°C). According to this study, the optimal temperatures of embryonic development probably lies within the range 28-34°C. The possible adaptive significance of incubation temperature effects on some life history characteristics of *A. impalearis* is discussed.

Key words: incubation temperature, embryonic development, sex determination, *Agama*

INTRODUCTION

The effects of temperature on embryonic development may constitute an important factor in the ecology and distribution of lizards (Licht & Moberly, 1965). In many species of reptiles, the incubation temperature of eggs can strongly influence a number of developmental traits. In this regard, several works have shown that temperature can induce variation in duration of incubation (Sexton & Marion, 1974; Miller, 1985; Gutzke & Packard, 1987; Van Damme, Bauwens, Braña & Verheyen, 1992), hatching success (Fitch, 1964; Rand, 1972; Venigar, 1973; Sexton & Marion, 1974; Van Damme *et al.*, 1992), sex proportion (Charnier, 1966; Langerwerf, 1983; Janzen & Paukstis, 1991) and growth of embryos (Sexton & Marion, 1974). Incubation temperature may also affect hatchling characteristics (Gutzke & Packard, 1987; Whitehead, Webb & Seymour, 1990; Van Damme *et al.*, 1992; Flores, Tousignant & Crews, 1994; Tousignant & Crews, 1995; Braña & Ji, 2000).

Sexual differentiation has been shown to be sensitive to the incubation temperature during a critical period of embryonic development in many species of reptiles (see Viets, Ewert, Talent & Nelson, 1994; Pieau, 1996 for review). This phenomenon has been termed

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temperature-dependent sex determination (Bull, 1980). It was suggested for the first time in an agamid lizard from Africa, *Agama agama* (Charnier, 1966). Subsequent studies showed that TSD is widespread but taxonomically biased in reptiles. Among the species studied so far, this phenomenon has been observed in all crocodilians, most turtles and several lizards (Bull, 1980; Raynaud & Pieau, 1985; Janzen & Paukstis, 1991; Viets *et al.*, 1994). Three TSD patterns have been recognized. *Pattern Ia*: Low temperatures yield 100% (or predominantly) males; high temperatures yield 100% (or predominantly) females. *Pattern Ib*: low temperatures yield 100% (or predominantly) females; high temperatures yield 100% (or predominantly) males. *Pattern II*: low and high temperatures yield 100% (or predominanatly) females, with intermediate temperatures yielding various ratios of males. Although TSD was reported in *A. agama* (Charnier, 1966), the sex determining mechanisms in other agamid lizards are still poorly known and only a few species of this group have been studied for their sex-determining pattern as a function of incubation temperatures (Langerwerf, 1983, 1988; Harlow & Shine, 1997, 1999). *A. agama* was reported as having pattern Ib of TSD (high temperatures yield males, low temperatures yield females; Ewert & Nelson, 1991), although only two incubation temperatures were tested (Charnier, 1966). However, studies at warmer temperatures than those previously employed might well

produce predominantly females in this species (Ewert, Jackson & Nelson, 1994).

It is likely that the microclimatic environment in the natural nest will vary during incubation such that the eggs are not exposed constantly to the optimal temperature for development, but rather to a range of temperatures that encompass this value (Beuchat, 1988). Because it is extremely difficult to locate lizard clutches in the field and consequently to determine the environmental conditions in the natural nesting sites, laboratory studies should provide precise information about the effect of temperature on embryos which can thus be used to establish the physiological limits during development and can help to define abiotic limits imposed on range extension and habitat occupation.

In the present work, using a large range of temperatures and a split clutch design to assign eggs from the same clutch to different treatments, we attempted to determine whether incubation temperature would affect sex determination in the North African agamid lizard, *Agama impalearis* (formerly *Agama bibroni*), a species closely related to *A. agama* (Joger, 1991). We also examined the effects of incubation temperatures on hatching time, embryo growth and survival, and size at hatching in this species.

MATERIALS AND METHODS

During the breeding seasons of 1997 and 1998, 77 gravid females of *A. impalearis* were collected by hand or with a noose in the central Jbilet mountains 25 km north of Marrakech, western Morocco (31°37'N 8°24'W, 580 m above sea level). They were measured for snout-vent length (SVL) and weighed (body mass; BM) to the nearest 0.1 mm and 0.1 g respectively. They were then kept in individual vivaria (70 x 50 x 50 cm) containing a sandy substrate and a pile of flat rocks (shale) that provided refuges for lizards and allowed them to climb. Food (mealworms dusted with commercial vitamin supplement) was offered in excess and water was provided *ad libitum* in small plastic dishes. A 100 W light bulb suspended 15 cm above the vivarium provided heat and light. The vivaria were sprayed with water each morning and lizards were checked for oviposition approximately every hour during the day. Each captive female produced one clutch after which it was returned to the field site.

Eggs freshly deposited were immediately removed and wiped. Post-laying females and their eggs were weighed to the nearest 0.1g and 0.01g, respectively. Eggs were scored for possible viability at oviposition and all eggs judged to exhibit reduced viability and to be at risk of hatching failure were not incubated. The eggs were then placed individually in covered plastic cups (150 ml) that contained moistened vermiculite (approximately -200 kPa water potential). Each clutch was distributed across thermostatically controlled incubators (Memmert, ± 0.1°C) at seven different incubation temperatures (20, 26, 28, 30, 32, 34 and 36°C). The positions of the cups in the incubators were shifted every three days to minimize the effects of possible thermal stratification within the incubators. The covers of the cups were removed daily and the eggs ventilated by fanning. The vermiculite moisture content was maintained close to the initial vermiculite/water ratio by adding water. Incubators were usually checked for hatchlings about twice a day. The newly-hatched lizards were weighed (BM to the nearest 0.01 g) and measured (SVL, to the nearest 0.1 mm). To determine the sex, hatched lizards were sacrificed and internally examined under a dissecting microscope. Testes were easily distinguishable from ovaries. Moreover, in males hemipenes were present and observable, whereas Müllerian ducts were regressed; in females, hemipenes were regressed whereas Müllerian ducts were present.

During the course of embryonic development at only two constant temperatures (26 and 34°C), sets of three to four eggs were randomly sampled and dissected at time intervals of 5-13 days according to the incubation temperatures. The length of the embryo was measured between the extremity of the head and the base of the tail at the level of the cloaca (to the nearest 0.1 mm).

To assess the sex ratios of hatchlings in nature, specimens were collected in late August (*n*=57) and late November (*n*=65), periods corresponding to the early and late clutches respectively. Young lizards (two months or less of age) presumably representing the late season clutches within the current annual cohort were segregated by their small size (50 mm SVL or less) during field sampling in November (El Mouden, Francillon-Vieillot, Castanet & Znari, 1997; El Mouden, Znari & Brown, 1999).

STATISTICAL ANALYSIS

Egg mass (log-transformed) was analysed as a dependent variable using multiple regression with the following independent variables: oviposition date (the relative laying date of every female was calculated each year using the first day of May as day 1), female SVL, female condition (estimated by the residuals derived from the regression of log [postpartum mass] and log [SVL]), and the relative clutch size (estimated as the residuals from the regression of log [clutch size] and log [SVL]). We estimated temperature effects on length of incubation by one-way ANOVA, and incubation temperature and sex effects on hatchling size (SVL and BM) using two-way ANOVA with initial egg mass entered as a covariate. Chi-square tests were used to test for differences in embryonic survival and sex ratios. For all tests, statistical significance was accepted for $P<0.05$.

RESULTS

CLUTCH CHARACTERISTICS

Mean clutch size was 13.7±0.27 eggs (range 9-23, *n*=77). Clutch size was positively related to maternal SVL and BM (excluding clutch mass) (SVL: $r=0.61$, $F_{1,71}=41.9$, $P<0.0001$; BM: $r=0.67$, $F_{1,72}=59.2$, $P<0.0001$). It increased by approximately one egg per 4 mm of female SVL.

TABLE 1. Effects of incubation temperature on hatching success, duration of incubation, sex ratio and size at hatching in *A. impalearis*. Values for duration of incubation are given as mean ± 1SD with sample size below. Sex ratios are given as 100x[male/(male+female)] with number of hatchlings examined in parentheses. Values for snout-vent length and body mass are given as mean ±1SD with range in parentheses and sample size the same as for sex ratio.

	Temperatures tested (°C)						
	20	26	28	30	32	34	36
Eggs incubated (no.)	51	95	116	83	59	43	75
Hatching success (%)	0	46.3	85	83.1	74.6	88.3	41.3
Duration of incubation (days)	-	83.3±6.3	67.3±4.1	54.5±2.9	45.5±2.4	43.5±1.3	41.2±2.2
Number of groups of eggs		9	6	8	8	5	5
Percentage male (male/female)	-	0%(0/44)	9%(9/90)	54%(37/32)	32%(14/30)	58%(22/16)	0%(0/31)
Size at hatching:							
snout-vent length (mm)	-	29.5±1.57	30.7±1.69	31.0±1.43	30.9±1.48	29.7±1.45	28.9±1.96
	-	(26.1-31.6)	(26.5-34.3)	(26.7-33.7)	(27.4-33.1)	(25.0-32.5)	(24.9-32.2)
body mass (g)	-	1.22±0.19	1.42±0.18	1.55±0.20	1.32±0.16	1.30±0.22	1.12±0.17
	-	(0.85-1.57)	(0.69-1.69)	(0.87-1.93)	(0.94-1.59)	(0.6-1.77)	(0.7-1.43)

Clutch mass (in g) was also positively and significantly related to SVL and BM (SVL: r=0.54, $F_{1,72}$=26.8, P=0.0001; BM: r=0.60, $F_{1,71}$=37.2, P=0.0001). Initial egg mass varied considerably among clutches of eggs (ANOVA: $F_{67,789}$=93.4, P=0.001). A multiple regression analysis indicated that only date of oviposition explained a significant amount of the variation in initial egg mass among clutches (partial regression coefficient; prc=-0.002, t=3.17, P=0.002). The oviposition date was negatively related to initial egg mass (r=-0.33, $F_{(1,70)}$=8.5, P=0.005). The examination of embryos at oviposition showed that their development starts before egg-laying; the eggs contained embryos at stage 28 of the developmental table established by El Mouden, Bons, Pieau, Renous, Znari & Boumezzough (2000) for the same species.

INCUBATION TIME AND EMBRYO SURVIVAL

Duration of incubation remained fairly constant within, but varied considerably between, temperature treatments (ANOVA: $F_{5,34}$=128.6, P=0.0001). Mean incubation times decrease at increasing temperature with a negative exponential relationship (Table 1). Temperature strongly affected embryo survival (χ^2=83.3, df=6, P<0.005) (Table 1). Hatching occurred in eggs kept at all temperatures tested except at 20°C. The low hatching success rates recorded at 26 and 36°C suggested that these temperatures are close to the lethal limits. Indeed, an analysis of embryos that did not hatch showed that the development at 36°C often stopped at early developmental stages, whereas those incubated at 26°C progressed to more advanced developmental stages.

SEX RATIO

The sex-ratio of hatched lizards varied significantly between different constant temperatures of incubation (χ^2=48.2, df=5, P<0,005). The 26 and 36°C incubation temperatures produced only females, whereas the male proportions were 9% at 28°C, 53.5% at 30°C, 32% at 32°C and 58% at 34°C (Table 1). Binomial tests showed that the deviations of the sex ratios from 1:1 were not significant only for egg samples incubated at 30 and 34°C (χ^2-test; P> 0.05 in both cases). The examination of the sex ratio within each group of eggs from the same clutch raised at 32°C, revealed the same responses as at 30 and 34°C. Thus, the sex ratio value which deviates significantly from 1:1 at 32°C is probably due to sample variability and can be considered similar to sex ratios at 30 and 34°C. Based on these findings, it appears that the sex of *A. impalearis* is influenced by incubation temperature. We noted that at 26°C all dead embryos were female, so this eliminates an alternative proposal of predetermined sex with differential mortality. However, at 36°C – as dead embryos were so young and their sexes could not then be identified – we were not able to check this hypothesis. Nevertheless, the examination of groups of eggs from the same clutch incubated at 30, 32 and 34°C revealed an among-family heterogeneity in sex ratio with, at the same temperature, some groups of eggs from the same clutch giving only males while others produced only females.

Under natural conditions, juvenile sex ratios were 75.4 and 36.9% respectively for early and late clutches (χ^2 = 18.2, df= 1, P< 0.05). The pooled samples provide a global sex ratio of 54.9% which is close to 1:1 (χ^2-test; P>0.05 in both cases). (χ^2= 1.18, df= 1, P>0.05).

GROWTH

During the entire embryonic period at the two incubation temperatures tested, 26 and 34°C, eggs increased in mass by a factor of 2.6 (mass just before hatching divided by mass at oviposition), and in length and width by factors of 1.2 and 1.5 respectively. This emphasizes the importance of water absorption for embryo development.
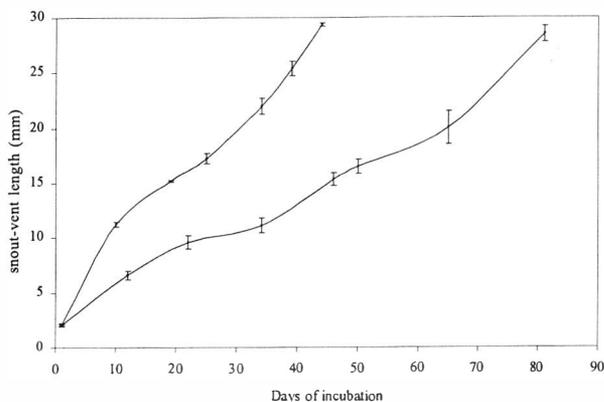
FIG. 1. Snout-vent length of *A. impalearis* embryos incubated at two different constant temperatures (26 and 34°C) as a function of age after oviposition.

The pattern of embryonic growth during incubation at 34 and 26°C in *A. impalearis* is shown in Fig. 1. The relative growth rates (i.e. proportional change in SVL per day) decrease throughout incubation in a similar manner at both temperatures. They are highest early in incubation and decrease thereafter for several days prior to hatching at both temperatures tested (Fig. 2). Therefore, embryonic growth is clearly not exponential. A polynomial fit to the relationship between SVL and age of embryos reveals significant quadratic terms (26°C: $F_{1,21}$=482.2 ; 34°C: $F_{1,18}$=433.1, P<0.0001 in both cases), indicating that growth is unlikely to be linear. Rather, absolute growth is a successively concave and convex downwardly curvilinear relationship and is best described by a polynomial regression. Embryonic growth rates decrease continuously until approximately 15 and 10 days of incubation at 26 and 34°C respectively, and become progressively higher as the body length increases at about 20 days of incubation.

SIZE AT HATCHING

Analysis of variance at each incubation temperature tested revealed that both SVL and BM of hatchlings varied significantly with the average initial individual egg mass of each clutch (one-way ANOVA, P<0.05 in all cases), indicating that the two parameters were related to the egg mass at oviposition. Two-way ANOVAs (within the transitional range that produced both males and females), with sex and temperature entered as fac-

tors, and initial egg mass as covariate, indicated no significant sex effect or sex x incubation temperature interaction for either SVL and BM (all P>0.05). Thus, more detailed one-way ANOVAs (all the temparatures tested) were used with initial egg mass as a covariate, to test for the incubation temperature effect on both variables. Incubation temperature significantly affected SVL and BM of hatchlings (SVL, $F_{(5,221)}$=6.6; BM, $F_{(5,233)}$=13.8, P=0.0001 in both cases). The Duncan's new multiple range test (*a posteriori* test, Kramer, 1956) showed that the eggs incubated at 36, 26 and 34°C produced hatchlings that had significantly lower SVLs and BMs than those incubated at intermediate temperatures (30, 32 and 28°C) (Table 1).
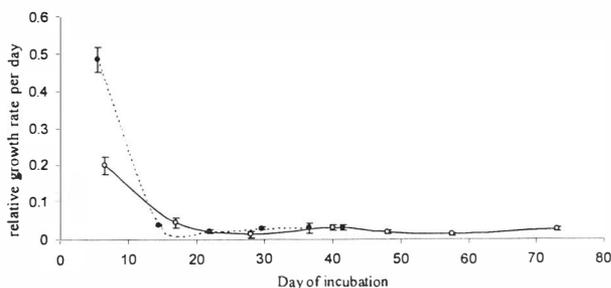
DISCUSSION

The present work provides evidence for significant effects of incubation temperature on several developmental characteristics of *A. impalearis* during the embryonic period, including duration of incubation, embryonic mortality, sex determination, embryonic growth, and hatchling size.

As in other lizards (e.g. Sexton & Marion, 1974; Muth, 1980; Packard & Packard, 1988; Van Damme *et al.*, 1992; Castilla & Swallow, 1996), our data indicate a negative exponential relationship between the length of embryonic development and the incubation temperature. The incubation times in *A. impalearis* are similar to those previously reported by Zbysek (in Rogner, 1997) and Znari, El Mouden & Boumezzough (1998), respectively of 45-54 and 45-58 days at 30°C. The value of 90 days found at 25°C by Santonja (1969) and Santonja & Bons (1972) for the same species is in accordance with the aforementioned trend for incubation duration relative to temperature. Nevertheless, Bons (1964) reported an incubation length varying from 75 to 95 days at 19°C, but the eggs were not placed in a thermostatically controlled incubator. These values are around those we obtained at 26°C. Moreover, all eggs incubated at a constant 20°C failed to develop. The incubation times found in this study are relatively close to those previously cited for an Australian agamid lizard, *Pogona* (=*Amphibolurus*) *barbatus* whose eggs, kept at 25°C hatched in 88-95 days (Bustard, 1966). In contrast, eggs of *A. agama* (Charnier, 1966) took less time to hatch than did those of *A. impalearis* incubated at similar incubation temperatures. On the other hand, in *Iguana iguana*, incubation times at 28°C (Ricklefs & Cullen, 1973) and 30°C (Licht & Moberly, 1965) were much longer at 99 and 73 days respectively. This indicates that the incubation time depends not only on incubation temperature but also on lizard species (e.g. Bustard, 1967; see Packard & Packard, 1988 for review).

The broad thermal tolerance range in *A. impalearis* embryos is comparable to that of certain temperate iguanians (Phrynosomatids) occupying similar habitats in arid areas of North America (Sexton & Marion, 1974; Muth, 1980). By contrast, *I. iguana*, a tropical lizard which normally develops in nests that are well buffered

FIG. 2. Relative growth rate of *A. impalearis* embryos during incubation at two different temperatures, 26°C and 34°C.

against temperature changes (Rand, 1972), has a very narrow tolerance range near 30°C (Licht & Moberly, 1965). This suggests adaptation of *A. impalearis* embryos to the variable temperatures likely to be encountered during the natural incubation period which lasts from mid-Spring to mid-Autumn (Znari & El Mouden, 1997). In nature, eggs of *A. impalearis* may hatch between 45 and 60 days (Znari & El Mouden, 1997). This value is close to that determined for eggs maintained at temperatures between 30 and 32°C.

Embryo mortality in *A. impalearis* varied considerably with incubation temperature. This is in accordance with findings in many other reptiles studied from this viewpoint (Sexton & Marion, 1974; Packard & Packard, 1988; Van Damme *et al.*, 1992). Our data indicate that *A. impalearis* eggs hatch over a temperature range with a lower limit between 20 and 26°C and an upper limit exceeding 36°C. The 16-day difference in incubation period between 26 and 28°C is considerably more than the two-day difference between mean values at 34 and 36°C. Similar results were obtained by Sexton & Marion (1974) in an iguanian lizard, *Sceloporus undulatus,* and they interpret this non-linear relationship as indicating that the upper temperature at which incubation was tested is near the thermal maximum for embryonic development.

Genotypic sex determination (GSD) has been reported for *Pogona vitticeps* (Viets *et al.,* 1994) and some other Australian agamids (Harlow & Shine, 1997). In contrast, *Laudakia* (*=Stellio*) *caucasica* (Langerwerf, 1983), *Laudakia* (*=Stellio*) *stellio* (Langerwerf, 1988) and *Chlamydosaurus kingii* (Harlow & Shine, 1999) were reported as having TSD. Two of the three TSD patterns that have been recognized from incubation of reptilian eggs at constant temperatures (Ewert & Nelson, 1991) were reported in agamid lizards (Charnier, 1966; Bull, 1980; Langerwerf, 1983, 1988; see also Viets *et al.* 1994). *L. caucasica* exhibits pattern II (Langerwerf, 1983) while *L. stellio* shows pattern Ib (Langerwerf, 1988). *A. impalearis* exhibits TSD pattern II which is common to the three major reptilian lineages (Ewert & Nelson, 1991). *A. agama*, a species closely related to *A. impalearis*, has been reported to have pattern Ib (Bull, 1980), although only two incubation temperatures were tested (Charnier, 1966). However, as suggested by Ewert *et al.* (1994), studies at warmer temperatures than those previously used might well yield predominantly females in this species as found in *A. impalearis*. In this study, we detected among-family heterogeneity for sex ratio within three incubation temperatures tested. This suggests the existence of genetic sex determination along with the incubation temperature effect, or between-family variability in thermal sensitivity or threshold levels within the ESD system. However, due to insufficient samples we were not able to test for between-family variation and consequently to confirm genetic variation in sex ratio in *A. impalearis,* as done for other reptiles (Rhen & Lang, 1998). The examination of sex ratios among hatchlings from natural populations revealed different values between early and late

clutches with a predominance of males and females respectively. This discrepancy in sex ratio between clutches could be attributed to differences in temperature ranges within nests, the average incubation temperatures being higher for the first clutches (incubated during the summer) than for the second clutches (incubated mostly during autumn).

Several authors have suggested a relationship between the mechanism of sex determination and sexual dimorphism (Charnov & Bull, 1977; Webb & Smith, 1984; Ewert & Nelson, 1991; Ewert *et al.*, 1994). In this regard, Harlow & Shine (1997) investigated the possible adaptation value of TSD by comparing life history characteristics in 20 species of Australian agamids. They found that highly territorial species with extreme sexual dimorphism and male combat are most likely to have TSD, while less territorial species with low or moderate sexual dimorphism do not. Ewert & Nelson (1991) and Ewert *et al.* (1994) pointed out that species with females smaller than males usually have pattern II TSD (pattern-dimorphism congruence hypothesis). In *A. impalearis*, males are very territorial (Bons, 1968, unpublished data) and larger than females (Znari & El Mouden, 1997) which well supports the pattern-dimorphism congruence hypothesis.

Embryonic growth has been investigated in several reptile species (Dmi'el, 1970; Ricklefs & Cullen, 1973; Ackerman, 1980; Renous, Rimblot-Baly, Fretey & Pieau, 1989; Guyot, Pieau & Renous, 1994), but there have been few attempts to model growth data allowing comparisons within or among taxa. Both sigmoidal and exponential growth curves have been observed (Andrews, 1982). Constants obtained by fitting sigmoidal equations to embryonic growth data, as in sea turtles (Ackerman, 1980), are difficult to interpret in lizards including *A. impalearis*, because the size of lizard embryos do not reach plateau levels prior to hatching and asymptotes would therefore be determined arbitrarily (Ricklefs, 1987). Simple exponential equations assume a constant relative growth rate, which is not the case for embryonic growth as was found in *A. impalearis*. To our knowledge, the only lizard species whose embryonic growth has been described mathematically is *I. iguana* (Ricklefs & Cullen, 1973). This species exhibits a growth curve (based on length of embryo) very similar to that observed in *A. impalearis*. However, according to Ricklefs & Cullen (1973) the iguana embryos grew at a constant exponential rate during most of the developmental period. Consequently, the whole growth curve of *A. impalearis,* and probably also that of *I. iguana*, are best fitted to a polynomial model. Although providing good fits to the data, polynomials are arbitrary functions that are difficult to compare across sample taxa since they do not incorporate biologically significant parameters (Ricklefs, 1987).

Embryos of *A. impalearis* grow more rapidly at a high temperature (34°C) than they do at a lower one (26°C). Indeed, developing embryos of numerous lizards complete incubation sooner in warm conditions than they

do in cooler conditions (e.g. Sexton & Marion, 1974; Muth, 1980; Packard & Packard, 1988; Van Damme *et al.*, 1992). This effect of temperature on growth rate in *A. impalearis* embryos is exerted particularly during the early extra-oviductal developmental stages, as also reported in other reptiles (Yntema, 1978; Pieau, 1982). Rapid growth and early completion of development at high temperatures presumably reflect higher rates of metabolism than at lower temperatures (Packard, Tracy & Roth, 1977; Packard & Packard, 1988).

The results from our study indicate that egg mass influences both SVL and BM at hatching. Moreover, the hatchlings from eggs incubated at lower and higher temperatures are on average smaller and lighter than those incubated at intermediate temperatures. Gutzke & Packard (1987) found a similar effect for snout-vent length, but reported a generally decreasing pattern for mass. Packard, Packard & Birchard (1989) did not find significant temperature effects. Burger (1990) considered only two incubation temperatures with increasing size effect. Temperature effects reported by Campos (1993) were evident in length whereas mass remained similar. In ectotherms, with a few exceptions (Atkinson, 1995), the size at a given developmental stage should be reduced by increased rearing temperature (Atkinson, 1994). In our samples, the occurrence of the same effect of incubation temperature on both SVL and BM indicates that the differences in body size are due to actual changes in growth. According to Packard & Packard (1988), the embryos developing at high temperatures may have a higher metabolic rate, but a reduced metabolic efficiency and consequently lower body size. In *Crocodylus johnstoni* hatchlings, residual yolk mass increases positively with incubation temperatures and this pattern of yolk utilization was explained by long incubation periods at low temperatures allowing embryos to metabolize more yolk with a consequent increase in body size (Manolis, Webb & Dempsey, 1987; Deeming & Ferguson, 1989). We did not evaluate residual yolk mass in *A. impalearis* hatchlings but larger sizes at intermediate temperatures suggest a different pattern of yolk utilization – probably with a maximal efficiency occurring at thermal metabolic optima.

An estimation of the optimal temperature of embryonic development for *A. impalearis* reveals that the experimental temperature range of 28–34°C produces the best balance between embryonic survival rate and hatchling size. This optimal thermal range includes the mean temperature recorded in natural nesting sites in early summer (30.4°C) (Znari *et al.*, 1998) and was relatively close to the body temperature selected by adult *A. impalearis* in a thermogradient (TP= 34±2.3°C; Znari & El Mouden, 1998). In addition, the results obtained for the survival rate within the range of incubation temperatures tested showed that the lower and upper thermal limits for embryonic development were much higher and lower respectively than the critical thermal minimum and critical thermal maximum of adults (CT_{max}=45.6°C and

CT_{min}=10.7°C) (Znari & El Mouden, 1998). These support the multiple optima hypothesis suggested by Huey (1982).

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