Parental thermal environment alters offspring sex ratio and fitness in an oviparous lizard

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Summary Statement: This interdisciplinary study demonstrates that the thermal environment experienced by parents influences the phenotype of their offspring.

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Abstract

The environment experienced by parents can impact the phenotype of their offspring (parental effects), a critical component of organismal ecology and evolution in variable or changing environments. Although temperature is a central feature of the environment for ectotherms, its role in parental effects has been little explored until recently. Here, parental basking opportunity was manipulated in an oviparous lizard with temperature-dependent sex determination, the jacky dragon (*Amphibolurus muricatus*). Eggs were incubated at a temperature that typically produces a 50/50 sex ratio, and hatchlings were reared in a standard thermal environment. Offspring of parents in Short Bask (SB) conditions appeared to have better fitness outcomes in captive conditions than those of parents in Long Bask (LB) conditions – they had greater growth and survival as a function of their mass. In addition, the sex of offspring (male or female) depended on the interaction between parental treatment and egg mass, and treatment impacted whether sons or daughters grew larger in their first season. The interactive effects of treatment on offspring sex and growth are consistent with adaptive explanations for the existence of TSD in this species. Moreover, the greater performance recorded in SB offspring may represent an anticipatory parental effect to aid offspring in predicted conditions of restricted thermal opportunity. Together, these responses constitute a crucial component of population response to spatial or temporal variation in temperature.

Introduction

The parental environment is a key driver of offspring phenotypic variation (Mousseau and Dingle 1991; Bernardo 1996; Bonduriansky and Day 2009). These nongenetic parental effects (e.g. maternal effects) can enhance offspring fitness when the parental environment provides a reliable cue for the offsprings' environment (anticipatory parental effects or transgenerational plasticity; Mousseau and Fox 1998; Galloway and Etterson 2007; Marshall and Uller 2007; Uller et al. 2013; Leimar and McNamara 2015). Alternatively, phenotypic changes can be neutral or disadvantageous for offspring if they arise as by-products of parental physiology or favour parental fitness at the expense of each offsprings' fitness (Marshall and Uller 2007). Because such phenotypic change occurs via nongenetic means and has uncertain heritability, parental effects impact evolutionary adaptation to variable or changing environments (Räsänen and Kruuk 2007; Badyaev and Uller 2009; Bonduriansky and Day 2009; Bonduriansky et al. 2012).

One major environmental factor that impacts living organisms is ambient temperature. Temperature may influence physiological rates, phenology, behaviour, and sexual development (for species with temperature-dependent sex determination (TSD), Janzen and Paukstis 1991; Parmesan and Yohe 2003; Visser and Both 2005; Schwanz and Janzen 2008; Angilletta 2009). Accordingly, variation in temperature (geographical, micro-habitat or temporal) has implications for demographic parameters and population persistence (Parmesan and Yohe 2003; Visser and Both 2005; Kearney and Porter 2009; Wapstra et al. 2009; Schwanz et al. 2010a; Sinervo et al. 2010; Boyle et al. 2014).

Temperature-related changes in parental behavior may represent a key process in determining offspring incubation temperatures and phenotypes across variable thermal environments (i.e. thermal parental effects), and be equally important as thermal adaptation and acclimation (Angilletta 2009; Sgro et al. 2016). For example, the timing and location of

oviposition in egg-laying animals often responds to variation in temperature, and strongly influences developmental conditions (oviposition effects; Parmesan and Yohe 2003; Doody et al. 2006; Schwanz and Janzen 2008; Refsnider and Janzen 2010; Schwanz et al. 2010a). Similarly, in viviparous ectotherms, parental thermoregulatory behaviour directly impacts embryonic developmental temperatures (Shine and Harlow 1993; Wapstra 2000; Robert and Thompson 2001; Wapstra et al. 2004; Burgess and Marshall 2011).

Thermal parental effects may also operate via pre-oviposition physiological mechanisms that do not impact offspring developmental temperature. In invertebrates, parental cold exposure or heat shock prior to oviposition can improve offspring tolerance of the same temperature stressor (Zhou et al. 2013; Valentina Zizzari and Ellers 2014). Similarly, damselfish can maintain metabolic performance (i.e. aerobic scope) at warm temperatures if their parents were acclimated to warm temperatures (Donelson et al. 2011).Across animals, experimental parental temperatures during egg production impact components of offspring fitness, often enhancing fitness in warmer compared to cooler parental temperatures (Gilchrist and Huey 2001; Shine 2006; Steigenga and Fischer 2007; Lorioux et al. 2012; but see Rodríguez-Díaz and Braña 2011; Guillaume et al. 2016). In several fish species, offspring growth at a given temperature is greatest when the parental temperature iss the same (Bownds, Wilson and Marshall 2010; Salinas and Munch 2012; Shama et al. 2014; Shama 2015). Moreover, the complexity of thermal nongenetic effects is becoming more apparent through evidence of persistence to the grand-offspring generation (Shama and Wegner 2014), discordant paternal and maternal effects (Shama and Wegner 2014; Guilluame et al. 2016), and possible bet hedging of offspring phenotype (Shama 2015).

Thermal parental effects are particularly important for species with TSD, as air temperature influences nest and cohort sex ratios and can lead to heavily biased adult sex ratios that hasten population demise (Schwanz et al. 2010a; Boyle et al. 2014). Thus, it is

particularly intriguing that temperatures experienced by parents can influence offspring sexual development independent of incubation temperature in species with TSD (Schwanz et al. 2010b; Warner et al. 2013). This transgenerational plasticity in sexual outcome could mitigate the negative impacts of climatic variation on population sex ratios and offspring fitness (i.e. frequency-dependent selection on sex; Schwanz et al. 2010a,b).

Surprisingly, there is a relative dearth of research on pre-oviposition parental effects due to temperature (Sgro et al. 2016). Yet these effects are likely to be common given the demonstrated importance of other pre-oviposition factors such as maternal diet, maternal age and clutch order on offspring size, sex, and behaviour (Mousseau and Dingle 1991; Fox et al. 2003; Warner et al. 2008; Radder *et al.* 2009, Paranjpe *et al.* 2013). Moreover, physiological mechanisms of parental effects in oviparous taxa are diverse (Jablonka and Raz 2009), including transfer of antibodies and antioxidants from mother to egg (Grindstaff et al. 2003; Berthouly et al. 2008), nutrient provisioning (Radder et al. 2009), deposition of yolk hormones (Groothuis et al. 2005; Radder 2007; Uller et al. 2007), and epigenetic mechanisms (Jablonka and Raz 2009). Thus, thermal parental effects have unexplored potential to alter offspring phenotypes and demand much greater empirical attention.

In this study, I examined whether parental thermal environment has an influence on offspring phenotype in a dragon lizard species with TSD. In the Jacky dragon (*Amphibolurus muricatus*), cold (below 25°C) and hot (above 33°C) temperatures produce all female offspring, while intermediate (27-28°C) temperatures produce a roughly 50:50 sex ratio (Harlow and Taylor 2000; Warner and Shine 2005). This form of sex determination appears to be adaptive, as males and females have highest lifetime fitness when incubated at their respective, natural temperatures (Warner and Shine 2008a). Natural nest temperatures have been recorded to average 23-28°C (Harlow and Taylor 2000; Warner and Shine 2008b).

For the present study, parental basking opportunities (number of hours with access to a basking lamp) during egg production were manipulated experimentally in a lab colony of jacky dragons. I examined whether parental treatment influenced offspring size, sex and survival. Importantly, eggs and offspring were reared in common conditions, with an egg incubation temperature that typically produces a 50:50 sex ratio. Thus, any differences between treatments can be attributed to pre-oviposition parental effects.

Material and Methods

Study Animal

All jacky dragons used in this study were wild-caught from near Canberra, Australia (Wamboin, New SouthWales; 35.25001S; 149.29171E; elev. ~800m) or were 2-year-old lab-born individuals. Animals were housed indoors at the University of New South Wales.

Experimental Design

Three females and one male were housed together in opaque plastic cages (500mm × 300mm × 300mm) with sand floors. Bark, branches, ceramic flower pots or tiles served as shelters and basking objects. Lizards were fed with 4-6 gut-loaded crickets, depending on apparent hunger, 3 times per week (dusted with vitamins 1-2 times per week) and had ad lib access to water. Each cage lid was aluminium mesh, with an external UV light and incandescent basking lamp. UV lights and room lights were adjusted every two weeks to match natural daylight hours.

Experimental treatments were established in late winter (August) by assigning either 7 hours (Short-bask, SB) or 11 hours (Long-bask, LB) of daily basking lamp duration to each cage. The temperature directly under the basking lamp can reach 56.6°C, while the far side of the cage averages 24°C. This creates substantial variation to obtain preferred body temperatures (~35°C in *A. muricatus*; Heatwole et al. 1973). The treatment durations were similar to common protocols for studies on captive lizards (4-10 hrs; e.g. Radder et al. 2007; Cadby et al. 2014) and constrained by previous research in the jacky dragon showing that 5 hours of basking lamp is insufficient for reproduction (Halstead and Schwanz 2015). The source population of the colony experiences daylengths of 12.5 to 14 hours during October and November. For lizard species that are active thermoregulators in thermally-heterogeneous habitats, body temperatures are often near preferred temperatures (or above 30°C) for the majority of sampled daylight hours (up to11 hours of the day; e.g. Huey 1982;

Christian and Weavers 1996; Andrews et al. 1997; Lara-Resendiz et al. 2015). Thus, although insufficient data are available on the thermoregulatory behaviour of wild *A. muricatus*, 11 hours seems likely to be near the maximum duration of daily thermoregulatory ability during the breeding season.

Cages were assigned to treatments so as to ensure similar average body sizes and ages of females in the two treatment groups. The treatments were initiated 2 weeks after being brought from brumation to room temperature and 1 week after first access to a basking lamp. A total of 43 females (22 LB and 21 SB) and 14 males were used for the study. One LB female had to be euthanized during production of her first clutch and was replaced approximately 1 month into the study with an additional female.

In the middle of the gravid season (October, Julian dates 280-289), basking behavior of lizards was measured by affixing iButton temperature loggers (wrapped in parafilm; DS1921G Thermochron iButton,Maxim Integrated, San Jose, CA, USA) to the lizards' upper dorsum with superglue. For ethical reasons, iButtons were only applied to lizards that exceeded 30g mass, ensuring the device was no more than 10% of the lizard's body mass. Temperature loggers were programmed to record temperature every 10 minutes. These temperatures combine thermal inputs from the lizard's body, ambient air and incident radiation; thus they are not strictly body temperature. Data were downloaded from the temperature loggers using 1-Wire data software (OneWireViewer ver. 3.15.50, Maxim Integrated, San Jose, CA, USA).

Females were inspected visually each day for signs of gravidity, followed by palpation if gravidity was suspected. Fully gravid females (those with eggs detected on palpation) were checked daily for egg deposition. Subsequently, the sand in the cage was searched for nests that had been constructed. If females did not lay their eggs within two

weeks of becoming fully gravid, they were induced to lay their eggs with peritoneal injection of 1-6 I.U. of oxytocin/100 g.

Eggs were cleaned of sand and other debris, weighed (to 0.001 g), and placed individually in 120:100 water:vermiculite by mass (approximately -200 kPA) within a labelled 125 ml glass jar (vermiculite from L&A Fazzini, Greenacre, NSW, Australia). Egg jars were covered with plastic cling wrap (GLAD® ClingWrap, Clorox Pty Limited, Padstow, NSW Australia), sealed with a rubber band, and incubated at a constant 28°C. For first clutches produced by each female, we attempted to draw up to 50-200 μl of yolk from two eggs for later hormone sampling (results not presented). Sampled eggs were reweighed following yolk sampling. A similar proportion of incubated eggs from each treatment were sampled for yolk (LB: 23.9% (N=117); SB: 27.2% (N=114)). This reduced the egg mass by an average of 0.14 ± 0.06 g (approximately 10% of egg mass). For later clutches by each female, two whole eggs were frozen.

Eggs were checked once daily for hatching. All post-hatch measurements were conducted blind to parental treatment. Hatchlings were blotted dry and weighed to the nearest 0.001 g. Snout-vent length was measured by stretching the hatchling against a clear ruler and recording SVL with digital calipers (nearest 0.01mm). Sex was recorded during the first week post-hatch by a single researcher (the author) via hemipenes eversion under a dissecting microsope (Harlow 1996). Accuracy of this procedure was assessed by re-examining sex in the first autumn and at approx. 1.5 years of age. Of 133 hatchlings re-examined at autumn, sex concurred with sex at hatching for 126 (94.7%). Forty-seven of these hatchlings were alive at 1.5 years, and all retained the same sex assignment (100%). Thus, the accuracy of sex assignment at hatching is 95%. Of the seven hatchlings whose sex assignments changed in the autumn, sex at 1.5 years matched the autumn assignment for two lizards and matched the original hatching assignment for two lizards (three were no longer alive).For the first week

after hatching, hatchlings were housed individually in pencil trays (305mm × 75mm × 50mm) with paper towel substrate, ad lib water and no food. UV lights and incandescent lights were suspended above the pencil trays. At approximately a week after hatching, hatchlings were given a unique toe-clip and group-housed in plastic bins (250mm × 300mm × 300mm) with a paper towel floor and a mesh lid. Hatchlings were provided with a UV light set to natural daylength, a basking lamp on 08:00 to 17:00 (9 hrs), basking and sheltering objects, and ad lib water. They were fed daily with 4-6 gut-loaded pinhead or weenie crickets (dusted with vitamins 3 times per week) for approximately the first month, then fed 5 times per week until approximately 3 months old, subsequently followed by 3 times per week. Five to eight hatchlings were housed together, with the oldest and youngest hatchlings no more than 1 week different in age. This variation in initial hatchling cage density was unavoidable due to the demand of keeping all hatchlings within 1 week of age. For all post-hatching traits, two cages with fewer than seven initial hatchlings were excluded to reduce noise associated with variable growth conditions.

Mass and SVL were recorded again at 1 month of age and before colony brumation in the autumn (27-28 April). Autumn weights were recorded to 0.01 g. Age variation within a cage could introduce natural dominance relationships, and the number of hatchlings in the cage (density) may influence levels of competition. To account for these complications, rank age within a cage (1=oldest) was assigned to each hatchling. Animals hatched on the same day were given the same rank. The average density was calculated starting when the last hatchling was added. Both calculations were weighted over time according to deaths within the cage. The weighted values for rank age and density were calculated for the first month and for the interval until autumn measurements.

Data Analysis

Basking behaviour. Temperature logger data were extracted between the hours of 7:00 and 19:00 (the hours of basking lamp access for the LB treatment group plus one additional hour to capture thermal inertia) over 10 days of recording. From these intervals, I calculated the mean temperature and summed the total number of records that were greater than or equal to 30°C and 35°C for each lizard. The first temperature (30°C) is the maximum recorded by iButtons placed on the coldest side of the cage, thus assesses the extent to which lizards are seeking warmer temperatures than baseline. The second temperature is the preferred body temperature of jacky dragons (Heatwole et al. 1973). The three response variables were examined in separate mixed-effect models with treatment and sex as factors and cage entered as a random effect. Sex was not significant in any model, so was removed.

Reproductive timing and output. The proportion of females breeding in each basking treatment (SB and LB) and number of clutches per female were analysed with likelihood-ratio chi-square tests. Clutch lay date (excluding the late-added female) and clutch size were analyzed with linear mixed effect models, including clutch order as a predictor (maternal ID was entered as a random effect to account for multiple clutches per female). Maternal SVL and its interaction with treatment were included in all models. The interaction was removed when p > 0.15, but SVL was retained in all models.

Egg and offspring traits. Response variables related to parental effects on individual offspring were examined in separate models and consisted of egg mass, 'hatching' traits (hatching success, and hatchling sex, SVL and mass), '1-month' traits (SVL and mass), 'autumn' traits (SVL and mass), and two intervals of post-hatch survival (hatching – 1 month; 1 month – autumn). Continuous response variables were examined using linear mixed effect models, whereas categorical response variables were examined with logistic mixed effect models. All models included parental treatment as a fixed effect and maternal ID as a

random effect to account for multiple offspring from the same mother. Additional covariates and cofactors were entered into each model based on *a priori* expectations of potential modifiers. Models were reduced via sequential model reduction ($p > 0.15$), starting with interaction terms. Treatment was always retained in the models.

The model of egg mass included additional predictor variables of maternal SVL, clutch lay date, and their interactions with parental treatment. For 'hatching' response variables, 16 frozen eggs were excluded. Five full-term embryos that were excised from their eggs dead or severely deformed (warranting immediate euthanasia) were considered failures for hatching success and were excluded from phenotypic analyses. Predictor variables entered into all 'hatching' models were maternal SVL, clutch lay date, egg mass, plus all interactions with treatment. The models for hatchling SVL and mass contained sex and the identity of the researcher measuring the traits due to potential researcher bias in measuring SVL (3 researchers measured hatchling and 1-month traits; 2 researchers measured autumn traits). In addition, hatchling SVL was included as a covariate in the model of hatchling mass; thus, the results should be interpreted as residual body mass after accounting for length. It was unclear *a priori* which measure of egg mass should be used as a covariate in these models: 1) final egg mass, or 2) initial egg mass (plus yolk sampling (Y/N) and the interaction). Complementary models for each egg mass option were initiated and the two final (reduced) models were compared with AICc. The preferred model is presented here, along with the ΔAICc compared to the less-preferred model.

Models of SVL and mass at 1-month and autumn as response variables included predictors of sex, initial residual mass (at the start of the survival interval), cage rank age, cage density and all interactions with treatment. The mass model additionally included concurrent SVL as a predictor. Mass and SVL at autumn included age (in days) as a covariate. Traits at 1-month used rank age and density within the cage in the first month,

whereas traits in the autumn used rank age and density across the active season. I excluded 11 hatchlings from two cages where the starting number of hatchlings was less than seven to reduce variation due to cage density.

Survival over the two time intervals was examined with additional predictors of sex, initial SVL, initial residual mass, cage rank age, cage density and all interactions with treatment. Residual mass was calculated from a linear regression of mass versus SVL using all live offspring (at hatch: $r^2 = 0.49$, $p < 0.0001$, $N = 161$; at 1-month: $r^2 = 0.74$, $p < 0.0001$, $N = 137$). All statistical analyses were conducted using JMP release 12 (SAS Institute Inc., Cary, North Carolina, USA).

Results

Basking behaviour

Owing to 4 hours of additional basking opportunity, lizards in the LB treatment had a higher mean body temperature between 7:00 and 19:00 (33.4 ± 0.8, $N = 20$) compared to SB lizards (31.4 ± 0.6, $N = 17$; $F_{1,12.91} = 34.2$, $p < 0.0001$). Similarly, across the 10 days of 12-hr intervals, LB lizards had a greater number of records above 30°C compared to SB lizards (LB, 628.4 ± 48.1, $N = 20$; SB, 457.1 ± 40.8, $N = 17$; $F_{1,12.45} = 80.1$, $p < 0.0001$). In both treatments, the quantity of these records for both treatments approximated the total number of samples with the basking lamp on (LB: 660 readings; SB: 420 readings), consistent with lizards actively thermoregulating for the duration of their basking lamp access. Record totals in excess of the maximum lamp access were likely caused by thermal inertia of the lizard, light bulb, and basking objects. Treatments did not differ in records above the preferred temperature (35°C; LB, 234.2 ± 99.8, $N = 20$; SB, 208.0 ± 59.5, $N = 17$; $F_{1,12.77} = 0.73$, $p = 0.41$).

Reproductive timing and output

A similar percent of females bred in each of the parental basking treatments (Table 1). Chemical induction of egg-laying was required for 26.3% (5/19) of clutches produced by LB females and 28.6% (6/21) of clutches produced by SB females. Parental treatment did not significantly influence total number of clutches, clutch lay date, or clutch size (Table 1).

Egg and Offspring traits

Egg mass. Mass of eggs decreased with increasing laying date, but did not differ between treatments (Table 2). Final egg mass following yolk sampling (for eggs not frozen) similarly did not differ between treatments (F (treatment) = 0.04, $p = 0.84$; F (clutch date) = 5.96, $p = 0.02$; F (date*treatment) = 2.07, $p = 0.15$; $N = 235$).

Hatching traits. Hatching success increased with egg mass and decreased if yolk was taken (Table 2). The majority of eggs less than 1.0 g was either lacking the red spot associated with fertilization or had turned yellow or hard prior to discovery. Hatching success did not depend on treatment or any of its interactions (LB, yolk unsampled: 71.9% (64/89); LB, yolk sampled: 65.4% (17/26); SB, yolk unsampled: 73.2% (60/82); SB, yolk sampled: 64.5% (20/31)).

LB females had a near-significant tendency to produce overall more daughters than SB females (Table 2, Fig. 1A). SB eggs were more likely to develop as daughters as egg mass increased compared to LB eggs (Table 2, Fig. 1B). This correlation was not driven by the correlation between lay date and egg mass, as lay date was eliminated from the model, and an alternative model excluding egg mass similarly eliminated lay date (results not shown). Sampling yolk (i.e. reducing egg mass) had a non-significant tendency to increase the chances of a male developing. In the less-preferred model of offspring sex, egg mass after yolk sampling interacted with parental treatment in the same fashion, but the interaction term was not significant.

Hatchling SVL was positively correlated with egg mass, but did not differ between treatments (Table 2). Hatchling mass increased with egg mass after accounting for the positive correlation with SVL (Table 2). Offspring sex, treatment, and their interaction did not explain variation in residual mass (Fig. 2A), but SB hatchlings gained more residual mass as egg mass increased than did LB hatchlings.

1-month traits. SVL at 1 month was greater for hatchlings with higher residual mass at hatching, and this effect was greater for SB offspring than LB offspring (Table 3). SVL at 1 month was also greater for older hatchlings within a cage and varied by treatment and sex − SB daughters and LB sons tended to be the longest ($p = 0.054$; Table 3). At 1 month, SB hatchlings had greater residual mass than LB hatchlings, and SB daughters and LB sons had

greater residual mass than their counterparts (Table 3; Fig. 2B). While higher density cages led to lower mass, this effect was weaker in LB offspring compared to SB offspring.

Autumn traits. SVL at autumn was positively related to age and negatively related to cage density, but was not related to treatment (Table 3). Residual mass at autumn still depended on the interaction between treatment and sex, with SB daughters and LB sons having the greatest residual mass (Table 3; Fig. 2C).

Survival. Survival rates were high overall (Table 3). Survival in the first month post-hatch was higher for hatchlings with high residual mass at hatching and for the oldest hatchlings within a cage (lowest rank age). The effect of residual mass on increased survival was greater for SB hatchlings than LB hatchlings (Fig. 3A).

Only 12 additional hatchlings died between 1 month and autumn (Table 3). Survival during this time was higher in less-dense cages and was greater for males. SB had greater survival probability as residual mass increased compared to LB (Fig. 3B). Because the hatchlings were different ages, survival until autumn is across a diversity of total number of days; however, reproductive timing and, hence, hatchling age did not differ between treatments.

Discussion

In this study of oviparous jacky dragons, duration of parental basking opportunity during egg development influenced offspring growth, sex and survival. These impacts were not driven by variation in the overall timing or energetic investment of reproduction by mothers, and thus represent direct alteration of offspring behaviour or physiology. Two main impacts were observed. First, the offspring of Short Bask (SB) parents appeared to get a boost in survival and growth compared to the offspring of Long Bask (LB) parents (Fig. 4). As energetic resources (egg mass) increased, offspring of SB parents gained more than those of LB offspring in fitness outcomes. They hatched with higher residual mass, and extra residual mass provided SB offspring with an increase in survival in their first month and through autumn. Greater residual mass also provided SB offspring with enhanced growth in the first month and led to greater mass overall at 1 month (which did not persist until autumn; Fig. 4). Consistently, SB offspring had better outcomes than LB offspring as their residual mass increased. This persistent interaction suggests that the boost observed in SB offspring was driven by physiological processes dependent on somatic body reserves; for example, through enhanced metabolic performance or efficiency.

The second major impact of parental basking opportunity was particularly intriguing. Parental treatment led to altered sexual outcome for offspring and a persistent sex-differential effect on offspring size (Fig. 4). LB parents tended to produce more daughters than SB parents, and there was an interaction between parental treatment and egg mass – SB offspring increasingly developed as females as egg mass increased, whereas LB offspring appeared more likely to develop as a male at greater egg masses.

Concomitantly, the sex of offspring that grew better differed between parental treatments. SB daughters were heavier than their brothers through the autumn, while LB sons were heavier than their sisters (Fig. 4). This interaction persisted after accounting for egg

mass, which indicates that SB daughters had higher residual mass than their brothers throughout the season not simply due to hatching from larger eggs, but also from having greater growth.

A temperature-by-sex effect on fitness is the hallmark of evolutionary hypotheses for temperature-dependent sex determination (i.e. sex allocation theory; Schwanz et al. 2006; Schwanz et al. 2016). In the present study, 'temperature' was experienced by parents rather than offspring, but may also represent thermal conditions during embryonic development and post-hatching. Adaptive hypotheses for TSD in lizards tie together incubation temperature, timing of hatching, and growth in the first active season (e.g. Warner et al. 2009a; Schwanz et al. 2016). Warm eggs hatch early and provide more time for hatchling growth before the winter. Similarly, a summer with more thermal opportunities should allow more growth. It is unclear, however, whether high growth benefits male or female hatchlings more in lifetime fitness. High growth (but not slow growth) may allow females to reach reproductive size in their first or second year, while males will not reproduce until several years later when they acquire a physically-competitive body size (Warner et al. 2009a; Schwanz et al. 2016). Alternatively, body size may be more important for reproductive success in males than females in each year that they breed (Warner and Shine 2008a).

In the present study, a restricted thermal environment (SB) was associated with producing large daughters and small sons, and an extended thermal environment (LB) was associated with large sons and small daughters. This supports a hypothesis that sons benefit more than daughters as growth conditions improve, assuming parental thermal conditions are predictive of thermal opportunities for hatchlings. That is, hatching from a large egg and developing in extended thermal conditions allows males to acquire a competitive body size, while the maximum size that could be obtained with large eggs in restricted thermal conditions is insufficient to produce competitive males. In contrast, starting large in a

restricted thermal environment or starting small in an extended thermal environment may have little effect on relative fitness in females. Only an experiment that simultaneously manipulates pre- and post-oviposition environment would allow testing this hypothesis.

This hypothesis, at first consideration, seems inconsistent with the tendency for LB parents (extended thermal conditions) to produce more daughters. The disparity may be explained by frequency-dependent selection on sex, which maintains sex ratios at an equilibrium (not necessarily 50:50; Charnov 1982). Given that nest temperatures in wild jacky dragons normally range from all-female cold conditions (24°C) to mixed sex ratio warm conditions (28°C; Harlow and Taylor 2000; Warner and Shine 2008b), natural cohort sex ratios are likely female-biased, and a warm year could lead to more sons than is evolutionarily stable. An increase in daughters developing at 28°C could reflect a parental strategy to restore the equilibrium sex ratio (assuming LB parental environments are associated with warm nests; Schwanz *et al.* 2010b). Sex ratios often vary across time due to climatic variation (e.g. Schwanz et al. 2010a; Wapstra *et al.* 2009), which can destabilize populations, cause local maladaptation, or counter-act the evolution of TSD (Schwanz and Proulx 2008; Boyle et al. 2014; Harts et al. 2014). If wild populations of jacky dragons plastically altered sexual development as a function of climate in such a way that sex ratio fluctuation was minimized, it would have positive implications for persistence under climate change.

The ecological and evolutionary impacts of this study's finding depend on the links between climate and parental basking opportunity, as well as between parental, nest and hatchling thermal conditions. Little is known about thermoregulation of wild jacky dragons, and body temperatures of actively-thermoregulating ectotherms are hard to predict from climate due to complex use of microhabitats. Unpublished data from jacky dragons in semi-natural enclosures indicates that they acquire preferred body temperatures whenever either

direct sun or warm air temperatures are available, up to 9 or 10 hours a day (direct sunlight restricted prior to 9am due to high enclosure walls, Schwanz, unpubl. data). These observations are consistent with long hours (up to 11 hours) in the preferred temperature ranges observed in other actively-thermoregulating lizards (Huey 1982; Christian and Weavers 1996; Andrews et al. 1997; Lara-Resendiz et al. 2015). In the present study, jacky dragons acquired body temperatures above 30°C for all hours that the basking lamp was on in both treatments, suggesting that basking opportunities will be sought whenever climate allows. However, it is important to note that warmer climates may not necessarily lead to greater hours of thermoregulation each day, as hot air temperatures can restrict lizards to cool refuges and restrict thermoregulatory opportunity (Huey et al. 2009; Sinervo et al. 2010).

The results of this study suggest interesting lines of inquiry as to the physiological mechanisms of thermal parental effects. First, the phenotypic response of offspring could be a direct result of differences in early embryonic developmental temperatures prior to oviposition. In oviparous squamates, the time that developing embryos are retained in utero represents a substantial proportion of total development time (25-40%; Shine 1983). The eggs of jacky dragons are laid at around stage 30, when embryonic squamates are typically 3-6 mm long and have limb buds, but thought to be prior to the temperature-sensitive phase (Shine 1983; Harlow 2001). However, the sex of jacky dragons is sensitive to experimental sex-reversal by hormones immediately following oviposition (Shine et al. 2007), so the possibility remains that sexual lability associated with temperature also occurs near the end of the uterine stage.

Second, steroid hormones in the yolk of birds and reptiles, such as testosterone and corticosterone, are known to influence post-hatching growth (Hayward and Wingfield 2004; Rubolini et al. 2006; Uller et al. 2007). Yolk hormones can also affect the growth in males and females differently (Tschirren 2015), thus are a likely candidate for producing the sex-

specific parental effects on size observed in the present study. Sexual outcome is also strongly influenced by hormones, especially for species with temperature-dependent sex determination. Sex reversal at a given temperature using exogenously-applied hormones has been a component of experimental research on TSD species for decades (e.g. Pieau 1996). In jacky dragons, corticosterone applied to the outside of eggs has been shown to depress post-hatching growth and lead to more daughters (Warner et al. 2009b), a combined effect observed in LB offspring in the present study. Moreover, poor maternal diet in jacky dragons decreases yolk testosterone but increases the proportion of sons produced (Warner et al. 2007).

From an evolutionary perspective, it is tempting to hypothesize that the boost in growth and survival recorded in SB offspring in a common thermal environment is adaptive based on the anticipation that offspring will be limited in thermal opportunity (anticipatory parental effects; Mousseau and Fox 1998; Uller et al. 2013; Burgess and Marshall 2014; Leimar and McNamara 2015). For example, experimental water temperature in sheepshead minnows strongly impacted offspring growth such that offspring grew the fastest when reared at temperatures that matched that of their parents (Salinas and Munch 2012). The present study standardized offspring environments to isolate parental effects, so a follow-up experiment that separates offspring into post-hatching environments that match or mismatch the parental environment is necessary to test an anticipatory hypothesis.

This study demonstrated clear parental effects due to thermal environment in a terrestrial vertebrate, contributing to the growing appreciation that transgenerational plasticity plays an important role in evolutionary ecology in thermally variable environments. Moreover, the interaction observed between parental thermal treatment and sex on offspring outcomes provides novel support for the evolutionary significance of temperature-dependent sex determination.

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Data Accessibility

The data presented in this paper will be made available in Dryad upon paper acceptance.

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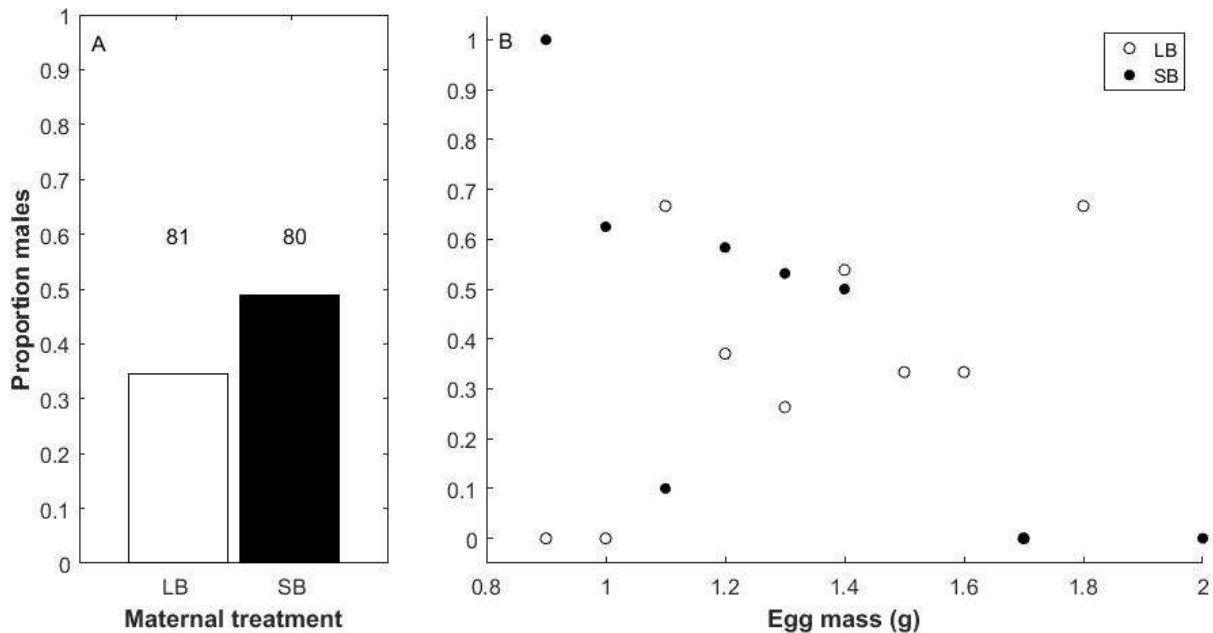
Figures

Figure 1. Effect of parental basking treatment – Short Bask (SB; 7 hours) and Long Bask (LB; 11 hours) – on offspring sexual outcome when incubated at 28°C. A) Total proportion of males that hatched from each parental treatment; numbers indicate sample size of individual offspring. B) Proportion of males that hatched, binned according to every 0.1g interval of egg mass (filled circles – SB; open circles – LB). In both panels, n(LB)=81 and n(SB)=80.
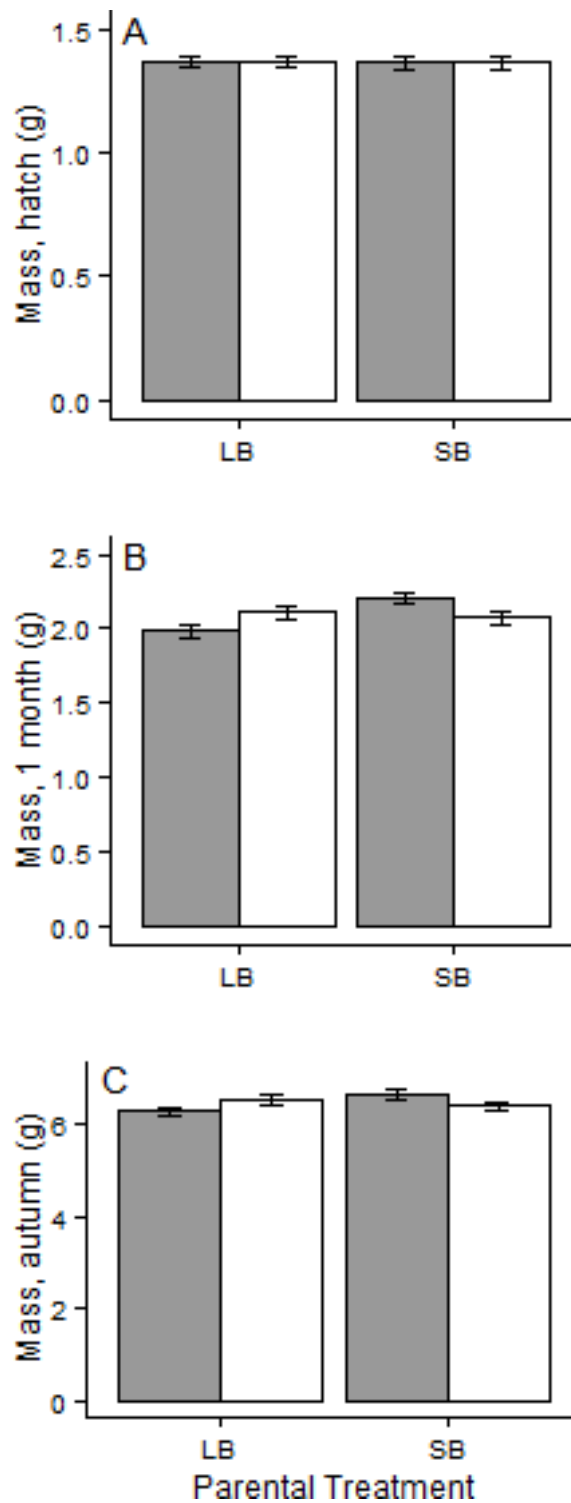
Figure 2. Residual offspring mass for daughters (grey bars) and sons (white bars) as a function of parental basking treatment (Short Bask, SB, 7 hours; Long Bask, LB, 11

hours). Data for A) day of hatching (n(LB)=81; n(SB)=80), B) 1 month old (n(LB)=68; n(SB)=69) and C) autumn (April; n(LB)=61; n(SB)=63). Bars show LSM from final statistical models ± SEM.
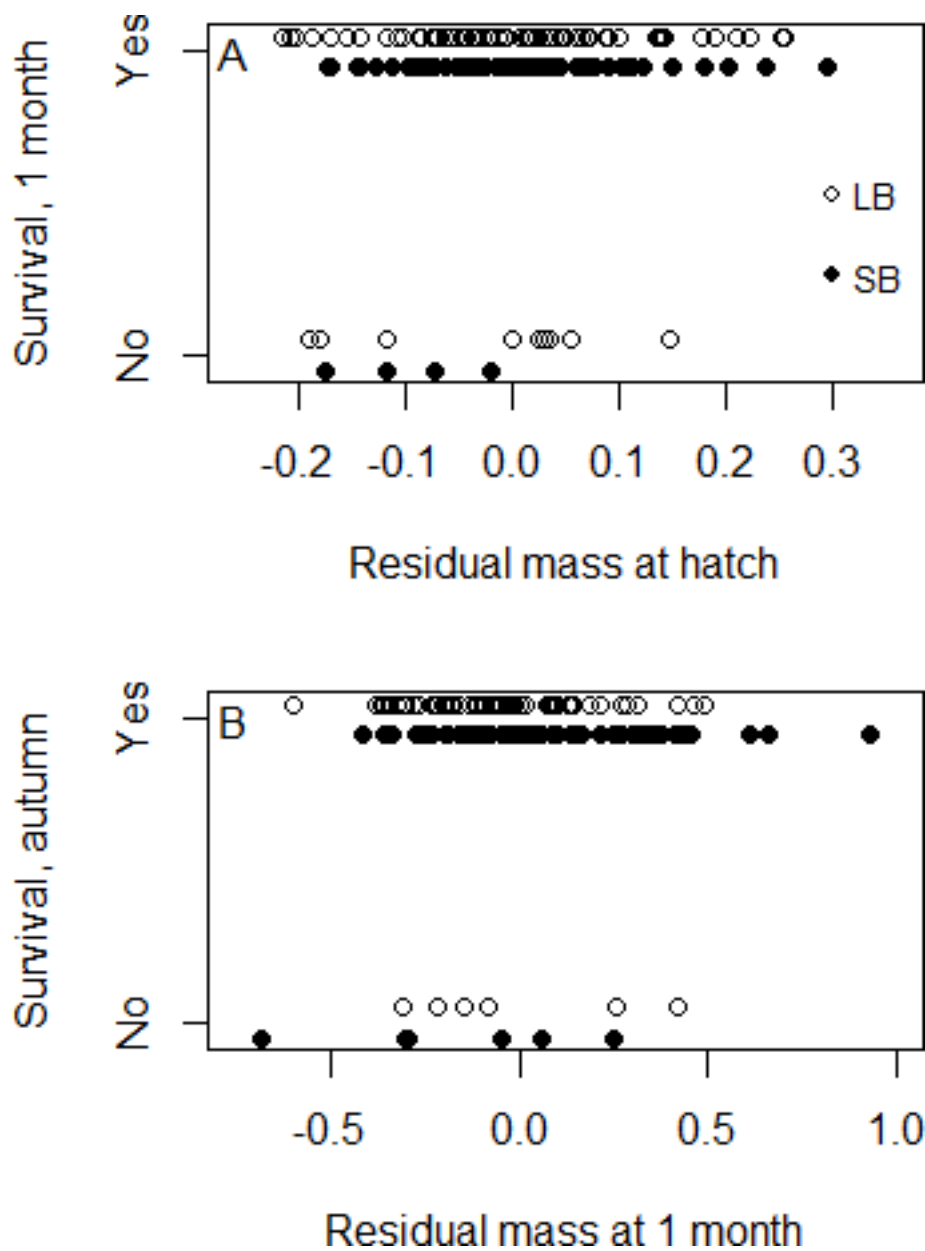
Figure 3. Effect of parental basking treatment – Short Bask (SB; 7 hours) and Long Bask (LB; 11 hours) – on offspring survival. Survival was measured A) until 1 month old (*n*(LB)=77; *n*(SB)=73), and B) between 1 month and autumn (April; *n*(LB)=68; *n*(SB)=69). Filled circles – SB; open circles – LB.

Tables

Table 1. Basic reproductive traits for the two parental treatments. For laying date (Julian date) and clutch size, maternal ID was additionally entered as a random effect. Mean ± SD presented.

Response	LB	SB	Treatment	SVL	Clutch Order
Percent breeding $N = 43$	63.6% (14/22)	76.2% (16/21)	$\chi^2 = 0.85$ p = 0.36	$\chi^2 = 1.94$ p = 0.16	—
Number of clutches $N = 30$	1: 71.4% 2: 21.4% 3: 7.1% (14)	1: 68.8% 2: 31.3% 3: 0% (16)	$\chi^2 = 1.88$ p = 0.39	$\chi^2 = 2.89$ p = 0.24	—
Clutch lay date[1,2] $N = 39$	280.2 ± 24.2 (18)	281.6 ± 15.7 (21)	F = 1.02 p = 0.32	F = 0.06 p = 0.82	**F = 51.01 p < 0.0001**
Clutch size $N = 40$	6.68 ± 1.53 (19)	6.10 ± 2.74 (21)	F = 0.81 p = 0.37	**F = 21.50 p = 0.0002**	F = 2.82 p = 0.09

[1] One successful female omitted as she was introduced to the breeding cages at a later date

[2] Data log-transformed for analysis

Table 2. Parental treatment effects on egg and hatchling traits. Maternal ID was entered as a random effect in all models, and predictor variables were removed when p > 0.15. 'Trt' = Treatment; 'Yolk' refers to whether yolk was drawn from the egg. Treatment averages are mean ± SD (*N*).

Response	LB	SB	Predictors retained Predictor	Statistic	p
Egg mass $N = 250$	1.22 ± 0.28 (127)	1.24 ± 0.26 (123)	Treatment	F = 0.01	p = 0.93
			Clutch lay date	F = 14.45	p = **0.0002**
Hatching success $N = 233$ ΔAICc = 36.1	69.2% (81/117)	69.0% (80/116)	Treatment	χ^2 = 0.11	p = 0.74
			Initial egg mass	χ^2 = 18.58	p < **0.0001**
			Initial egg mass*Yolk	χ^2 = 47.37	p < **0.0001**
			Yolk	χ^2 = 9.11	p = **0.003**
Hatchling sex $N = 161$ ΔAICc = 2.52	34.6% males (28/81)	48.8% males (39/80)	Treatment	χ^2 = 3.17	p = 0.08
			Initial egg mass*Trt	χ^2 = 4.52	p = **0.03**
			Yolk	χ^2 = 3.29	p = 0.07
Hatchling SVL $N = 161$ ΔAICc = 2.37	31.68 ± 1.22 (81)	31.51 ± 1.31 (80)	Treatment	F = 0.24	p = 0.63
			Final egg mass	F = 40.06	p < **0.0001**
			Researcher	F = 10.43	p < **0.0001**
			Sex*Trt	F = 2.89	p = 0.09
Hatchling mass $N = 161$ ΔAICc = 2.74	1.36 ± 0.14 (81)	1.35 ± 0.15 (80)	Treatment	F = 0.02	p = 0.90
			Clutch lay date	F = 3.10	p = 0.08
			Final egg mass	F = 51.26	p < **0.0001**
			Final egg mass*Trt	F = 10.65	p = **0.001**
			Researcher	F = 4.72	p = **0.01**
			SVL at hatch	F = 47.54	p < **0.0001**

Table 3. Survival and growth of hatchlings[1] from day of hatch to autumn. Maternal ID was entered as a random effect in all models, and predictor variables were removed when p > 0.15. 'Trt' = Treatment. Treatment averages are mean ± SD (*N*).

Response	LB	SB	Predictors retained Predictor	Statistic	p
Survival to 1 month *N* = 149	88.3% (68/77)	94.5% (69/73)	Treatment	$\chi^2 = 3.08$	p = 0.08
			Rank age	$\chi^2 = 6.80$	p = **0.009**
			Residual mass	$\chi^2 = 7.96$	p = **0.005**
			Residual mass*Trt	$\chi^2 = 4.16$	p = **0.04**
SVL,1 month (mm) *N* = 137	35.83 ± 2.58 (68)	36.04 ± 2.71 (69)	Treatment	F = 0.0002	p = 0.99
			Rank Age	F = 5.62	p = **0.02**
			Rank Age*Trt	F = 2.88	p = 0.09
			Residual mass at hatch	F = 6.46	p = **0.01**
			Residual mass*Trt	F = 5.82	p = **0.02**
			Sex*Trt	F = 3.77	p = 0.054
Mass, 1 month (g) *N* = 137	1.96 ± 0.46 (68)	2.09 ± 0.54 (69)	Treatment	F = 6.64	p = **0.02**
			Density	F = 32.65	p < **0.0001**
			Density*Trt	F = 4.17	p = **0.04**
			Researcher	F = 8.68	p = **0.0003**
			Residual mass at hatch	F = 2.39	p = 0.13
			Sex*Trt	F = 13.02	p = **0.0004**
			SVL	F = 523.65	p < **0.0001**
Survival from 1 month to autumn *N* = 137	91.2% (62/68)	91.3% (63/69)	Treatment	$\chi^2 = 0.09$	p = 0.77
			Density	$\chi^2 = 4.70$	p = **0.03**
			Residual Mass 1mo*Trt	$\chi^2 = 5.47$	p = **0.02**
			Sex	$\chi^2 = 5.17$	p = **0.02**
			SVL 1mo	$\chi^2 = 3.05$	p = 0.08
SVL autumn (mm) *N* = 124	52.10 ± 5.16 (61)	52.63 ± 4.42 (63)	Treatment	F = 1.67	p = 0.22
			Age	F = 8.91	p = **0.004**
			Density	F = 20.30	p < **0.0001**
			Rank Age	F = 3.64	p = 0.06
Mass autumn (g) *N* = 124	6.23 ± 1.86 (61)	6.70 ± 1.78 (63)	Treatment	F = 0.71	p = 0.41
			Age	F = 3.02	p = 0.09
			RankAge	F = 2.39	p = 0.13
			Residual mass at 1mo	F = 2.98	p = 0.09
			Sex*Trt	F = 6.72	p = **0.01**
			SVL at autumn	F = 1197.64	p < **0.0001**
			SVL*Trt	F = 3.08	p = 0.08

[1]Excluded 11 hatchlings initially placed in cages with fewer than 7 total hatchlings