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Does the hydric environment affect the incubation of small rigid-shelled turtle eggs?

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ABSTRACT

Hydric environments are hypothesized to have minor effects on the embryonic development of rigid-shelled turtle eggs due to the low water permeability of the eggshell. However, the water reserve in the eggs may still influence their resistance to environmentally induced dehydration. We incubated rigid-shelled turtle eggs (*Pelodiscus sinensis*) on different moist substrates (from −12 to −750 kPa) to test the hypothesis that small rigid-shelled eggs would be sensitive to hydric environments. The hydric treatment significantly affected the incubation period, with eggs incubated in the moistest and driest substrates taking longer to hatch than those on the medium-moisture substrates. Hatching success was slightly lower for eggs incubated in dry conditions than those in wet conditions, but the difference was not statistically significant. The heart rates of early embryos were lower on moist substrates than those on dry substrates, but this difference disappeared in late embryos. Hatchlings from the moistest substrate were larger (in carapace length and width) and heavier than those from drier substrates. However, the dry body mass of the hatchlings did not differ among the hydric treatments. The functional performance (righting response) of the hatchlings was affected by the hydric environment. The time to right was shorter for the hatchlings from the substrate of −12 kPa than those from −220 kPa. These results are consistent with the hypothesis that the hydric environment may significantly affect developing embryos and the resulting hatchlings in turtle species, such as *P. sinensis*, with small rigid-shelled eggs.

1. Introduction

The phenotypes of offspring are determined not only by the genetic basis derived from their parents but also by environmental factors experienced during development (Via and Lande 1985). The phenotypic variation induced by the developmental environment (e.g., developmental plasticity) may impose selection on a number of ecological processes in both mother and offspring, e.g., female nest-site selection and hatchling survival (Via et al. 1995; Birchard and Deeming 2004; Shine 2004). Developmental plasticity is especially prevalent in oviparous species, whose embryogenesis is completed outside of the mother's body, and is thus strongly affected by environmental factors (Deeming 2004). Therefore, the consideration of developmental plasticity is critical to an understanding of the adaptation of these species to their environment.

Developmental plasticity in oviparous reptiles has attracted increasing attention from ecologists (See review by Shine 2004). Despite the large quantity of literature on the phenotypic responses of the embryonic development and hatchling traits of reptiles to environmental factors such as moisture (Deeming 2004), conclusions about hydric effects on egg incubation are not universal among species. Significant effects are found in certain species of reptiles but not in others (Packard 1999; Ji and Du 2001; Booth 2002; Shine and Brown 2002; Brown and Shine 2006). This among-species discrepancy may reflect interspecific variation in the embryonic response to the hydric environment due to the diversity of egg traits such as the eggshell and the water content. Oviparous reptiles produce eggs with either a rigid or a flexible shell (Packard and DeMarco 1991). The difference in water permeability between rigid and flexible shells is profound due to the differences in physical properties between these types of shells. A rigid shell has a thick mineral layer and is relatively impermeable to water vapor, whereas a flexible shell has a thin mineral layer and is relatively permeable to water vapor (Packard 1991; Thompson and Speake 2004). Consequently, embryonic development in rigid-shelled eggs relies primarily on the availability of sufficient water in the eggs at oviposition and is generally insensitive to the external hydric environment (Packard 1999; Booth 2002; Booth and Yu 2009), whereas embryonic development in flexible-shelled eggs relies primarily on the water absorbed during incubation and is relatively sensitive to the hydric environment (Ji and Braňa 1999; Du and Shine 2008). Nevertheless, the water content of eggs would affect the sensitivity of embryonic development of rigid-shelled eggs to external hydric environments, with small eggs less resistant to environmentally induced dehydration than large eggs (Packard 1999). In this case, one might expect that small rigid-shelled eggs would tend to be

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sensitive to the hydric environment. However, this hypothesis has not been tested empirically.

Traditional egg-incubation experiments focus on the effects of incubation environments on hatchling traits, with less emphasis on embryos *per se* due to logistical difficulties. Recent advances in methodology provide an opportunity to elucidate the physiological basis of environmental effects on developing embryos (Du et al. 2010b). An interesting aspect of hydric effects on reptilian eggs is that eggs incubated on wet substrates tend to take longer to hatch (Packard 1999; Brown and Shine 2006). The physiological mechanisms underlying the variation in incubation period have not been determined but may be related to (1) different rates of embryonic development, i.e., embryos on a wet substrate develop more slowly than those on a dry substrate; and (2) a facultative shift in the degree of embryogenesis completed prior to hatching (Shine and Olsson 2003; Du et al. 2010b). Given that heart size, heart rate, and stroke volume are related to cardiac output, an important determinant of the rate of embryonic development (Kam 1993; Burggren and Keller 1997; Pearson et al. 2000), differences in heart rate or heart size would be expected if the embryos have different developmental rates under various hydric treatments. Alternatively, a wet substrate could induce eggs to delay hatching because a longer period of embryonic development within the egg facilitates energy conversion from yolk to hatchling tissues (Booth and Yu 2009). If the embryos adopt this avenue of physiological regulation, eggs from a wet substrate would produce larger hatchlings with small residual yolk than their counterparts from a dry substrate.

In the present study, we experimentally incubated small rigid-shelled turtle eggs (*Pelodiscus sinensis*) at a range of substrate water contents (from −12 to −750 kPa) to quantify the hydric effects on embryonic heart rates, incubation periods, hatchling body sizes, heart mass, and performance. We applied this system to answer two questions. Are small rigid-shelled eggs sensitive to the hydric environment? Is the moisture-induced variation in the incubation period due to the different developmental rate of embryos (reflected by different heart rate) or the facultative shift in the relative amount of development that occurs within the egg (reflected by the ratio of tissue to yolk in the hatchling)?

2. Materials and methods

2.1. Egg collection and incubation

In May 2009, a total of 77 freshly laid *P. sinensis* eggs (fertilized eggs with a white patch on the shell surface, average egg mass = 2.9 g) were collected from a private farm in Hangzhou city of Zhejiang Province, China. The eggs were weighed to ±1 mg using an electronic balance (Mettler Toledo AB135-S) and individually numbered with a pencil on the eggshell for later identification. Because the maternity of the eggs was unknown, we randomly assigned these eggs to different treatments to minimize maternal effects. The eggs were individually incubated (half buried) in a 60-mL jar filled with vermiculite at four different levels of moisture: −12 (2 g water/1 g vermiculite), −220 (1 g water/1 g vermiculite), −500 (0.63 g water/1 g vermiculite), and −750 (0.38 g water/1 g vermiculite) kPa, following an empirically derived calibration curve linking the water potential to the mass ratio of water to dry vermiculite (M. Thompson, unpublished data) and previous studies (Ji and Braňa 1999). The jars were covered with plastic wrap (sealed with a rubberband) and then placed in an FPQ incubator (Ningbo Life Science and Technology Ltd, China) at a constant temperature of 28 °C, an optimal incubation temperature for this species (Du and Ji 2003). Each jar with moist vermiculite was weighed at the beginning of the experiment and was then reweighed twice a week after removing the egg. Water was added to balance the water loss due to evaporation and absorption by the eggs and thereby maintain the water potential of the incubation substrate at a relatively constant level.

In May 2012, we further determined the influence of hydric condition on water exchange of *P. sinensis* eggs during incubation. A total of 102 freshly laid eggs (average egg mass = 4.3 g) were incubated at the four hydric treatments of −12, −220, −500, and −750 kPa. We weighed the eggs once a week to estimate the water exchange during incubation. The setup and procedure of egg incubation in 2012 were the same as those in 2009.

2.2. Heart rate of embryos

We measured the heart rates of embryos twice during incubation (days 10 and 30). The heart rates (beats per minute, bpm) were measured at 28 °C using an infrared heart rate monitor (Buddy system, Avian Biotech; see detailed procedures in Du et al. 2009 and Du et al., 2010b).

2.3. Incubation period, hatchling morphology and performance

Toward the end of incubation, the jars were monitored once a day for newly emerging hatchlings. The days elapsed between the beginning of incubation and the emergence of the hatchlings was recorded as the incubation period. After emergence, the hatchlings were maintained in the cup until the yolk had been entirely absorbed. The turtles were then weighed and individually kept in cages placed in a temperature-controlled room at 28 ± 1 °C and with a 12-h light/12-h dark cycle. One week later, we assessed the righting response, a fitness index in freshwater turtles, in the temperature-controlled room. Each turtle was placed upside down in an open area (250 × 200 × 40 mm). A digital camera (SONY, DCR-SR220E) was used to record the trials. Each turtle was tested five times, and the time to right (defined as the time required for a turtle to right itself after it began to move; Delmas et al., 2007) was collected *a posteriori* from the videotapes. After that the turtles were euthanized, and oven dried at 65 °C for 48 h to determine hatchling dry mass.

2.4. Data analysis

The normality of distributions and the homogeneity of variances were tested with a Kolmogorov–Smirnov test and a Bartlett's test, respectively. We conducted a G test to determine the effect of the hydric environment on hatching success. An analysis of variance (ANOVA) and a repeated-measures ANOVA were used to determine the influence of the hydric environment on the incubation period and heart rates of embryos, and water exchange during incubation. An analysis of covariance (ANCOVA) or MANCOVA, with initial egg mass or hatchling carapace length as the covariate, was used to analyze hydric effects on the body size and righting response of hatchlings. A Tukey post hoc multiple comparisons test was used to detect differences among treatments.

3. Results

During incubation, eggs incubated at −12 kPa gained a small amount of water, whereas eggs incubated at drier conditions from −220 kPa to −750 kPa lost water ($F_{18,588} = 24.11$, $P < 0.0001$). Water loss by the eggs increased as substrate moisture decreased (Fig. 1).

Hatching success was not affected by the hydric treatment ($G = 0.55$, $df = 3$, $P > 0.05$), with 85%, 69%, 62% and 61% success for eggs incubated on substrates with a water potential (WP) of −12, −220, −500 and −750 kPa, respectively. The incubation period differed among different hydric treatments ($F_{3,47} = 3.99$, $P = 0.01$), with longer periods for hatchlings from the moistest (−12 kPa, 52.5 ± 0.5 d) and driest (−750 kPa, 52.1 ± 0.6 d) substrates than from the two medium substrates (−220 and −500 kPa, 50.5 ± 0.8 and 50.3 ± 0.6 d).