Direct effects of incubation temperature on morphology, thermoregulatory behaviour and locomotor performance in jacky dragons (Amphibolurus muricatus)

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ARTICLE INFO

ABSTRACT

Incubation temperature is one of the most studied factors driving phenotypic plasticity in oviparous reptiles. We examined how incubation temperature influenced hatchling morphology, thermal preference and temperature-dependent running speed in the small Australian agamid lizard Amphibolurus muricatus. Hatchlings incubated at 32 °C grew more slowly than those incubated at 25 and 28 °C during their first month after hatching, and tended to be smaller at one month. These differences were no longer significant by three months of age due to selective mortality of the smallest hatchlings. The cooler incubation treatments (25 °C and 28 °C) produced lizards that had deeper and wider heads. Hatchlings from 28 °C had cooler and more stable temperature preferences, and also had lower body temperatures during a 2-h thermoregulatory behaviour trial. Locomotor performance was enhanced at higher body temperatures, but incubation temperature had no measurable effect either independently or in interaction with body temperature. Our study demonstrates that incubation temperature has direct effects on morphology and thermoregulatory behaviour that appears to be independent of any size-dependent effects. We postulate a mechanistic link between these two effects.

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1. Introduction

Phenotypic plasticity, or changes in phenotype induced by the environment, plays an important part in many organisms' response to their environment. Many examples of phenotypic plasticity may be adaptive if the phenotype in question develops in a manner that enhances its functionality in the environment that is affecting it (Ghalambor et al., 2007; Losos et al., 2007; Shine, 2004). While there is much debate as to whether phenotypic plasticity reduces evolutionary responses to selection or if it provides new phenotypes for selection to act on (Via et al., 1995), it appears clear that plasticity itself is a property of the genotype, and therefore capable of adaptive evolution (Scheiner, 1993).

Aspects of the current environment in which an organism lives are not the only possible triggers for phenotypic plasticity. Maternal and early-life (e.g. incubation) conditions can play an important part in the future phenotype of the offspring (Monaghan, 2008). One of the most-studied factors known to drive phenotypic

plasticity in oviparous reptiles is egg incubation temperature during embryonic development (Deeming, 2004). Incubation temperature can affect hatchling growth (Alberts et al., 1997; Janzen, 1993; Roosenburg and Kelley, 1996; Shirley et al., 1987), body shape (Braña and Ji, 2000; Pan and Ji, 2001), locomotor performance (Braña and Ji, 2000; Burgess et al., 2006; Janzen, 1993; Van Damme et al., 1992), pigmentation (Deeming and Ferguson, 1989), thermal choice (Blumberg et al., 2002; Goodman and Walguarnery, 2007; Lang, 1987; O'Steen, 1998), behaviour (Burger, 1990; Flores et al., 1994) and even cognitive ability (Amiel and Shine, 2012). However, perhaps the most remarkable effect incubation temperature has is through determining whether an embryo develops into a male or a female, a phenomenon known as temperature-dependent sex determination or TSD (Janzen and Paukstis, 1991; Shine, 1999; Valenzuela and Lance, 2004). Moreover, maternal behaviours such as nest-site selection, egg care or basking preferences can strongly influence incubation temperature (Robert and Thompson, 2001; Schwanz et al., 2010), so that resulting phenotypic effects are also an important component of non-genetic maternal effects on offspring phenotype (Mousseau and Fox, 1998; Uller, 2008).

The functional implications of these plasticities and maternal effects are still unclear. A given phenotypic state (e.g. fast running speed) may have an advantage across all environments. In contrast, incubation temperature may permanently set future temperature

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preferences and temperature-dependent performance or represent a maternal effect to "anticipate" the thermal environment of hatchlings (Burger, 1990; Marshall and Uller, 2007). We know almost nothing about whether incubation temperature influences performance at different ambient temperatures. For example, do cool-incubated individuals perform better at cool temperatures compared to warm temperatures? Understanding the effects of incubation temperature on the phenotypes of an organism can provide insight into several aspects of the functional ecology of plasticity, such as thermal preference, maternal nesting behaviour and sex determining mechanisms. In addition to these evolutionary questions, the information will allow better predictions of the response of individuals and populations to thermal environment changes, caused by habitat modification or climate change (Clusella-Trullas et al., 2011; Crozier et al., 2008; Hulin et al., 2009; Mitchell et al., 2010; Visser, 2008).

The Australian jacky dragon (*Amphibolurus muricatus* (White, 1790)) is an ideal system to experimentally test these hypotheses. The jacky dragon is a medium-sized (70–110 mm SVL) agamid lizard that inhabits forests and forest edges along the southeastern edge of Australia. This species exhibits TSD, producing a higher proportion of females at low (23–25 °C) and high (30–33 °C) incubation temperatures and approximately equal proportion of females and males at intermediate (25–30 °C) temperatures (Harlow and Taylor, 2000; Warner and Shine, 2005). Other factors such as temperature fluctuation (Warner and Shine, 2010), maternal nutrition (Warner et al., 2007) and corticosterone levels during embryological development (Warner et al., 2009) also contribute to sexual outcome. Extensive research has been done regarding the effects of incubation and nest conditions on hatchlings of this species during the last decade. Incubation temperature has a temporary effect on hatchling body size, but no measurable direct effect on running speed (Warner and Shine, 2005). Time of hatching has significant effects on growth and survival, with faster growth rates and better per-week survival in early hatched lizards (typically incubated at warmer temperatures) (Warner and Shine, 2005, 2007, 2009). Moreover, although the mechanism is unknown, high and low (female-producing) temperatures promote higher fitness in females than do intermediate temperatures, while intermediate temperatures promote higher fitness in males compared to extreme temperatures (Warner and Shine, 2008a), supporting an adaptive explanation for TSD in this species (Charnov and Bull, 1977).

Here we use a split-clutch design to incubate hatchlings at three temperatures (25, 28 and 32 °C constant temperature) similar to natural nest temperatures (range in nest temperatures=20–36 °C, Warner and Shine, 2008b). We sought to determine the direct effects of incubation temperature (independent of time of hatching) on phenotype in hatchlings across different juvenile stages. We were particularly interested in determining whether incubation temperature influences body size and shape, thermal preferences and temperature-dependent performance. We looked at morphology (size and multiple components of shape), thermoregulatory behaviour, and locomotor performance of the hatchlings at the same age, irrespective of time of hatching, which has been well studied during the last decade in jacky dragons.

2. Materials and methods

2.1. Animal collection and care

Gravid jacky dragon females were collected in the austral spring (September–November) from two sites near Canberra (Australian Capital Territory), separated by approximately 50 km: Wamboin, New South Wales (35.2500°S; 149.2917°E; elev. ~800 m) and Burra, NSW (35.5500°S; 149.2167°E; elev. ~815 m). Lizards were located

on road verges and noosed with an extendable fishing pole. Following capture, lizards were brought back to a lab at The Australian National University, where mass (nearest 0.5 g) and SVL (nearest 0.5 mm) were recorded. Lizards were housed in cages (500–600 $L \times$ 300–400 mm $W \times$ 300 mm H) with a sand substrate and multiple basking and sheltering objects. They were provided with UV light (Outback Max 10% UVA/UVB 18 W lamps, Ultimate Reptiles Suppliers, Adelaide, South Australia, Australia) and basking lamp (40 W incandescent spotlight) for approximately 12 h a day. Water was provided ad libitum and food was provided 3 times/ week as crickets or occasional mealworms (dusted with vitamins once/week).

Cages were checked every afternoon for clutches. Only a few females laid their clutches in the cages. Females that appeared fully gravid for two weeks were injected with 1–4 units/100 g of oxytocin once per week and placed in a shoebox with damp paper towel for up to 7 h to induce ovipositing. If the female did not lay her clutch following oxytocin induction, she was returned to her cage and inducement was attempted again the following week.

Laid eggs were blotted dry, weighed to the nearest 0.001 g and placed individually in 125 mL glass mason jars half-buried in Grade 2 vermiculite (L&A Fazzini, Greenacre, NSW, Australia) at approximately −200 kPA water potential (120 g water/100 g dry vermiculite, following Harlow, 2001). Jars were covered with Cling Wrap and placed in incubators set at constant 25 °C, 28 °C or 32 °C. Eggs from a single clutch (4–11 total eggs per clutch) were allocated equally across the three temperature treatments. Jars were rotated within each incubator weekly. Near the anticipated hatch date, eggs were checked daily for pipping.

On the day of hatching, hatchlings were brushed clean of vermiculite and weighed to the nearest 0.001 g. Snout-vent length (SVL) was measured to the nearest 0.01 mm and sex was determined by checking for the presence of hemipenes under a stereoscope (Harlow, 1996). Hatchlings were housed independently in plastic cages (200 mm $L \times$ 150 mm $W \times$ 70 mm H) with ad libitum water for their first 5–14 days of life and given small crickets starting at week one. Following this confinement period, hatchlings were given a unique toe-clip for individual identification and placed in small groups in a communal cage (410 mm $L \times$ 310 mm $W \times$ 230 mm H) with other hatchlings that were within approximately 10 days of age. In the communal cages, a paper towel substrate and multiple basking and sheltering objects were provided. Light conditions were the same as for adults. Hatchlings were provided small crickets at least three times per week and water ad libitum. Mass and SVL were recorded again at one month and three months of age. At three months, we also recorded a suite of morphological traits (tail length; left hind limb length; left 4th toe length, left forelimb length; axilla-groin distance, head height, head width; head length).

2.2. Experimental procedures

We performed a thermoregulatory behaviour assay and a running performance test. First, we placed the hatchlings in thermoregulatory cages (410 mm $L \times$ 310 mm $W \times$ 230 mm H). These cages had sand floors and a 4.5 cm tall rectangular wooden perch intended as a basking perch running the middle of the cage. Two 40 W reflecting clamp lamps were provided above the perch, one near the edge of the cage, 19 cm above the floor and the other 34 cm above the floor in the centre of the cage. Each cage had nine iButtons (DS1921G Thermochron iButton, Maxim Integrated, San Jose, CA, USA) placed at evenly-spaced intervals on the substrate (sand or wood) and were programmed to record temperature every 5 min.

To initiate the assay, hatchlings were placed at the coolest end of the cage on the perch, and the room temperature was set to

20 °C. After 30 min of acclimation, we observed hatchling location from behind a blind and recorded the iButton that the hatchling was closest to. This was repeated every 30 min thereafter for a total of 2 h in the cage. iButton temperatures were later downloaded to convert locational data of the hatchlings to local cage temperature data. These temperature values do not represent preferred body temperatures of hatchling jacky dragons, given the brevity of the experiment and the lack of repeated body temperature data; however, they provide a measurement of thermoregulatory behaviour in a novel environment. At the end of the experiment, hatchling skin temperature (as an estimate of body temperature) was immediately measured without handling by pressing a thermocouple against the animal's flank.

Second, we measured sprinting speed at two body temperatures, 20 °C and 30 °C. Hatchlings were placed in small cages within incubators (20 °C and 30 °C) to acclimate for at least 1 h before each sprint test. Sprinting was measured in a 25 °C room on a 1 m race track with a rugged rubber surface on the floor and walls on the sides, with diodes at every 0.25 m recording passage. Lizards were removed from their incubator, immediately placed at the start of the track and encouraged to run with a paintbrush. Each lizard was sprinted three times per temperature and the fastest time to cover 0.25 m was used as the performance variable. We randomly assigned half of the hatchlings to run first at 20 °C body temperature, and the other half at 30 °C. Immediately after the first test, hatchlings were moved to the incubator of the other treatment and again acclimated for approximately 1 h. Body temperature was estimated at the end of the sprints by placing a thermocouple against the hatchling's flank. Hatchling body temperature changed by no more than 2 °C across the sprints. All sprinting and thermoregulation assays were run in the afternoon (approx. 13:00–14:00 h).

2.3. Data analysis

Differences in hatchability of eggs and sex of hatchlings according to incubation temperature were assessed with a chi-square test. We examined the influence of incubation temperature on mass and SVL at: hatching, one month and three months using linear mixed-effect models, with sex and incubation temperature entered as fixed effects and maternal ID entered as a random effect to account for non-independence within clutches. For the measurements at hatching, egg mass was entered as a covariate. Where the effect of incubation temperature on SVL changed over hatchling age, we used the same mixed-effect models as above to determine whether growth rates (mm/day) differed among treatment groups. As an alternative explanation, we examined whether selective mortality had occurred with a chi-square test (predictors: incubation temperature, sex, initial SVL).

We tested for incubation effects on body shape by performing a Principal Components Analysis (PCA) on all of the raw morphometric measurements to explore and visualize the variation and reduce the number of shape variables. PC1 is considered to largely represent size, so we used PCs 2 and 3 for interpretation of shape variation. Following assessment of shape variation with PCA, we also performed mixed effect linear models on PC2 and PC3 with incubation temperature, sex and maternal Id (random effect) as factors. We then performed separate mixed effect linear models on all of the morphometric measurements taken at three months of age with SVL as a covariate and the same factors mentioned above. We additionally performed mixed effect models on SVL and mass on the day of hatching and at one month of age with egg mass as covariate (for day of hatch) and incubation temperature, sex and maternal Id (random effect) as factors.

To test the effect of incubation temperature on fastest sprinting time at the two body temperatures, we analyzed a mixed effect model on fastest running time over 0.25 m, with incubation temperature, running temperature and the interaction of these factors as fixed factors, and hatchling ID as a random effect to account for the repeated measures of individuals, and SVL at 3 months as a covariate. By comparing the AICc (corrected Akaike Information Criterion) of the models with and without the random effects, we could assess that these random effects generally had some influence on the model. However we also checked if the 95% CI of the random effect included 0, which would indicate that the random effect is unimportant.

Thermoregulatory behaviour was assessed with a mixed effect model, using the temperatures from the iButton closest to each hatchling at observation. This regional temperature at each observation time (30 min, 1 h, 1.5 h, 2 h) was the response variable, with time of observation, incubation temperature, the interaction between time and incubation temperature, hatchling mass and sex as fixed effects. Hatchling ID was entered as a random effect to account for the repeated measures made on each hatchling. Body temperature at the end of the experiment was analyzed in an ANCOVA with incubation temperature, mass and sex as predictor variables.

All statistical analyses were performed using JMP. All the response variables were tested for normality and when significant deviations from normality were found, the data was log-transformed. In the case of principal component scores with no normal distribution, which include negative values, a $\log(x+2)$-transformation was applied.

3. Results

A total of 82 eggs from 11 clutches (11 females) was produced. There was no statistical difference in hatchability among the temperatures (25 °C: 54% of 26; 28 °C: 71% of 31; 32 °C: 60% of 25; L–R $\chi^2 = 1.86$, $p = 0.40$, $N = 82$). As expected, incubation duration of eggs was shorter for lizards incubated at warmer temperatures (25 °C: 69.9 ± 3.3; 28 °C: 51.1 ± 1.8; 32 °C: 38.4 ± 1.8; ANOVA, $F = 679.1$, $p < 0.0001$, $N = 51$). The sex ratio differed among temperatures consistent with previous reports of jacky dragons, with 8% males at 25 °C ($N = 13$), 41% males at 28 °C ($N = 22$), and 27% males at 32 °C ($N = 15$; L–R $\chi^2 = 5.08$, $p = 0.08$, $N = 50$).

3.1. Body size and shape

Mass on the day of hatching was positively correlated with egg mass (see Table 1). Hatchling snout-vent length (SVL) at hatching was not influenced by incubation temperature, sex or egg mass. During the first month after hatching, hatchlings from 32 °C tended to grow less in length (Change in length per day: Incubation temperature, $F_{2,37.7} = 2.59$, $p = 0.09$; Sex, $F_{1,40.4} = 0.11$, $p = 0.74$,

Table 1
Effects of incubation temperature and sex on SVL and mass of hatchlings at hatching, 1 month and three months old.; 95% CI on parameter estimate for Mom ID all spanned zero.

Variable (N)	Inc. Temp.			Sex		
	F	df	p	F	df	p
SVL Hatch (48)[a]	1.36	2,41.1	0.27	0.02	1,39.8	0.89
Mass Hatch (45)[b]	1.96	2,37.6	0.16	0.03	1,36.0	0.85
SVL, 1 mo (46)	3.12	2,39.0	0.055	0.01	1,37.2	0.92
Mass, 1 mo (45)	1.43	2,38.9	0.25	0.38	1,37.5	0.54
SVL, 3 mo (41)	1.11	2,36.0	0.34	0.18	1,34.0	0.67
Mass, 3 mo (41)	1.58	2,35.7	0.22	0.10	1,33.5	0.75

[a] Egg mass also entered into model: $F_{1,17.3} = 0.03$, $p = 0.83$.
[b] Egg mass also entered into model: $F_{1,18.2} = 11.2$, $p = 0.004$.

$N=45$) and be smaller in SVL at one month of age (compared to hatchlings from cooler temperatures; Table 1); however, these effects were weak and not significant at the $\alpha=0.05$ level. By three months of age, there were no significant differences in size (Table 1 and Fig. 1). This was not due to compensatory growth by 32 °C hatchlings between one month and three months (Change in length per day: Incubation temperature, $F_{2,35.9}=0.75$, $p=0.48$; Sex, $F_{1,33.8}=0.68$, $p=0.42$, $N=41$), but rather due to selective mortality. Smaller hatchlings and females were more likely to die during this time interval compared to their counterparts (1-month survival: SVL, $\chi_1^2=14.8$, $p=0.0001$; Incubation temperature, $\chi_2^2=4.99$, $p=0.08$; Sex, $\chi_1^2=5.39$, $p=0.02$).

PCA performed on raw morphological traits (tail length; left hind limb length; left 4th toe length, left forelimb length; axilla-groin distance, head height, head width; head length) showed that PC1 explained 78.1% of the total variance. As expected, PC1 is highly correlated with snout-vent length ($R^2=0.93$, $p<0.0001$) and so primarily represents differences in body size. Despite having a high eigenvector for SVL, all others morphometric measurements had similar eigenvectors on PC1 (see Table 2). PC2 and PC3 represent 6.51% and 5.07% of the total variance and reflect differences in shape. PC2 had high positive eigenvectors for tail length and hind limb length and opposite direction (negative) high values for fourth toe length and head height. Therefore, individuals with high PC2 scores have long tails and hind limbs, shorter fourth toes and shallower heads. PC3 had high positive eigenvectors for fourth toe length and tail length and high negative eigenvectors for axilla-groin distance and forelimb length. In this component, individuals with high scores have long fourth toes and tails and short axilla-groin distances and forelimbs.

There were no strong differences in principal components among hatchlings from different incubation temperatures (Fig. 2;
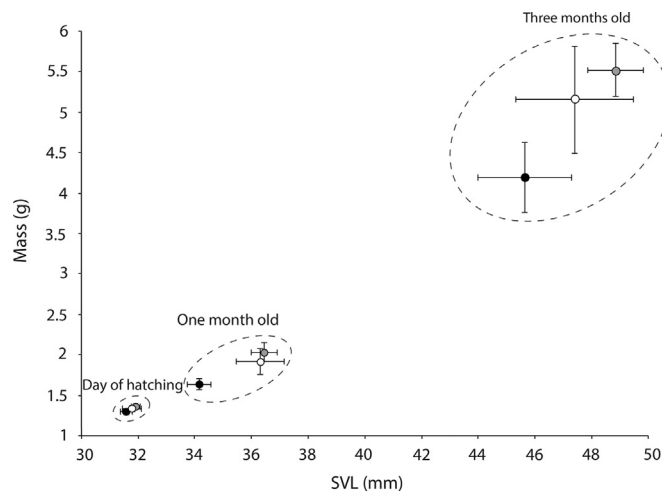
Table 3). PC2 tended to be higher for the hatchlings from 32 °C (Table 3). The mean PC2 score for these hatchlings was positive (0.41), and negative for hatchlings from 28 °C (-0.19) and 25 °C (-0.24). These results can be interpreted as hatchlings from 32 °C tending to have relatively longer tails and hind limbs and shorter fourth toes and shallower heads than the hatchlings incubated at cooler temperatures.

When considering the morphological measurements separately, only head shape was important when comparing incubation treatments (Table 3). Heads were significantly shallower and narrower in hatchlings from 32 °C. Sex has only a significant effect on head length, with males exhibiting longer heads than females (male mean$=13.1 \pm 1.32$; female mean$=12.88 \pm 1.38$).

3.2. Thermoregulatory behaviour

Across four observations during 2 h of hatchling thermoregulatory behaviour, hatchlings were observed in regions of the test cage with temperatures ranging from 21 °C to 51 °C. Mean temperature for the hatchlings' preferred position in the cage ranged from 21 °C to 49.4 °C. Thermoregulatory behaviour changed across the course of the observation period, with hatchlings in general choosing cooler regions for the '2-h' observation compared to earlier observations. However, incubation temperature influenced how temperature choice varied across time (strongly significant interaction between incubation temperature and observation time)—hatchlings from 25 °C and 32 °C showed considerable variation in temperature choice across the observation period, whereas hatchlings from 28 °C were relatively invariant in temperature choice over time (Fig. 3). In general, hatchlings from 28 °C
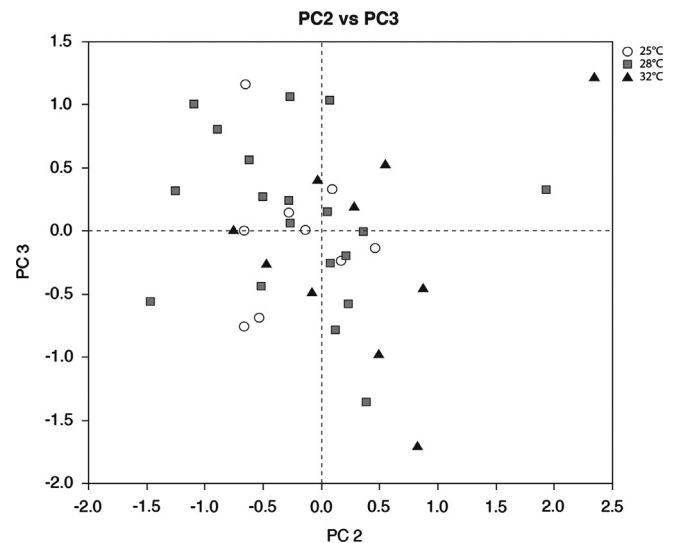

Fig. 1. Bivariate plot of hatchling SVL vs. mass at different ages and incubation temperature treatments. White circles, individuals incubated at 25 °C; grey circles, individuals incubated at 28 °C; black circles, individuals incubated at 32 °C.

Fig. 2. Scatter plot of PC2 vs. PC3. Open circles, individuals incubated at 25 °C; grey squares, individuals incubated at 28 °C; black triangles, individuals incubated at 32 °C.

Table 2
Results of Principal Components Analysis for nine morphometric measurements. Captions are PC, Principal Component; %variation, percentage of variation explained; SVL, snout-vent length; TL, tail length; HLL, hind limb length; 4T, fourth toe length; FLL, forelimb length; AGD, axilla-groin distance; HH, head height; HW, head width; HL, head length.

PC	Eigenvalue	%variation	Eigenvectors								
			SVL	TL	HLL	4T	FLL	AGD	HH	HW	HL
1	7.029	78.1%	0.363	0.290	0.329	0.308	0.337	0.327	0.326	0.351	0.362
2	0.586	6.512%	0.019	0.726	0.390	-0.342	0.083	-0.141	-0.36	-0.19	-0.105
3	0.457	5.073%	-0.076	0.278	0.077	0.626	-0.468	-0.506	0.069	0.178	-0.090

Table 3
Sample sizes (*n*), means and standard deviations (SD) for different morphometric measurements and principal component scores. All linear measurements are in (mm). Additionally, the results of mixed effect models on PC2, PC3 and all three month old hatchling morphometric measurements (these latter variables have SVL as a covariate) are presented. Captions are the same as in Table 1. Bold values are statistically significant (*p* < 0.05).

Variable	25 °C (N=10)	28 °C (N=20)	32 °C (N=11)	Inc. temp.		Sex		SVL	
	Mean ± SD	Mean ± SD	Mean ± SD	F	p	F	p	F	p
PC2[a]	−0.24 ± 0.42	−0.19 ± 0.75	0.41 ± 0.87	3.06	0.06	0.025	0.88	–	–
PC3[a]	−0.03 ± 0.57	0.09 ± 0.66	−0.16 ± 0.82	0.24	0.79	0.005	0.94	–	–
TL[a]	96.22 ± 24.01	102 ± 17.65	97.6 ± 23.1	1.16	0.33	0.07	0.80	33	**< 0.0001**
HLL	26.6 ± 4.9	26.70 ± 3.18	26.1 ± 3.14	2.21	0.13	2.91	0.10	79.76	**< 0.0001**
4T	11.4 ± 1.35	11.48 ± 1.39	10.53 ± 1.29	0.52	0.60	1.29	0.26	47.60	**< 0.0001**
FLL	20.5 ± 2.8	20.63 ± 1.91	19.73 ± 2.2	0.76	0.48	3.7	0.06	154.50	**< 0.0001**
AGD	23.4 ± 3.27	22.91 ± 2.41	21.6 ± 3.07	1.63	0.21	0.43	0.52	61.95	**< 0.0001**
HH	8.14 ± 0.83	8.38 ± 0.57	7.58 ± 0.7	3.62	**0.04**	0.42	0.52	72.17	**< 0.0001**
HW	11.58 ± 1.36	11.92 ± 0.96	10.71 ± 1.1	4.47	**0.02**	1.49	0.23	181.34	**< 0.0001**
HL	13 ± 1.66	13.20 ± 1.13	12.4 ± 1.38	2.44	0.10	8.30	**0.01**	450.28	**< 0.0001**

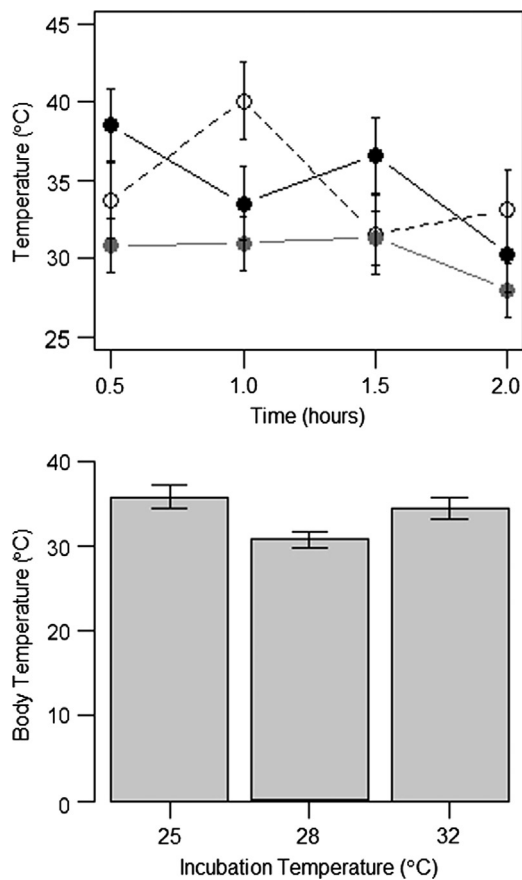[a] Sample size were 9, 19, and 10 due to hatchlings having tails too-kinked to measure.

Fig. 3. Thermoregulatory behaviour of hatchlings. (a) Thermal preferences across 2 h in a test cage varied across time and among incubation treatments (25 °C=open symbols; 28 °C=grey symbols; 32 °C=black symbols). (b) At the end of the 2-h thermal preference test, hatchlings from the 28 °C incubation treatment had a lower skin temperature than those from the other treatments.

Table 4
Thermoregulatory behaviour of hatchlings across 2 h of observation (four observations total). Hatchling ID was entered as a random effect to account for the repeated measures. N=163. Bold values are statistically significant (*p* < 0.05).

Predictor	F ratio	df	Prob > F
Incubation temp.	2.7603	236.03	0.0767
Time	4.5359	3113.2	**0.0048**
Time × Incubation Temp.	2.9889	6113.2	**0.0095**
Sex	0.4454	136.04	0.5088
Mass	0.1284	135.95	0.7222

3.3. Running performance

Hatchlings running at 30 °C body temperature had faster sprint times compared to 20 °C; however sprint time was not affected by incubation temperature or by the interaction of incubation temperature and running temperature (incubation temperature, $F_{2,35}=0.27$, $p=0.76$; running temperature, $F_{1,36}=5.35$, $p=0.03$; interaction, $F_{2,36}=0.31$, $p=0.74$; SVL, $F_{1,35}=2.83$, $p=0.10$).

4. Discussion

In many organisms, early-life environment can greatly impact phenotypes (Deeming 2004). To understand the evolution of this plasticity and associated maternal reproductive traits (e.g. nesting behaviour), we must first gain some insight into the fitness consequences of environmentally-induced variation in offspring phenotypes (Marshall and Uller, 2007). In our study of jacky dragons, we found that incubation temperature of eggs influenced aspects of hatchling body size and shape and thermoregulatory behaviours but had no effect of temperature-specific locomotor performance. Below we discuss each of these results in turn.

4.1. Body size and shape

We found weak evidence that hatchlings incubated at warm temperatures were smaller and grew more slowly, in agreement with previous findings (Warner and Shine, 2005). This difference was not evident at three months of age due to selective mortality of the smaller hatchlings from 32 °C. These results, which are based on hatchlings at the same age, compliment previous studies that examined size and survival on a seasonal basis. Warner and Shine (2005), (2007) have shown that time of hatching is important for size in jacky dragons (Warner and Shine, 2007). They demonstrated that early-hatching lizards (warm-incubated or

tended to prefer cooler locations within the thermoregulatory cage, although this effect failed to reach statistical significance (Table 4).

At the completion of the thermoregulatory trials, hatchlings from 28 °C had a lower body temperature than those from the other two incubation temperatures, while body mass and sex were not significant (Fig. 3; incubation temperature, $F_{2,38}=5.76$, $p=0.01$; Body mass, $F_{1,39}=0.14$, $p=0.71$; Sex, $F_{1,39}=2.21$, $p=0.15$; $r^2=0.27$, $N=41$).

early-laid) have lower survival before winter, but because they had more time to grow, these survivors ended up being larger than the late-hatched ones. Our results indicate that reduced pre-winter survival of warm-incubated hatchlings may be due in part to a lower survival rate per unit time as well as a greater time period in which to die.

Indirect effects of temperature on size, mediated by time of hatching, have been shown in many lizard species (Andrews et al., 2000; Shine, 1999; Sinervo and Doughty, 1996). The fact that we found only weak effects of incubation temperature on morphology at a set age suggests that the most important influence of incubation temperature on hatchling morphology is its influence on timing of hatching and subsequent growth opportunities (Warner and Shine, 2007),

Across reptile studies, there is no universal pattern in the correlation between incubation temperature and growth rate and size, which makes understanding the physiological mechanism difficult. The correlation appears to be positive in some cases (Alberts et al., 1997; Roosenburg and Kelley, 1996) but negative in most cases (Booth et al., 2004; Braña and Ji, 2000; Burgess et al., 2006; O'Steen, 1998; Van Damme et al., 1992). Our results suggest that the relation is more complex and not linear given that lizards from the intermediate temperature tended to be the largest.

We also observed that the two lower temperatures produced hatchlings with deeper and wider heads, and the smallest head dimensions in male and female lizards from 32 °C, a generally female-producing temperature. This coincides with the observation that adult male jacky dragons have larger heads in all three dimensions (Harlow and Taylor, 2000). Given our experimental design however, we cannot truly separate the effects of temperature and sex on head size. The possibility of the variation being caused by temperature per se rather than sex-specific differences is quiet intriguing in an evolutionary sense (O'Steen, 1998). If large head size is beneficial for males due to intrasexual competition (Peters and Ord, 2003), then developing as a male primarily at temperatures that lead to large heads (i.e. TSD) could be favoured evolutionarily.

4.2. Thermoregulatory behaviour

We found that incubation temperature affected hatchling thermoregulatory behaviour. Hatchlings from 28 °C were observed at fairly constant and low temperatures over a 2-h trial, in comparison with hatchlings from the other incubation treatments, which were regularly observed near the voluntary maximum body temperature for adult jacky dragons (38.4 °C, Heatwole and Firth, 1982). Indeed, hatchlings were observed at temperatures high enough (e.g. $> 40\,°C$) to assume that some must have been moving between hot and cold locations repeatedly. Consistent with these behavioural observations, hatchlings from 28 °C had lower body temperatures at the end of the trial. It is unclear what explains this variation or what its implications would be in the wild. Body size did not appear to be an important predictor of thermoregulatory behaviour or body temperature in our statistical models. Clearly, further experiments are needed to examine (1) thermoregulatory behaviour across greater time and social contexts, and (2) the relationship between thermoregulatory behaviour and fitness in this species.

While the temperatures that hatchlings were observed at are similar to those collected for adults in thermal gradient tests (Heatwole and Firth, 1982), we caution against using the current data to draw conclusions about body temperature preference in hatchling jacky dragons in general for three reasons: (1) the thermal gradient in our thermoregulatory cage was steeper than is ideal for a test of thermal preference, (2) we only measured body temperature once per individual, and (3) ibuttons under

spotlights have the potential to record higher temperatures than a hatchling lizard would experience. None of these caveats are capable of generating spurious results related to hatchling incubation temperature, as they apply equally to tests with all hatchlings; however, they should be considered when comparing our results with thermal preference data.

Temperature choice of an individual can affect several life history traits, including growth rate (Sinervo and Adolph, 1989, 1994), diet, locomotor performance, habitat choice and even possibly survival rate and fecundity (Adolph and Porter, 1993). Across reptile taxa, the influence of incubation temperature on thermal preference also exhibits conflicting patterns. As with most variables studied, the correlation between incubation temperature and thermal preference can be positive (Lang, 1987) or negative (O'Steen, 1998; Qualls and Andrews, 1999; Rhen and Lang, 1999). Nevertheless, as with size and shape, our findings on temperature preference do not exhibit a linear relationship between incubation and preferred temperatures, instead they show that intermediate temperatures produce the most stable temperature preference.

4.3. Locomotor performance

Our results suggest that neither incubation temperature nor sex have an effect on locomotor performance in jacky dragons, similar to one previous study in this species (Warner and Shine, 2005) while conflicting with another (Warner and Shine, 2010). We also examined whether performance was influenced by concurrent body temperature and whether temperature-dependent performance was linked to incubation temperature. The hypothesis that the thermal optima for individuals will be set by the temperature at which the embryo develops (Burger, 1990) has been tested in lizards before without support (Elphick and Shine, 1998). We found that higher body temperature led to faster running regardless of incubation treatment, therefore we similarly found no evidence to support this hypothesis.

5. Conclusion

Incubation temperature in jacky dragons affected their thermal preference, and some aspects of size and shape. However, it apparently did not affect locomotor performance. We postulate that there could be a mechanistic link between the effects of incubation temperature on growth and on temperature choice if hatchlings that prefer warmer temperatures are able to grow more. The size differences we found are apparently short lived, but they remain a focus of future research to determine if differences in shape and thermoregulatory behaviour are persistent through adult life. Even temporary effects can have a profound impact on fitness for short-lived species such as *A. muricatus*. Future directions on this subject should include testing the persistence of the differences in shape and thermoregulatory behaviour we found through the adult life of jacky dragons, and determine the significance these effects have on their reproductive fitness.

Wales Office of Environment and Heritage (permit SL101003). The research received ethical approval from the Australian National University Animal Experimentation Ethics Committee (A2012/12) and was funded by an Australian Research Council Discovery Early Career Researcher Award (LES) and a Becas Chile-Conicyt scholarship (DE).

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