Experimental assessment of winter conditions on turtle nesting behaviour

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Methods: We performed a 25-year observational study of painted turtle nesting, and explored whether the timing of nesting (phenology) was related to ice-off dates recorded in the region. With a complementary experiment, we exposed female painted turtles to conditions simulating different ice-off dates and monitored nesting phenology, nest-site choice, and nest depth.

Results and conclusions: Our study identified a significant, positive correlation between ice-off dates and nesting phenology of turtles, with turtles initiating nesting later in years where winter conditions persisted for longer. Contrary to expectations, however, turtles did not differ in nesting behaviours between the ‘early’ and ‘late’ ice-off treatments in the experiment. Thus, variation in these traits in the wild likely is driven by other factors. These results highlight the complexity of understanding how animal behaviours will be altered as climate changes.

Keywords
hibernation, maternal effects, nest depth, nest-site choice, phenology

Disciplines
Animal Sciences | Behavior and Ethology | Ecology and Evolutionary Biology | Terrestrial and Aquatic Ecology

Comments

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Experimental assessment of winter conditions on turtle nesting behaviour

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ABSTRACT

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INTRODUCTION

Many organisms modify their reproductive behaviours, especially phenology, in response to various environmental cues (Post, 2003; Todd et al., 2011). For example, reproduction occurs relatively early during warm years in many organisms. However, global climate change has reduced the ability of some environmental factors to serve as reliable cues, which causes ecological mismatches (Saino et al., 2011). For example, climate warming is becoming more pronounced during winters (Grimm et al., 2013), which may cause some hibernating animals to initiate reproduction earlier than they normally would (Inouye et al., 2000).

Reptiles may be especially susceptible to changing climate (Urban et al., 2014). In particular, many reptiles have temperature-dependent sex determination (TSD), where nest temperature during incubation determines offspring sex. Nest temperature is primarily a function of ambient environmental conditions and, consequently, directional shifts in climate could ultimately yield skewed population sex ratios (Janzen, 1994). Several maternal behaviours (e.g. phenology, nest-site choice, nest depth) influence nest temperatures, and changes in these traits are correlated with climate as well (Schwanz and Janzen, 2008; Telemeco et al., 2009; McGaugh et al., 2010; Refsnider et al., 2013). Understanding how these maternal behaviours respond to changing climate is essential to predict population dynamics under future climate scenarios.

The painted turtle (Chrysemys picta) is an excellent organism for examining plasticity in nesting behaviour for several reasons. Painted turtles have TSD, and nest temperature is influenced by some maternal nesting behaviours. Long-term research on one population has demonstrated that after colder winters, turtles nest later in spring and in somewhat sunnier microhabitats (Schwanz and Janzen, 2008; McGaugh et al., 2010). These studies included examining the relationship between air temperature and phenology, but because adult painted turtles hibernate in water, air temperature is unlikely to have a direct influence on phenology. We hypothesize this correlation arises because air temperature influences ice duration. Ice-on is the formation of ice on a water body, while ice-off is the eventual thawing and disappearance of that ice (Livingstone et al., 2010), and together these times mark the ice duration of a body of water. While a water body is completely covered in ice, aquatically hibernating turtles are unable to breathe air, bask, or forage, and water temperatures tend to be fairly stable under the ice. For these reasons, we suggest that ice cover may be a biologically important variable linked to turtle reproduction, and we chose to further explore the role of ice cover on the nesting behaviours of turtles.

The aims of this project were to identify the influence of ice-off date on nesting behaviours of painted turtles through a field study and complementary experiment. To establish an environmental connection between winter thermal conditions and nesting phenology, we examined the relationship between ice-off date and onset of the nesting season in a wild population of painted turtles over 25 years. In the complementary experiment, we released turtles housed in common lab hibernation conditions into a fenced pond on different dates to simulate variation in ice-off. We predicted that simulating an earlier spring would result in earlier phenology, and shifts towards shadier and/or deeper nests (McGaugh et al., 2010; Refsnider et al., 2013).
METHODS

Study organism

Painted turtles occupy wetlands, lakes, and slow-moving rivers across much of the United States and southern Canada (Ernst and Lovich, 2009). Adult females initiate vitellogenesis during the fall, but yolk is not deposited during the winter months (Callard et al., 1978). Adult painted turtles hibernate aquatically, and across much of their range most aquatic habitats freeze over during winter, remaining covered in ice for months (Ernst and Lovich, 2009). Water temperatures under ice are fairly stable, and field studies have suggested that adult painted turtles experience temperatures between around 3–6°C, and often anoxic conditions while under the ice (Taylor and Nol, 1989; Crawford, 1991). After the ice melts, turtles begin breathing air, feeding on vegetation and basking. They continue vitellogenesis until it is complete, at which point turtles can oviposit (Callard et al., 1978). Subsequently, painted turtles nest in early summer (late May to early July). Consequently, the duration of ice cover on the aquatic habitats of turtles is biologically important because ice limits their ability to feed and to thermoregulate by basking. Many factors influence ice formation and ice melt on water bodies (e.g. wind, snowfall, solar radiation, water flow), but temperature is strongly correlated with ice duration (i.e. the duration of ice tends to be longer in colder winters) (Livingstone et al., 2010).

Field study

Our study population of painted turtles is located in the backwaters of the Mississippi River at the Thomson Causeway Recreation Area (TCRA) in Thomson, Illinois. We leveraged a 25-year painted turtle nesting phenology dataset to assess whether ice-off dates and nesting phenology are correlated. Nesting phenology has been carefully observed at the TCRA since 1989 by systematic visual surveys that begin prior to the nesting season, such that first nesting date is reliably observed (Schwanz and Janzen, 2008). Reliable ice-off dates are not recorded at our field site, but consistently defined ice-on and ice-off dates have been recorded from Lake Mendota in Madison, Wisconsin (~140 km northeast of the TCRA). The Wisconsin State Climatology office visually inspects lakes, and defines ice-off consistently with oral tradition, which allows a dataset of ice-on and ice-off dates dating back to 1852 (Wisconsin State Climatology Office: http://www.aos.wisc.edu/~sco/lakes/msnicesum.html).

Substantial spatial autocorrelation in meteorological factors is well known, and so we assumed the ice-off dates for Lake Mendota approximated those at the TCRA. To explore our expectation that winter air temperature influences turtles by affecting ice duration, we regressed first nesting dates for painted turtles at the TCRA over a 25-year period (1989–2013) on spring ice-off dates for Lake Mendota. We tested for temporal autocorrelation visually with a correlogram and statistically using a Durbin-Watson test for time lags of 1 to 7 years.

Experimental methods

We also performed an experiment to investigate the influence of ice-off dates on nesting behaviour. From 8 to 23 June 2010, we captured 71 adult turtles at the TCRA either by hand
or with aquatic hoop and fyke traps. We marked these turtles and measured left and right hind limb length. Within a week of capture, we transported turtles to an outdoor experimental pond (14.5 × 19 × 1.2 m) enclosed by a fence at the Horticultural Research Station (HRS) at Iowa State University. Turtles had access to floating basking objects and also basked on the pond shore, and had ad libitum access to natural food in the pond (invertebrates, anurans, vegetation). We supplemented this diet with Mazuri aquatic turtle food twice a week. The pond area was enclosed by a silt fence (25 × 55 m) that was buried ∼12 cm below the surface of the ground and held upright by steel T-posts. This arrangement allowed the turtles to have considerable terrestrial habitat outside of the pond that was suitable for nesting while preventing escape. To provide additional shade cover in the nesting area, ten evenly distributed artificial structures were erected using 1 × 2 m shade cloths affixed horizontally to steel T-posts, 1 m above the ground. Natural vegetation around the pond also provided some shade. These turtles were used for a different experiment from 2010 through the fall of 2011. They lived outdoors at the HRS during the summer of 2010, were hibernated in the laboratory from late October 2010 through early April 2011, and then returned to the pond in the summer of 2011.

On 17 November 2011, we drained the pond, captured 41 females and 10 males (which represents a subset of the turtles used in the 2010–2011 experiment), and transported them to the laboratory for overwintering. This date was selected for logistical reasons; it was as late as possible while reducing the risk of the pond freezing naturally and obstructing our ability to retrieve turtles. At this point, painted turtles have ceased feeding (Callard et al., 1978) and in some populations are already encountering ice-on. Since our research question focused on female reproductive behaviours, we minimized the number of males in the project but included some males to enhance the chances of female reproduction. We randomly distributed turtles among 53-litre plastic tubs filled with tap water, placing 4–5 females and one male in each tub. We stored tubs in a walk-in cold room maintained at 4°C and with a photoperiod that mimicked the natural light cycle at the HRS. This temperature is within the range of temperatures measured from hibernating turtles in the field (Taylor and Nol, 1989).

On 25 March 2012, we removed 20 females and 5 males from the cold room, painted a number on the carapace of each turtle with nail polish to allow identification from a distance, and released them into the pond. These turtles comprised the ‘early’ treatment, which simulated a winter with early ice-off (this release marks the first opportunity for turtles to bask, feed, and thermoregulate). We similarly marked and released the other 21 females and 5 males on 13 April 2012. This ‘late’ treatment simulated a year with late ice-off. These dates are within the range of observed ice-off dates at Lake Mendota, and therefore simulate environmentally relevant ice-off dates experienced in the wild. Thus, turtles in the early treatment had an additional 19 days to feed and bask than those in the late treatment. For this period, we obtained data from the National Climate Data Center for a weather station (Ames 8 WSW, IA, USA) near the HRS. During the first 11 days, air temperatures were warm enough for substantial turtle activity and basking; this period was warmer than the long-term daily average at the HRS. However, a cold front saw air temperatures drop for the final 8 days of the experiment, with minimum temperatures falling to 0°C or lower on all but one of the final 5 days, which was below the long-term average (Fig. 1). Throughout the spring, turtles had access to natural food and also received turtle food.

From mid-May through late June, we regularly monitored the area for nesting turtles. When we encountered a nesting turtle, we visually identified it from the number on its carapace,
and allowed it to complete nesting. We recorded the date the nest was constructed (oviposition date) and nest depth. We also used hemispherical photography and Gap Light Analysis software to quantify canopy cover (% canopy open) and incident radiation (i.e. transmitted total radiation) received at the nest (Doody et al., 2006). Furthermore, we took hemispherical photographs in a 2×2 m grid across the entire available nesting area to obtain background canopy cover and incident radiation measurements. In the first week of July 2012, we drained the ponds and released the turtles back to the TCRA.

Statistical analyses

We documented 42 total nests in the experiment from 41 females, yet were able to positively identify the mother for only 25 of those nests (early \( n = 13 \), late \( n = 12 \)). Thus, we only analysed data for these 25 nests. We performed an analysis of covariance (ANCOVA) to explore whether treatment (early or late) influenced phenology, nest-site choice, or nest depth with mean hind limb length as a covariate. We used the Akaike Information Criterion (AIC) to determine whether to include hind limb length as a covariate, and it improved model fit in all cases, except with transmitted total radiation where it was equivocal. To assess whether nest sites differed from the available habitat with respect to canopy cover and transmitted radiation, we used an ANOVA to compare maternally selected nest sites (pooled across treatments) to the entire available nesting area (measured from our grid).
We were unable to ascertain maternal identity for 17 nests. Because some turtles in our experiment may have nested twice and others not at all, the first clutch for some females may have been missed resulting in inaccurate assignment of the first nesting date for some individuals. To account for this possibility, we performed a simulation in R v.3.2.3 (R Development Core Team 2015), which randomly assigned nests of unknown origin to a plausible mother, given information on her nesting history and biological constraints. Specifically, we generated a pool of plausible mothers from which a random mother was assigned to each unknown nest. That pool changed for each nest, after specifying an inter-clutch interval of 16 days (Bowden et al., 2004). For example, a particular mother was removed from the pool for any unknown nests laid 16 days before or after a date on which she was known to have nested (or assigned to an unknown nest). This simulation approach generated a complete simulated dataset that included a randomly assigned mother from a pool of plausible mothers for each of the 17 unknown nests, along with the 25 known nesting events. We ran this simulation through 999 permutations. From each simulated dataset, we calculated treatment means and obtained the difference between the two values by subtracting the late value from the early value. We then compared the results to those from our original analysis that excluded the missing data. This simulation allows us to verify that the values from original analyses fell within the range of plausible outcomes.

RESULTS

Field study

The first nesting date of painted turtles from the 25-year observational field study at the TCRA was positively correlated with ice-off date at Lake Mendota ($r^2 = 0.32$, $P = 0.0031$, $N = 25$; first nest date $= 120.32 + 0.33 \times$ ice-off date). In other words, later ice-off dates corresponded to later initiation of the nesting season (Fig. 2). Our data were not temporally autocorrelated (Durbin-Watson value range 1.50–2.13; all $P$-values $> 0.14$).

Nesting

In analyses that excluded the missing maternal data, treatments did not differ in oviposition date (phenology), percentage canopy openness, transmitted total radiation, or nest depth (Table 1). Turtles nested randomly with respect to the available habitat, as canopy openness and transmitted radiation did not differ between maternally selected sites and available sites (canopy openness: $F_{1,168} = 0.82$, $P = 0.36$; incident radiation, $F_{1,168} = 0.16$, $P = 0.68$). Additionally, the first nest in the experiment at the HRS was observed on 18 May 2012, only 3 days after the onset of nesting at the TCRA that same year.

Comparison with simulated data

The simulated datasets produced a distribution of plausible outcomes. The actual difference between the early and late treatment (obtained from Table 1) fell within the interquartile range of the difference between the early and late treatment for the simulated datasets (Table 2). Thus, results from our analyses that excluded the missing data were not outliers given the range of plausible nesting scenarios from the simulated dataset. This outcome suggests the missing data did not bias our results.
DISCUSSION

The opening of the ice is likely a significant event for many aquatic organisms because it substantially expands their opportunity to feed, thermoregulate, and access atmospheric oxygen. We identified a correlation between the onset of the nesting season in a wild population of painted turtles and ice-off dates at a nearby lake. However, by experimentally quantifying the influence of simulated ice-off dates on the subsequent nesting behaviour

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**Table 1.** Comparison of nesting behaviours between *C. picta* turtles from the early and late spring treatments (mean ± SD)

<table>
<thead>
<tr>
<th>Trait</th>
<th>Early (n = 13)</th>
<th>Late (n = 12)</th>
<th>Covariate</th>
<th>Statistic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oviposition date (Julian date)</td>
<td>159 ± 10</td>
<td>160 ± 9</td>
<td></td>
<td>$F_{1,21} = 2.75$, $P = 0.11$</td>
</tr>
<tr>
<td>Canopy openness (%)</td>
<td>78.5 ± 10.5</td>
<td>74.7 ± 13.8</td>
<td></td>
<td>$F_{1,21} = 1.01$, $P = 0.32$</td>
</tr>
<tr>
<td>Incident radiation (MJ·m⁻²·day⁻¹)</td>
<td>9.15 ± 0.84</td>
<td>8.60 ± 1.55</td>
<td></td>
<td>$F_{1,21} = 0.76$, $P = 0.39$</td>
</tr>
<tr>
<td>Nest depth (cm)</td>
<td>8.4 ± 0.6</td>
<td>8.4 ± 0.6</td>
<td></td>
<td>$F_{1,21} = 1.26$, $P = 0.27$</td>
</tr>
</tbody>
</table>

*Note:* Hind limb length was used as a covariate for nest depth.
of painted turtles, we found no evidence that ice-off date influences the key behaviours of nesting phenology, nest-site choice, or nest depth.

We predicted that turtles in the early treatment would nest earlier and in shadier and/or deeper sites, yet we observed no treatment differences. The lack of any significant differences in phenology between early and late wintering treatments has several potential biological explanations, which are not mutually exclusive. First, turtles from the early treatment were released in the pond 19 days before those in the late treatment. Air temperature fell substantially over days 11–19 (Fig. 1), which may have minimized any advance in reproductive physiology gained had the weather remained warm. Second, the treatment differences may have been insufficient to induce a substantial biological difference for the turtles. Increasing the difference in time between our treatments would have still been ecologically relevant (Lake Mendota has a range of 65 days in ice-off date over the past 150 years), and may have elicited a pronounced behavioural effect. A third possibility is that the weather immediately after ice-off is less important for phenology relative to the weather later in spring. In our experiment, all turtles experienced a common environment from 13 April onwards, a period when the majority of basking and nutrient acquisition likely occurs. Future work should focus on improving our understanding of the relationship between egg development, temperature, and nutrient acquisition in turtles, which would clarify our understanding of proximate factors influencing phenology.

Given no detectable differences in phenology between treatments in the experiment, the lack of differences in the other two behaviours (nest-site choice and nest depth) is not surprising. Whereas phenology exhibits substantial sensitivity to seasonal weather in wild painted turtles, nest-site choice and nest depth show weaker trends (McGaugh et al., 2010; Refsnider et al., 2013). Although the overall area of our nesting enclosure was smaller than what a wild turtle would have to select from, the available habitat had substantial variation in canopy cover. Nonetheless, nest-site choice did not differ from the available habitat with respect to canopy openness and transmitted total radiation. This result suggests the available habitat was generally suitable for nesting, since turtles typically exhibit fine-tuned nest-site choice (Mitchell et al., 2013).

The results of our experiment do not exclude the possibility that ice duration during winter is important to nesting behaviours, but certainly suggest that winter temperatures may have less of a direct effect on phenology than previously thought (Schwanz and Janzen, 2008). Given that longitudinal studies demonstrate that phenology is sensitive to weather, our experiment instead suggests that conditions during more defined time periods may be more important to nesting turtles. This assertion could be assessed with additional experiments,

<table>
<thead>
<tr>
<th>Trait</th>
<th>IQR of simulated differences</th>
<th>Actual difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oviposition date (Julian date)</td>
<td>0.81, −1.71</td>
<td>−1</td>
</tr>
<tr>
<td>Canopy openness (%)</td>
<td>0.03, 4.52</td>
<td>3.8</td>
</tr>
<tr>
<td>Incident radiation (MJ·m⁻²·day⁻¹)</td>
<td>0.07, 0.60</td>
<td>0.55</td>
</tr>
<tr>
<td>Nest depth (cm)</td>
<td>−0.30, 0.06</td>
<td>0</td>
</tr>
</tbody>
</table>

*Note: The actual differences calculated from the original analysis (early, late from values in Table 1) are included for comparison, and fall within the interquartile range of the simulations.*
detailed analyses of longitudinal datasets, and/or the development of models that link reproductive physiology with nutrition and temperature. Many inter-correlated variables likely contribute to variation in nesting phenology and other maternal behaviours, and experimental approaches like the one described here will provide excellent insight into which factors are most critical. Information of this sort will help inform predictions of how organisms might respond to environmental change.

ACKNOWLEDGEMENTS

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REFERENCES


