Research Article

ACTA ZOOLOGICA BULGARICA
Acta zool. bulg., Suppl. 10, 2017: 139-145

History of the reintroduced population of the European Pond Turtle, *Emys orbicularis* (L., 1758) (Emydidae), at the Betlém site, South Moravia, Czech Republic

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Abstract: Between 1989 and 1994, a total of 30 (9 males, 21 females) European Pond Turtles *Emys orbicularis* (L., 1758), originally from the Romanian part of the Danube Delta, were gradually released into the protected Betlém wetlands, South Moravia. The site (N 48°54′23.414″, E 16°35′17.288″) is situated in the former inundation area of the Dyje River. The locality for this reintroduction was chosen primarily because this region had been inhabited by the European Pond Turtle in the 19th century. After the releases, the turtles have been systematically monitored since 1995. This is the first study to present data on how turtles have lived up to the present day. Turtles regularly reproduce in and around wetlands, enabling us to acquire a great deal of information concerning reproduction, including what is needed to place a nest, incubation success rates, predation pressure or the ways in which young spend the winter. Here, I present the first retrospective phenological and ecological data on the European Pond Turtles from the Czech Republic, where no autochthonous or other viable population of this species occurs. In 2010–2016, a total of 247 individuals were caught and tagged by making an incision in their marginal plate. All were measured, weighed and photographs were taken of the colouring on their plastron and carapace. Active measures are regularly taken to protect nests from predators in order to assure the successful survival of the population. All the obtained data show that the reintroduction process has been running successfully.

Key words: reptiles, *Emys orbicularis*, reintroduction, reproduction, protection, Betlém wetlands, Czech Republic

Introduction

The target area for the reintroduction of European Pond Turtles, *Emys orbicularis* (L., 1758), was deliberately located in South Moravia, in the basin of the river Dyje. According to locals from the village Mušov, the locality was still inhabited by turtles during the 19th century (Z. Kux, pers. comm.). This and adjacent areas have been regarded as a place where individual descendants from the original population might still occur (Randík et al. 1971, Baruš et al. 1992). However, during the last fifty years (c. 1930–1989), no occurrence has been documented, suggesting that the indigenous population is extinct. Therefore, a reintroduction program was initiated. From 1989 to 1994, a total of 30 (21 females, 9 males) adult European Pond Turtles, all originally from the Romanian part of the Danube Delta, were released into the wild at Betlém in South Moravia (Šebela 1994, 2012, 2015). Before being released, all were measured, weighed, photographed and tagged with a visible metal rivet mounted on the marginal plate. Since the release, the turtles in the wetlands were systematically monitored; phenological data and additional information on how they live were collected and recorded.

Materials and Methods

The Betlém site (N 48°54′23.414″, E 16°35′17.288″) is situated in South Moravia, in the former inundation area of the Dyje River. In 1979, the middle reservoir of three artificial lakes of Nové Mlýny (3,000 ha water area) was constructed very next to it. During the construction of the dams, half of the site was destroyed and the area was inundated with soaking water in 1979. As a result, a natural succession of plants and animals appeared and wetlands of approx. 4.5 ha came into being. Currently, the maximum depth is 1.20 m, the average being about 0.7 m and 75% of the area not exceeding 0.5 m. The character of the site has strongly influenced the natural development of the littoral vegetation (*Phragmites australis*, *Typha angustifolia*) covering approx. 2/3 of the wetlands. The water surface is totally overgrown with water plants (*Ceratophyllum demersum*). There are 15 satellite pools very next to the main wetlands. In the north wetlands pass into the riverine forest; on the contrary, their southern bank rises by approx. 4 m to the level of the original sandy gravel alluvium. This part of some 4 ha has the character of the forest-steppe with scattered tree and bush vegetation and compact grass cover.

Between 2010 and 2016, a total of 247 individuals were caught in life traps, some individuals repeatedly (see below). The life trap is an adapted fishpot with total basket length 1.5 m, diameter 1 m, wing length 1.5 m and height 1 m (Fig. 1). It has been made from wire mesh with holes of 3 cm diameter, including an air chimney towering above the water surface. The trap was installed in the depth of approx. 1 m, during the whole research period at the same place in the middle of the wetlands, from May to September. The captured turtles were measured and tagged using a notch into marginal shield and released immediately at the place of capture. The identification of specific individuals was ensured by photographing their plastron. I successfully determined the sex of 229 individuals (131 males, 98 females; 18 unidentified). The sex was determined on the basis of external features, particularly carapace and plastron shape. This method can be reliably used to determine sex on most specimens whose body length is more than approx. 80 mm. Iris coloration, tail length, shell height and other morphological features were also used to complement the sex determination. According to the number of growth zones (one zone per year) and carapace size, the animals were divided into three groups: juveniles (up to 3 years), subadults (4–7 years) and adults (more than 7 years) (see Results below). All the specimens caught were measured, weighed with a spring scale (±1 g), and photographed. The relative abundance and all bionomic manifestations (duration of active season, mating, laying eggs, influence of predators) were observed directly on the site and (or) supplemented

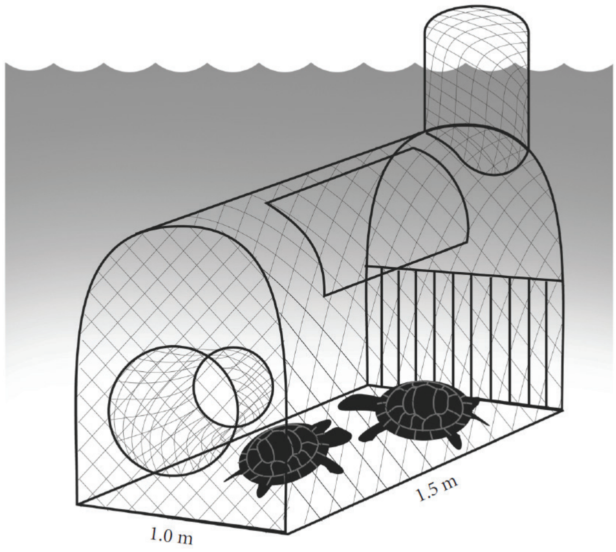

Fig. 1. Scheme of a life trap. Drawing by Jan Dungel

with capture-recapture method. The size of the population was estimated through the Jolly-Seber method. The collected morphometric data allowed to monitor the animals' growth and to appraise the age structure of the population. The analysis of protected nests made it possible to estimate to success rate of the reproduction. When nests are found they are protected with wire mesh to keep out predators. The total of the protected nests was 37 during 1989–2016.

Results

Sex ratio

The sex ratio for all the specimens caught using life traps and whose sex could be determined (229 specimens) was 1.33 : 1 in favour of males. The ratio between males and females caught in traps during the season changed only in the month of May (3.16 : 1), which is clearly caused by higher spatial activity amongst males during this reproductive period. It is also worth mentioning the fact that while in 2010–2012 and 2014 the sex ratio was in favour of males (1.11–1.87 : 1), in 2013 there was a predominance of females (1 : 1.22). Between 1989 and 2016 a total of 128 juvenile individuals were caught.

Spatial activities

For the whole time that the turtle population was monitored (1989–2016), the majority of adults remained in the main part of the wetlands and in the satellite pools, i.e. within an area of approx. 5 ha. The turtles' strong affinity to this relatively small territory is supported by recurring catches of an adult female in 2011 and 2013, an individual from the original import, released in 1994. The fact that it has remained in its new environment for almost twenty years suggests that the wetlands offer ideal living conditions. Further support of the affinity to this locality also lies in the high number of specimens tagged (163) in 2010–2013 and 95 re-captured in 2014. The turtles move around regularly in the wetlands and satellite pools throughout the season. These migrations usually occurred in the first half of the season and were limited to just a few days or weeks. Movements of adults outside the wetlands can be divided up into two types:

- regular, short-distance seasonal movements of females seeking places to lay their eggs: occurred every year between the main body of water and the land on the southern shore. These migrations led them to the same places every year and kept within a maximum distance of 250 m from the shore. Only in two cases females were observed searching for a suitable place to lay their eggs near the dam of the middle reservoir of the Nové Mlýny Waterworks (approx. 150 m from the shore of the wetlands);
- long-distance, irregular migrations concerning only a limited number of specimens (n = 15) were observed occasionally at various periods in different localities up to 5 km away from Betlém. The small number of specimens migrating outside the wetlands and the abundance of the turtle population proved by recaptures show clearly that most turtles stay faithful to the wetlands in which they were born.

Active season and hibernation

Usually around mid-March the turtles came out of their winter hibernation and, only on sunny days, when the air temperature reaches around 10°C, began to bask in the sun on beds of reeds and floating tree trunks. At this time, the water in the wetlands only reached a temperature of around 5–6°C. The earliest observations of two adult specimens basking were made on 23.2.2014, during an unusually very warm weather during the second half of February. The average numbers of turtles observed basking culminates towards the end of April. As the water gradually gets warmer and the number of warm and sunny days increases during June, the number of turtles basking decreases, as the turtles spend more time in the water. The highest numbers were observed during the last ten days of April and at the beginning of May and then only under optimal weather conditions. The maximum numbers recorded during a particular season do not reflect actual numbers and serve merely as a relative indicator (2013 – 73 specimens, 2011 – 56 specimens). The end of the active season is heralded by fewer basking turtles; due to less sunshine the turtles stay more in water the temperature of which in September can still reach 15 to 19°C. From the middle of September, turtles bask only sporadically and there are intervals of a few days between sightings of individual turtles. The last basking turtles were most frequently observed during the last ten days of September. A unique situation occurred at the end of 2010, when the warm and sunny weather (water temperature of around 10°C) prolonged the turtles' active season until the end of October. According to observations of last basking turtles, most of them hibernate every year in several traditional places in the main part of the wetlands with the depth of 60–80 cm, always in close proximity to tufts of reed and bulrushes. In three places approx. 10 × 15 m in size, the turtles regularly pass the winter in groups of 15 or more specimens. It was only in two cases that turtles hibernated in the two satellite pools. In the places where the turtles hibernate, water temperatures on the bottom reach around 2°C (0.7–3.5°C).

Population density

The average estimation of the population was 247 specimens (Jolly-Seber method: N2 – 84.21, N3 – 301.62, N4 – 177.81, N5 – 638.52, N6 – 88.85). The number of captured individuals is comparable – some 300 individuals including juvenile animals that had not been caught with life traps.

Growth

The number of scute growth rings and grooves (growth ridges) and carapace and plastron plates were used to estimate age and to determine the relevant age groups (Tables 1–3), as are used by many other authors (Auer & Taskavak 2004, Meeske 2006, Mitrus & Zemanek 2004, Schneeweiss 2003, 2004) when estimating the age of this species. Therefore, the results detected and analysed must be considered applicable for the study population. The large variability in the body length (min. 23.0, max. 30.0, average 26.3 mm) of new-born specimens also evidently determines their subsequent growth, which eventually results in a relatively large size range within the different age classes. The abdominal plates (pectoralia, abdominalia, femoralia) are the most suitable means of assessing evident growth ridges, where the structure of the growth zones lasts longer than on the carapace plates. In males, 8 to 9 apparent growth ridges are generally formed, which are then followed by grooves packed together. In one isolated case, a female was found to have 14 discernible growth rings, but the maximum number is generally around 10 to 12. Using growth rings to divide up the various

Table 1. Growth (LC in mm, weight in g) – young animals of both sexes

		LC		weight	
number of growth rings	n	range	median	range	median
2	9	59–76	69.9	65–110	85.0
3	14	77–98	86.1	90–220	135.0

Table 2. Growth (LC in mm) – age groups according to growth rings

	males			females		
number of growth rings	n	range	median	n	range	median
4	18	89–116	102.2	17	87–119	100.4
5	35	93–138	117.9	26	99–135	111.7
6	33	109–152	130.2	23	105–148	128.6
7	22	128–150	142.3	29	130–159	145.2
without growth rings	71	132–169	154.3	64	140–197	173.0

Table 3. Growth (weight in g) – age groups according to growth rings

	males			females		
number of growth rings	n	range	median	n	range	median
4	17	120–320	198.2	14	100–270	189.3
5	37	160–440	287.3	24	180–355	301.0
6	41	260–510	390.6	22	200–510	376.8
7	19	340–660	485.3	21	370–765	566.4
without growth rings	52	420–670	543.9	72	470–1150	843.9

age groups enabled me to exactly determine the age of the specimens caught up to a maximum of seven years of age. In the third season of their active life some young turtles reach sizes exceeding 85 mm, at which point secondary sexual characteristics begin to form on their bodies, enabling their sex to be determined. Most of all, this is a concave curvature of the plastron in males, which is the most suitable external morphological feature for determining the sex of an individual.

The representation of different age groups in the sample indicates only the approximate population structure, as the catching equipment used was selective and did not catch the youngest specimens. Nevertheless, more than a half (n = 168) of the specimens caught are sub-adults aged 4–7 years, which indicates that this is a very healthy and viable population that reproduces successfully on a regular basis (Figs. 2 and 3).

Reproduction

Between 2003 and 2016, reproductive behaviour and mating turtles were recorded in a total of eight cases. Males were observed to approach females in aerial basking places, but mating occurred in the water. Mating was observed during the last third of April (in 5 cases) and in May in the rest (Fig. 4). A total of 62 pieces of direct evidence of laying (nesting females and all types of nests) were found in the immediate vicinity of the wetlands. These findings show that eggs are laid from the middle of May to the first ten days of July, and from the way the data are distributed during this period it is clear that laying peaks during the first ten days of June (46.8% of all broods). All females lay just one brood during the season. Only one nest, found destroyed by a predator on 31.07.2003, may indicate a second brood as described by Rössler (2000b) and Kotenko (2000).

From 1998 to 2016, a total of 23 females were encountered searching for a suitable place to dig their nest hole or laying eggs. The average size of laying females (body length = 173.3 mm, min. 154.0 mm; max. 188.0 mm, n = 12) matches the boundary for sexually mature females (e.g. Meeske 2006). Further data about laying were acquired from 25 freshly-laid broods, found the morning after they had been laid. The character of the location of all the nest chambers found implies that their placement is governed by a number of rules. In particular, a nest hole was never dug out in a shady spot beneath a tree or a bush. If a nest was near a tree, for example, it always faced the south, i.e. so that no shadow would

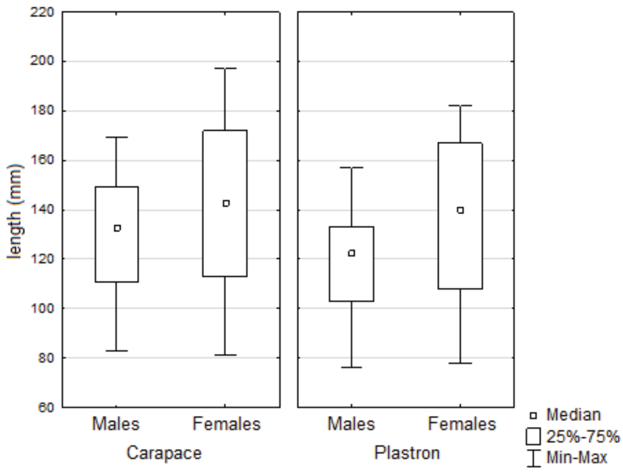

Fig. 2. Differences in the carapace and plastron size according to sex (n = 130 males, 83 females)

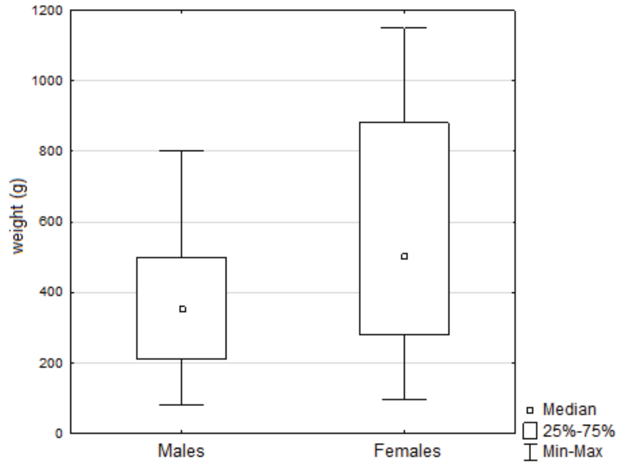

Fig. 3. Differences in weight according to sex (n = 94 males, 77 females)

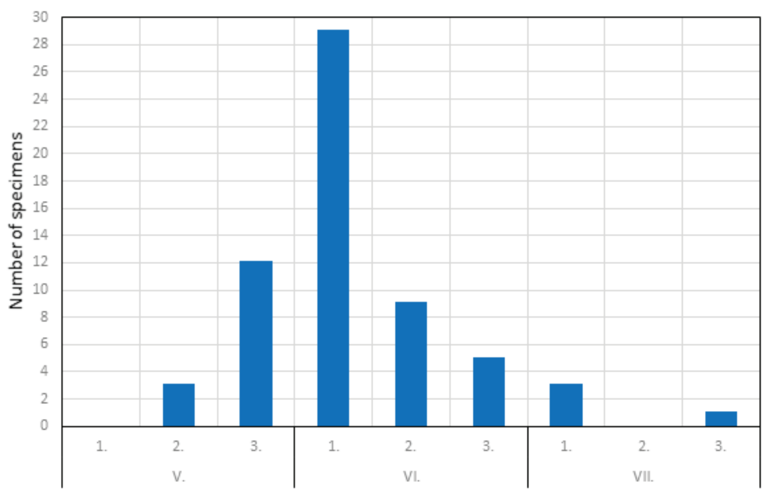

Fig. 4. Distribution of laying eggs during May–July (n = 62)

be cast over it. On slightly undulated terrains nests were always positioned on eastern or southern facing side. In all cases the sites most preferred consisted of hard-packed clay and sandy soil, with sparse herbaceous vegetation.

All the identified nests (n = 109) were used to gauge the distance of the nest holes from the water. In over half the cases the females dug their nesting holes within a distance of 80 m from the bank (min. = 6 m, max. = 250 m).

The eggs were counted in the nests after hatching as well as in all the nests where incubation did not happen. The average number of eggs per nest was 8.2 (range 6–13; SD = 1.8; n = 32). All not damaged and for various reasons not incubated eggs were measured during the whole research. The average egg size was 33.5 × 19.4 mm (min. 31.0 × 18.5 mm; max. 34.0 × 21.0 mm, SD = 1.14). Only in 21 nests (65.6%; n = 32) viable young hatched, averaging 7.3 juvenile specimens per clutch. These nests had a relatively high hatching success rate (85.3%). Unfertilised eggs and dead young were also found in these nests (14.7%). For all 32 nests, 145 juveniles (56.2%) successfully hatched from 257 eggs.

In protected nests, up to 2010 juveniles did not leave the nest until the following April–May. Almost half of these cases occurred in the last ten days of April. While monitoring juvenile turtles leaving the nest, it was found that the very earliest date was 8.4.2007 and the latest was 20.5.1999. Juveniles that overwintered successfully left the earliest on 8 April (2007) and the latest on 20 May (1999). Only in three cases hatched turtles left the nest hole the same year (two cases on 1.10.2011 and on 12.09.2013), similar to other European localities (Gay & Lebraud 1998, Mitrus & Zemanek 1998, Rössler 1999, Kotenko 2000). Both these seasons had very warm summers with above-average temperatures lasting until the end of September. A major reasons for hatchlings dying in the nest is several days long prolonged severe frost (below -15°C) when the ground is not yet covered by a layer of snow. In one case, all the young had died as roots had penetrated deep into the nest.

The local predators that abound in the vicinity also greatly influence the success rate of hatching. A total of 84 dug-out nests and destroyed broods were found (65.4% of all nests). In 48 of these cases the species of predator could be determined from the way the eggs were dug out, from damage to the shells and from paw prints. The highest number of nests destroyed was by the Red Fox (*Vulpes vulpes*) – 28, and the Badger (*Meles meles*) – 19. One nest was accidentally destroyed by a Common Mole (*Talpa europaea*) – the eggs were not eaten. I did not identify nests disturbed by a Wild Boar (*Sus scrofa*) or Raccoon Dog (*Nyctereutes procyonoides)*, which also occur in this area. An overview of broods disturbed during the year clearly shows that the vast majority of nests were dug when the broods were being laid or during the following weeks – a total of 71 broods (i.e. 84.5%) during May–July.

Discussion

When comparing our results to other reintroduction projects in Europe, the results are not at all unequivocal. All previous documented attempts carried out in the Czech Republic have failed (Pecina 1995, Mikátová et al. 2001, Moravec et al. 2015), as well as the introduction project in western Slovakia (Burešová et al. 2001). In contrast, in its first two years the reintroduction project in Savoy (Cadi & Miquet 2004) had a very positive start and excellent results were also attained in one of the first introduction projects carried out after 1950 at Moulin-de-Vert in the Rhone basin in Switzerland (Mosimann & Cadi 2004). Reintroduction projects are not currently being implemented in the Czech Republic, as in general terms the conditions governing reintroduction are very strictly defined in relation to the systematic status of each subspecies and their alleged differing environmental and climatic requirements (Fritz 1996, 1998, Lenk et al. 1999). Far more common are various rescue programmes (Fritz & Chiari 2013) aimed at protecting the surviving remnants of the original populations, which combine elements of both legislative and active protection of localities, including revitalisation work, reducing agricultural usage, or directly protecting brooding grounds and individual clutches (Mitrus 2000, Maciantowicz & Najbar 2004).

Our reintroduction effort with individuals from the supposed subspecies to occur in Czech Republic but taken from approx. 2,000 km away demonstrates that the individuals can successfully survive, reproduce and establish a new population. On the contrary, some results relating to growth or reproduction indicate a high degree of the adaptability of this species required for the newly established population to develop successfully.

This is a good point to compare the specimens, which have been transferred from the Danube Delta to Betlém and which were part of the origin of this population: 8 females with an average body length of 150.8 mm (min. 137 mm, max. 164 mm). Despite being smaller in size, they showed evident features of older specimens and some were already sexually adult with fertilised eggs in their bodies, as they laid eggs immediately after being released into the wild and their reproduction continued in the following years. The oldest specimens born in Betlém are now aged up to 25 years old and measurements show that their maximum and average body lengths are greater than their parents. If we exclude the possibility that some other, large turtles were released into the research locality at the beginning of this project – and this has been clearly ruled out by systematic catches over a period of five years – we must admit that trophic and climate conditions can have a fundamental influence on the specimens' growth and morphometric parameters. Even though the original set of released specimens is relatively small, these preliminary results indicate that the environment and real "ecomorph" development play a significant role, just as similar differences in the sizes of turtles in southern and northern populations in Dagestan (Bannikov 1954).

The successful long-term survival of the reintroduced population in South Moravia also disproves theories (Mikátová et al. 1995) concerning the unsuitability of the climate in these latitudes for the reproduction of this species, or concerning the lack of suitable localities (Zavadil & Moravec 2003). The success of this reintroduction is due not only to the choice of a suitable locality but also to the high adaptability of this species. At the same time, changes in the reproductive cycle (hatchings leaving the nest earlier as in late summer in 2011 and 2013) or the shortening of the winter hibernation period in mild winters are due to the rising average annual temperatures in recent years, as documented by the data of the Czech Hydrometeorologic Institute (+0.9ºC and +1.2ºC compared to long year average in 1961–1990).

Active protection of this population primarily relies on limiting human disturbance throughout the locality by fencing that prevents people from entering (but not turtles and other animals), coupled with regular checks. When nests are found, they are protected with wire mesh to keep out predators as well as with a layer of thermal insulation during heavy frosts. Due to the gradual succession of vegetation cover in the places where clutches were laid regularly and the declining quality of these areas, work commenced in 2013 to build a clay rampart that should offer suitable conditions for laying eggs. Since 2014, turtles already have laid eggs there. At the same time, two shallow pools were also created, which should provide the right environment for young turtles in particular to survive in.

Acknowledgements: I am grateful to Prof. J. Gaisler (†), Z. Řehák (Masaryk University, Brno) and my colleague M. Černý for their help with the elaboration of the manuscript. I thank R. Suchánek (†) and K. Lisal for the support of the project directly on the site.

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Accepted: 14.11.2017