
Enikő Horváth¹, Peter Havaš², Stanislav Danko², Martin Bona³, Milan Novotný², Adriana Burešová², Peter Kaňuch¹,⁴ & Marcel Uhrin¹*

¹Department of Zoology, Faculty of Science, P. J. Šafárik University in Košice, Moyzesova 11, 040 01 Košice, Slovakia; E-mail: t.eni432@gmail.com, marcel.uhrin@gmail.com
²Fauna Carpatica, Maďarská 5, 040 13 Košice, Slovakia; E-mail: peter.havas@seznam.cz
³Department of Anatomy, Faculty of Medicine, P. J. Šafárik University in Košice, Moyzesova 11, 040 01 Košice, Slovakia; E-mail: martinbonask@gmail.com
⁴Institute of Forest Ecology, Slovak Academy of Sciences, L. Štúra 2, 960 53 Zvolen, Slovakia; E-mail: kanuch@netopiere.sk

Abstract: Environmental conditions strongly influence the activity patterns of reptiles having behavioural thermoregulation. This study examined the influence of two weather characteristics (average daily air temperature and daily sunshine duration) on the onset of the nesting period of *Emys orbicularis* in the conditions of the Tajba National Nature Reserve in eastern Slovakia. Using data collected over 18 years, we found that the period of almost two weeks before the first egg-laying date is the most critical in the progression of the onset of nesting. During these days, the mean daily temperature was ca. 16°C and the mean sunshine duration was eight hours. Inter-annual deviations from the mean values in both daily weather measures caused significant shifts in the first egg-laying date. Intrauterine embryonic development that undergoes in turtle females may explain the observed pattern in their thermoregulatory behaviour during this vulnerable period. The obtained knowledge is necessary for launching subsequent conservation actions for this endangered species.

Key words: European Pond Turtle, egg-laying, climate, thermal ecology, temperature, sunshine, Pannonian Plain

Introduction

Heliothermic ectotherms are temperature-dependent animals and thus rely on environmental sources to gain heat for almost all of their life processes. They need to concentrate their daily activities within a range of temperatures that optimizes their physiological and behavioural functions. Body temperature influences many physiological processes in such ectotherms and determines their fitness (Angilletta et al. 2002). Among them, heliothermic reptiles in particular exhibit various thermoregulatory behaviours to regulate their body temperature by taking advantage of sunshine (Vitt & Caldwell 2014). For freshwater turtles occurring in temperate climates, such as the European Pond Turtle *Emys orbicularis* (Linnaeus, 1758), sun-basking is used to regulate their body temperature within the optimal range (Bulté & Blouin-Demers 2010, Vitt & Caldwell 2014). For example, optimal body...
temperature is essential for earlier stages of embryogenesis (follicular development, ovum production) in reproductively active freshwater female turtles (Shine 2005, Carrière et al. 2008). Furthermore, weather conditions also play a significant role in the nesting behaviour of these species, and the timing of breeding could be critical for reproductive success of the population (Bowen et al. 2005, Shine 2005).

Exposure of fertilized female freshwater turtles to adequate environmental temperatures and insolation rates are necessary for their successful reproduction (Mitrus et al. 2012). To date, however, there is little knowledge about the link between weather conditions and the time of nesting, as most studies have focused on embryonic development, incubation success and temperature-dependent sex determination only (Pieau & Dorizzi 1981, Pieau et al. 1998, Schneeweiss et al. 1998, Girondot 1999, Schneeweiss 2004). *Emys orbicularis* is a widely distributed freshwater turtle occurring from north-western Africa through the Iberian Peninsula, inhabiting much of the European continent and extending into western Asia (Fritz 2001, 2003, Rogner 2009). The nesting behaviour of this species is well documented from several countries of its distribution, including data about the beginning of the nesting period. Throughout the European part of the distribution area, the onset of the nesting period varies in a fairly broad range (from the end of April to the final third of June) (Fritz 2001, 2003, Kuzmin 2002). In Europe, the species is often in regression or locally extinct, and in Slovakia, as the only autochthonous turtle species, it is classified as critically endangered (Kminiak 1992, Fritz & Chiari 2013, Urban & Kautman 2014). Nowadays, its occurrence is limited to only two isolated regions in Slovakia, i.e. the Východoslovenská Nížina Lowland (with a long-standing population in Tajba) and the Danubian Lowlands (Jablonski et al. 2015). The main factors contributing to the decline of the species are habitat disturbance (fluctuation of the water level, loss of nesting sites), nest predation, destruction of nests by agricultural activities and mortality of females and juveniles during migration to nesting sites or water, respectively. Thus, the most important actions for conservation of this species is to preserve their habitat and maintain reproduction undisturbed (Havaš & Danko 2009). Continuous enrichment of the local population with young individuals is necessary for its survival. However, in the conditions of the Tajba National Nature Reserve (Tajba NNR) in eastern Slovakia most of the nests are destroyed by predators (mainly by Red Foxes and Badgers) on the same day or a day after oviposition (Havaš & Danko 2009, Fritz & Chiari 2013). Therefore, for successful protection of the nests and eggs it is thus necessary to know the location of clutches but also the exact timing of egg-laying.

In order to understand how actual weather conditions may shift the expected onset of nesting in *E. orbicularis*, we analysed the available long-term data (18 years) on the nesting activity of turtles in the Tajba NNR. It could be hypothesised that prolonged hibernation or cold weather at the beginning of season will delay nesting (Kminiak 1992, Drobenkov 2012) while, in contrast, warmer spring temperatures will accelerate this behaviour (Meeske 1997). Hence, we attempted to quantify ambient energy input that is necessary during the spring period to induce the start of nesting activity. The values of two weather characteristics – average air temperature and duration of sunny hours – were used as a proxy for energy input that could be obtained from the environment, and they were employed to predict the onset of nesting in an isolated but persisting population.

**Materials and Methods**

**Study area**

The study was conducted in the Tajba NNR in south-eastern Slovakia, which is located in the central part of the *E. orbicularis* range. The reserve is located in the Východoslovenská Nížina Lowland (part of the Pannonian Plain), one kilometer northeast of the village Streda nad Bodrogom at an altitude of about 100 m (N 48°23”, E 21°47”). Besides the marsh (a 2.5 km long and 100–150 m wide former oxbow of the Bodrog River), the Tajba NNR also includes 100 m of surrounding riparian zone with a total area of 27.4 ha. The study area is characterized by four habitat types: (1) marsh densely covered by vegetation; (2) slopes of the Roháč hill covered by several tree species; (3) sandy slopes with xerophilous flora south of the marsh and (4) fields north of a water body used for agriculture (Fig. 1; see Novotný et al. 2004 for details). *Emys orbicularis* in the Tajba NNR is the only known long-term reproducing population in Slovakia, with estimated abundance of ca. 80 individuals (authors’ unpublished data).

**Data collection**

Data on nesting activity of *E. orbicularis* in the study area were collected at four nesting sites, mostly in sandy slopes (Fig. 1), during the 18-year period 1999–2016. For analysis of the timing of first egg-laying, the start of the nesting activity in each season (Table 1) was defined alternatively as (1) date of the
The Effect of Two Weather Parameters on the Timing of Nesting in a Critically Endangered Population...

first observation of egg-laying female turtles at the nesting site, (2) the first observation of migrating female turtles at the nesting site, and/or (3) the first observation of destroyed nests at the nesting site. In case of the observation of destroyed nests, the date of the start of the nesting period was set one day earlier than the exact finding. To determine the beginning of nesting activity, regular daily visual observations were taken in the area that covered the expected time of nesting (from mid-May onwards) at known nesting sites (app. 40 ha). To avoid disturbing the females during the egg-laying and migration, observations were made between 20:00–22:00 h and to detect potentially destroyed nests, nesting sites were visited between 08:00–10:00 h (UTC +2 h). Climate data were provided by the Slovak Hydrometeorological Institute (Košice) from the regional weather station in Milhostov, which is ca. 40 km distant from the study area. This weather station and the study area occur in the same climatic subregion of Slovakia (Lapin et al. 2002).

**Statistical analysis**

Two weather characteristics, daily measures of the average temperature and the length of sunshine, were used to describe the local climatic conditions and to test for plastic responses in the starting time of egg-laying by the turtles in the study area. In order to make a non-arbitrary choice of the appropriate climatic time window that may induce egg-laying activity, we searched for the period that provided the best correlation between the annual first egg-laying date and the two selected weather characteristics. We calculated Pearson’s correlation coefficients between the first egg-laying dates and both the average daily temperature and the length of sunshine, as determined using sliding time windows. The considered window size ranged from 1 to 23 days; thus 23 different time periods prior to the first egg-laying date in each year (n = 18 years). The used range of 23 days is consistent with the total variation in the first egg-laying date that was observed in the field within the whole study period (18 years; Table 1) whereas longer time windows were not considered as there was increased risk of artificial or biologically implausible correlations. The period for which the selected weather characteristics provided the highest correlation with the first egg-laying date was taken to represent the best description of the local climatic conditions that influence the behaviour of the turtles.

Analyzed data did not deviate from normality (Shapiro-Wilk normality test, p > 0.05), thus the first egg-laying date was modelled as a linear function of the year-specific climatic conditions in regression models using both weather characteristics as explanatory variables (due to the low sample size, we could not combine the two weather characteristics into a single model). This conservative approach with single explanatory variables was used also because selected variables were supposed to be collinear to each other as spring sunny days should have higher average temperature. Furthermore, using linear regression we also tested for a possible effect of the year on a continuous shift in the weather characteristics and egg-laying date. Data was analysed in R 3.3.0 environment for statistical computing (R Core Team 2016).

**Fig. 1.** A sketch of the study area showing the main habitat types, where four nesting sites (represented by turtle symbols) of the European Pond Turtle in the Tajba NNR (south-eastern Slovakia) occurred. Map source: OpenStreetMap.org
Results

During 18 years of observations (1999–2016), the mean date of the first egg-laying was 23 May (range: 15 May – 6 June). The earliest egg-laying was detected in 2002 (15 May), when a nesting female was observed. On the other hand, a considerably delayed start of the nesting activity was observed in 2006 (6 June), when a freshly destroyed nest was found (Table 1). The sliding windows approach showed that a time period of 13 days prior to the date of the first egg-laying provided the highest (negative) correlations between the first egg-laying date and both daily measures of the weather, the average temperature ($r = -0.63, P = 0.005$) and the length of sunshine ($r = -0.64, P = 0.004$; Fig. 2). During this period, the mean temperature was 15.7°C (range: 12.6–17.8°C) and the mean length of sunshine was 8.1 h (range: 3.4–11.2 h) per day. Climatic conditions did not change significantly over the years (average temperature, $P = 0.61$; length of sunshine, $P = 0.55$), and the same was also found in the mean first egg-laying date ($P = 0.83$). However, linear regression models revealed that the first egg-laying date significantly shifts as a plastic response to both the average daily temperature ($b = -1.96 \pm 0.61 \text{ day}^{-1} \cdot ^\circ\text{C}^{-1}, R^2 = 0.39, F_{1,16} = 10.4, P = 0.005$, Fig. 3a) and the length of sunshine ($b = -1.41 \pm 0.43 \text{ day} \cdot h^{-1}, R^2 = 0.41, F_{1,16} = 11.0, P = 0.004$, Fig. 3b) when the local climatic conditions of the last 13 days before egg-laying were evaluated.

Discussion

The presented study found significant effects of particular weather characteristics on the nesting behaviour of the European Pond Turtle. The energetic cost of the nesting process is very high for turtles; therefore, among others, acquisition of sufficient thermal energy for the nesting process is vital (Fossette et al. 2012). The basking behaviour of these heliothermic animals depends on solar radiation and provides the most important energy source for their activity (Di Trani & Zuffi 1997). We found a significant influence of the length of sunshine on the onset of the nesting period. A decrease or increase of sunshine duration per day of about one hour during the pre-nesting period induced the onset of nesting one and a half days later or earlier, respectively, comparing with the long-term average. A parallel effect was observed in the average daily temperature, as a difference of one-degree Celsius shifted nesting by approximately two days. Although the interaction effect of the explanatory variables was not tested, the former variable naturally has an impact on the second. Moreover, greater model estimates suggest that the temperature has the final determining influence on the nesting activity of European Pond Turtles.

Earlier nesting and compressing of the nesting season as behavioural responses to higher ambient temperature has already been observed in marine turtles, where sea surface temperatures were the determining factor (Weishampel et al. 2004, 2010, Pike et al. 2006). The temperature of the water column also affects the behaviour of freshwater turtles; it determines the beginning and the end of the hibernation period, which can be indirectly connected to the onset of the nesting season (Novotný et al. 2008). However, in freshwater turtles inhabiting temperate zones, detailed information about the influence of weather characteristics shortly before nesting is still insufficient. In populations of some North American species occurring under similar climatic conditions as those of the European Pond Turtle, the initiation of nesting activity was inversely correlated with ambient spring temperatures; warmer spring months lead to earlier nesting of Chelydra serpentina (Congdon et al. 1987, Obbard & Brooks 1987, Iverson et al. 1997), Chrysemys picta (Iverson & Smith 1993, Rowe et al. 2003), Emydoidea blandingii (Congdon et al. 2003), and Trionyx species (Ogada & Ducey 2012). The temperature of the water column also affects the behaviour of freshwater turtles; it determines the beginning and the end of the hibernation period, which can be indirectly connected to the onset of the nesting season (Novotný et al. 2008). However, in freshwater turtles inhabiting temperate zones, detailed information about the influence of weather characteristics shortly before nesting is still insufficient.

Table 1. The first egg-laying dates of the European Pond Turtle in the Tajba NNR in the period 1999–2016 and the type of evidence of nesting behaviour

<table>
<thead>
<tr>
<th>Year</th>
<th>Date</th>
<th>Evidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>1999</td>
<td>May 28</td>
<td>migrating female</td>
</tr>
<tr>
<td>2000</td>
<td>May 23</td>
<td>nesting female</td>
</tr>
<tr>
<td>2001</td>
<td>May 21</td>
<td>nesting female</td>
</tr>
<tr>
<td>2002</td>
<td>May 15</td>
<td>nesting female</td>
</tr>
<tr>
<td>2003</td>
<td>May 20</td>
<td>migrating female</td>
</tr>
<tr>
<td>2004</td>
<td>May 25</td>
<td>nesting female</td>
</tr>
<tr>
<td>2005</td>
<td>May 28</td>
<td>nesting female</td>
</tr>
<tr>
<td>2006</td>
<td>June 6</td>
<td>destroyed nest</td>
</tr>
<tr>
<td>2007</td>
<td>May 20</td>
<td>nesting female</td>
</tr>
<tr>
<td>2008</td>
<td>May 25</td>
<td>nesting female</td>
</tr>
<tr>
<td>2009</td>
<td>May 19</td>
<td>destroyed nest</td>
</tr>
<tr>
<td>2010</td>
<td>May 23</td>
<td>nesting female</td>
</tr>
<tr>
<td>2011</td>
<td>May 22</td>
<td>destroyed nest</td>
</tr>
<tr>
<td>2012</td>
<td>May 24</td>
<td>nesting female</td>
</tr>
<tr>
<td>2013</td>
<td>May 20</td>
<td>destroyed nest</td>
</tr>
<tr>
<td>2014</td>
<td>May 25</td>
<td>nesting female</td>
</tr>
<tr>
<td>2015</td>
<td>May 20</td>
<td>migrating female</td>
</tr>
<tr>
<td>2016</td>
<td>May 25</td>
<td>nesting female</td>
</tr>
</tbody>
</table>
et al. 1983) and *Graptemys ouachitensis* (Geller 2012). Furthermore, our results show that the period shortly before nesting is critical for the initiation of the nesting process. It also showed plastic and very sensitive responses in the starting time of egg-laying among freshwater turtles that live in a relatively cold climate. Such ability may thus ensure effective reproduction in harsh conditions at the range margins of the species (Sommer et al. 2007).

In the Tajba NNR, the first successful copulation was observed in mid-April or early May, but actual fertilization can occur later than the time of insemination; sperm can be stored from the time of mating until just prior to ovulation (Gist et al. 1990, Sarkar et al. 2003, Novotný et al. 2004, Roques et al. 2004). In general, turtles cease mating about two weeks prior to ovulation, which precedes nesting by about two weeks (Miller & Dinkelacker 2008). After fertilization, the embryos develop to middle gastrulation in the oviduct and then enter diapause until after oviposition (Ewert 1979, 1991). This intrauterine period is variable among species; the minimum period between ovulation and oviposition for marine turtles is about 14 days (Miller & Dinkelacker 2008). According to Ewert (1979), some freshwater turtle species of the Emydidae family also fit this general pattern. The duration of this pre-nesting development overlaps well with our results and thus may explain the pattern we observed. Considering such a reproductive strategy, we suggest that during the period of two weeks prior to the date of egg-laying, the embryos are undergoing sensitive developmental changes. This is probably the shortest period when ambient weather conditions can have the required effect on embryonic development.

Knowledge about quantified ambient energy...
input before the onset of the nesting period provides a starting point for further studies. These findings will enable the effects of climatic variables to be modelled and thus make it possible to predict the onset of the nesting season. Once in possession of such information we will be able to regulate the start of land-management work and coordinate the presence of field workers in the area for the effective protection of the nests.

Acknowledgements: We are thankful to the Slovak Hydro- and Aerological Institute Košice for providing the required climatic data used in this study. This study was funded by the Slovak Scientific Grant Agency VEGA (1/0077/17) and by the Internal Scientific Grant System of Faculty of Science of P. J. Šafárik University in Košice (#VVGS-PF-2017-282).

References


MITRUS S. & ZEMANEK M. 2004. Body size and survivorship of the


Schneeweiss N. 2004. Climatic impact on reproductive success of *Emys orbicularis* at the northwestern border of the species’ range (Germany). Biologia, Bratislava 59 (Suppl. 14): 131–137.


Accepted: 30.10.2017