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# Short-term Water Potential Fluctuations and Eggs of the Red-eared Slider Turtle (*Trachemys scripta elegans*)

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## ABSTRACT

We exposed eggs of the red-eared slider turtle (*Trachemys scripta elegans*) to short duration (i.e., 48 hours) changes in water potential at two embryonic ages (20 and 40 days). Survivorship to hatching did not differ by substrate water potential or among treatments. Net change in egg mass, a measure of net water exchange between the egg and substrate, was affected by treatments. However, treatments had no effect on hatchling mass, carcass mass, yolk mass, or incubation period. Eggs and embryos are able to exploit beneficial short-term increases in water potential and withstand adverse ones.

## INTRODUCTION

The microclimate within natural turtle nests varies on a daily and seasonal basis (e.g., Packard et al., 1985; Ratterman and Ackerman, 1989; Cagle et al., 1993). Such variation can influence survivorship of embryos and phenotypes of hatchlings (e.g., Cagle et al., 1993; Packard et al., 1993). This phenotypic variation is biologically significant because variation in hatchling size, incubation period, and survivorship results.

Cagle et al. (1993) found that significant variation in water potential can occur due to short-term phenomena such as a single heavy rainfall. They also found water potential to be the best predictor of water exchange by eggs, of the length of incubation, and of hatchling body size in natural nests despite the daily and seasonal variation in nest temperatures (Cagle et al., 1993).

We exposed eggs of the red-eared slider (*Trachemys scripta elegans*) to short-term (i.e., 48 h) fluctuations in water potential by moving eggs from dry to wet substrates or vice versa. We investigate responses of embryos after 20 and 40 days of incubation. We ask whether short duration fluctuations in water potential are sufficient to influence hatchling phenotypes.

## METHODS AND MATERIALS

Twenty female turtles were collected between 4 and 8 May, 1995 on their nesting migrations at Stump Lake, Jersey County, Illinois (Tucker, 1997). We induced oviposition with intramuscular injections of oxytocin (Ewert and Legler, 1978). After the eggs were patted dry, they were uniquely numbered with carbon ink and weighed on an electronic balance to 0.01 g.

We prepared 20 individual plastic boxes (32.5 X 19.4 X 10 cm). Eight boxes contained the wetter substrate, a mixture of 150 g perlite and 170 ml water (-60 kPa) and twelve boxes received a dry mixture made from 150 g perlite and 27 ml water (-189 kPa). Water potential for the mixtures was determined by thermocouple psychrometry (Tucker et al., 1998). We prepared more dry boxes because we anticipated that mortality would be higher on drier substrates.

Each clutch of 12 eggs was placed into one of the 20 boxes. Thus, one box contained all 12 eggs from a single clutch. Aluminum foil placed under the box lid retarded moisture loss. All eggs in each box were reweighed seven times during incubation with the final measurement made after 52 days incubation.

We maintained hydration in each box by periodically reweighing the box and its contents. Water was added to replace water absorbed by the eggs along with water lost to evaporation. During incubation, all boxes were kept together at the same vertical height. Boxes were rotated to spread potential effects of undetected thermal gradients over all boxes (and clutches). Incubation temperature fluctuated as it does in natural nests. We recorded temperatures daily with maximum-minimum thermometers. Incubation temperature averaged about 29°C.

Three experimental treatments were conducted on eggs in each box. Four eggs were designated as controls and remained at the same water potential throughout incubation. Four eggs were either moved from a wet substrate to a dry one or vice versa for 48 h after eggs had incubated for 20 days (= 20-day eggs). Four other eggs were either moved from a wet substrate to a dry one or vice versa for 48 h after eggs had incubated for 40 days (= 40-day eggs). Eggs from each clutch were randomly assigned to each treatment.

Eggs were moved to boxes containing newly made-up substrate of the correct kPa for 48 h. Eggs were then returned to their original boxes to continue incubation. We excluded all eggs that failed to whiten (Ewert, 1985); unfertilized eggs fail to whiten.

Once the first egg pipped, we placed a bottomless waxed paper cup over each egg (Janzen, 1993). We recorded pip date and defined incubation period as initial date minus pip date (Gutzke et al., 1984). We weighed hatchlings with an electronic balance to 0.01 g. Hatchlings were then killed by freezing. The carcass and yolk were separated. We weighed residual yolk with an electronic balance (to 0.01 g), and determined carcass mass by subtracting the mass of residual yolk from hatchling mass.

Statistical analyses were conducted using SAS 6.12 (SAS Institute, 1996). We used Fisher's exact test (two-tailed) to test for differences in survivorship due to substrate and treatment effects. We calculated least significant difference (LSD) for Figs. 1 and 2 using harmonic means because cell sizes differed (Snedecor and Cochran, 1986). We cannot distinguish clutch from box effects. However, Packard and Packard (1993) previously found that clutch effects generally are more important than box effects. Moreover, box and clutch effects were not variables of interest in this experiment.

For both experiments, we used a mixed model for analysis. The model specified treatment (i.e., control, 20-day, or 40-day) as the fixed effect. Clutch and the interaction between clutch and treatment were identified as random effects. Each variable (i.e., net change in egg mass during incubation, incubation period, hatchling mass, carcass mass, and yolk mass) was entered into the General Linear Model (GLM) Procedure and the Mixed Procedure (SAS Institute, 1996) following the method of Packard et al. (1999).

The Mixed Procedure estimated variance components using the restricted maximum likelihood method (REML) and Satterthwaite's approximation to correctly compute denominator degrees of freedom. The GLM Procedure was needed to obtain least squares means to assess the magnitude of differences observed among treatments for each variable. The GLM Procedure was also required to assess the significance levels for the covariance parameters of random effects derived from the Mixed Procedure. The GLM model statement included treatment, clutch, their interaction, and initial egg mass as the covariate. Treatment, clutch, and their interactions were also included as random effects with the 'test' option of SAS version 6.12 selected.

## RESULTS AND DISCUSSION

Survivorship to hatching did not differ by substrate water potential. For eggs on dry substrate, 94.29% of all eggs (n = 72) hatched whereas 91.67% of all eggs (n = 72) hatched on the wet substrate (Fisher's exact test, $X^2 = 0.372$, $p = 0.745$). We also found that combined survivorship for all clutches among treatments did not differ significantly ($X^2 = 0.468$, $p = 1.00$ for dry substrate and $X^2 = 4.364$, $p = 0.154$ for wet substrate). Survivorship by substrate and treatment ranged from 83.33% of all eggs (n = 24) for 20-day treatment eggs on wet substrate to 100% of all eggs (n = 24) for the control eggs on wet substrate. Survivorship for the control eggs on the dry substrate was 95.45% of all eggs (n = 24).

Change in egg mass during 52 days of incubation, a measure of water exchange between eggs and substrate, varied with clutch (or box) and treatment for the 12 clutches on the drier substrate (Table 1, Fig. 1). In contrast, neither clutch nor treatment had a significant influence on any variable for the eight clutches on the wetter substrate (Table 1, Fig. 2). Control eggs which remained on dry substrate throughout incubation gained significantly less mass than did eggs moved to wetter substrate at 20 or 40 days (Table 2).

The results of our experiments with *Trachemys scripta elegans* resemble those of Gutzke and Packard (1986) with the painted turtle (*Chrysemys picta*) and of Packard and Packard (1988) with the snapping turtle (*Chelydra serpentina*). Even though we used a shorter treatment interval and wetter substrates, we found that eggs on dry substrates could add water even in this short period. Thus, compensatory water exchanges (Gutzke and Packard, 1986) can occur during short-term fluctuations in water potential.

The rapidity of response for eggs that we observed may be biologically significant even though phenotypes of the hatchlings did not vary in our experiment. The ability to rapidly absorb substrate water should be advantageous for species that develop in nests with unpredictable water potentials (Packard et al., 1985; Ratterman and Ackerman, 1989; Cagle et al., 1993). Our finding that eggs and embryos can exploit short-term increases in water potential (Fig. 1) but withstand short-term decreases in water potential (Fig. 2) is important given the environmental variation experienced by turtle embryos in natural nests (Cagle et al., 1993).

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Table 1. Variance components for random effects (Clutch and Treatment*Clutch) and Type III *F*-value for fixed effect (Treatment) for incubation period, net change in egg mass, hatchling mass, carcass mass, and yolk mass for 20 clutches of red-eared slider (*Trachemys scripta elegans*) eggs. Significance levels (in parentheses) were determined using 'test' option in the General Linear Model Procedure of SAS 6.12. Measures of mass are given in g, whereas incubation period is in days.

| Source | DF | Δ egg mass | Incubation Period | Wet Mass | Carcass Mass | Yolk Mass |
|---|---|---|---|---|---|---|
| **Dry substrate** | | | | | | |
| Treatment | 2 | 18.0 | 0.72 | 1.82 | 1.24 | 1.40 |
| | | (0.0001) | (0.4900) | (0.1733) | (0.2979) | (0.2568) |
| Treatment*Clutch | 22 | 0.001 | 0.006 | 0.023 | 0.046 | 0.006 |
| | | (0.7803) | (0.3369) | (0.1076) | (0.0742) | (0.1729) |
| Clutch | 11 | 0.032 | 0.818 | 0.032 | 0.000 | 0.013 |
| | | (0.0001) | (0.0001) | (0.1007) | (0.4714) | (0.0383) |
| **Wet substrate** | | | | | | |
| Treatment | 3 | 0.56 | 1.23 | 1.09 | 0.38 | 0.13 |
| | | (0.5750) | (0.3003) | (0.3450) | (0.6887) | (0.8800) |
| Treatment*Clutch | 21 | 0.000 | 0.015 | 0.000 | 0.000 | 0.000 |
| | | (0.3829) | (0.1411) | (0.1193) | (0.3585) | (0.4500) |
| Clutch | 7 | 0.000 | 0.970 | 0.139 | 0.078 | 0.015 |
| | | (0.8505) | (0.0001) | (0.0001) | (0.0001) | (0.0090) |

Table 2. Least squares means for treatments (control, 20-day, 40-day) conducted on wet and dry substrates for 20 clutches of red-eared slider (*Trachemys scripta elegans*) eggs. Standard error is in parentheses. Measures of mass are given in g, whereas incubation period is in days.

| Source | Δ egg mass | Incubation Period | Wet Mass | Carcass Mass | Yolk Mass |
|---|---|---|---|---|---|
| **Dry (to Wet)** | | | | | |
| Control | 0.46(0.07)* | 58.4(0.17) | 7.50(0.08) | 6.57(0.11) | 0.93(0.04) |
| 20-day | 0.72(0.06) | 58.5(0.17) | 7.70(0.07) | 6.75(0.10) | 0.95(0.04) |
| 40-day | 0.89(0.06) | 58.7(0.17) | 7.57(0.07) | 6.54(0.10) | 1.03(0.04) |
| **Wet (to Dry)** | | | | | |
| Control | 1.36(0.10) | 58.8(0.15) | 8.11(0.06) | 7.12(0.08) | 0.99(0.04) |
| 20-day | 1.42(0.10) | 58.9(0.16) | 8.20(0.07) | 7.20(0.09) | 1.01(0.04) |
| 40-day | 1.50(0.10) | 58.5(0.16) | 8.23(0.06) | 7.22(0.08) | 1.01(0.04) |

* Control < 20-day and 40-day, $P < 0.006$; no other pairwise comparisons different.

Figure 1. Egg mass through incubation for eggs of the red-eared slider (*Trachemys scripta elegans*) incubated on dry substrate and either moved to a wetter one at 20 and 40 days incubation or kept on dry substrate throughout incubation (control). Least significance difference (LSD) is shown as a vertical line.

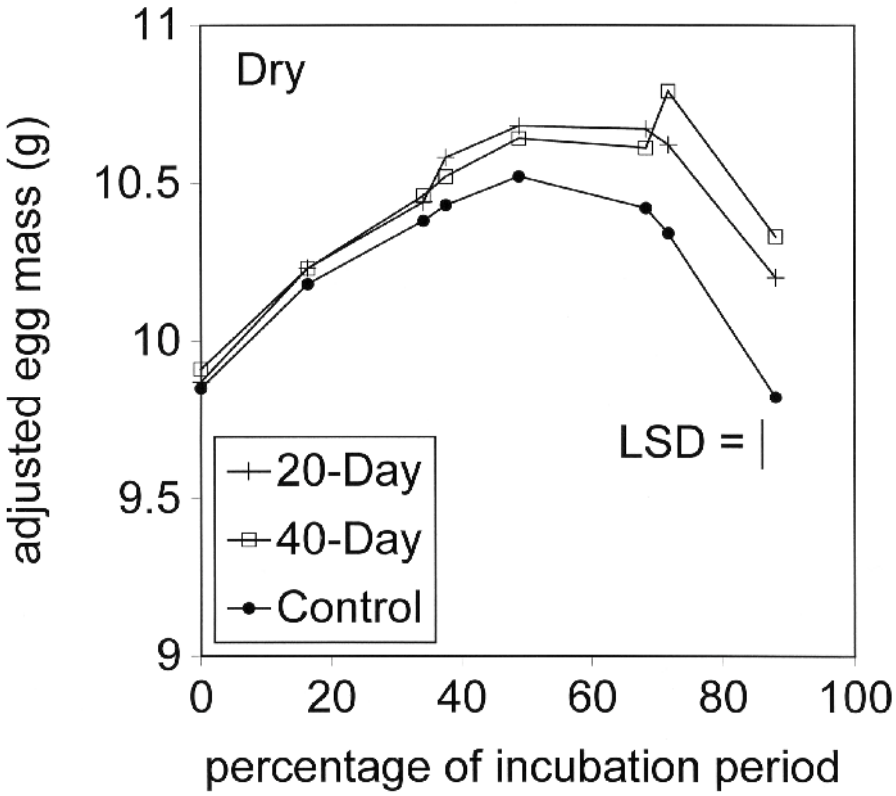

Figure 2. Egg mass through incubation for eggs of the red-eared slider (*Trachemys scripta elegans*) incubated on wet substrate and either moved to a drier one at 20 and 40 days incubation or kept on wet substrate throughout incubation (control). Least significance difference (LSD) is shown as a vertical line.