Research Article

ACTA ZOOLOGICA BULGARICA
Acta zool. bulg., Suppl. 10, 2017: 43-47

Thermal Conditions in Nest Chambers of the European Pond Turtle, *Emys orbicularis* (L., 1758), in Tajba National Nature Reserve, Slovakia

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Abstract: The European Pond Turtle (*Emys orbicularis*) in eastern Slovakia lays eggs from the end of May to the last decade of June. Incubation of the eggs takes place in the nest chambers and the hatchlings mostly stay in the nests until next year's spring. For two seasons (2008/2009 and 2009/2010) we recorded ten nest temperatures in Tajba National Nature Reserve, south-east Slovakia, using automated data loggers. For clarity of presentation, according to literature data and the temperature profiles obtained, we divided the nesting period into four consecutive parts. During the first two and half to three months egg incubation takes place. The post-incubation period is characterized by the gradual reduction of the nest temperature down to 0°C. Then, the length of the overwintering period depends on the climatic conditions of particular year: 61 days in 2008 and 93 in 2009. The post-overwintering period ends when the hatchlings leave the nest. However, exceptions exist – for the first time in Slovakia we recorded turtles leaving during the post-incubation (two nests in 2009). The highest temperature recorded in a nest was 34.5°C (01 June 2009 in nest #1 (2009)), while the lowest temperature was -9°C (25 January 2010 in nest #8 (2009)). Long-term measurement of nest temperatures can provide information on the current status of hatchlings in the nest, depending on the outside temperature at the site of the nest. That can be useful in human-aided thermal protection of nests during extreme outdoor temperatures but also for determination of ideal places for egg laying or egg replacement. These results have practical implications such as providing necessary information when selecting optimal places for (re)introduction of *E. orbicularis* and for recovery of nest places and reproduction management of existing populations.

Key words: incubation, overwintering, data logger, hatchlings

Introduction

The European Pond Turtle *Emys orbicularis* (L., 1758) lays eggs in a pear-shaped nest dug in sandy soils. After laying the eggs the female closes off the hole, creating the nest chamber where the "nest period" takes place. This period comprises incubation and, depending on the locality, overwintering of hatchlings; therefore, it can vary from approximately five to eleven months. Depending on the climatic conditions at the individual location the hatchlings leave the nest either in their first autumn (Rovero & Chelazzi 1996, Tertyšnikov & Gorowaja 1984) or in the next spring (Servan 1983, Duguy & Baron 1998).

The European Pond Turtle is one of the few native freshwater turtles that live in Europe and the only native turtle species in Slovakia. At many locations in Europe, the occurrence of this turtle is very rare, and in many places it is a highly endangered species. Only one population of this species reproduces in Slovakia, located in a pond within the Tajba National Nature Reserve (NNR) (Novotný et al. 2004). It is also the only reptile classified by the Slovak Red List

as "Critically endangered" (Kautman et al. 2001).

Many factors affect nest success, such as predation, human activities, soil humidity, and climatic conditions. The main climatic factor is undoubtedly environmental temperatures, including soil temperature, which affect the duration of incubation (Kminiak 1992) and survival rate during overwintering in nest (Schneeweiss et al. 1998).

To establish the thermal conditions in the nest chambers during the nest period at this single locality in Slovakia, we used automated temperature data loggers to record temperature data inside a total of ten nests in the seasons 2008/2009 and 2009/2010. Using these data, we evaluated the impact of thermal conditions on eggs and hatchlings and survival during the whole nest period. The results will be useful in the management of *E. orbicularis* in general, and particularly for more effective protection of the pond turtles of the Tajba NNR.

Materials and Methods

The study was conducted in the Tajba National Nature Reserve (N48˚23'11.58" E21°46'27.89"), situated in south-eastern Slovakia, ca. 1 km northeast of the town of Streda nad Bodrogom. The protected area covers 27.36 ha and includes Tajba pond (2 km long and 100–150 m wide, oxbow shaped) and a 100 m wide buffering area.

In the nest seasons 2008/2009 and 2009/2010, we measured the temperature in a total of ten nest chambers of the European Pond Turtles living at this locality in 3-h and 4-h, respectively, intervals. We used miniature automated data loggers (DS1921G Thermochron iButton Device, Maxim Integrated, USA; dimensions 1.7 × 0.6 cm, measurement range -40 to +85°C, accuracy ±1°C, resolution 0.5°C, up to 2048 samples). All the nests we have chosen for the experiment, were found during egg laying period and, depending on the circumstances, data loggers were placed either directly in the nest chamber during the egg laying, or in the immediate vicinity of the nest (ca. 25 cm) afterward. To evaluate potential differences caused by various placements of data loggers, we compared the data using t-test (in the nest / immediate vicinity) and we did not find any significant variations in measurements. All the nests were situated on the meadows in sandy soil with practically no canopy over them and only sparse grassland vegetation around. All nests were regularly checked for any visible changes as required by the nature of the experiment. Each nest was open at the end of nest period (next summer) for evaluation the nesting success according the condition of eggs, embryos in eggs, hatchlings or eggshells. In the season 2008/2009 we measured temperature in six nests focused on post-incubation, hibernation and post-hibernation period with sampling rate every 3 hours. Season 2009/2010 included measurements during incubation period with a sampling rate every 4 hours; we measured temperature in 4 nests.

For clarity of presentation, according to literature data and the temperature profiles (Fig. 1) we divided the nest period into four consecutive parts: incubation, post-incubation, overwintering, and post-overwintering period (Fig. 1). The length of incubation period was established at 75 to 90 days after egg laying based on data for the same locality (Novotný et al. 2004). The post-incubation period is conservatively starting from the ninetieth day and ending on

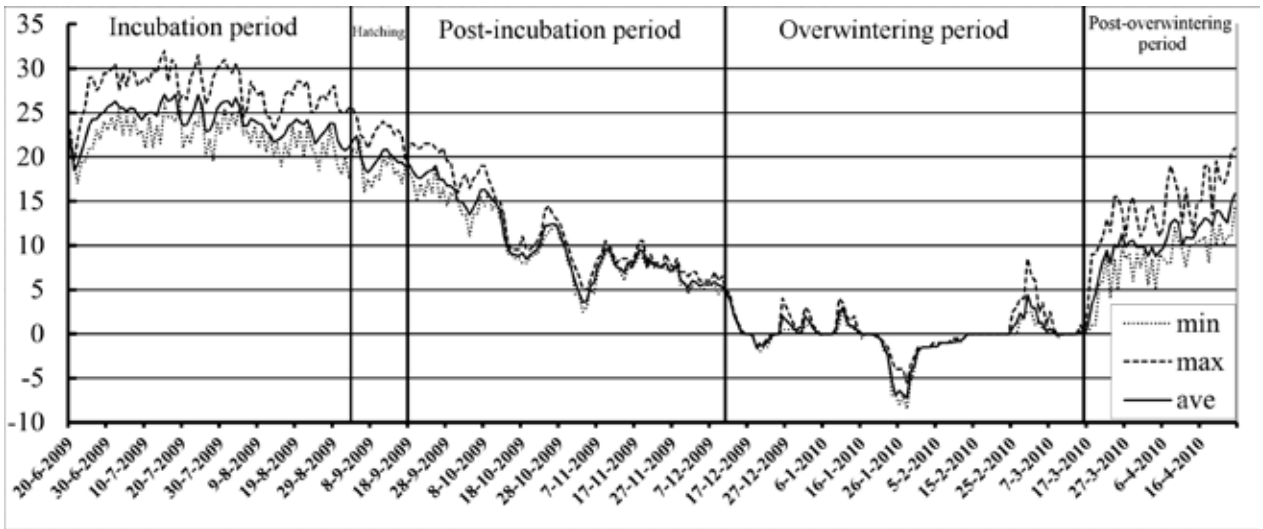

Fig. 1. Thermal graph divided into nesting periods, showing maximum, minimum and average daily temperatures within ten nests of *Emys orbicularis* in Slovakia

Table 1. Temperatures (°C) of monitored nests during the incubation period in 2009. Max. = maximum temperature, Min. = minimum temperature, Ave. = average temperatures, SD = standard deviation

Nest #	Max.	Min.	Ave.	SD
#1 (2009)	34.5	13.0	22.81	4.35
#2 (2009)	32.5	13.5	23.70	3.67
#7 (2009)	29.5	15.0	21.53	2.93
#8 (2009)	32.0	16.0	23.43	3.22

the first date on which 0°C was measured inside the nest. The overwintering period then is defined as the days between the first and the last recorded 0°C. It is followed by the post-overwintering period, which, along with the whole nest period, ends when the hatchlings leave the nest.

Results

For the first time we established temperatures inside nesting chambers of free-ranging *E. orbicularis* in Slovakia. Below we present detailed results for each separate period and the overall survival success of all monitored nests.

Incubation period

This period is characterized by a slowly decreasing temperature with large daily dispersion (Fig. 1). During incubation, average temperatures per nest ranged from 21.5 to 23.7°C (Table 1). The average incubation temperature of all nests was 22.87 ± 0.83°C (4 nests in 2009, 540 measurements each). The highest overall temperatures in nests were measured in July, while the lowest in September (Fig. 2). Nevertheless, the lowest absolute temperature (13°C) was recorded on 01 July in nest #1 (2009) and the highest absolute temperature (34.5°C) was recorded in same nest on 01 June. Based on visual observations we found that nest #2 (2009) was destroyed by a predator. After evaluation of the data obtained by the logger, we concluded that it was on 21 August between 08:00 and 12:00 h.

Post-incubation period

A relatively strong decrease in temperature is typical for this period. In the beginning, daily temperatures fluctuate widely, similar to the incubation period, but as time progresses the fluctuation is greatly reduced (Fig. 1). Such rapid reduction in fluctuation was observed on 18 November 2008 and 09 October 2009. In 2008 Post-incubation period lasted 112.7 ± 11.2 days (n = 6); in 2009 – 114 days for nest #1 (2009) and 90 days for nest #8 (2009). The average temperature in 2008 was 8.26 ± 0.26°C, while in 2009 it was 12.23°C for nest #1 (2009) and 10.28°C for nest #8 (2009) (Table 2).

In 2009, we observed hatchlings leaving the nest during this period for the first time in Slovakia. We first observed an opening of nest #7 (2009) on 08 October; on 06 November, after we opened the nest and examined the contents, we estimated based on the empty eggshells three hatchlings had left the nest. Another 13 hatchlings were still in eggs, but 9 of them were already dead. The mean temperature in nests in October 2009 was 11.3 ± 2.22°C. In addition, an estimated twelve hatchlings left a nest #4 (2009) on 05 October. However, this nest was not equipped with a data logger and is not included in the other results.

Overwintering period

We measured a relatively constant temperature with low daily fluctuation (Fig. 1). In 2008, the period's mean length was 65.83 ± 6.19 days, while in 2009 it was 93 days in both nests. As well as the length of this period, the temperatures vary from year to year. In the season 2008, the mean temperature of nests was 0.33 ± 0.21°C (from -4°C to 9°C) while in the season 2009 it was considerably colder (0.03 ± 1.74°C and -0.3 ± 2.06°C). The lowest temperature measured in 2009 was -9°C.

Post-overwintering period

We recorded a sharp temperature rise with a wide variation between the maximum and minimum daily temperatures, similar to the incubation period. Because the precise day when the hatchlings leave the nest is very hard to observe and it is also impossible to estimate it based on the measured temperatures, we managed to define this only for one nest (#6 (2008)), with hatchlings leaving it on 14 April 2009. In this case the post-overwintering period lasted for 47 days, with an average temperature of 6.96 ± 4.3°C.

Survival successes

All monitored nests, except #2 (2009) and #7 (2009) (see above), remained intact until the hatchlings left the nest. After evaluating the nesting success based on the condition of eggs, embryos in eggs, hatchlings or eggshells post-hatching we found out that eggs in two nests have not been properly incubated and one nest has been lost due to forestry activities in spring. The remaining five nests produced 7.4 hatchlings in average, with overall success rate of 60% (Table 4).

Table 2. Duration (Days) and Temperatures (°C) of monitored nests during the post-incubation period during the seasons 2008/2009 and 2009/2010. Max. = maximum temperature, Min. = minimum temperature, Ave. = average temperatures, SD = standard deviation

Nest #	Duration	Max.	Min.	Ave.	SD
#2 (2008)	111.0	17.5	0.5	8.60	4.62
#3 (2008)	125.0	18.5	0.5	8.13	4.77
#5 (2008)	120.0	17.0	0.5	8.06	4.76
#6 (2008)	121.0	17.5	0.5	8.29	4.74
#8 (2008)	101.0	17.0	0.5	7.95	4.43
#10 (2008)	98.0	21.5	0.5	8.51	5.20
#1 (2009)	114.0	32.0	0.5	12.23	6.66
#8 (2009)	90.0	22.5	1.0	10.28	4.81

Table 3. Duration (Days) and Temperatures (°C) of monitored nests during the overwintering period during the seasons 2008/2009 and 2009/2010. Max. = maximum temperature, Min. = minimum temperature, Ave. = average temperatures, SD = standard deviation

Nest #	Duration	Max.	Min.	Ave.	SD
#2 (2008)	79.0	6.5	-4.0	0.27	1.98
#3 (2008)	65.0	7.0	-3.0	0.08	1.70
#5 (2008)	66.0	7.0	-3.5	0.08	1.80
#6 (2008)	61.0	6.5	-2.5	0.55	1.69
#8 (2008)	63.0	6.0	-2.5	0.44	1.61
#10 (2008)	61.0	9.0	-3.5	0.58	1.95
#1 (2009)	93.0	7.5	-6.0	0.03	1.74
#8 (2009)	93.0	8.5	-9.0	-0.30	2.06

Table 4. Survival success of monitored nests of *Emys orbicularis* in Slovakia. All hatchlings from successful nest left post-wintering, except nest #7 (2009)

Nest #	Living/ dead	Note
#2 (2008)	12/0	
#3 (2008)	7/5	
#5 (2008)	0/11	died in autumn 2008
#6 (2008)	8/2	
#8 (2008)	0/15	died in autumn 2008
#10 (2008)	6/4	
#1 (2009)	4/7	
#2 (2009)	?	destroyed by predator in 2009
#7 (2009)	7/9	left on 08 October 2009
#8 (2009)	?	lost in spring 2010

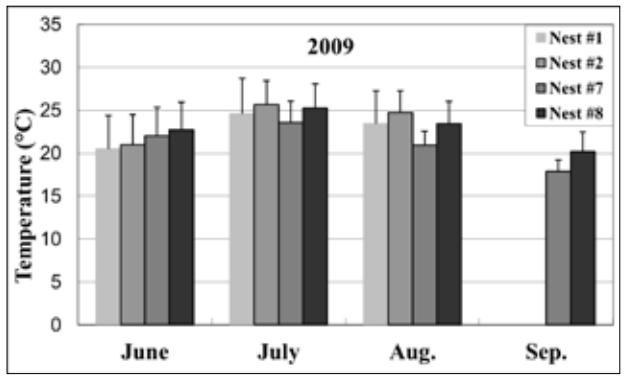

Fig. 2. Mean monthly nest temperatures of *Emys orbicularis* during incubation in 2009

Discussion

The European Pond Turtle is a reptile with a temperature-dependent sex determination (TSD) (Pieau 1974). At incubation temperatures below 27.51°C only males are produced, while at temperatures above 29.51°C the hatchlings are predominantly females; between 27.51–29.51°C both sexes can develop, while the pivotal temperature was established at 28.51°C (Hulin et al. 2009). Under natural conditions, however, temperatures are not nearly as ideal and certainly not constant. It is estimated that the sex of 83 percent of hatchlings in natural conditions is affected by genetic predisposition or maternal influence (Girondot et al. 1994).

In our study, in 2009, the average incubation temperature in all nests was 22.87 ± 0.83°C, with a maximum of 34.5°C. Similar temperatures were recorded in Austria and Germany (Rössler 1999, Schneeweiss et al. 1998).

The end of the incubation period is critical for the hatchling's survival because the nest becomes more olfactory and acoustically attractive for predators as the turtles are hatching. This theory is supported by our finding of the depredated nest #2 (2009).

During the post-incubation period hatchlings leave the nest chambers in some southern locations (Krasavzev 1941, Tertyšnikov & Gorowaja 1984). Likely due to lower temperatures in Slovakia and other northern latitudes hatchlings leave the nest during the post-hibernation period. However, for the first time since monitoring of nests and hatching were initiated in 1999 in Slovakia, we observed two cases of hatchlings leaving the nest early, in the post-incubation period. We also found that in this period the hatchlings from two nests #5 (2008) and #8 (2008) died still in the eggshells, possibly due to issues during incubation. Although we did not

measure incubation temperature in these nests, we recorded the lowest mean temperatures during the post-incubation period. So we can assume low temperatures even during the incubation period.

The length of the overwintering and survivorship of hatchling during this period are strongly influenced by the climatic conditions in a given year. Despite the different climatic conditions, we recorded successfully overwintered individuals for two years. Schneeweiss et al. (1998) observed successfully overwintering individuals in range between -9°C and -5°C, while they observed 100% mortality at -12°C. De Pari (1996) mentioned -10°C as survival temperature limit in nest chambers of *Chrysemys picta*. In our work the lowest measured temperature in the nest was -9°C; unfortunately, we are not able to confirm survival of the hatchlings because the nest #8 (2009) was lost in spring 2010. However, we can confirm successful overwintering at a temperature of -6°C in nest #1 (2009).

The post-overwintering period is characterized by abandonment of nests in most of the northern populations (Fritz & Gunther 1996, Schneeweiss et al. 1998, Mitrus & Zemanek 2000). In Tajba NNR hatchlings always left in this period since 1999, except for the two cases described in this paper. This was probably caused by the extremely favourable conditions during the year 2009.

Our results show that more studies are needed for some important phases of the nest period. We stipulate that measures to guarantee artificial nest protection during egg laying and hatching are key for decreasing predator pressure and increasing the hatching success. Increased sampling effort in Slovakia is needed during post-incubation, to estimate more accurately the climatic and other drivers that may lead to hatchling's early leaving of the nest. Long-term studies of nest temperatures with an increased sample size will help devise additional successful conservation measures to be utilized in other locations, including in the only population in Slovakia of reproducing *E. orbicularis*.

Acknowledgements: This publication has been undertaken within the framework of the projects VVGS PF 20/2007/B, VVGS PF 2/2008/B, VVGS PF 01/2009/B. The authors are grateful to CHKO Latorica. Special thanks to Yurii Kornilev for patience and help with editing the manuscript. We are also grateful to the Ministry of the Environment for granting permission (3757/2007-2.1).

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Accepted: 28.10.2017