RESEARCH ARTICLE

Rigid Shells Enhance Survival of Gekkotan Eggs

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ABSTRACT

The majority of lizards and snakes produce permeable parchment-shelled eggs that require high moisture conditions for successful embryonic development. One clade of gekkotan lizards is an exception; females produce relatively impermeable rigid-shelled eggs that normally incubate successfully under low moisture conditions. I tested the hypothesis that the rigid-shell increases egg survival during incubation, but only under low moisture conditions. To test this hypothesis, I incubated rigid-shelled eggs of *Chondrodactylus turneri* under low and under high moisture conditions. Eggs were incubated with parchment-shelled eggs of *Eublepharis macularius* to insure that incubation conditions were suitable for parchment-shelled eggs. *Chondrodactylus turneri* eggs had very high survival (>90%) when they were incubated under low moisture conditions. In contrast, eggs incubated under high moisture conditions had low survival overall, and lower survival than those of the parchment-shelled eggs of *E. macularius*. Mortality of *C. turneri* and *E. macularius* eggs incubated under high moisture conditions was the result of fungal infection, a common source of egg mortality for squamates under laboratory and field conditions. These observations document high survival of rigid-shelled eggs under low moisture conditions because eggs escape from fungal infection. Highly mineralized rigid shells also make egg survival independent of moisture availability and may also provide protection from small invertebrates in nature. Enhanced egg survival could thus compensate for the low reproductive output of gekkotans that produce rigid-shelled eggs. *J. Exp. Zool. 323A:607–615, 2015.* © 2015 Wiley Periodicals, Inc.

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The great majority of squamate reptiles (lizards and snakes) produce parchment-shelled eggs that are highly permeable to water vapor; eggs desiccate rapidly when exposed to low moisture conditions. Squamate eggs do not normally desiccate during incubation because they are buried underground where high moisture conditions favor a net uptake of water from the environment (Fig. 1A; Skulan, 2000). Indeed, uptake of water from the nest environment during incubation may be necessary for successful development for all but the largest parchment-shelled eggs (Ackerman et al., '85; Packard and Packard, '88). In accord, females select nest sites carefully and may dig a number of pilot nests before they oviposit (Warner and Shine, 2008). Nests are typically in or on the ground and range from shallow in tropical rain forests (Andrews, '88) to as much as a meter below the soil surface in arid environments (Cohn, '26; Sherbrooke, 2002; Andrews et al., 2008; Doody et al., 2014). Eggs are also placed in humus, rotten logs, tree holes, piles of compost, and termite mounds (e.g., King and Green, '99; Löwenborg et al., 2012).

One clade of gekkotan lizards is a notable exception to the dependence on humid nest sites by squamates. More than 1,200 species of sphaerodactylid, phyllodactylid, and gekkonid geckos (Gamble et al., 2012) produce highly calcified rigid-shelled eggs (Andrews, 2012; Pike et al., 2012). These eggs are relatively impermeable to water vapor (Deeming and Thompson, '91; Andrews, 2012; Andrews et al., 2013). Like bird eggs, rigid-shelled eggs of gekkotans are typically placed in aboveground nests where eggs are exposed to the relatively low water vapor pressure of the atmosphere (Rahn et al., '77; Osadnik, '84; Henkel

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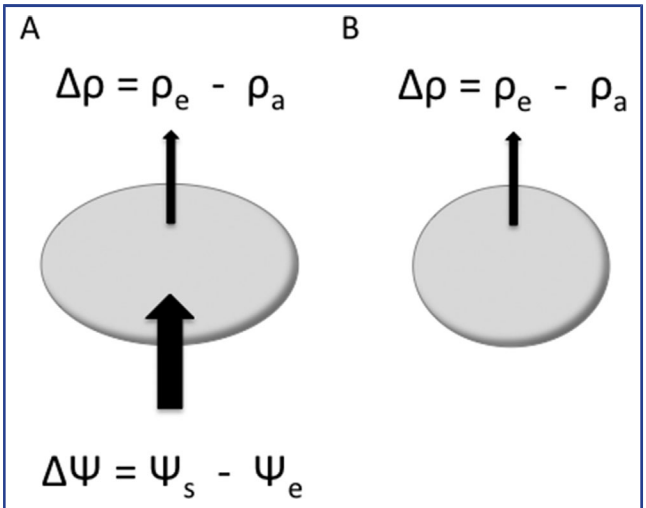

Figure 1. The likely direction and magnitude (arrows) of water exchange for two types of squamate eggs given incubation conditions used in this study (after Tracy et al., 1978; Ackerman et al., 1985). **A**. Parchment-shelled egg buried in a high moisture substrate (28°C); some fraction of the egg surface is in contact with the substrate and some exposed to the nest atmosphere. The physical driver for exchange of liquid water is the difference between the water potential of the substrate (Ψ_s) and of the egg (Ψ_e). The physical driver for exchange of water vapor is the difference between the vapor density of the egg (ρ_e) and of the atmosphere (ρ_a). Typically, uptake of water from the substrate exceeds loss to the atmosphere and eggs increase in mass (e.g. $\Psi_s = -250$kPa, $\Psi_e = -800$ kPa). Water exchange with the atmosphere would be relatively low because the vapor densities of eggshells and the atmosphere are both near saturation (e.g. ρ's ≈ 28 g/m^3). **B**. Rigid-shelled egg exposed to a low moisture atmosphere (28°C). The driver for exchange of water vapor is the difference between the vapor density of the egg (ρ_e) and of the atmosphere (ρ_a). Typically, eggs decrease in mass because the vapor density of the eggshell is near saturation and the vapor density of the atmosphere is substantially lower (e.g. $\rho_e = 28$ g/m^3, $\rho_a = 7$ g/m^3).

and Schmidt, '95; Ineich, 2010; Mateo and Cuadrado, 2012); low moisture conditions favor a net loss of water during incubation (Fig. 1B).

The rigid-shelled egg appears to be a key innovation (*sensu* Bond and Opell, '98) that is associated with the adaptive radiation of sphaerodactylid, phyllodactylid, and gekkonid geckos (Gamble et al., 2012). Nonetheless, a direct connection between rigid-shelled eggs and the numerical success of gekkotans that produce these eggs has yet to be identified. To the contrary, the production of rigid-shelled eggs appears to come with significant fitness costs.

Rigid-shelled eggs of gekkotans are small relative to the body size of the female (Kratochvíl and Frynta, 2006; Pike et al., 2012). Rigid-shelled eggs are spherical to minimize the amount of calcium; their diameter is limited so that they can pass through the pelvic aperture of the female. In contrast, parchment-shelled eggs of gekkotans are elongate, and thus, for the same diameter, have a larger volume than spherical rigid-shelled eggs. Because rigid-shelled eggs lose water during normal development, eggs must be provisioned initially with sufficient water to accommodate diffusive losses. As a result, eggs have relatively more water than parchment-shelled lizard eggs (Belinsky et al., 2004), and hatchlings are small relative to the size of the egg (Pike et al., 2012). Small hatchlings are poor competitors, grow to reproductive size slowly, and have low survival relative to large hatchlings (Ferguson and Fox, '84; Sinervo et al., '92; Uller and Olsson, 2010; Warner et al., 2010), although other factors such as the seasonal timing of hatchling can complement or override the costs of small hatchling size per se (Warner and Shine, 2007). Incubation length is longer for rigid-shelled than parchment-shelled eggs (Pike et al., 2012). Long incubation periods extend generation time, and thus reduce the intrinsic rate of population growth. In aggregate, these costs should reduce reproductive output of gekkotans that lay rigid-shelled eggs relative to those gekkotans that produce parchment-shelled eggs.

The observation that gekkotans that produce rigid-shelled eggs are so successful despite constraints on reproductive output suggest that some other aspect of their biology provides compensatory benefits. One possibility is that the rigid shell increases egg survival during incubation (Pike et al., 2012). The relatively impermeable shell makes eggs less vulnerable to desiccation and might deter predation by small invertebrates and reduce microbial infection or both. Fungal infection is an important source of mortality for reptile eggs in the laboratory and field (Tracy '80; Brown and Duffy, '92; Phillott and Parmenter, 2001; Moreira and Barata, 2005; Huang, 2006) and therefore appropriate to isolate as a mortality factor in laboratory experiments. Given, however, the association between rigid-shelled eggs and utilization of exposed nest sites, reduction in egg mortality due to fungal infection may be contingent on the nest environment itself.

I therefore conducted laboratory experiments to test the hypothesis that rigid-shelled eggs have a survival advantage over parchment-shelled eggs in their respective normal nest environments, but not overall. To do this, I incubated rigid-shelled eggs of *Chondrodactylus turneri* Gray (Gekkonidae) under their normal low moisture conditions (Andrews, 2012) and under the high moisture conditions required for the successful incubation of parchment-shelled eggs. Eggs of *C. turneri* were incubated along side parchment-shelled eggs of *Eublepharis macularius* Blyth (Eublepharidae) to insure that the high moisture conditions were indeed suitable for parchment-shelled eggs. To additionally assess the affect of moisture conditions on development, I also

determined several phenotypic attributes of *C. turneri* hatched from eggs incubated under low and high moisture conditions.

MATERIALS AND METHODS

Source of Geckos and Husbandry Protocols

Eggs were obtained from breeding colonies of geckos maintained in my laboratory. *Chondrodactylus turneri* (7 males, 7 females) were housed in pairs in screen cages 76 cm H, 40 cm W, 43 cm D. Cages were provided with upright sheets of Ondura® corrugating roofing material for shelters and tubs of dry sand for oviposition. *Eublepharis macularius* (8 females, 6 males) were housed in groups of 1–2 females and one male in plastic tubs (9 cm H, 27 cm W, 16 cm D). Tubs were provided with bark slabs and pieces of Ondura for shelter and plastic storage containers partially filled with moist coco peat (processed coir fiber) for oviposition sites. Cages of both species had dishes for water and for calcium supplements (ground cuttlebone and Rep-Cal Calcium). Ambient lighting was provided by east and south facing windows and by full spectrum Vita-Lite (0800–1700 h) fluorescent bulbs hung over each cage. Thermal gradients for basking were provided by 100-watt spotlights (0900–1600 h) focused on the Ondura sheets (*C. turneri*) and heat strips under one end of tubs (*E. macularius*). Geckos were watered daily and fed 3 days a week on canned cat food (*E. macularius* only), crickets dusted with Rep-Cal Calcium and Herptivite Multi-vitamin supplements, mealworms, and cockroaches.

Cages were checked daily for eggs. Eggs of *C. turneri* were buried in moist coco peat overnight (2012) or for 1–2 hr (2013) so that sand adhering to the shell could be removed easily (Andrews, 2012). Eggs were then weighed and assigned to experimental treatments. Eggs of *E. macularius* were weighed and immediately allocated to experimental treatments. Some eggs were sampled at oviposition to determine the dry mass of eggshells.

Experimental Design

Observations were made on eggs produced from March through July 2012 and from January through April 2013. *Chondrodactylus turneri* and *E. macularius* females produce 1–2 egg clutches at 2–3 week intervals. Because of low egg production, data collected in 2012 and 2013 were combined. Eggs were allocated such that the eggs produced by each female would be represented equally in all treatments (that is, in each moisture by temperature combination).

Eggs of *C. turneri* were incubated under low and high moisture conditions whereas eggs of *E. macularius* were incubated only under high moisture conditions (eggs are not viable under low moisture conditions). Because incubation success varies as a function of temperature (Andrews and Schwarzkopf, 2012), eggs were incubated at 25 (2012 only), 28, and 31°C. Optimal incubation temperature for *C. turneri* is not known although it is probably about 30°C like that of other gekkotans; the optimal incubation temperature of *E. macularius* is 32°C (sources from Andrews and Schwartzkopf, 2012). Mortality at 25° was high and incubation was so prolonged that eggs were shifted to the 28°C treatment at 44–67 d (*C. turneri*) and at 51–57 d (*E. macularius*) to allow surviving embryos to complete development. Results from incubation at 25°C are reported for qualitative comparisons only.

Experimental Protocols

Chondrodactylus turneri eggs incubated under low moisture conditions were placed on the surface of a layer of dry sand in small petri dishes. Dishes were placed in plastic storage boxes with loose ports that allowed circulation of air. *Chondrodactylus turneri* and *E. macularius* eggs incubated under high moisture conditions were buried alternately in moistened coco peat in plastic shoeboxes. The coco peat was moistened to a water potential of −250 kPa (1 part coco peat and 1.75 parts distilled water). Boxes were sealed with several layers of food wrap to regulate water loss.

Egg boxes were placed in one of three Percival (Perry, IA, USA) programmable incubators according to their temperature treatment (25, 28, or 31°C). Temperature and relative humidity (RH) in the low moisture boxes were monitored every hour (2012) or every four hours (2013) with Onset data loggers (Bourne, MA, USA, Hobo U23-001 Pro v2) during the entire incubation period. Mean temperatures were 25.3, 28.1, and 31.0°C in 2012 and 28.3 and 30.9°C in 2013. Respective mean RHs (water vapor densities) were 39, 40, and 31% (9.0, 11.2, and 9.6 g/m^3) in 2012 and 21 and 16% (6.0 and 5.0 g/m^3) in 2013. Ambient laboratory RH in January-April 2013 was lower than ambient RH in March-July 2012; hence the lower RH (and water vapor densities) in the low moisture egg boxes in 2013 than 2012. Temperature in the high moisture boxes would have been the same as measured in the low moisture boxes; water vapor densities in the moist substrate would have been near saturation.

Eggs were weighed 1–2 times a week during incubation. Boxes with the high moisture substrate were weighed at these times and distilled water added to return moisture levels to initial values before replacing eggs. Microbial infection was noted when eggs were weighed; if infected eggs had decreased in mass, they were removed from the egg boxes and opened. The embryos were dead in all cases.

Several days before anticipated hatching, eggs were shifted to individual containers with treatment appropriate conditions; eggs were weighed one or two times a day until hatching. When eggs hatched, yolk remaining in the eggshell or extending from the umbilicus was removed and weighed. Eggshells were rinsed, dried at 40°C overnight, and then weighed to the nearest 0.1 mg. Net water exchange of eggs during incubation was the difference between the final mass of the egg measured <24 h prior to pipping or hatching and the initial mass of the egg. Fluid loss associated with hatching was the difference between the final mass of the egg and the live mass of the hatchling plus the dry mass of the eggshell and the wet mass of external yolk.

Hatchlings were weighed to the nearest mg and their snout-vent length (SVL) and tail length (TL) were measured to the nearest 0.5 mm. They were toe clipped for permanent identification and paint marked for visual identification. Hatchlings were housed under the same cage conditions as adults, but fed 6–7 times a week. Initially, 4–6 similarly sized individuals were housed together; numbers per cage were reduced as individuals grew. Groups were reformed to balance body sizes at these times if necessary.

Chondrodactylus turneri individuals were weighed every 1–2 weeks after hatching. Growth in mass was assessed as the slope of the regression between Log10 mass and age for observations made between hatching and 60 d. Selected body temperatures (T_b) were measured 2–4 times a week in home cages between 1000 and 1500 h using a Fluke 574 infrared thermometer (Everett, WA, USA) using precautions suggested by Hare et al. (2007). Measurements were made after lifting the Ondura sheets to expose the sheltering hatchlings. Body temperatures were measured only when hatchings were in their original position or if they had shifted position by no more than a body length. Because hatchlings became very active after a few seconds of exposure, only one or two individuals could be measured per cage per day. For statistical analyses, the T_b of each individual was the average of its multiple observations (Mean n = 17, Range = 9–33). At the end of the study, juveniles were euthanized with an overdose of isoflurane gas.

Animal Care Approval

The Virginia Tech Institutional Animal Care and Use Committee approved the research protocols for this study (IACUC Nos. 10-041-BIOL and 13-020-BIOL).

Analyses

Parametric analyses were two-factor ANOVAs or ANCOVAs with moisture level (low or high) and temperature (28 or 31°C) as class variables (*C. turneri*) or one-factor ANOVAs or ANCOVAs with temperature (28 or 31°C) as the class variable (*E. macularius*). The covariate for analyses of net water exchange, hatching water loss, hatchling mass, and hatchling SVL was initial egg mass. The covariate for hatchling tail length was hatchling SVL.

While both *C. turneri* and *E. macularius* exhibit temperature-dependent sex determination (TSD), sex was not used as a factor in analyses because sex was determined only in 2013 (the majority of hatchings were males). Previous observations on species with TSD indicate that temperature, not sex, affects the performance variables assessed in this study (Rhen and Lang, '95; Warner and Shine, 2011). Results of analyses of hatchling performance therefore are unlikely to be biased by sex.

Parametric statistical analyses were conducted using JMP software (JMP®, Version 11, SAS Institute Inc., Cary, NC, 1989–2007). Preliminary models incorporated interaction terms. Because no interactions were significant ($P > 0.05$), they are not included in the models presented. Non-parametric analyses were based on Siegel ('56).

RESULTS

Egg Mass and Shell Mass at Oviposition and at Hatching

Egg masses at oviposition did not differ among treatments (*C. turneri*: $F_{3,25} = 0.15$, $P = 0.93$; *E. macularius*: $F_{1,33} = 1.49$, $P = 0.23$, ANOVAs) with overall means of 1.65 and 3.24 g, respectively. Masses of dry eggshells at oviposition were 0.129 ± 0.0017 g and 0.086 ± 0.0039 g, respectively. Masses of dry eggshells at hatching did not differ among treatments (*C. turneri*: $F_{3,28} = 0.19$, $P = 0.90$; *E. macularius*: $F_{1,29} = 2.37$, $P = 0.13$, ANOVAs) with overall means of 0.122 and 0.096 g, respectively. Dry masses of eggshells at oviposition did not differ from those at hatching (*C. turneri*: $F_{1,21} = 3.00$, $P = 0.10$; *E. macularius*: $F_{1,32} = 0.26$, $P = 0.61$, ANCOVAs). Because the dry mass of eggshells was the same at the time of oviposition and hatching, changes in egg mass during incubation were due to net water exchange.

Egg Survival

Patterns of survival differed between the two species of geckos when eggs were incubated under conditions normal for each species (Table 1). *Chondrodactulus turneri* eggs incubated under low moisture conditions had greater than 90% survival at all incubation temperatures, and survival did not differ at 28 and 31°C ($P = 1.0$, Fisher Exact Test). In contrast, survival of *E.*

Table 1. Survival of rigid-shelled eggs of *Chondrodactylus turneri* and parchment-shelled eggs of *Eublepharis macularius* incubated under low (Low) and high (High) moisture conditions at three incubation temperatures.

	Survival, % (initial number of eggs)		
Temperature	*C. turneri* – Low	*C. turneri* – High	*E. macularius* – High
25°C	100 (3)	0 (3)	45 (11)
28°C	100 (12)	42 (12)	48 (25)
31°C	93 (15)	64 (11)	96 (24)

See text for details.

macularius eggs incubated under high moisture conditions was temperature dependent; survival was lower at 28°C (48%) than at 31°C (96%) ($X^2 = 11.5$, $P < 0.001$, Chi-squared test).

Chondrodactylus turneri incubated under low moisture conditions had higher survival than *E. macularius* incubated under high moisture conditions at both 25°C (100 vs. 45%) and at 28° (100 vs. 48%, $P = 0.002$, Fisher Exact Test), while at 31°C survival did not differ (93% vs. 96%, $P = 1.0$, Fisher Exact Text).

Chondrodactylus turneri eggs incubated under "abnormal" high moisture conditions had lower survival than those incubated under low moisture conditions, and substantially so at the lowest incubation temperature (Table 1). At 25°C, all three eggs in the low moisture conditions survived while none of the three eggs in high moisture conditions did so. At 28°C, survival was significantly higher under low (100%) than high moisture conditions (42%) ($P = 0.005$, Fisher exact probability test). At 31°C, egg survival was also higher under low (93%) than high moisture (64%) conditions but not significantly so ($P = 0.128$, Fisher exact probability test). For the 28 and 31°C treatments combined, 26 of 27 (96%) of eggs survived under low moisture conditions while only 12 of 23 (52%) survived under high moisture conditions ($X^2 = 10.8$. $P < 0.01$, Chi-squared test).

Under high moisture conditions, the pattern of survival of *C. turneri* and *E. macularius* eggs was similar: survival was temperature dependent and survival under high moisture conditions was the greatest at 31°C (Table 1). Survival did not differ at 28°C ($P = 1.0$, Fisher exact probability test) and *C. turneri* eggs had lower survival than *E. macularius* eggs at 31°C (64 vs. 96%, $P = 0.026$, Fisher exact probability test).

Egg mortality was associated with fungal infection. Hyphae were first seen in small patches on the shells of eggs that died, but eventually covered their surfaces and penetrated the shells. Mortality could have been direct (fungi infected embryos) or indirect (fungi infected eggs with embryos that had died as a result of inimical incubation conditions). Because egg survival was near 100% under optimal incubation conditions (*C. turneri*: low moisture, all temperatures; *E. macularius*: high moisture, 31°C), mortality was unlikely to be related to infertility or embryo non-viablity.

Incubation length, Water Exchange During Incubation, and Water Loss at Hatching

Incubation length of *C. turneri* eggs was affected by both temperature and by moisture (Table 2). Incubation length was 2 d shorter under low than high moisture conditions and 21 d shorter at 31 than at 28°C. For *E. macularius*, incubation length was 11 d shorter at 31 than at 28°C. Despite their smaller eggs, *C. turneri* had substantially longer incubation lengths (28 and 31°C: 70 and 49 d, respectively) than *E. macularius* (28 and 31°C: 51 and 38 d, respectively).

Patterns of net water exchange (difference between the final and initial masses of eggs) differed substantially between *C. turneri* and *E. macularius* (Table 2). For *C. turneri*, net water exchange was related to moisture but not temperature. Eggs decreased substantially in mass under low moisture conditions (−0.254 g) and increased slightly in mass (0.006 g) under high moisture conditions. These values represent a respective 15% decrease and a 0.4% increase relative to mean initial egg mass. For *E. macularius*, eggs incubated at 28 and 31°C had respective net increases in mass of 0.92 and 0.69 g during incubation. These values represent respective increases of 28 and 21% relative to mean initial egg mass.

Rigid-shelled eggs are more resistant to volume expansion than parchment-shelled eggs (Adams et al., 2010). This is

Table 2. The effect of moisture and temperature on incubation length, net water exchange, and hatching water loss of rigid-shelled eggs of *Chondrodactylus turneri* and parchment-shelled eggs of *Eublepharis macularius*.

Species	Moisture	Temperature	LS Means (± 1 SE)
C. turneri			
Incubation length (d)	**$F_{1,34} = 9.6$, $P = 0.004$**	**$F_{1,34} = 1476.6$, $P < 0.0001$**	Low = 58.4 ± 0.33, High = 60.2 ± 0.47, 28°C = 69.7 ± 0.42, 31°C = 48.8 ± 0.37
Net water exchange (g)	**$F_{1,25} = 375.9$, $P < 0.0001$**	$F_{1,25} = 0.9$, $P = 0.36$	Low = −0.254 ± 0.009, High = 0.006 ± 0.010
Hatching water loss (g)	**$F_{1,24} = 75.1$, $P < 0.0001$**	$F_{1,24} = 0.7$, $P = 0.41$	Low = 0.124 ± 0.024, High = 0.454 ± 0.029
E. macularius			
Incubation length (d)	–	**$F_{1,28} = 956.3$, $P < 0.0001$**	28°C = 50.5 ± 0.33, 31°C = 38.0 ± 0.23
Net water exchange (g)	–	**$F_{1,27} = 11.5$, $P = 0.002$**	28°C = 0.918 ± 0.055, 31°C = 0.691 ± 0.038
Hatching water loss (g)	–	**$F_{1,23} = 7.2$, $P = 0.014$**	28°C = 1.418 ± 0.081, 31°C = 1.155 ± 0.054

Low and High indicate low and high moisture conditions, respectively. Significant effects are indicated with bold font.

presumably why almost half of *C. turneri* eggs incubated under high moisture conditions developed conspicuous cracks. Of the 23 eggs in the combined 28 and 31°C high moisture treatments, 10 cracked (43%) while none of the 27 eggs in the low moisture treatment cracked ($X^2 = 11.6$, $P < 0.001$). Eggs that cracked in the moist treatment were less likely to hatch (3 of 10 eggs hatched) than eggs that did not crack (9 of 13 hatched) ($P = 0.10$, Fisher exact probability test).

Hatching was associated with a substantial loss of fluid from the egg for both species (Table 2). For *C. turneri*, fluid losses were affected by moisture, but not temperature. Eggs lost 0.124 g in the low moisture treatment and 0.454 g in the high moisture treatment, representing 8 and 28% of the mean final mass of eggs (1.49 and 1.64 g, respectively). For *E. macularius*, fluid losses were affected by temperature. Fluid losses were 1.42 and 1.16 g at 28 and 31°C, respectively, representing 34 and 23% of the mean final mass of eggs (4.20 and 4.97 g, respectively).

Hatchling Performance of *Chondrodactylus turneri*

Hatching size was affected by moisture conditions, but not temperature. Hatchlings from eggs incubated under normal low moisture conditions weighed more and had longer SVLs than those from eggs incubated under high moisture conditions (Table 3). Hatchlings from eggs incubated under high moisture conditions were relatively small, in part, because of failure to utilize all the yolk in the egg prior to hatching. Of the 12 hatchlings from high moisture conditions (28 and 31°C combined), eight (67%) left yolk in the shell or had external yolk attached to the umbilicus at hatching whereas all of the 26 hatchlings from eggs incubated under low moisture conditions had internalized yolk prior to hatching ($X^2 = 6.47$, $P < 0.02$).

Tail length was affected by temperature but not moisture. Individuals from the 31°C treatment had longer tails than those from the 28°C treatment. The non-significant moisture effect indicates that the tails of hatchlings did not differ from that expected from their SVLs, the covariate in analyses.

Selected body temperatures of *C. turneri* hatchlings were affected by both incubation temperature and moisture (Table 3). Hatchlings selected higher body temperatures if they were incubated under low rather than high moisture conditions (32.0 and 31.3°C, respectively) and if they were incubated at 28 rather than at 31°C (31.9 and 31.4°C, respectively). In contrast, growth rates over the first 60 d of life were not related to either moisture or temperature (overall ANCOVA: $F_{2,23} = 0.99$, $P = 0.416$, mass at hatching as the covariate).

DISCUSSION

Hatching Success

My objective was to evaluate the hypothesis that the rigid-shell of gekkotan eggs increases egg survival, but that the benefit is situation dependent. Experimental results support this hypothesis. Rigid-shelled eggs of *C. turneri* eggs had very high survival (>90%) when they were incubated under normal low moisture conditions. In contrast, *C. turneri* eggs incubated under the high moisture conditions critical for hatching success of parchment-shelled squamate eggs, had low survival overall, and lower survival than the parchment-shelled eggs of *E. macularius*. Mortality was particularly high at the two lowest incubation temperatures, presumably because fungal growth outpaced embryonic development. Nonetheless, at 31°C, the experimental temperature selected to match optimal incubation temperatures of *C. turneri* and *E. macularius* eggs, *C. turneri* eggs had lower survival than *E. macularius* eggs, 64 and 96%, respectively.

The water relations of *C. turneri* eggs were greatly altered by incubation under high moisture conditions and, as a consequence, egg survival was reduced. Normally, rigid-shelled eggs of gekkotans, including *C. turneri*, decrease in mass during incubation as the result of diffusive water losses (Fig. 1B). In contrast, diffusive water loss under high moisture conditions would have been very low (Fig. 1A). Indeed, the mass of *C. turneri* eggs increased only slightly (0.4%) whereas the mass of the parchment-shelled eggs of *E. macularius* increased by 21–28%.

Table 3. The effect of moisture and temperature during incubation on mass, SVL, tail length, and selected body temperature (T_b) of *Chondrodactylus turneri* hatchlings.

Trait	Moisture	Temperature	LS means (± 1 SE)
Mass (g)	**$F_{1,25} = 5.8$, $P = 0.024$**	$F_{1,25} = 0.1$, $P = 0.76$	Low = 1.162 ± 0.021, High = 1.098 ± 0.025
SVL (mm)	**$F_{1,25} = 6.2$, $P = 0.020$**	$F_{1,25} = 0.2$, $P = 0.70$	Low = 33.1 ± 0.28, High = 32.0 ± 0.34
Tail length (mm)	$F_{1,31} = 3.2$, $P = 0.09$	**$F_{1,31} = 4.3$, $P = 0.047$**	28°C = 26.8 ± 0.42, 31°C = 28.0 ± 0.39
Tb (°C)	**$F_{1,25} = 6.3$, $P = 0.019$**	**$F_{1,25} = 4.3$, $P = 0.047$**	Low = 32.0 ± 0.18, High = 31.3 ± 0.19, 28°C = 31.9 ± 0.19, 31°C = 31.4 ± 0.18

Low and High indicate low and high moisture conditions, respectively. Significant effects are indicated with bold font.

The increased volume and resultant cracking of *C. turneri* eggs incubated under high moisture conditions made eggs more vulnerable to fungal infection presumably through enhanced access to the shell membrane by fungal spores and hyphae.

Effects of High Moisture Conditions During Incubation on *Chondrodactylus turneri* Hatchlings

Survival of *C. turneri* eggs during incubation under high moisture conditions was reduced compared to incubation under normal low moisture conditions. Did detrimental effects extend to hatchlings? Observations on hatchlings suggest that this was the case. Hatchlings from eggs incubated under high moisture conditions had smaller bodies, non-internalized yolk, and longer incubation periods compared to hatchlings from eggs incubated under normal low moisture conditions. Eggs incubated under high moisture conditions contained almost four times more fluid just prior to hatching than eggs incubated under low moisture conditions. These observations suggest that embryos from eggs incubated under high moisture conditions competed for space in the egg with its fluid contents and, as a consequence, developed more slowly and converted less yolk to body mass than embryos from eggs incubated under low moisture conditions.

In contrast, observations over the following 60 d indicate that individuals had recovered from a seemingly bad start in life (Radder et al., 2007). All hatchlings from eggs incubated under high moisture conditions survived to the end of the 60 d experimental period. While incubation conditions had a long-term affect on thermal behavior, how such differences would affect fitness is not known.

Water Relations of Rigid-shelled Eggs

Reptiles that lay rigid-shelled exhibit dichotomous patterns of nesting ecology and water exchange of eggs (Deeming and Unwin, 2004). On the one hand, the rigid-shelled eggs of crocodilians and turtles are buried in nests underground where the atmosphere is, or is often, saturated with moisture. Eggs have relatively high mass specific water vapor conductance but exchange relatively small amounts of water with the nest environment. Hydric conditions have either a negligible effect on hatching success and hatchling quality or these metrics are enhanced by high moisture availability (Booth, 2002; Packard, '91). For example, when rigid-shelled eggs of the turtle *Pelodiscus sinensis* were half-buried in substrates with water potentials of −12 to −750 kPa, they increased in mass by only 25 mg (0.7% of an initial egg mass of 3.5 g) on the wettest substrate and decreased in mass by only 125 mg (3.6% of initial egg mass) on the driest (Zhao et al., 2013). Despite considerable variation in substrate water potentials, egg survival and hatchling dry mass did not differ among treatments in the Zhao et al. (2013) study. While hatchling wet mass and linear dimensions were the largest on the wettest substrate, this was the result of greater water content, not greater dry tissue mass. Finally, hydric conditions affected incubation length and hatchling righting performance but effects did not vary systematically with substrate water potential.

On the other hand, the rigid-shelled eggs of gekkotans and birds are placed in nests exposed to the atmosphere or in substrates where water potential is low (large negative values). Eggs have relatively low mass specific water vapor conductance and decrease in mass by roughly 15–20% during incubation as a result of diffusive losses of water (Ar and Rahn, '80; Rahn et al., '77; Andrews, 2012). For both birds and gekkotans, hydric conditions affect hatching success and hatchling quality; when the water vapor density of the nest environment is high, diffusive water loss from eggs is reduced with attendant negative effects on egg survival (this paper, Ar, '91).

Rigid-shelled eggs of gekkotans and birds exhibit poor hatching success under physical conditions that, superficially at least, are associated with high hatching success of crocodilians and turtles. Bird eggs may have poor hatching success at high water vapor densities because they do not lose enough water to form the air cell that the embryo uses to inflate its lungs prior to hatching (Ar, '91). Gekkotan eggs have poor hatching success because eggs become vulnerable to microbial infection. These observations suggest that for both gekkotans and birds the evolution of a relatively impermeable shell and an egg provisioned with water stores sufficient to accommodate substantial water loss during incubation may have been associated with the use of exposed nests above the ground or low moisture substrates.

For gekkotans that produce rigid-shelled eggs, observations of reduced hatching success, reduced hatchling size, and increased incubation length under high moisture incubation conditions indicate that concurrent shifts in nest site and eggshell structure would have increased reproductive success relative to that of squamates that produce permeable parchment-shelled eggs. My observations specifically document enhanced survival under low moisture conditions because eggs escape from fungal infection. The highly mineralized shell also makes egg survival independent of moisture availability and may provide physical protection from small invertebrates in nature. Enhanced egg survival could thus compensate for the relatively low reproductive output of gekkotans that produce rigid-shelled eggs.

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