The influence of the pre- and post-natal environment on Tokay gecko (*Gekko gecko*, Squamata, Gekkonidae) development in captivity

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Birgit Szabo [a, b] *

[a] Division of Behavioural Ecology, Institute of Ecology and Evolution, University of Bern, Bern, Switzerland

[b] Centre for Research on Ecology, Cognition and Behaviour of Birds, University of Gent, Belgium

*Correspond to: Birgit Szabo, Sint-Pietersnieuwstraat 33, 9000 Gent, Belgium; email: birgit.szabo@gmx.at,

ORCID BS: 0000-0002-3226-8621

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Abstract

In captivity, reptiles often experience unnatural incubation and rearing conditions due to limited nest site choices, which can negatively impact individual development and welfare. Incubation temperature and humidity are especially critical for reptile development, influencing factors such as sex, growth, and morphology. However, the conditions experienced after hatching, such as social housing, are far less often considered. Here, I studied the effects of both the incubation environment (temperature and humidity) and social rearing compared to isolation rearing on the development of captive bred tokay geckos (*Gekko gecko*). I find that incubation temperature but not humidity are associated with phenotypic sex, hatchling size and incubation duration. Furthermore, females selected nest sites with higher temperature and lower humidity. After hatching, rearing group size did not affect snout vent length, growth or body condition. My findings indicate an association between incubation temperature and phenotypic sex despite the presence of sex chromosomes in this species. Therefore, care should be taken when raising tokay geckos in captivity to provide nest site options and ensure optimal development of all offspring.

Keywords: Captive rearing, enrichment, husbandry, reptile, social housing, welfare

Introduction

In captivity, reptiles often only have limited choice as to the environment their offspring will incubate in and juveniles are raised in isolation to ensure healthy growth and easy health monitoring (Burghardt and Layne-Colon 2023). How deviations from natural conditions before and after hatching or birth affect the development of captive individuals, and in turn, their welfare and suitability for reintroduction programs, remains poorly understood (Crates et al. 2023).

One of the most important factors shaping development in reptiles is incubation temperature. Suboptimal thermal incubation environments, either too low or too high, can influence incubation time and lead to reduced survival as well as body deformities at birth and after hatching (Burghardt and Layne-Colon 2023; Noble et al. 2018). Importantly, suboptimal incubation temperatures can have long-term effects on morphology and growth rate for up to one year after hatching (Noble et al. 2018). Apart from temperature, incubation humidity has also been shown to influence development in egg laying species with a similar effects on morphology and survival at suboptimal levels, too wet or dry (Bell et al. 2025). Incubation mimicking natural condition in captivity has been shown to improve development (Hall and Warner 2020), produce important phenotypic variation (Shine et al. 1997a) and should, therefore, be considered important in captive breeding.

Incubation temperature also plays an important role in sex-determination in many reptile species. Lizards exhibit a large diversity in sex chromosomes and sex determining modes which indicates a high lability in sex determination mechanisms (Ezaz et al. 2009). Within lizards, even closely related species might demonstrate genotypic sex determination (GSD) or temperature dependent sex determination (TSD), and some research suggests a high presence of transitional stages between GSD and TSD (Ezaz et al. 2009; Shine et al. 2002; Quinn et al. 2007). For example, central bearded dragons (*Pogona vitticeps*; Ahl 1926) have ZZ/ZW micro-sex chromosomes. At most incubation temperatures, equal numbers of males and females are hatching. However at incubation temperatures above 32°C, more females are hatching due to sex reversal (feminised males; Ezaz et al. 2005; Holleley et al. 2015; Quinn et al. 2007). A second mechanism that might lead to hatchling sex bias, apart from sex reversal, is sex biased mortality (Burghardt and Layne-Colon 2023; Gutzke and Crews 1988; Nemesházi and Bókony 2025). If environmental conditions override genotypic sex or affect pre-natal mortality are still unanswered questions in most species of lizards (Holleley et al. 2016). Such information is important to incorporate into captive breeding programs to ensure the produced animals are healthy.

In mammals and birds, it is generally assumed that natural rearing in social groups is more beneficial for the development of offspring compared to hand rearing by humans (Burghardt and Layne-Colon 2023). While in reptiles it is common to separate neonates after birth or hatching to be raised in simplified environments that facilitate cleaning and welfare checks (Burghardt and Layne-Colon 2023). In lizards, group living after hatching is uncommon but not absent and should, therefore, be considered when raising individuals of such social species in captivity (Burghardt and Layne-Colon 2023). On the one hand, rearing in isolation can negatively influence development of behaviour such as in juvenile veiled chameleons (*Chamaeleo calyptratus*; Duméril and Duméril 1851) which showed deficits in social and feeding behaviour when raised alone compared to group rearing (Ballen et al. 2014). However, on the other hand, it should be noted that social housing might also have negative effects. Housing lizards in groups of siblings or with parents can increase competition and if dominant or larger individuals exclude subordinates from resources, it might affect the development of neonates and juveniles. For example, housing neonatal chameleons (*Chamaeleo* spp.) in groups with limited vertical space caused smaller individuals to be forced to the ground, where they reduced their feeding and drinking, to keep their distance from others (Castle 1990). Furthermore, in tree skinks (*Egernia striolata*; Peters 1870) housing with a social partner reduced growth and subordinate individuals were more likely to suffer tail loss (Riley et al.

2017). Consequently, group housing can be beneficial, but care should be taken in captivity that all individuals have enough space and access to important resources such as food, water, shelter and a thermal and humidity gradient (Burghardt and Layne-Colon 2023).

While the importance of temperature and humidity for captive breeding are well known in reptiles (Burghardt and Layne-Colon 2023; While et al. 2018), far less attention has been given to the social environment, both pre- and post-hatching. Tokay geckos (*Gekko gecko*; Linnaeus 1758) are a large, nocturnal gecko species in which adults perform biparental care towards offspring (Grossmann 2007). Nonetheless, due to their facultative social nature, offspring can be raised isolated from adults. However, it is yet unclear what consequences such socially deprived rearing has on the development of offspring or if different levels of competition (number of siblings) might influence growth. Additionally, tokay geckos exhibit XX/XY sex chromosomes (Solleder and Schmid, 1984). However, incubation temperature can affect sex development in some lizard species with genetic sex determination. So far, studies looking at a potential influence of incubation temperature on hatchling phenotypic sex-ratio are missing in this species (Holleley et al. 2016; Nemesházi and Bókony 2022; 2025) and so is information on how the incubation environment influences developmental time and post hatching growth.

Therefore, the aim of the current study was to quantify the effects of the pre-natal incubation environment (humidity and temperature) and the post-natal social environment (group size) on the development of tokay geckos in captivity. Consequently, this study fills an important gap regarding best practice in tokay gecko captive rearing that takes their social organisation into account, to ensure best possible welfare in captive bred individuals of this species (Burghardt and Layne-Colon 2023). Additionally, this study provides important new data on the effect of the incubation environment on the development of phenotypic sex in a species with sex chromosomes, data that is scarce in the literature (Holleley et al. 2016; Nemesházi and Bókony 2025). I hypothesise that both the pre- and post-natal environment will have an effect on development. I predict that (1) incubation temperature will be associated with a phenotypic sex-ratio bias towards females at low temperatures (Gamble 2010; Quinn et al. 2011; Viets et al. 1994), similar to what was found in *Gekko japonicus* (Duméril and Bibron 1836; Tokunaga 1985). (2) Higher incubation temperatures will be associated with shorter incubation time (e.g. Elphick and Shine 1998; Noble et al. 2018; Shine et al. 1997b) and (3) smaller hatchling size due to shorter incubation time (e.g. Van Damme et al. 1992). Furthermore, I predict that rearing in social isolation will have a positive effect on growth due to reduced competition (e.g. Riley et al. 2017).

Methods

Animals

33 captive bred tokay geckos, 21 females and 12 males, were included in this study. Phenotypic sex was determined by looking at the presence (for males) or absence (for females) of femoral glands (Grossmann 2007). All animals originated from 16 breeding pairs (see details below) and hatched between May 2022 and March 2023 (first round of breeding) and May and November 2024 (second round of breeding) at the Ethological Station, University of Bern, Bern, Switzerland.

I established 16 breeding pairs from our captive population of 39 adult, captive bred geckos. All adults were purchased from different breeders or bred in house and were between 2 and 8 years old (average = 3.17 years, two females were 8 years old). Adults were randomly paired in January 2022 (first round of breeding) and December 2023 (second round of breeding) and stayed in pairs for a minimum of one year. Females produced their first clutches (Fig. 1A) in February 2022/ 2023 and continued to produce clutches approximately every 30 days. All eggs and their location were recorded upon discovery of a clutch. Across breeding pairs, 19 offspring hatched from a first clutch, six offspring from a second clutch, one from a fourth clutch, two from a sixth clutch, two from a seventh clutch, and one each from an eighth, tenth and eleventh clutch. This distribution was based on hatching success (some eggs did not develop and were removed by females). More specifically, egg mortality occurred in 8 out of 23 clutches. In 6 of these cases the sex of the individual that successfully hatched from the clutch was female, in two cases it was male. After hatching, three offspring died, two (both female) in the first breeding of 22 offspring total (20 surviving) and one (sex undetermined) in the second breeding of 11 offspring total (10 surviving). All clutches incubated within the home enclosure (see below) in the presence of the parents to ensure natural incubation.

Isolation and group rearing

From around 90 days of incubation, I started checking for hatchlings daily. Offspring started hatching after 78 to 138 days (range) of incubation. After hatching, offspring were allocated to either stay within the home enclosure to be raised with their parents (Figure 1B) or were removed immediately to be raised alone without adults or siblings. Offspring that stayed with the parents either had no siblings, one sibling or two siblings. Therefore, group sizes ranged from one (isolation, N = 7), three (adult parents only, N = 7), four (adult parents plus one sibling, N = 13) to five (adult parents and two siblings, N = 6). Offspring were raised with parents until they were six months of age and then moved to be housed alone.

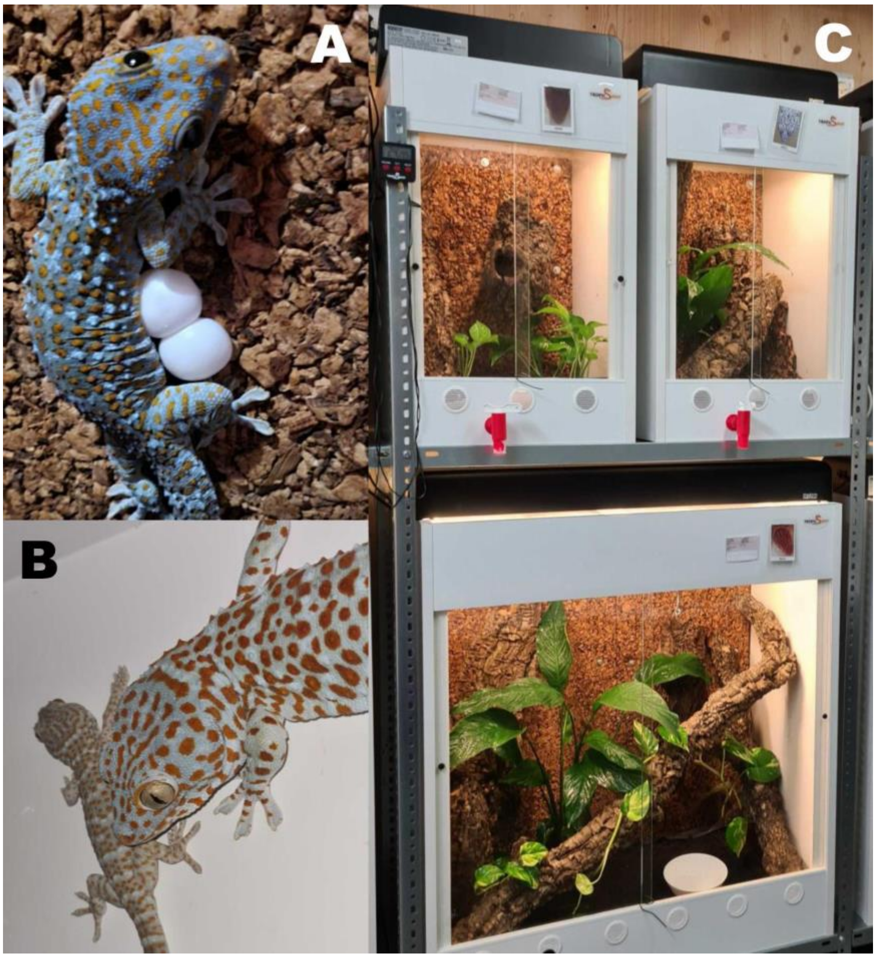

Figure 1. A) Female tokay gecko curling around eggs attached to the cork back wall of the enclosure (shelter removed). B) Male tokay gecko next to one of his offspring (social rearing condition). C) Tank set up showing shelters on the back wall, branches, life plants and a water bowl (picture from 2021). Bottom enclosure for group rearing, top enclosures for single housed females (outside of breeding).

Captive condition

Offspring single housing

Animals were housed in terraria of the size 30 L × 45 W × 45 H cm (only suitable for short term housing in scientific contexts), made of rigid foam slabs with a net top and glass front doors. Terraria were fitted with a compressed cork wall fixed to the back, cork branches cut in half that were hooked on the back (functioning as shelters and nests), cork branches allowing lizards to climb, and life plants as enrichment. Each terrarium was equipped with a drainage layer of expanded clay, separated by a mosquito mesh from the soil placed on top (organic tropical forest soil; Dragon BIO-Ground). I spread sphagnum moss and autoclaved red oak leaves on the soil as shelter and food for the isopods that decompose the faecal material of the lizards. Terraria were organized on shelves in three layers. To simulate natural environmental conditions, the room environment was controlled by an automatic system. Animals were exposed to a reversed 12h:12h photo period (i.e. light from 6 pm to 6 am, dark from 6 am to 6 pm) and the system imitated sunrise and sunset, which were accompanied by changes in temperature reaching approximately 25 °C during night and 31°C during day. In addition, an UVB light (Exo Terra Reptile UVB 100, 13 W) was provided on top of the terraria during the daylight phase. A red light (PHILIPS TL-D 36W/15 RED) invisible to geckos (Loew 1994) was kept on for 24h to provide minimal visibility and enable experimenters to work with the lizards. Furthermore, lizards were able to thermoregulate to their optimal body temperature at any time by means of a heat mat (TropicShop) attached to the right outer wall of each enclosure which locally increased the temperature by 4-5 °C. Humidity was kept at 50 %, but every 12 hours, at 5pm and 4am, 30 seconds of rainfall (with reverse osmotic water) briefly increased humidity to 100 %.

Offspring group housing

Terraria for group housing measured 90 L × 45 B × 100 H cm in size (only suitable in scientific contexts; Fig. 1C). Except for their size, they were set up exactly the same as described above. Additionally, large enclosures included a larger number of branches and shelters on the back wall, larger plants as well as larger water bowls proportional to the larger enclosure size (Fig. 1C). To prevent small offspring from drowning in these water bowls, I added a large stone to ensure easy escape of small individuals.

Husbandry

Offspring were fed five times per week, with 10-15 small to large sized house crickets (*Acheta domesticus*) using scatter feeding. The size of the crickets was adjusted to the changing head size while growing. Adult geckos were fed 3-5 adult house crickets (first round of breeding) or cockroaches (*Nauphoeta cinerea*l; second round of breeding) using 25 cm long forceps to monitor food intake. In order to provide optimal nutrition to our animals (vitamin D and calcium), the insects were fed with high protein dry cat food (various brands, e.g. Purina, Leonardo), cricket mix (reptile planet LDT), and fresh carrots. Fresh water for geckos was supplied ad libitum in water bowls. Moreover, adult geckos were weighted (± 1g) every month and measured (SVL - snout vent length, ±0.5 cm) approximately every three months, to track their body condition. Adult geckos weight (6 month of age and above) was measured by capturing an individual inside a transparent box (22.5 L × 16.5 W × 7.5 H cm), weighing them on a scale and subtracting the weight of the box from the total weight of the box with the lizard inside. Adult geckos SVL was measured by taking a ventral picture while sitting on the glass front doors of their enclosure next to a ruler as a reference (for more details of how the pictures were processed see below).

Measurement of growth and body condition

Every two weeks, offspring (less than 6 months of age) were carefully captured by hand and placed in a transparent box (22.5 L × 16.5 W × 7.5 H cm). The box was placed on its side to expose the ventral body surface of each individual. I then placed a ruler next to the offspring on the outside of the box to take a picture from straight in front (to avoid distortions). These pictures were then used to measure SVL using ImageJ 1.53k (Schneider et al., 2012) with the ruler as a reference. In ImageJ, I first drew a line along the ruler of known length (e.g. 14cm) and then set this measure as the scale. Next, I drew a line from the snout tip of the lizard to the cloaca along the centre of the body while accounting for bends using the segmented line tool. Finally, ImageJ calculated the length of the line along the body based on the reference resulting in the SVL of the individual. After the picture was taken, offspring were released back into their home enclosure. Hand capture did not lead to tail autotomy in any of the individuals included in this study. At six months of age, offspring were switched to the adult schedule of weighing once a month and measurement of SVL every three months.

Measurement of incubation temperature and humidity

For 32 out of the 33 individuals included in this study, I determined average incubation temperature (day and night pooled), average day and night incubation temperature (separate), average incubation humidity (day and night pooled) as well as average day and night incubation humidity (separate) using 1-Wire® Hygrocgron™ iButtons (DS1923#F5) encased in a waterproof case. iButtons were placed as close to clutches as possible by tying them to enclosure furnishings with string. Temperature and humidity were automatically recorded for a minimum of one week and taken every 15 minutes continuously. In those cases in which measurements were recorded for longer than one week, I extracted averages from a randomly chosen seven consecutive days. As our system kept the humidity and temperature changes stable across time, these seven days were close representative values for the whole incubation period although small changes that might have occurred were not captured with this method. Finally, I also determined average temperature and humidity in a control location for 10 breeding pairs in breeding round one that was at the same height as the clutch and covered by a shelter but where no eggs were deposited. This was done to find out if there are microclimatic conditions (temperature and humidity) that geckos prefer when selecting an egg deposition site. iButtons were again tied to enclosure furnishings to match the height of clutches within the same enclosure.

Ethical statement

The experimental procedure applied in this study was strictly non-invasive and followed the guidelines provided by the Association for the Study of Animal Behaviour/ Animal Behaviour

Society for the treatment of animals in behavioural research and Teaching (2023). Experiments were approved by the Swiss Federal Food Safety and Veterinary Office (National No. 33232, Cantonal No. BE144/2020, BE9/2024). Captive conditions were approved by the Swiss Federal Food Safety and Veterinary Office (Laboratory animal husbandry license: No. BE4/11). Three offspring died of natural causes (pathology was inconclusive) during the course of this study. One around 16 weeks and two around six weeks after hatching. During pair formation, we monitored adults closely for 12h to prevent harm. If any aggression occurred, we immediately separated the male and female to avoid injurie. Males were then paired with a different female (N = 17 attempted pairings total) until we established stable pairs that did not show any aggression towards each other. Similarly, after hatching, we monitored hatchlings that stayed with their parents closely and removed one hatchling (G033; included in the data as raised in isolation) due to concerns of insufficient parental care.

Statistical analyses

All statistical analyses were run in R version 4.4.2 (R Core Team 2025). I used Bayesian linear mixed models (LMM) using the package *brms* (Bürkner 2017; 2018; 2021). For all models, I ensured that model Rhat was 1, that the ESS was above 2000 and checked the density plots and correlation plots to ensure that the models had sampled appropriately. Additionally, I used posterior predictive checks to ensure good model fit and leave-one-out cross validation to select best fitting models (Vehtari et al 2017). I used non-informative priors (see provided R code for details) and ran 4 chains per model of 5000-7000 iterations each and a thinning interval of 1 (default settings). I used Bayes factors (BF) to evaluate the inclusion of predictors by determining BF from marginal likelihoods using the package *brms*. In accordance with Schmalz and colleagues (2023), I report results as follows: BF above 1 but below 3 are reported as a weak support for inclusion, BF larger than 3 but below 10 are reported as support for inclusion, BF larger than 10 but below 30 are reported as strong support for inclusion, and values larger than 30 are reported as very strong support for inclusion. Data generated during this study and the analysis code are available for download from the Open Science Framework (OSF, https://doi.org/10.17605/OSF.IO/GDQ8Y).

The influence of the pre-natal incubation environment

I investigated the relationship of average incubation temperature and humidity (response variables in two separate linear mixed models) with offspring phenotypic sex, incubation time (in days) and SVL at hatching (in cm) (fixed effects). Incubation time was log transformed as it was not showing a linear relationship with incubation temperature and humidity. In both models, I included a random effect of parent identity to account for the fact that females produced multiple offspring with different males.

I also investigated if the average temperature and humidity differed across locations in which geckos deposited their eggs and a control location. I ran two separate linear mixed models, one for the temperature and one for the humidity (response variable) and used location as the only fixed effect. Again, I included parent identity as a random effect.

The influence of the post-natal environment

I investigated the relationship of offspring SVL (response variable), taken every two weeks until six months of life, with rearing group size, phenotypic sex, incubation time and clutch number (fixed effects) using a linear mixed model. To understand if animals showed different growth rates based on rearing group size or phenotypic sex, I included the interactions of measurement number and rearing group size as well as measurement number and phenotypic sex as additional fixed effects. However, model selection based on leave-one-out cross validation indicated that the best fitting model did not include any interactions. Therefore, I report the results of the simpler model below. Furthermore, I included a random effect of offspring identity nested in parent identity to account for repeated measures of SVL within individuals and offspring origin.

To understand the potential long-term effects of the post-natal environment, I also ran a linear mixed model with the body condition at six months of age as the response variable. To do so, I calculated the Scaled Mass Index (SMI) as the measure for body condition following Peig and Green (2009):

$$SMI_i = M_i \left[\frac{L_{av}}{L_i}\right]^{b_{SMA}}$$

SMI_i represents the scaled mass index of individual i, M_i and L_i represent the mass and SVL of individual i, respectively. L_{av} represents the average SVL of the study population and b_{SMA} the scaling exponent estimated by a standard major axis regression (package *smatr*; Warton et al. 2012) of mass on SVL.

Offspring that were raised within a family group remained in these family groups for the first six month of their lives. I included rearing group size, SVL at hatching, phenotypic sex, incubation time and clutch number as fixed effects. Again, I included a random effect of parent identity.

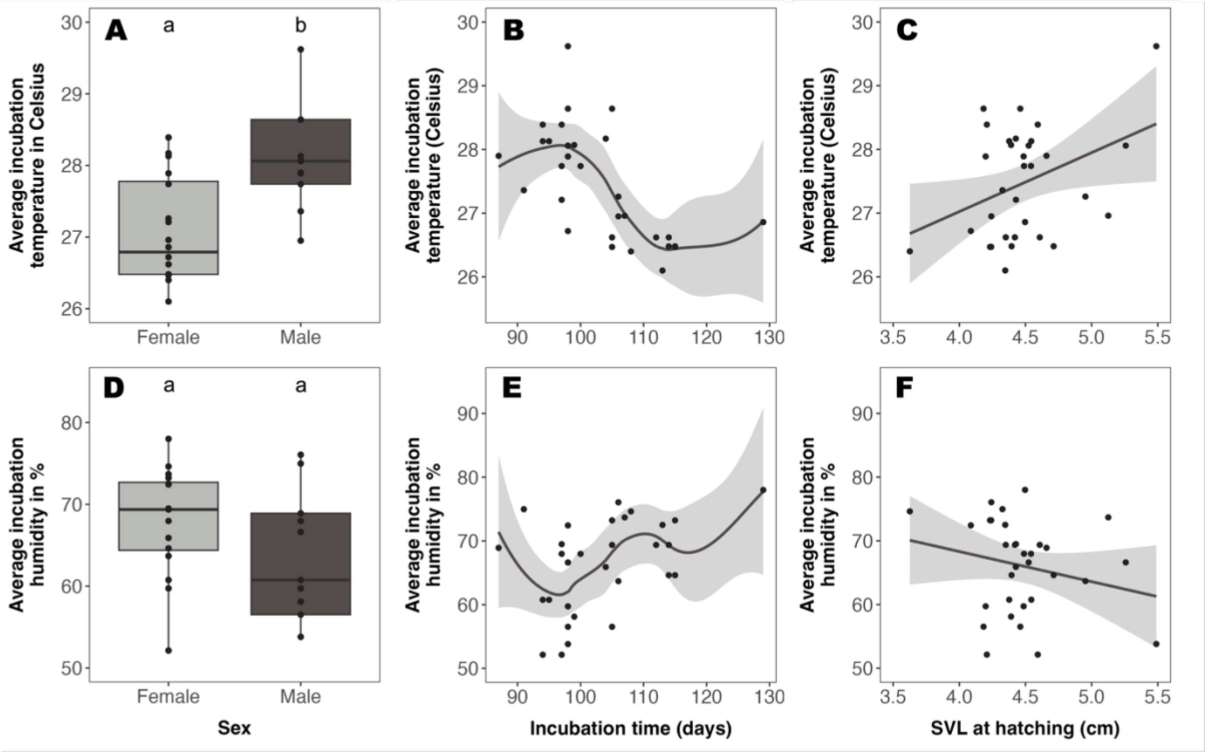

Figure 2. A) Average incubation temperature (in degree Celsius) at which males and females hatch. The bold line within the boxes indicates the median, the upper and lower box edges indicate the upper and lower quartile, and the top and bottom whisker ends indicate the maximum and minimum. B) Relationship between average incubation temperature and incubation time (days). C) Relationship between average incubation temperature and SVL at hatching (cm). D) Average incubation humidity (%) at which males and females hatch. E) Relationship between average incubation humidity and incubation time. F) Relationship between average incubation humidity and SVL at hatching. Across plots, dots represent individual data points and the shaded area around the predicted line represents the 95% confidence interval. a, b – dissimilar letters indicate a difference based on credible intervals.

Results

The influence of the pre-natal incubation environment

More males than females hatched at higher average incubation temperatures, although the 95% and 90% credible intervals overlapped zero (Table 1, Fig. 2A). However, the 80% credible interval excluded zero, and model comparison supported the inclusion of phenotypic sex in the model (BF = 3.711, Table 1). Shorter incubation time was associated with higher average incubation temperatures as the 95% credible interval did not include 0 and model comparison strongly supported the inclusion of incubation time in the model (BF = 28.565, Table 1, Fig. 2B). Finally, higher average incubation temperature was also associated with larger hatchling size. Again, the 95% credible interval did not include 0 and model comparison very strongly supported the inclusion of incubation time in the model (BF = 44.238, Table 1, Fig. 2C).

The posterior estimate suggested no association of phenotypic sex, incubation time or hatchling size with average incubation humidity. Neither, the 95%, 90% nor 80% credible interval excluded 0 for all three parameters (Table 1). However, model comparison supported the inclusion of all three predictors in the model to different degrees (BF_{sex} = 132.676, Fig. 2D; $BF_{incubation\ time}$ = 43.010, Fig. 2E; $BF_{hatchling\ size}$ = 7.576, Fig. 2F).

Average incubation temperature was higher and humidity was lower at locations where clutches were found (Table 1). In both models, the 95% credible interval did not include 0 and model comparison very strongly supported the inclusion of location in the model (temperature: BF = 83.964; humidity: BF = 62.217).

Table 1. Parameter estimates and test statistics for the Bayesian linear mixed models looking at the relationship of phenotypic sex, incubation time, hatchling size and location with average incubation temperature and humidity. Parameters that show evidence for an effect (based on credible intervals) are highlighted in bold.

Response variable	Parameter	Estimate	95% credible interval	90% credible interval	80% credible interval
Average incubation temperature	Intercept	36.146	24.32, 49.45	26.36, 46.96	28.34, 44.24
	Male	0.287	-0.119, 0.722	-0.045, 0.642	**0.029, 0.552**
	Incubation time	**-2.652**	**-5.499, -0.174**	**-0.910, -0.582**	**-4.353, -1.025**
	Hatchling SVL	**0.793**	**0.276, 1.322**	**0.364, 1.228**	**0.460, 1.126**
	Intercept	26.636	26.05, 27.26	-	-
	Location clutch	**0.557**	**0.219, 0.906**	-	-
Average incubation humidity	Intercept	75.625	-103.8, 224.6	-68.03, 203.2	-31.99, 177.1
	Male	-1.758	-6.518, 3.260	-5.665, 2.363	-4.789, 1.400
	Incubation time	-0.813	-32.41, 38.50	-28.18, 30.36	-22.74, 22.62

Hatchling SVL	-1.249	-7.887, 4.903	-6.579, 3.918	-5.241, 2.687
Intercept	72.242	69.11, 74.99	-	-
Location clutch	**-3.147**	**-5.777, -0.577**	-	-

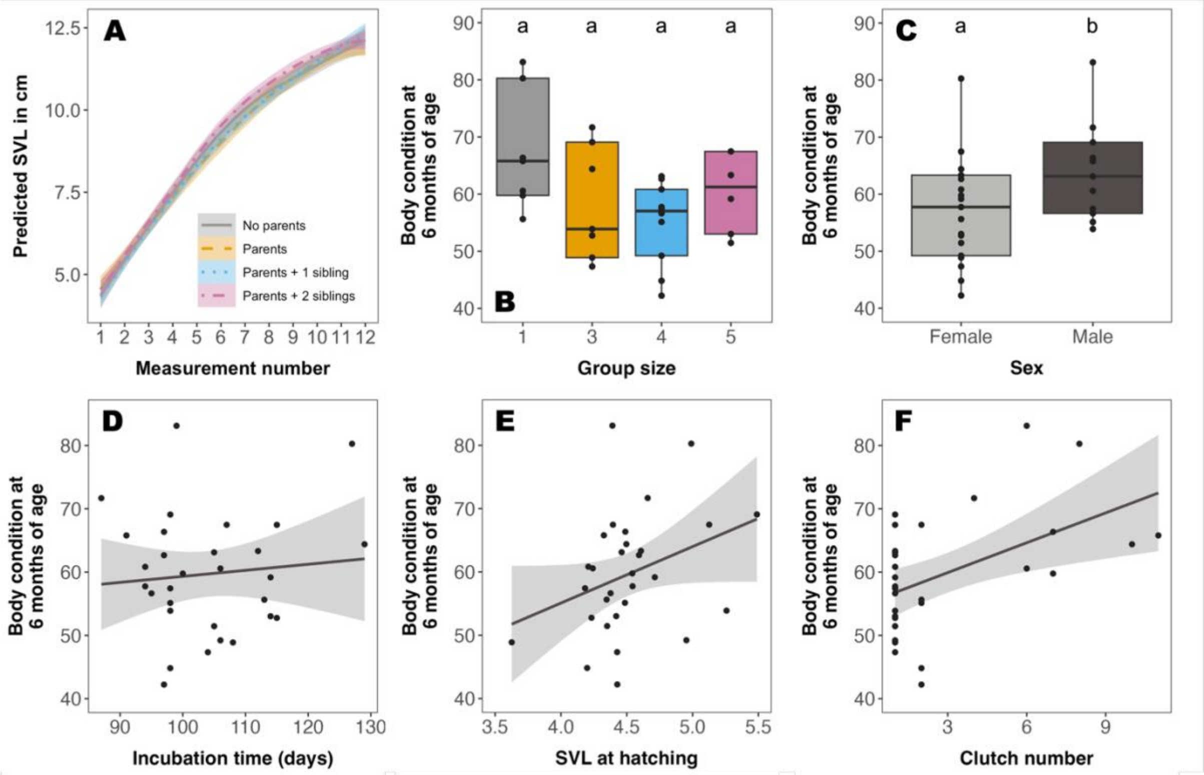

Figure 3. A) Predicted change in SVL across the first six months of life split into the different rearing group sizes. B) Body condition at six months of age split into rearing group sizes. Group sizes ranged from one (isolation, N = 7), three (adult parents only, N = 7), four (adult parents plus one sibling, N = 13) and five (adult parents and two siblings, N = 6). The bold line within the boxes indicates the median, the upper and lower box edges indicate the upper and lower quartiles, and the top and bottom whisker ends indicate the maximum and minimum. C) Body condition of males and females at six months of age. D) Relationship between body condition at six months and incubation time. E) Relationship between body condition at six months and SVL at hatching. F) Relationship between body condition at six months and clutch number. Across plots, dots represent individual data points and the shaded area around the predicted line represents the 95% confidence interval. a, b – dissimilar letters indicate a difference based on credible intervals.

The influence of the post-natal environment

SVL increased with increasing measurement number supported by the 95% credible interval not including 0. Model comparison also very strongly supported the inclusion of measurement number in the model (BF = $6.80*10^{211}$, Table 2, Fig. 3A). However, the posterior estimate suggested no relationship of SVL with rearing group size as neither the 95%, 90% nor 80% credible interval excluded 0 and model comparison did also not support the inclusion of this parameter (BF = 0.679, Table 2, Fig. 3A). In addition, the posterior estimate suggested no relationship of SVL with phenotypic sex, incubation time and clutch number. Again, neither the 95%, 90% nor 80% credible interval excluded 0 for all three parameters (Table 2). Model comparison did not support the inclusion of incubation time and clutch number ($BF_{\text{incubation time}}$ = 0.035, $BF_{\text{clutch number}}$ = 0.183) but the inclusion of phenotypic sex (BF = 5.123).

The posterior estimate suggested no relationship of rearing group size with body condition at six month of age as neither the 95%, 90% nor 80% credible interval excluded 0. However, model comparison did very strongly support the inclusion of rearing group size (BF = 2832, Table 2, Fig. 3B). Body condition at six month of age was positively associated with phenotypic sex and clutch number. Males had higher body condition than females and individuals hatching from later clutches also had higher body condition. The 95% credible interval did not include 0 in both cases and model selection provided strong support for the inclusion of both phenotypic sex and clutch number (BF_{sex} = 56.229, $BF_{\text{clutch number}}$ = 12.737, Table 2, Fig. 3C, F). Moreover, body condition increased with increasing incubation time, although the 95% and 90% credible intervals overlapped zero (Table 2, Fig. 2D). However, the 80% credible interval excluded zero, and model comparison weakly supported the inclusion of incubation time in the model (BF = 1.601, Table 2). Finally, body condition also increased with increasing hatchling size. Although the 95% credible intervals overlapped zero, both the 90% and 80% credible interval excluded zero (Table 2, Fig. 3E). Furthermore, model comparison very strongly supported the inclusion of hatchling size (BF = 38.940).

Table 2. Parameter estimates and test statistics for the Bayesian linear mixed models looking at the relationship of measurement number, group size, phenotypic sex, incubation time, and hatchling size with snout vent length and body condition. Parameters that show evidence for an effect (based on credible intervals) are highlighted in bold.

Response variable	Parameter	Estimate	95% credible interval	90% credible interval	80% credible interval
Snout vent length	Intercept	3.867	0.903, 7.020	1.374, 6.445	1.933, 5.833
	Measurement number	**0.711**	**0.692, 0.730**	**0.695, 0.727**	**0.699, 0.724**
	Group size – 3	0.125	-0.514, 0.774	-0.393, 0.654	-0.281, 0.530
	Group size – 4	0.150	-0.617, 0.863	-0.470, 0.747	-0.315, 0.613
	Group size – 5	0.394	-0.484, 1.250	-0.333, 1.108	-0.158, 0.952
	Male	0.238	-0.272, 0.747	-0.185, 0.654	-0.089, 0.567
	Incubation time	0.002	-0.026, 0.029	-0.021, 0.024	-0.016, 0.019
	Clutch number	0.041	-0.051, 0.132	-0.034, 0.116	-0.017, 0.099
Body condition at 6 month of age	Intercept	1.183	-48.86, 53.58	-40.93, 43.80	-31.68, 33.76
	Group size – 3	-4.380	-11.64, 2.653	-10.33, 1.342	-8.850, 0.039
	Group size – 4	-4.218	-25.24, 6.765	-13.19, 4.834	-11.14, 2.694
	Group size – 5	-1.228	-11.73, 9.201	-9.799, 7.561	-7.865, 5.492
	Hatchling SVL	6.203	-0.918, 13.17	**0.297, 12.05**	**1.674, 10.69**
	Male	**6.156**	**0.160, 12.43**	**1.142, 11.33**	**2.265, 10.03**
	Incubation time	0.260	-0.091, 0.583	-0.032, 0.534	**0.040, 0.476**
	Clutch number	**1.102**	**0.073, 2.135**	**0.248, 1.965**	**0.448, 1.756**

Discussion

In this study, I aimed to gain insights into how incubation temperature and humidity as well as the social environment after hatching influence tokay gecko development. I found that higher incubation temperature was associated with a higher number of phenotypic males hatching. Higher temperatures during incubation were also associated with larger hatchling SVL and lower incubation time. However, my analysis did not support an association of phenotypic sex, incubation time and hatchling SVL with average incubation humidity. I also found that geckos selected nest sites with higher temperature and lower humidity. After hatching, hatchling growth rate did not differ across rearing group sizes. Furthermore, I found no relationship between SVL and rearing group size, incubation duration, clutch number or phenotypic sex. Finally, body condition at six months of age, when offspring where still with parents and siblings, was not related to rearing group size, but body condition differed between the sexes, was positively related to hatchling SVL, incubation time and clutch number.

According to my prediction, I found that higher incubation temperatures were related to faster development in offspring which is a general trend across all groups of reptiles (Noble et al. 2018). All eggs incubated within the enclosures of the parents, and therefore, did not experience any extreme temperatures (measured range of average temperature = 26.1°C to 29.6°C). Despite the small range of only 3.5°C, I found a difference in incubation duration of 51 days. None of the offspring hatched with deformities (Burghardt and Layne-Colon 2023; Noble et al. 2018). Therefore, I suggest that these incubation temperatures may have been within a suitable developmental range. Importantly, I am unaware of a single study investigating the naturally occurring range of incubation temperatures in the wild in tokay geckos. This important information could inform future, more targeted investigations related to development at normal and abnormal temperatures and the effects of rising temperatures due to global warming.

Also according to my prediction, I found that a higher proportion of phenotypic females was observed hatching at lower incubation temperatures (or, conversely, a higher proportion of phenotypic males at higher temperatures), similar to a closely related species, the Schlegel's Japanese Gecko (Tokunaga 1985). Based on their observations, Tokunaga (1985) concluded that *G. japonicus* demonstrates temperature dependent sex determination. However, in the light of more recent evidence and the fact that both tokay geckos and *G. japonicus* possess sex chromosomes (Solleder and Schmid, 1984), this pattern could potentially be explained by mechanisms such as sex-biased mortality or sex reversal at certain temperatures (Burghardt and Layne-Colon 2023; Gutzke and Crews 1988; Nemesházi and Bókony 2025). Currently, I have no genetic data available to determine the genetic sex of the animals included in the current study. Therefore, I am unable to confirm if some females that hatched at lower temperatures are sex-reversed males or if male biased mortality occurred at lower temperatures. In some clutches incubated at lower temperatures (4/11), only one individual hatched due to mothers removing the second egg. This could point towards sex-biased mortality, but could also be an effect of disturbance. Future studies should investigate if sex reversal or rather sex biased mortality is causing the skewed sex-ratio at different temperatures. Again, due to incubation within enclosures, no extremely high incubation temperatures were observed, and therefore, I am unable to determine if the sex-ratio would have become female biased again at higher temperatures as was found in *G. japonicus* (Tokunaga 1985). Finally, despite the statistical model only showing weak evidence for a relationship between phenotypic sex and incubation temperature, both the raw data and model comparison support the association. The inability of the model to detect a stronger effect was likely due to high variability and low sample sizes.

Contrary to my prediction, incubation at higher temperatures was associated with larger hatchling SVL possibly because more males hatched at these temperatures which were larger than females. According to the literature, in many species no difference in hatchling SVL was found at different incubation temperatures (e.g. Elphick and Shine 1998; Shine et al. 1997b; Tiatragul et al. 2017). To disentangle the effect of incubation temperature and sex on hatchling SVL in tokay geckos, future studies should incubate eggs under controlled conditions using temperatures that produce both males and females enabling a direct comparison within each sex.

Based on my model estimates, incubation humidity was not associated with phenotypic sex, incubation time or hatchling SVL as the confidence intervals did cross 0. Humidity is not often considered as an additional factor aside temperature in reptile development, but a recent meta-analysis showed a positive relationship between substrate moisture and hatchling SVL with larger hatchlings under moister conditions (Bell et al. 2025). In the current study, model comparison strongly supported the inclusion of all three parameters which suggest that there might be a subtle effect that is not detected with the current sample size. Future studies using larger sample sizes or more controlled experimental manipulations could help clarify if humidity has an effect on tokay gecko development or not.

Contrary to my last prediction, my models did not detect differences in post-natal development between rearing group sizes under the conditions and sample size of the current study (size, growth rate and body condition at six month of age). Offspring were housed in similar, naturalistic enclosures no matter if raised alone or in a family group. Therefore, offspring raised alone only experienced lower social complexity and smaller space compared to offspring raised within a family group. I used scatter feeding of insects during the first six months adjusting the amount to the group size which might have decreased competition. Nevertheless, despite model estimates showing no differences across rearing group sizes, the raw data as well as model comparison both suggest that at least body condition at six month of age differed across rearing group sizes. This might be explained by the low sample size and imbalanced design caused by logistical constraints (e.g. space for enclosures, low number of breeding pairs). In another study, I demonstrated that socially raised individuals were bolder and varied more in their associative learning ability (Szabo and Ringler 2025). Both studies together provide some evidence that more impoverished environments influence lizard development (physical and/or behavioural), similar to what has been found in birds and mammals (e.g. Meehan and Mench 2002; Schrijver et al. 2002; 2004). Generally, more studies are needed to investigate the effects of different housing conditions during development in reptiles (but see Almli and Burghardt 2006; Burghardt and Layne-Colon 2023). Such studies would greatly improve our ability to create optimal captive conditions to produce healthy adult individuals (Burghardt and Layne-Colon 2023). This is not just important in terms of captive welfare but also for reintroduction programs that breed animals in captivity for future release (Crates et al. 2023).

Finally, I was also interested in understanding if tokay geckos selected nest sites in captivity despite the lower variability in environmental factors typical for captive environments. As discussed above, it is critical for females to select an appropriate nest location both regarding temperature and humidity to ensure optimal offspring development. In the wild, reptiles show plasticity in their choice of nest site to compensate for unfavorable climatic conditions (e.g. Doody et al. 2006; 2020; Du, et al. 2023) but experiments on nest site selection under more impoverished captive conditions are missing. My results show that geckos select sites with higher temperature and lower humidity. I also observed that the same nest sites were used by different females across years. Both a temperature and humidity gradient were present in the enclosures, giving females a variety of nest site choices. Future studies should investigate what conditions geckos prefer when selecting a nest site. Such information would help design better captive environments that meet the animals' needs under multiple contexts.

Conclusion

I found that the pre-natal incubation environment but less so the post-natal social environment were associated with lizard development. In the future, it could be interesting to extend investigations regarding the influence of the social environment to also include the pre-natal environment. Tokay geckos produce a clutch of 1-2 eggs every month which are placed closely together, often attached to each other (Grossmann 2007). Such adjacent incubating eggs, could be a source of social influence affecting development which could be investigated in the future. Furthermore, in the current study, all eggs incubated in the presence of parents, and it would be interesting to investigate if the absence of parents influences development and hatching success. Thirdly, I have previously shown that females increase basking before egg production (Szabo 2024). This maternal thermal incubation environment embryos experience before oviposition is rarely considered in oviparous reptiles (e.g. McDonald and Schwanz 2018; Schwanz 2016). Future studies could investigate how decreased basking opportunity for females before egg laying influences offspring development which could highlight the need for appropriate basking opportunities for breeding females in captivity. Importantly, contrary to tree skinks (Riley et al. 2017), I only observed tail loss caused by interactions within the family group in two individuals. Raising multiple offspring without parents, as was done in tree skinks, could shed further light on how sociality might affect juvenile development. Together, my results provide important insights into captive breeding in tokay geckos but many unanswered questions remain.

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References

Ahl E (1926) Neue Eidechsen und Amphibien. Zoologischer Anzeiger 67: 186-192.

Almli LM, Burghardt GM (2006) Environmental enrichment alters the behavioral profile of ratsnakes (Elaphe). Journal of Applied Animal Welfare Science 9(2): 85-109. https://doi.org/10.1207/s15327604jaws0902_1

ASAB Ethical Committee, ABS Animal Care Committee (2023) Guidelines for the treatment of animals in behavioural research and teaching. Animal Behaviour 195: I-XI. https://doi.org/10.1016/j.anbehav.2022.09.006

Ballen C, Shine R, Olsson MM (2014) Effects of early social isolation on the behaviour and performance of juvenile lizards, *Chamaeleo calyptratus*. Animal Behaviour 88:1-6. https://doi.org/10.1016/j.anbehav.2013.11.010

Bell C, Raynal RS, Noble DW, Schwanz LE, Warner DA, Pruett JE, Riley JL (2025) The effect of moisture during development on phenotypes of egg-laying reptiles: A systematic review and meta-analysis. Journal of Experimental Biology 228(25): JEB249960. https://doi.org/10.1242/jeb.249960

Bestion E, Teyssier A, Aubret F, Clobert J, Cote J (2014) Maternal exposure to predator scents: offspring phenotypic adjustment and dispersal. Proceeding of the Royal Society B 281. https://doi.org/10.1098/rspb.2014.0701

Burghardt GM, Layne-Colon DG (2023) Effects of Ontogeny, Rearing Conditions, and Individual Differences on Behaviour: Welfare, Conservation, and Invasive Species Implications. In: Warwick C, Frye FL, Murphy JB (Eds) Health and welfare of captive reptiles. Cham: Springer International Publishing, 287-321.

Bürkner P-C (2017) brms: An R Package for Bayesian Multilevel Models Using Stan. Journal of Statistical Software 80(1): 1-28. https://doi.org/10.18637/jss.v080.i01

Bürkner P-C (2018) Advanced Bayesian multilevel modeling with the R Package brms. The R Journal 10(1): 395-411. https://doi.org/10.48550/arXiv.1705.11123

Bürkner P-C (2021) Bayesian Item Response Modeling in R with brms and Stan. Journal of Statistical Software 100(5): 1-54. https://doi.org/10.48550/arXiv.1905.09501

Castle E (1990) Husbandry and breeding of chameleons, *Chamaeleo* spp. at Oklahoma City zoo. International Zoo Yearbook 29:74-84. https://doi.org/10.1111/j.1748-1090.1990.tb03333.x

Crates R, Stojanovic D, Heinsohn R (2023) The phenotypic costs of captivity. Biological Reviews 98(2): 434-449. https://doi.org/10.1111/brv.12913

Doody JS, Guarino E, Georges A, Corey B, Murray G, Ewert M (2006) Nest site choice compensates for climate effects on sex ratios in a lizard with environmental sex determination. Evolutionary Ecology 20: 307-330. https://doi.org/10.1007/s10682-006-0003-2

Doody JS, McGlashan J, Fryer H, Coleman L, James H, Soennichsen K, Rhind D, Clulow S (2020) Plasticity in nest site choice behavior in response to hydric conditions in a reptile. Scientific Reports 10(1): 16048. https://doi.org/10.1038/s41598-020-73080-6

Duméril AMC, Bibron G (1836) Erpetologie Générale ou Histoire Naturelle Complete des Reptiles. Librairie encyclopédique de Roret, Paris, 528.

Duméril AMC, Duméril AHA (1851) Catalogue méthodique de la collection des reptiles du Muséum d'Histoire Naturelle de Paris. *Gide and Boudry*, 224.

Du WG, Li SR, Sun BJ, Shine R (2023) Can nesting behaviour allow reptiles to adapt to climate change? Philosophical Transactions of the Royal Society B 378(1884): 20220153. https://doi.org/10.1098/rstb.2022.0153

Elphick MJ, Shine R (1998) Longterm effects of incubation temperatures on the morphology and locomotor performance of hatchling lizards (*Bassiana duperreyi*, Scincidae). Biological Journal of the Linnean Society 63(3): 429-447. https://doi.org/10.1111/j.1095-8312.1998.tb01527.x

Ezaz T, Sarre SD, O'Meally D, Marshall Graves JA, Georges A (2009) Sex chromosome evolution in lizards: independent origins and rapid transitions. Cytogenetic and genome research, 127(2-4): 249-260.

Ezaz T, Quinn A, Miura I, Sarre S, Georges A, Marshall Graves J (2005) The dragon lizard Pogona vitticeps has ZZ/ZW micro-sex chromosomes. Chromosome Research 13: 763-776.

Gamble T (2010) A review of sex determining mechanisms in geckos (Gekkota: Squamata). Sexual Development 4(1-2): 88-103. https://doi.org/10.1159/000289578

Grossmann W (2007) Der Tokeh: *Gekko gecko*. Natur und Tier Verlag, Münster.

Gutzke WHN, Crews D (1988) Embryonic temperature determines adult sexuality in a reptile. Nature 332: 832-834. https://doi.org/10.1038/332832a0

Hall JM, Warner DA (2020) Ecologically relevant thermal fluctuations enhance offspring fitness: biological and methodological implications for studies of thermal developmental plasticity. Journal of Experimental Biology 223(19): JEB231902.

Holleley CE, O'Meally D, Sarre SD, Marshall Graves JA, Ezaz T, Matsubara K, Azad B, Zhang X, Georges A (2015) Sex reversal triggers the rapid transition from genetic to temperature-dependent sex. Nature 523: 79-82.

Holleley CE, Sarre SD, O'Meally D, Georges A (2016) Sex reversal in reptiles: reproductive oddity or powerful driver of evolutionary change? Sexual Development 10(5-6): 279-287.

Linnaeus C (1758). Systema naturæ per regna tria naturæ, secundum classes, ordines, genera, species, cum characteribus, differentiis, synonymis, locis. Tomus I. Editio decima, reformata. Laurentii Salvii, Holmiæ, 824.

Lindström J (1999) Early development and fitness in birds and mammals. Trends in Ecology & Evolution 14(9): 343-348. https://doi.org/10.1016/S0169-5347(99)01639-0

Loew ER (1994) A third, ultraviolet-sensitive, visual pigment in the Tokay gecko (*Gekko gecko*). Vision Research 34(11): 1427-1431. https://doi.org/10.1016/0042-6989(94)90143-0

McDonald S, Schwanz LE (2018) Thermal parental effects on offspring behaviour and their fitness consequences. Animal Behaviour 135: 45-55. https://doi.org/10.1016/j.anbehav.2017.11.007

Meehan CL, Mench JA (2002) Environmental enrichment affects the fear and exploratory responses to novelty of young Amazon parrots. Applied Animal Behaviour Science 79: 75-88. https://doi.org/10.1016/S0168-1591(02)00118-1

Monteith KM, Andrews C, Smiseth PT (2012) Post-hatching parental care masks the effects of egg size on offspring fitness: a removal experiment on burying beetles. Journal of Evolutionary Biology 25(9): 1815-1822. https://doi.org/10.1111/j.1420-9101.2012.02567.x

Nemesházi E, Bókony V (2025) Interplay of Genotypic and Thermal Effects on Sex Determination Shapes Climatic Distribution in Herpetofauna. Global Ecology and Biogeography 34(7): e70096. https://doi.org/10.1111/geb.70096

Nemesházi E, Bókony V (2022) Asymmetrical sex reversal: Does the type of heterogamety predict propensity for sex reversal? BioEssays 44(7): 2200039. https://doi.org/10.1002/bies.202200039

Noble DW, Stenhouse V, Schwanz LE (2018) Developmental temperatures and phenotypic plasticity in reptiles: A systematic review and meta-analysis. Biological Reviews 93(1): 72-97. https://doi.org/10.1111/brv.12333

Peig J, Green AJ (2009) New perspectives for estimating body condition from mass/length data: the scaled mass index as an alternative method. Oikos, 118(12): 1883–1891. https://doi.org/10.1111/j.1600-0706.2009.17643.x.

Peters WCH (1870) Eine Mitteilung über neue Amphibien (Hemidactylus, Urosaura, Tropidolepisma, Geophis, Uriechis, Scaphiophis, Hoplocephlaus, Rana, Entomogossus, Cystignathus, Hylodes, Arthroleptis, Phyllobates, Cophomantis) des Königlich-zoologischen Museums. Monatsberichte der Deutschen Akademie der Wissenschaften zu Berlin, 641-652.

Quinn AE, Georges A, Sarre SD, Guarino F, Ezaz T, Graves JAM (2007) Temperature sex reversal implies sex gene dosage in a reptile. Science 316: 411. https://doi.org/10.1126/science.1135925

Quinn AE, Sarre SD, Ezaz T, Marshall Graves JA, Georges A (2011) Evolutionary transitions between mechanisms of sex determination in vertebrates. Biology Letters 7(3); 443-448.

R Core Team (2025). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/. Accessed February 2025

Riley JL, Noble DW, Byrne RW, Whiting MJ (2017) Early social environment influences the behaviour of a family-living lizard. Royal Society Open Science 4(5): 161082. https://doi.org/10.1098/rsos.161082

Schmalz X, Biurrun Manresa J, Zhang L (2023) What is a Bayes factor? Psychology Methods 28(3) 705-719. https://doi.org/10.1037/met0000421

Schneider CA, Rasband WS, Eliceiri KW (2012) NIH Image to ImageJ: 25 years of image analysis. Nature Methods 9(7): 671-675. https://doi.org/10.1038/nmeth.2089

Schrijver NCA, Bahr NI, Weiss IC, Würbel H (2002) Dissociable effects of isolation rearing and environmental enrichment on exploration, spatial learning, and HPA activity in adult rats. Pharmacology, Biochemistry, and Behavior 73: 209-224. https://doi.org/10.1016/S0091-3057(02)00790-6

Schrijver NCA, Pallier PN, Brown VJ, Würbel H (2004) Dissociable effects of social and environmental stimulation on spatial learning and reversal learning in rats. Behavioural Brain Research 152: 307-314. https://doi.org/10.1016/j.bbr.2003.10.016

Schwanz LE (2016) Parental thermal environment alters offspring sex ratio and fitness in an oviparous lizard. Journal of Experimental Biology 219(15): 2349-2357. https://doi.org/10.1242/jeb.139972

Shine R, Elphick M, Donnellan S (2002) Co-occurrence of multiple, supposedly incompatible modes of sex determination in a lizard population. Ecology Letters 5: 486-489. https://doi.org/10.1046/j.1461-0248.2002.00351.x

Shine R, Elphick MJ, Harlow PS (1997a) The influence of natural incubation environments on the phenotypic traits of hatchling lizards. Ecology 78(8): 2559-2568. doi.org/10.1890/0012-9658(1997)078[2559:TIONIE]2.0.CO;2

Shine R, Madsen TR, Elphick MJ, Harlow PS (1997b) The influence of nest temperatures and maternal brooding on hatchling phenotypes in water pythons. Ecology 78(6): 1713-1721. doi.org/10.1890/0012-9658(1997)078[1713:TIONTA]2.0.CO;2

Solleder E, Schmid M (1984) XX/XY-sex chromosomes in *Gekko gecko* (Sauria, Reptilia). Amphibia-Reptilia 5(3-4): 339-345. https://doi.org/10.1163/156853884X-005-03-14

Szabo B (2024) Changes in enclosure use and basking behaviour associated with pair housing in Tokay geckos (G*ekko gecko*). Applied Animal Behaviour Science 272: 106179. https://doi.org/10.1016/j.applanim.2024.106179

Szabo B, Ringler E (2025) Does the Post-Natal Social Environment Influence Cognitive Development in a Social Gecko? Ecology and Evolution 15(6): e71560. https://doi.org/10.1002/ece3.71560

Tiatragul A, Kurniawa A, Kolbe JJ, Warner DA (2017) Embryos of non-native anoles are robust to urban thermal environments. Journal of Thermal Biology 65: 119-124. https://doi.org/10.1016/j.jtherbio.2017.02.021

Tokunaga S (1985) Temperature-Dependent Sex Determination in *Gekko japonicus* (Gekkonidae, Reptilia). Development, Growth & Differentiation 27(2): 117-120. https://doi.org/10.1111/j.1440-169X.1985.00117.x

Uller T (2008) Developmental plasticity and the evolution of parental effects. Trends in Ecology & Evolution 23(8): 432-438. https://doi.org/10.1016/j.tree.2008.04.005

Van Damme R, Bauwens D, Braña F, Verheyen RF (1992) Incubation temperature differentially affects hatching time, egg survival, and hatchling performance in the lizard *Podarcis muralis*. Herpetologica 48(2): 220-228. https://www.jstor.org/stable/3892675

Vehtari A, Gelman A, Gabry J (2017) Practical Bayesian model evaluation using leave-one-out cross-validation and WAIC. Statistics and Computing 27(5): 1413-1432. https://doi.org/10.1007/s11222-016-9696-4

Viets BE, Ewert MA, Talent LG, Nelson CE (1994) Sex-determining mechanisms in squamate reptiles. Journal of Experimental Zoology 270(1): 45-56. https://doi.org/10.1002/jez.1402700106

Warton DI, Duursma RA, Falster DS, Taskinen S (2012) smatr 3 - an R package for estimation and inference about allometric lines. Methods in Ecology and Evolution 3(2): 257-259.

While GM, Noble DW, Uller T, Warner DA, Riley JL, Du WG, Schwanz LE (2018) Patterns of developmental plasticity in response to incubation temperature in reptiles. Journal of Experimental Zoology Part A: Ecological and Integrative Physiology 329(4-5) 162-176. https://doi.org/10.1002/jez.2181