

Water Vapor Permeability of the Rigid-Shelled Gecko Egg

ROBIN M. ANDREWS*

Department of Biological Sciences, Virginia Tech, Blacksburg, Virginia



ABSTRACT

The vast majority of squamate reptiles (lizards and snakes) produce parchment-shelled eggs that absorb water during incubation, and thus increase in mass, volume, and surface area. In contrast, females from a single monophyletic lineage of gekkotan lizards produce rigid-shelled eggs. These eggs are functionally comparable to those of birds, that is, at oviposition, eggs contain all the water needed for development, and their mass decreases during incubation via the diffusion of water vapor through the shell. I determined patterns of water loss and shell permeability to water vapor from oviposition to hatching for the rigid-shelled eggs of the gekkonid *Chondrodactylus turneri* and compared permeability of *C. turneri* eggs to those of birds and other squamates. *Chondrodactylus turneri* eggs incubated at 28.5°C and 40% relative humidity (RH) decreased in mass by 14% over the course of a 68-day incubation period. The rate of water loss varied during incubation; egg mass decreased rapidly during the first 8 days of incubation, declined at a low constant rate during the next 35 days, and then decreased rapidly during the final 25 days of incubation. Overall permeability was 0.17 mg/day/kPa/cm². Percent water loss of rigid-shelled gecko eggs during incubation is similar to that exhibited by birds, but water vapor permeability is about one-third that of bird eggs and several orders of magnitude lower than that of parchment-shelled squamate eggs. In general, the water economy of their eggs may be associated with the adaptive radiation of the rigid-shelled sphaerodactylid, phyllodactylid, and gekkonid geckos. *J. Exp. Zool.* 317A:395–400, 2012. © 2012 Wiley Periodicals, Inc.

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The vast majority of squamate reptiles (lizards and snakes) lay parchment-shelled eggs that absorb water during incubation, and thus increase in mass, volume, and surface area. The water dynamics of these eggs are complexly related to the physical environment of the egg and to factors intrinsic to the egg and the developing embryo (Ackerman et al., '85; Adams et al., 2010). Nonetheless, successful hatching depends on a net water uptake by eggs (Thompson and Speake, 2004). Because shells of parchment-shelled eggs are so highly permeable to water vapor (Deeming and Thompson, '91), to avoid desiccation, eggs must be placed within substrates (e.g., soil) where the atmosphere is near saturated. In contrast, some gekkotan lizards produce rigid-shelled eggs that are highly resistant to desiccation, and are thus are not dependent on moist substrates for successful incubation.

Rigid-shelled eggs are produced only by members of a single monophyletic lineage comprising the sphaerodactylid, gekkonid, and phyllodactylid geckos (Gamble et al., 2008). Shell structure

of the eggs of these geckos is morphologically and functionally comparable to that of birds. Shells are heavily mineralized and, like bird eggs, have a pore system through which gas exchange with the atmosphere occurs (Packard and Hirsch, '89). Rigid-shelled gecko eggs contain all the water needed for embryonic development at oviposition, and egg mass decreases during incubation via the diffusion of water vapor through the shell (Deeming and Unwin, 2004). For birds at least, the amount

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*Correspondence to: Robin M. Andrews, Department of Biological Sciences, Virginia Tech, Blacksburg, VA 24061. E-mail: randrews@vt.edu

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diffusive water loss must be regulated to be “just right” for normal development (Paganelli, '91). On the one hand, because the rate at which water diffuses out is proportional to the rate at which oxygen diffuses into the egg, eggs must lose some amount of water during incubation to accommodate embryonic metabolism. On the other hand, if water loss is too high, the embryo will not have sufficient water to complete development, and to hatch successfully.

Despite the novelty of their shell type within squamates, water dynamics during incubation are not known for any gecko that lays rigid-shelled eggs. Published data concern static one-time measurements of water vapor permeability only. This situation contrasts greatly to that for birds for which patterns of water loss during the course of incubation have been examined for a wide range of species (reviewed by Booth and Rahn, '90; Ar, '91). The objectives of this paper are thus to (1) determine the rate of water loss from oviposition to hatching for eggs of the gekkonid *Chondrodactylus turneri* and (2) compare eggshell permeability of *C. turneri* with that of other squamates and birds.

MATERIALS AND METHODS

Source and Captive Husbandry of Geckos and Animal Care Approval

Four pairs of *C. turneri* were obtained from a commercial dealer in July 2010. Observations reported here are from eggs produced between November 2010 and June 2011. Pairs were housed in screen cages 76 × 43 × 40 cm. Cages were provided with sheets of Onduline corrugating roofing material for refuges, tubs of dry sand for oviposition, dishes for water and for calcium supplements (ground cuttlebone and other sources of calcium). Ambient lighting was provided by east and south facing windows. VitaLites (0800–1700 hr) and a 100 W spotlight (0900–1600 hr) hung over each cage provided additional light and heat for basking. Geckos were watered daily and fed 3 days a week on crickets dusted with Rep-Cal Calcium and Herptivite Multivitamin supplements, mealworms, and cockroaches. Cages were checked daily for eggs. Oviposition was always obvious because females mounded sand over the oviposition site. Eggs were always covered by a thin, but firmly attached, layer of sand. Females presumably applied the sand to the shell surface just after oviposition; other gekkonids that bury their eggs display this behavior, and may do so as a means of camouflaging their eggs (Henkel and Schmidt, '95).

The Virginia Tech Institutional Animal Care and Use Committee approved the research protocol for this study on April 6, 2010 (IACUC No. 10-041-BIOL).

Egg Incubation

Eggs were weighed at oviposition after gently removing loose sand with a make-up brush and placed on top of the substrate in an uncovered specimen jar partially filled with dry sand. Eggs were incubated at 28.5°C in a constant temperature incubator

calibrated against an NBST standard. A pan of water in the incubator kept the relative humidity at 40%, the relative humidity of bird nests (Rahn et al., '77). A nylon mesh cover was placed over the top of jars after 60 days of incubation to keep hatchlings from escaping. Hatchlings were weighed and their snout-vent lengths (SVLs) and tail lengths measured. At oviposition, embryos were at Dufaure and Hubert ('61) stage 26 (R.M. Andrews, unpublished data).

Experimental Protocols

The amount of sand adhering to shells was determined for nine eggshells immediately after hatching and for four freshly oviposited eggs. Shells and cracked eggs were placed in 1N hydrochloric acid overnight to remove calcium carbonate and the residue was ashed in a muffle furnace to remove organic material. Dry egg masses were obtained for three eggs that were weighed at oviposition and then dried at 40°C for 3 days and then reweighed.

Eggs decrease in mass during incubation as the result of the diffusion of water vapor from the interior of the egg into the atmosphere. To assess water loss during incubation, the relationship between egg mass and time was determined for nine eggs that were weighed at regular intervals from oviposition to a day or two prior to hatching. While egg mass decreased monotonically, the curve relating mass and time did not conform to any biologically related function (see Fig. 1). Mass decreased linearly during the middle phase of incubation, but nonlinearly initially and toward the end of incubation. The rate of water loss was therefore estimated separately during these three phases of incubation. The length of the middle linear phase was determined for each egg by iteratively removing observations of mass from the beginning and end of incubation such that the value of R^2 was maximized. Observations of mass from the beginning to end day of the resultant sequence were used to characterize the rate of water loss using linear regression. The length of the first and last portions of incubation were the difference between the beginning day of the linear portion and the day of oviposition and the difference between the end day of the linear portion and the day of hatching, respectively. The change in mass for each portion was divided by time in days. These values were corrected to standard pressure by multiplying them by 707/760 where 707 mmHg is the average barometric pressure at Blacksburg, Virginia. To calculate permeability, the partial pressure (kPa) of water vapor in the inner shell membrane was assumed to be the partial pressure of saturated water vapor at 28.5°C and the partial pressure external to the egg was 40% of that value. The surface area of eggs was estimated from the relationship $A = 4.835 M^{0.662}$ where M is the initial sand-free mass of the egg in grams and A is the surface area in square centimeter (Paganelli et al., '74). Permeability was calculated similarly for the entire incubation period as well. Permeability was expressed as $\text{mg} \cdot \text{day}^{-1} \cdot \text{kPa}^{-1} \cdot \text{cm}^{-2}$. Because water loss and permeability

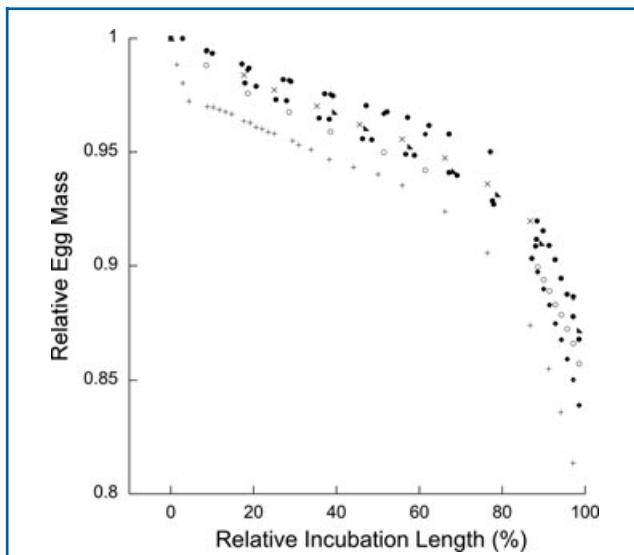


Figure 1. Decrease in mass of nine *Chondrodactylus turneri* eggs during incubation. Relative mass (observed mass divided by initial mass) is plotted as a function of the relative length of the incubation period (%). Unique symbols designate observations for five of the nine eggs to illustrate the general pattern of water loss by eggs; observations for the other four eggs are represented by black dots.

were based on observations up to, but not including hatching, water loss associated with hatching is not accounted for in calculating water loss and permeability. Observations of permeability are thus directly comparable with those on bird eggs (Ar and Rahn, '80).

Analyses

Statistical analyses were conducted with JMP Software (Copyright 2007, SAS Institute, Cary, NC). Means are given \pm 1 standard error (SE).

RESULTS

The mean mass of sand adhering to eggs was 0.15 g (\pm 0.006, $n = 13$). The amount of sand did not differ between eggs sampled at oviposition and at hatching (0.149 and 0.151 g, respectively, $F_{1,11} = 0.03$, $P = 0.86$, one-factor ANOVA). Sand adhering to eggs was thus firmly attached to the shell; the amount of sand on eggs at oviposition was the same as that at hatching, and observations of water loss were not biased by sand shed during incubation.

Overall egg mass at oviposition was 1.89 g (\pm 0.014, $n = 53$). Sand-free eggs would therefore have had a mean mass of 1.74 g (1.89–0.15 g). The overall mean mass of hatched shells was 0.27 g (\pm 0.01, $n = 9$) of which sand was 52.8% and the calcified

shell was 47.2%. Sand-free shells therefore weighed 0.13 g or 7.3% of sand-free egg mass. The dry mass of eggs was 27.5% of their initial mass excluding sand ($n = 3$). On average, therefore, the initial water content of eggs would have been 1.26 g (0.725×1.74 g).

Egg masses used to assess the shape of water loss curves were corrected for adhering sand by subtracting the estimated mass of sand at oviposition from initial egg mass at oviposition and from all subsequent measurements. The correction factor for each egg was based on the relationship between the mass of sand adhering to eggs and the initial mass of the egg including sand as: $\text{SandMass} = 0.032 + 0.063 \text{ EggMassInitial}$ ($F_{1,9} = 3.51$, $P = 0.094$, $R^2 = 0.20$, $n = 11$).

Overall, eggs lost 0.25 g of water during incubation (SE = 0.021, $n = 6$) or 14.3% of their initial sand-free mass. The rate of water loss, however, changed over time (Fig. 1 and Table 1). For the first 8 days of incubation on average, permeability was relatively high; eggs lost 10% of the total water lost during incubation during this phase. For the next 35 days, mass decreased linearly as a function of time, and permeability was relatively low; eggs lost 25% of the total water lost during incubation during this phase. During the final 25 days of incubation, permeability was very high, and eggs lost 65% of the total water lost during incubation during this phase.

Incubation length averaged 66 days ($n = 16$) and ranged from 62 to 70 days. For the subset of nine eggs that were weighed regularly during the entire incubation period, incubation length averaged 68 days (Table 1). Hatching success was 100%, and all hatchlings were viable. Hatchlings had mean masses of 1.23 g (\pm 0.013, $n = 18$), SVLs of 33.7 mm (\pm 0.16, $n = 14$), and tail lengths of 29.0 mm (\pm 0.62, $n = 12$). They were not sexed at hatching.

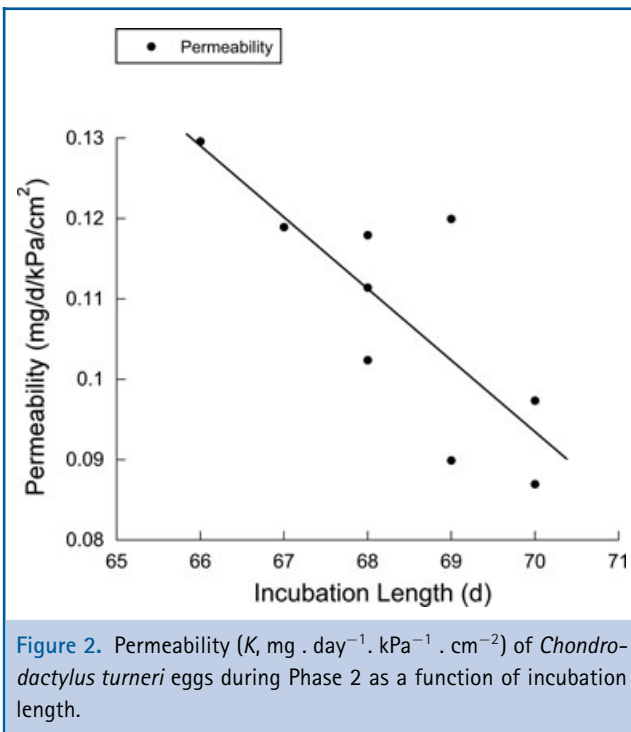
Permeability of the shell to water vapor was negatively related to incubation length (IncLen) during Phase 2 (Fig. 2), but not during Phases 1 or 3, or overall (P 's > 0.05). For Phase 2, the relationship was

$$K = 0.72 - 0.0089\text{IncLen} (F_{1,7} = 12.0, P = 0.011, R^2 = 0.63).$$

Table 1. Permeability ($\text{mg} \cdot \text{day}^{-1} \cdot \text{kPa}^{-1} \cdot \text{cm}^{-2}$) and the magnitude of water loss of *Chondrodactylus turneri* eggs during incubation.

	K (SE, n)	End of phase, day (SE, n)	Water loss, g (%, n),
Phase 1 (8 days)	0.135 (–, 9)	8.3 (1.98, 9)	0.025 (9.7, 9)
Phase 2 (35 days)	0.108 (0.005, 9)	43.2 (1.34, 9)	0.066 (25.6, 9)
Phase 3 (25 days)	0.370 (0.023, 6)	68.3 (0.44, 9)	0.167 (64.7, 6)

Permeability for Phase 1 is expressed as the median value because of one egg for which K was very high (0.91) because it lost a normal amount of water, but did so over a very short period of time.



DISCUSSION

Water dynamics of the rigid-shelled eggs of the gekkonid *C. turneri* exhibit striking parallels with those of the rigid-shelled eggs of birds. For example, eggs of *C. turneri* lost an average of 14% of their initial mass during incubation. This is similar to the average 15% decrease in mass exhibited by bird eggs (Ar and Rahn, '80) and is similar to that of the Pied Flycatcher (20%, Kern et al., '92) whose egg mass is the same as that of *C. turneri* (1.7 g), and the Pearly-eyed Thrasher (15%, 8.0 g, Arendt, 2005). For bird eggs, the net water loss prior to external pipping results in the formation of an air cell; water in the egg at hatching thus represents the balance between the internal generation of water through metabolism and loss as the result of diffusion (Ar and Rahn, '80). Water balance of the rigid-shelled eggs of geckos must be similarly obtained, although whether or not rigid-shelled gecko eggs develop an air cell during normal development is not known. Unlike birds, however, rigid-shelled eggs of geckos hatch successfully without appreciable water loss if they are incubated at a high relative humidity (Thompson and Russell, '99). Birds apparently require the development of an air cell in the egg for successful hatching, and incubation at high relative humidity precludes its formation (Rahn et al., '77).

Eggs of *C. turneri* also exhibited distinct changes in the rate of water loss during incubation in parallel with that exhibited by some birds. For bird eggs in general, the rate of water loss may be relatively constant during incubation or vary depending on

environmental conditions, including brooding behavior of the parents, and on developmental processes such as the thinning of the shell via transfer of calcium from the shell to the embryo and the increasing heat generated by the embryo as development proceeds (Ar, '91; Packard and Clark, '96). For example, the rate of water loss by bird eggs increases when brooding begins because of the increased temperature differential between eggs and the environment (Kern et al., '92; Arendt, 2005). While water loss of gecko eggs is not influenced by parental behavior, processes intrinsic to the egg itself appear to influence patterns of water loss.

During the first 8 days (range 0–14 days) after oviposition, permeability was relatively high, but the rate of water loss decelerated with time. This observation suggests some “maturational” process of the shell itself. One possibility is that, because the pores of the shell are filled with water at oviposition, the high initial rate of water is associated with drying of pore spaces in the outer layers of the shell (Thompson, '85), and that the resistance to water loss increases thereafter as a result of smaller and less continuous pore spaces in the inner layer of the shell (Packard and Hirsch, '89).

During the second, and longest, phase of incubation, the rate of water loss was relatively low and constant, presumably due to an unvarying water vapor pressure gradient between the inner and outer surfaces of the egg. The rate of water loss during this period was, however, inversely related to incubation length suggesting that the rate of water loss is linked to embryonic growth, perhaps via variation in the heat production by embryos (Ackerman et al. '85).

In contrast, during the third phase of incubation, the rate of water loss was accelerated. The egg tooth in geckos does not become mineralized until shortly before hatching (stages 39–40, R.M. Andrews, personal communication). This means that movements of embryos that could abrade the shell and/or the shell and embryonic membranes do not occur until much later in development than the observed increase in water loss. More likely explanations involve increasing heat production of the embryo and/or thinning of the shell via the transfer of calcium from the shell to the embryo that occur late in development. While both of these physiological processes are best documented in birds, they also occur in reptiles (reviewed by Ar, '91; Packard, '94; Packard and Clark, '96).

In parallel with birds, eggs of *C. turneri* lost a substantial amount of water during hatching per se. Assuming that the proportional wet mass of eggs and of hatchlings of *C. turneri* is the same as it is in birds (Ar and Rahn, '80), then, of the 1.26 g of water initially in *C. turneri* eggs, 0.89 g was incorporated into the embryo, and 0.25 g lost to diffusion. The remaining 0.12 g, or 32% of water lost overall, would thus represent water lost during hatching. This is similar to the estimate that 20–40% of the total water lost from bird eggs occurs after external pipping (Ar, '91).

Table 2. Observations of water vapor permeability (K , $\text{mg} \cdot \text{day}^{-1} \cdot \text{kPa}^{-1} \cdot \text{cm}^{-2}$) during incubation of rigid- and parchment-shelled reptile eggs.

Taxon (egg mass)	Shell type	K	Source
<i>Chondrodactylus turneri</i> (1.7 g)	Rigid	0.17	This paper
<i>Sphaerodactylus</i> sp. (0.14 g)	Rigid	0.20	Dunson and Bramham ('81)
<i>Lepidodactylus lugubris</i> (0.23 g)		0.26	Dunson ('82)
<i>Hemidactylus garnoti</i> (0.34 g)		0.26	
<i>Gehyra variegata</i> (0.52 g)		0.20	R.M. Andrews (unpublished data)
Birds (<4 g, 6 species)	Rigid	0.74	Ar et al. ('74)
Lizards (0.1–3.8 g, 22 species)	Parchment	211.0	Deeming and Thompson ('91)

Species and groups are listed in order of increasing K .

For comparative purposes, water loss of eggs is best expressed in terms of permeability that accounts for the magnitude of water lost as a function of time, egg surface area, and the difference in vapor pressure between the inside of the shell and the environment. Permeability of *C. turneri* eggs is similar to that measured for eggs of other geckos that lay rigid-shelled eggs (Table 2). In contrast, mean water vapor permeability of bird eggs is about three times higher than that of rigid-shelled gecko eggs (Table 2). This difference in permeability is likely related to the shorter incubation periods of birds than lizards—weeks rather than months of incubation, respectively. In turn, the water vapor permeability of the rigid-shelled eggs of birds and geckos is three orders of magnitude lower than that of parchment-shelled eggs of squamates. The rigid shell thus comprises a formidable barrier to the movement of water vapor out of the egg and explains why rigid-shelled eggs can incubate successfully in nests fully exposed to the air (Dunson and Bramham, '81), while parchment-shelled eggs must incubate within a substrate where RH approaches saturation (Deeming and Unwin, 2004). In fact, the water economy of their rigid-shelled eggs may explain the astounding success of sphaerodactylid, phyllodactylid, and gekkonid geckos (Pike et al., 2011). While clades with parchment-shelled eggs are the most basal on the gekkotan phylogeny (Gamble et al., 2008), the clades with rigid-shelled eggs make up about 80% of the 1,200+

species of all gekkotans. Because rigid-shelled eggs contain all of the water necessary for embryonic development and highly calcified shells that limit water loss from the egg, eggs can be laid in nest sites not available to other squamates; nests are often aboveground where eggs are placed loose in cavities or glued to exposed surfaces of trees or rocks or attached to overhanging rock faces (Dunson '82; Henkel and Schmidt, '95; Deeming and Unwin, 2004). In turn, use of aboveground nest sites may be associated with the radiation of geckos into arboreal niches as a reptilian parallel with the radiation of birds into aerial niches.

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